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Virus-infected bacteria in oligotrophic open waters of the East Sea, Korea

Chung Y. Hwang, Byung C. Cho*

Molecular and Microbial Ecology Laboratory, School of Earth and Environmental Sciences and Research Institute of Oceanography, Seoul National University, Seoul 151-742, Korea

ABSTRACT: Viruses are now known to be significant contributors to bacterial mortality in the marine environment; however, to date, most studies have focused on only the more productive coastal areas. The impact of viruses in oligotrophic environments is still poorly understood, but current literature implies that the percentage of bacteria visibly infected with viruses in these areas is generally low and may often be below the detection limit normally achieved by transmission electron microscopy (i.e. <0.3%). However, from calculations based on typical bacterial abundance, abundance ratios of viruses to bacteria, and plausible turnover times of bacteria and viruses, we hypothesized that the frequency of visibly infected bacteria (FVIB) should be frequently above the detection limit even in oligotrophic environments. We tested this hypothesis in the oligotrophic open waters in the East Sea, Korea, in summer. FVIB ranged from 1.2 to 2.2% and burst size varied from 12 to 15. Viral production ranged from 0.4 to 13.7 $\times$ 10^8 viruses l^{-1} d^{-1}. Estimated viral mortality of bacteria was on average 13.1% of bacterial production, indicating that viruses could be significant contributors to bacterial losses in oligotrophic waters. Comparison of grazing by heterotrophic nanoflagellates and lysis by viruses suggests that, overall, these processes cause comparable losses of bacteria in the oligotrophic East Sea; however, taken together, they accounted for only half of the total mortality.

KEY WORDS: Viral production · Oligotrophy · Bacteriophage · Bacterial mortality · Burst size · East Sea

INTRODUCTION

Over the past 12 yr viruses have been shown to be an abundant and dynamic component of the marine plankton and an important source of bacterial mortality (Wommack & Colwell 2000). In some cases, it has been estimated that losses of bacteria due to viral lysis were comparable to grazing by heterotrophic nanoflagellates (Fuhrman & Noble 1995, Steward et al. 1996). By lysing bacterial cells, viruses are thought to significantly influence the production of dissolved organic carbon (DOC) and the recycling of nutrients in microbial food webs (Bratbak et al. 1994, Wilhelm & Suttle 1999).

In oligotrophic waters, a large fraction of marine primary productivity is ultimately consumed by heterotrophic bacteria (Ducklow & Carlson 1992, Hoppe et al. 2002). Bacteria also comprise a major fraction of biomass in the oceans, especially in oligotrophic environments where bacterial biomass carbon can exceed that of the primary producers (Cho & Azam 1990, Gasol et al. 1997). Determining the fate of bacteria is therefore crucial to understanding the mechanisms by which carbon and nutrients are recycled in oligotrophic ocean environments. Until now, studies in oligotrophic waters have mainly focused on bacterial mortality due to nanoflagellate grazing (Hagström et al. 1988, Christaki et al. 1999, Sakka et al. 2000, Calbet et al. 2001). The contribution of viruses to bacterial mortality in these environments is still poorly understood.

*Corresponding author. Email: bccho@plaza.snu.ac.kr

A few studies done in ultra-oligotrophic or oligotrophic freshwaters showed that virus-like particles (VLP) were abundant (1.0 to 3.3×10^9 viruses l^{-1}; Klut & Stockner 1990, Laybourn-Parry et al. 2001). Laybourn-Parry et al. (2001) suggested a pivotal role for viruses in carbon cycling based on a positive relationship between VLP and DOC. In addition, Corpe & Jensen (1996) reported that ca. 17% of the prokaryotic host cells in an oligotrophic lