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Reconsidering transmission electron microscopy based estimates of viral infection of bacterioplankton using conversion factors derived from natural communities

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ABSTRACT: The frequency of virus infected bacterial cells (FIC) was estimated in surface waters of the Mediterranean Sea, the Baltic Sea and the North Sea using the frequency of visibly infected cells (FVIC) as determined by transmission electron microscopy (TEM) and published average conversion factors (average 5.42, range 3.7 to 7.14) to relate FVIC to FIC. A virus dilution approach was used to obtain an independent estimation of FIC in bacterioplankton, and we provide evidence for the reliability of this approach. Across all investigated environments, FIC ranged from 2.4 to 43.4%. FIC data using both approaches were well correlated; however, the values were higher using the virus dilution approach. This indicates that the TEM approach has the potential to reveal spatiotemporal trends of viral infection; however, it may underestimate the significance of viral infection of bacteria when average conversion factors are used. Using data from the virus dilution approach and the TEM approach, we calculated new conversion factors for relating FVIC to FIC (average 7.11, range 4.34 to 10.78). Virally caused mortality of bacteria estimated from published FVIC data of marine and freshwater systems and using the new conversion factors ranged from not detectable to 129%, thus confirming that viral infection is a significant and spatiotemporally variable cause of bacterial cell death.

KEY WORDS: Phage · Virus · Mortality · Lysogeny · Latent period

INTRODUCTION

The incorporation of the role of viruses into the microbial food web has refined our understanding of the ecological and biogeochemical role of microorganisms in aquatic systems (Fuhrman 1999, Wilhelm & Suttle 1999, Wommack & Colwell 2000). For example, viral lysis of microbial cells converts biomass into dissolved organic matter, and thus elements such as C, P, N, S and Fe should be retained longer in the euphotic zone. This lysed biomass is diverted from higher trophic levels and shunted into the dissolved organic matter pool. Such viral lysis products seem to sustain bacterial production and respiration and, by that, the cycling of energy and matter mediated by bacteria. Also, viral lysis may be a main mechanism influencing bacterial community composition (Fuhrman 1999, Wommack & Colwell 2000).

Viral production and virally caused mortality of bacteria (VMB) are crucial parameters for assessing the detailed role of viruses in food webs. Efforts have been made to measure these processes *in situ* using methods

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such as quantifying the net increase of viral abundance over time (Bratbak et al. 1990), adding the virus size fraction of seawater and assessing the effect on bacterioplankton (Proctor & Fuhrman 1992), estimating viral DNA synthesis by radiolabeled substrates (Steward et al. 1992), using fluorescent labeling of viruses and measuring tracer dilution rates (Noble & Fuhrman 2000), quantifying decay rates of infectivity (Suttle & Chen 1992) or particles (Heldal & Bratbak 1991, Wilhelm et al. 1998), via a virus dilution approach (Wilhelm et al. 2002) or by assessing the frequency of visibly infected cells (FVIC) using transmission electron microscopy (TEM) (Proctor et al. 1988, Proctor & Fuhrman 1990, Bratbak et al. 1992, Weinbauer et al. 1993). Direct estimation of FVIC with TEM was done using thin sectioning (Proctor & Fuhrman 1990) or whole cell inspection (Bratbak et al. 1992, Weinbauer et al. 1993). A virus dilution approach was also used to estimate the percentage of lysogens in a bacterial community, i.e. the number of cells containing a functional prophage genome (Weinbauer & Suttle 1996).

One of the very first reports suggesting that viruses are significant players in microbial food webs estimated that up to 7% of the bacteria in marine systems contained mature virus particles corresponding to up to 70% infected bacteria (Proctor & Fuhrman 1990). These data were based on the assumption that mature phage particles are only visibly during the last 10% of the latent period. Later these authors performed an electron microscopy study with isolated phage-host systems to obtain more reliable conversion factors (average 5.42, range 3.7 to 7.14) for relating FVIC to the frequency of virus infected bacterial cells (FIC) (Proctor et al. 1993). They also developed a model to relate FIC to VMB. This model assumes that a mechanism that removes 50% of the cells is responsible for 100% of the mortality in steady state. Thus, FIC has to be multiplied by 2, in order to get an estimate of viral mortality of bacteria. This 'factor of 2' rule was later replaced by a more rigorously defined model (Binder 1999).

This study was performed to compare viral infection frequencies of bacteria using a TEM and a virus dilution approach in various environments. On the basis of these data, we calculated conversion factors for relating FVIC to FIC based on whole community investigations. Also, we used these conversion factors to re-evaluate published data on FVIC, FIC and VMB from bacterioplankton.

MATERIALS AND METHODS

Study sites and sampling. The study sites were 2 stations in the Mediterranean Sea (Stn B1 in the center of the Bay of Villefranche-sur-mer, 43° 41′ N, 7° 19′ E, and Stn Dyfamed, 30 nautical miles off Nice, 43° 25′ N, 7° 51′ E) and 2 stations in the Baltic Sea (Stn BY15 in the Gotland Deep, 57° 11′ N, 20° 18′ E, and Stn Teili1, 59° 16′ N, 21° 18′ E), as well as several stations in the North Sea. In the Mediterranean Sea, samples were obtained in July 1998, June 1999 and December 1999, and samples from the offshore station were collected on board the RV 'Thetys II'. In the Baltic Sea, water was collected during a cruise from September 14 to 25, 1998, with the RV 'Aranda'. Samples from the North Sea were obtained during 2 cruises from July 10 to 21, 2000 (Plume and Bloom I [PBI]), and from September 11 to 21, 2000 (PBII), with the RV 'Pelagia' at Stns 112 (53° 34′ N, 4° 15′ E), 124 (52° 50′ N, 2° 50′ E), 132 (53° 36′ N, 4° 30′ E), 138 (53° 36′ N, 4° 30′ E), 205 (53° 15′ N, 3° 30′ E), 209 (52° 45 ′N, 3° 30 ′E), 213 (53° 41′ N, 0° 56′ E), 217 (53° 50′ N, 0° 10′ E) and 228 (53° 31′ N, 3° 46′ E). All samples were obtained from surface waters (2 to 5 m) using surface pumps or Niskin bottles.

Estimation of viral and bacterial abundance, burst size and FVIC. Bacteria and viruses from 0.8 to 2 ml formaldehyde (2% final concentration) preserved samples were collected onto 0.02 µm pore-size Anodisc filters, stained with SYBR Green I (10 000× in dimethyl sulfoxide [DMSO]; Molecular Probes, Chemical No. S-7567) and enumerated using epifluorescence microscopy as described in Noble & Fuhrman (1998). FVIC and the burst size, i.e. the number of phages set free during lysis of cells, were determined for each sample by TEM (Weinbauer & Suttle 1996) in the bacterial concentrates used for the virus dilution approach (see below). Burst size can be estimated as the average number of viral particles in all FIC (minimum) or as the average number of viral particles in all visibly infected cells completely filled with viral particles (maximum burst size). Electron micrographs of cells filled completely or partially with viruses can be found in Figs. 2 & 3, respectively, of Weinbauer & Peduzzi (1994). In the present study, we used the maximum burst size determined from at least 20 visibly infected cells to obtain a reliable burst size estimate.

Estimation of viral infection of bacterioplankton. The FVIC as determined by TEM was related to FIC using published conversion factors (average 5.42, range 3.7 to 7.14) (Proctor et al. 1993) as well as the model of Binder (1999) with the following equation:

$$FIC = 7.1\ FVIC - 22.5\ FVIC^2 \quad (1)$$

where data are expressed as percentages. To obtain an independent estimate of FIC, we used virus dilution approaches developed for estimating lysogeny (Weinbauer & Suttle 1996) and viral production (Wilhelm et al. 2002). In one of these virus dilution approaches (Weinbauer & Suttle 1996), bacterial concentrates were

made from seawater after prefiltration through 10 µm pore-size Nitex screening by using a 0.2 µm pore-size polycarbonate cartridge (Nuclepore). In the North Sea, water samples were prefiltered through 0.8 µm pore-size polycarbonate filters, and bacteria were concentrated using a tangential flow system (Pellicon, Millipore) equipped with a 0.2 µm pore-size polycarbonate cartridge. To obtain virus-free seawater, 0.2 µm pore-size filtered seawater was passed through spiral cartridges (Amicon S1Y100; 100 kDa cutoff; Amicon S1Y30, 30 kDa cutoff; Millipore) using a tangential flow ultrafiltration system (Amicon M12) or a peristaltic pump (6045; Watson-Marlow Bredel). Bacterial concentrates were brought up to the original volume with virus-free seawater (virus dilution-bacterial concentrates [VD-BC]). In the other approach (Wilhelm et al. 2002), prefiltered seawater was filled into a filtration unit equipped with a 0.2 µm pore-size polycarbonate filter. Bacteria were kept in suspension with a pipette while the viruses were washed out by maintaining the water volume with virus-free, ultrafiltered seawater (virus dilution-rinsing [VD-R]). The rationale behind both virus dilution approaches is to reduce virus abundance, in order to stop new viral infection. Thus, the viruses produced originate from already infected cells.

Lysogeny was estimated with the virus dilution approach (VD-BC) and by using mitomycin C (Sigma Chemical Co, No. M-0503, final concentration 1 µg ml^{-1}) as the inducing agent of the lytic cycle in lysogens; untreated samples served as controls (Jiang & Paul 1996, Weinbauer & Suttle 1996).

Incubations were done in duplicate 50 ml Falcon tubes, and samples for the enumeration of viruses and bacteria were taken periodically. Temperature was kept within ±2°C of the original water temperature by flow-through seawater or incubation chambers.

Estimation of VMB. VMB was estimated using the model of Binder (1999) where

$$VMB = (FIC + 0.6\ FIC^2)/(1 - 1.2\ FIC) \quad (2)$$

and data were expressed as percentages. In this equation it is assumed that infected and uninfected bacteria are grazed at the same rate and that the latent period equals the bacterial generation time (Proctor et al. 1993, Guixa Boixereu 1996). Also, steady state is assumed in this model.

RESULTS AND DISCUSSION

The model and data for estimating lytic and lysogenic viral production by using the VD-BC approach are shown in Fig. 1. Lytic viral production is the difference between viral abundance in the stationary phase of the control incubations and viral abundance at the start of the experiment. Lysogenic viral production is the difference between viral abundance in the mitomycin C treated and viral abundance in the control incubations. Dividing virus production by the burst size gives an estimate of the number of infected and induced cells, respectively, and thus of the percentage of FIC and lysogeny, respectively.

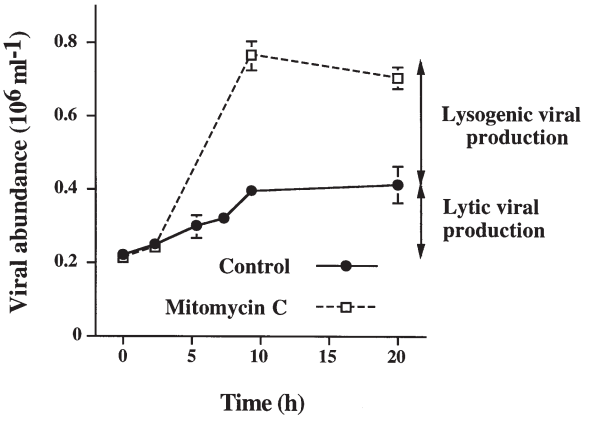

Fig. 1. Model and data for estimating lytic viral production and prophage induction. Data are from samples collected at the Dyfamed station in the Mediterranean Sea on July 22, 1998. Data are mean values and error bars the range of duplicate incubations. When error bars are not shown, they are smaller than the symbol

It is known from isolated bacterial lysogens that rapid growth can induce the lytic cycle (Calendar 1970, Gottesman & Oppenheim 1994). Moreover, Ripp & Miller (1997) have suggested that pseudolysogeny can be seen as the phenomenon where there is not enough energy available in starved cells for the phage to enter either the lytic or the lysogenic life cycle. Supplying nutrients could then be the trigger for forcing viruses to enter one of the 2 cycles. Both mechanisms could affect the estimates of lytic viral infection and lysogeny using virus dilution approaches, since a fraction of the bacteria is lost during the experimental procedure (likely by adsorption of cells to filters), and this dilution of bacteria might stimulate growth rates.

To check the potential bias induced by these mechanisms in the virus dilution approach, we added bacterial concentrates in decreasing concentrations to virus-free seawater. The expected increased growth rate due to a decrease of bacterial abundance could act as inducing agent or force a lytic or lysogenic response upon bacteria infected in a pseudolysogenic state. In these growth rate experiments (Table 1), bacterial concentrates were added back to virus-free seawater at abundances corresponding to 200 and 20%, respectively, of the original volume or to ca. 90–145% and 9–15%, respectively, of the *in situ* abundances. In the virus dilution experiments (see Table 3), bacterial re-

covery was calculated as the percentage of the number of bacteria in the experiments compared to the abundance of bacteria *in situ* and averaged 58.3% (range 45.2 to 75.0%) in the VD-BC approach and 21.9% (range 18.2 to 25.6%) in the VD-R approach. Thus, these values from the virus dilution experiments fall within the range tested in the growth rate experiments. The growth rates as determined from a linear increase of ln-transformed bacterial abundance with time increased, on average, 3.8-fold (range 3.1 to 5.6) due to a reduction of cell density (Table 1). In contrast, FIC and lysogeny values changed only slightly with the treatment, and the ratio averaged 1.06 (range 0.87 to 1.31) for FIC and 1.12 (range 1.10 to 1.14) for lysogeny (Table 1). In additional experiments performed with coastal and offshore seawater in the Mediterranean Sea in July 1999, bacterial concentrates were added to virus-free ultrafiltrate at ca. 100, 50, 10 and 5% of the *in situ* abundance, and bacterial production was estimated by ^{3}[H]-thymidine incorporation. The cell-specific bacterial production increased with the dilution factor (data not shown), thus further supporting the finding that the dilution stimulated bacterial growth.

Overall, these results indicate that an increasing growth rate was not a significant inducing agent in our experiments, and pseudolysogeny was of minor importance. This corroborates previous findings that an increase of bacterial growth rate due to dilution is no significant mechanism for prophage induction (Wilcox & Fuhrman 1994, Weinbauer & Suttle 1996). Our data do not rule out the occurrence of prophage induction by enhanced growth rates or pseudolysogeny; however, these mechanisms did not seem to interfere with the virus dilution approach. Thus, the virus dilution approach for estimating FIC (and lysogeny) appears to be robust against changes in bacterial growth rate.

Table 1. Effect of dilution of bacterial abundance on growth rates, frequency of infected cells (FIC) and lysogeny. Experiments were performed in the Mediterranean Sea. Growth rates were estimated as linear increase of bacterial abundance over time after ln transformation. Bacterial concentrates were added to virus-free seawater at abundances similar to the natural community (Reconc.) or at ca. 10% of the natural community (Diluted). NA: not applicable; ND: not determined

Environment	Growth rate (d^{-1})			FIC (%)			Lysogeny (%)		
	Diluted	Reconc.	Ratio	Diluted	Reconc.	Ratio	Diluted	Reconc.	Ratio
Coastal, July 1998	0.049	0.016	3.08	13.2	11.8	1.12	ND	5.2	NA
Offshore, July 1998	0.092	0.025	3.74	8.1	9.4	0.87	ND	9.2	NA
Coastal, June 1999	0.122	0.038	3.19	15.2	17.3	0.88	6.3	5.7	1.10
Offshore, June 1999	0.126	0.023	5.55	10.9	9.5	1.15	21.9	19.2	1.14
Coastal, December 1999	0.088	0.025	3.52	6.2	4.7	1.31	ND	24.1	NA

Table 2. Water temperature, salinity, burst size and bacterial and viral abundance at the study sites

Environment	Temperature (°C)	Salinity (psu)	Bacteria (10^6 ml^{-1})	Viruses (10^6 ml^{-1})	Burst size
Mediterranean Sea					
July 1998, B1	24.5	38.0	0.55	5.2	21
July 1998, Dyfamed	24.4	38.7	0.51	5.5	27
June 1999, B1	22.1	38.0	1.07	23.1	18
June 1999, Dyfamed	21.3	38.4	1.12	29.6	22
December 1999, B1	16.8	38.1	0.78	2.8	23
Baltic Sea					
BY15	14.9	6.7	2.15	23.4	32
Teili1	15.3	6.7	1.91	32.9	27
North Sea					
Stn 112	14.5	34.3	1.3	27.1	47
Stn 124	14.7	34.4	1.3	22.9	28
Stn 132	14.7	34.3	0.93	22.1	35
Stn 138	14.8	34.3	1.1	27.7	23
Stn 205	17.0	34.3	0.69	30.5	26
Stn 209	17.4	34.9	1.43	16.3	29
Stn 213	15.1	34.7	0.95	17.4	22
Stn 217	14.2	34.2	1.39	17.1	40
Stn 228	17.2	34.2	0.93	6.8	21

Bacterial and viral abundance in the investigated environments ranged from 0.5×10^6 to 2.2×10^6 ml^{-1} and from 5.2×10^6 to 32.9×10^6 ml^{-1}, respectively (Table 2). Estimates of FIC obtained by the VD-R approach were 0.97 to 1.3 times of those obtained with the VD-BC approach, suggesting that similar data were obtained by the 2 virus dilution approaches (Table 3). The finding that the VD-R approach leads to essentially the same values of viral infection as the VD-BC approach indicates that the potentially increased contact rates between viruses and bacteria during concentration of bacteria did not result in significant infection. Across environments, FIC values estimated by the virus dilution approach ranged from 3.7 to 43.4%, thus covering almost the entire range of FIC values typically found in marine environments (Wommack & Colwell 2000) (Table 4). FIC data obtained with the TEM approach and the virus dilution approach correlated well (Table 3, Fig. 2). This indicates that both approaches should reveal the same trends in space and time of viral infection of bacteria. However, at high FIC values (ca. ≥20%), TEM based values were lower than data based on the virus dilution approach. The 2 ways to calculate FIC from TEM based FVIC resulted in similar values with slightly higher values using the model of Binder (1999) (Table 3, Fig. 2).

We calculated conversion factors for relating FVIC to FIC by using FIC data determined by the VD-BC approach and FVIC data determined by the TEM approach. The FVIC data were obtained from the same bacterial concentrates used in the VD-BC approaches for estimating FIC, which makes a direct comparison possible. The conversion factors ranged from 4.34 to 10.78 (average ± standard deviation [SD], 7.11 ± 1.729) (Table 3). Proctor et al. (1993) reported conversion fac-

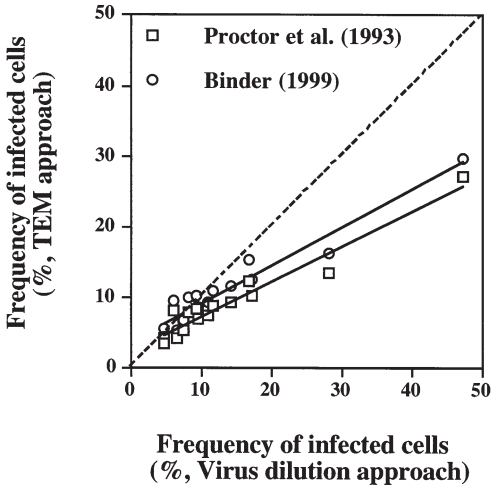

Fig. 2. Relationship between the frequency of infected cells (FIC) determined by the transmission electron microsopy (TEM) and the virus dilution approach. For the TEM approach, an average conversion factor of 5.42 (Proctor et al. 1993) was used to relate FVIC to FIC as well as Eq. 1 of Binder (1999). The dotted line shows the 1:1 relationship

Table 3. Comparison of methods for estimating percent viral infection of bacterioplankton. For the transmission electron microscopy (TEM) approach, data are from average conversion factors, and data in brackets are from the range of published conversion factors using data of Proctor et al. (1993) (TEM-P) or the model (Eq. 1) of Binder (1999) (TEM-B). Conversion factors in the table were calculated from the frequency of visibly infected cells (FVIC) and FIC as determined from the virus dilution approach

Environment	FVIC	FIC				Conversion factor
		TEM-P	TEM-B	VD-R	VD-BC	
Mediterranean Sea						
July 1998, B1	1.64	8.9 (6.1–11.7)	11.0	–	11.8	7.18
July 1998, Dyfamed	1.54	8.3 (5.7–11.0)	10.4	–	9.4	6.12
June 1999, B1	1.89	10.2 (7.0–13.5)	12.6	17.2	17.3	9.14
June 1999, Dyfamed	1.29	7.0 (4.8–9.2)	8.8	9.7	9.5	7.36
December 1999, B1	0.65	3.5 (2.4–4.6)	4.5	6.1	4.7	7.25
Baltic Sea						
BY15	1.75	9.5 (6.5–12.5)	11.7	–	14.4	8.20
Teili1	1.48	8.0 (5.5–10.6)	10.0	–	8.3	5.60
North Sea						
Stn 112	1.39	7.5 (5.1–9.1)	9.4	–	6.9	4.97
Stn 124	0.98	5.3 (3.6–7.0)	6.7	–	7.1	7.24
Stn 132	0.96	5.2 (3.6–6.9)	6.6	–	6.6	6.91
Stn 138	4.99	27.0 (18.5–35.6)	29.8	–	43.4	8.70
Stn 205	0.82	4.4 (3.0–5.9)	5.7	–	3.7	4.56
Stn 209	0.79	4.3 (2.9–5.6)	5.5	–	6.5	8.23
Stn 213	2.51	13.6 (9.3–17.9)	16.4	–	27.1	10.78
Stn 217	1.43	7.8 (5.3–10.2)	9.7	–	6.2	4.34
Stn 228	2.33	12.6 (8.6–16.6)	15.3	–	16.9	7.25

Table 4. Summary of published FVIC data from marine and freshwater environments and calculated FIC and virally caused mortality of bacteria (VMB) values. FIC values were estimated from FVIC using the average conversion factor of 7.11 determined in this study

Environment	Technique	FVIC (%)	FIC (%)	VMB (%)	Source
Marine					
Long Island Sound	TS	4.1	25.3	41.9	Proctor & Fuhrman (1990)
E Caribbean Sea	TS	3.9	24.3	39.3	Proctor & Fuhrman (1990)
W Caribbean Sea	TS	2.8	16.1	25.7	Proctor & Fuhrman (1990)
Sargasso Sea	TS	0.9	6.2	7.0	Proctor & Fuhrman (1990)
Gulf Stream	TS	4.3	26.4	44.7	Proctor & Fuhrman (1990)
Bering/Chukchi Sea	WC	0.2–3.3	1.4–21.0	1.4–31.6	Steward et al. (1996)
Santa Monica pier	TS	2.3	15.1	20.2	Fuhrman & Noble (1995)
N Adriatic Sea	WC	0–4.2	0–25.9	0–43.3	Weinbauer & Peduzzi (1995)
NW Mediterranean Sea	WC	0.7–1.9	4.5–12.6	4.9–16.0	This study
North Sea	WC	0.8–5.0	5.5–29.8	6.0–54.8	This study
Baltic Sea	WC	1.5–1.8	10.0–11.7	12.1–14.6	This study
Solar saltern	WC	0–3.8	0–23.5	0–43.3	Guixa-Boixereu et al. (1996)
Sinking particles	TS	0.7–3.7	4.9–23.2	5.3–36.6	Proctor & Fuhrman (1991)
Freshwater					
Lake Erie	WC	1.6	11.0	13.5	Wilhelm & Smith (2000)
Gössenkollesee	WC	0.9–2.3	6.2–15.1	7.0–20.2	Hofer & Sommaruga (2001)
Lake Constance	TS	0–1.7	0–11.4	0–14.1	Hennes & Simon (1995)
Plußsee	TS	1.5–3.3	10.3–21.0	12.5–31.6	Weinbauer & Höfle (1998)
Plußsee	WC	1.3–2.5	8.8–16.5	10.4–22.5	Weinbauer & Höfle (1998)
Danube backwater	WC	1–4	6.9–24.8	7.8–40.6	Mathias et al. (1995)
Danube backwater	WC	2.8–9	18.1–45.7	25.7–128.8	Fischer & Velimirov (2002)
Reservoir	WC	2	13.3	17.1	Šimek et al. (2001)

tors ranging from 3.7 to 7.14 (average 5.42) for relating FVIC to FIC using thin sectioning and isolated phage-host systems. Using a 1-sample *t*-test, the average conversion factor (5.42) and the lowest conversion factor (3.7) of Proctor et al. (1993) were significantly ($p = 0.0014$ and $p \leq 0.0001$, respectively) lower than the conversion factors of this study, whereas the highest conversion factor (7.14) did not differ significantly ($p = 0.9539$). Fig. 3 shows the empirical relationship between FVIC and FIC (as determined by the virus dilution approach), as well as the relationship between FVIC and FIC, using Eq. (1) and the conversion factor of 7.11. The equation for the empirical relationship is:

$$FIC = 9.524\ FVIC - 3.256 \quad (3)$$

with data given as percentages.

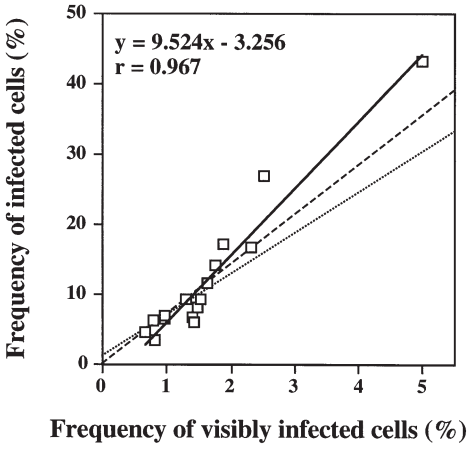

Fig. 3. Relationship between frequency of visibly infected cells (FVIC) as determined by the TEM approach and FIC as determined by the virus dilution approach. The broken line indicates the model of Binder (1999) to relate FVIC to FIC as shown in Eq. (1) and the dotted line the use of the average conversion factor of 7.11 determined in this study

One explanation for our comparatively high conversion factors might be that it is more difficult to detect mature viral particles in cells with the whole cell approach than with the thin sectioning approach, particularly when there are only a few viruses present (Fuhrman & Noble 1995, Weinbauer & Höfle 1998). It is also possible that visibly infected cells were disrupted during ultracentrifugation and sample preparation. This could result in lower FVIC values using the whole cell approach. Moreover, the difference might also be due to the fact that our conversion factors were derived from natural communities, which may show a different relationship between FVIC and FIC from isolated phage-host systems. The estimates of FIC and the conversion factors shown in this study might even be conservative, since only cells that were completely filled with viruses were used to assess burst size. Thus, the use of the published conversion factors might have

resulted in an underestimation of viral infection frequencies. This could be one of the explanations why estimates of VMB based on the TEM approach are occasionally lower than estimates based on other approaches (Fuhrman & Noble 1995, Guixa-Boixereu et al. 1999a,b). However, it has to be considered that the determination of burst size is prone to errors even if the maximum burst size is used (Weinbauer & Peduzzi 1994). Considering the potential problems with TEM based FVIC, FIC and burst size estimates (Fuhrman & Noble 1995, Weinbauer & Höfle 1998), the overall similarity of conversion factors and models is striking and supports the use of TEM for estimating FIC and VMB.

Using the approach presented in this paper, it is now possible to determine conversion factors for communities in specific environments. The conversion factors averaged 7.41, 6.90 and 7.00, for surface waters of the Mediterranean, the Baltic and the North Sea, respectively. These values are similar to the grand average of 7.11 (or 7.10 when environment-specific conversion factors were averaged), indicating that a single conversion factor may be used for a variety of environments; however, this has still to be confirmed.

The virus dilution approach should be more reliable than the TEM approach for estimating FIC, since the only additional parameter needed, i.e. the burst size, can be estimated in the samples, whereas TEM based data rely on conversion factors that may vary between phage-host systems (Proctor et al. 1993) and samples (Table 3). However, in sub-oxic or anoxic environments, the TEM approach has advantages, since keeping the oxygen partial pressure constant is logistically difficult for the virus dilution approach. Other logistic problems with the virus dilution approach may arise, if a large set of samples has to be processed, since virus-free seawater has to be made separately for each sample. Although it might be possible to use 1 virus-free ultrafiltrate for similar environments, it is unlikely that 1 ultrafiltrate can be used for contrasting environments such as surface versus deep marine waters.

The FIC values determined in this study by the virus dilution approach were not influenced by grazing, since grazers were removed or strongly reduced during prefiltration and did not increase in abundance or size during the incubation (data not shown). This is a prerequisite for calculating conversion factors, since grazers probably also feed on infected cells, which would reduce virus production and consequently lead to an underestimation of FIC using the virus dilution approach. Binder (1999) pointed out that the model for estimating VMB from FVIC is very sensitive to the relative length of the latent period and to conversion factors for estimating FIC from FVIC. The estimation of FIC using the virus dilution approaches and conversion factors derived from natural communities are means to improve the reliability of VMB estimates.

FIC and lysogeny data can be used to estimate the proportion of cells with lytic and lysogenic viral genomes. In surface waters of the Mediterranean Sea, we estimated that ca. 20 to 30% of the bacteria contained a functional viral genome.

In Table 4, published FVIC values and estimated FIC values using Eq. (1) and calculated VMB values using Eq. (2) are summarized. Published FVIC values range from not detectable to 9%. Estimated FIC values range from not detectable to 46% and estimated VMB range from not detectable to 129%. Overall, the data confirm that viral infection can be a significant but variable cause of bacterial mortality. The overall similarity of the new and published conversion factors may allow for a stronger confidence in the obtained TEM based data on the role of viral infection and its variability in aquatic systems.

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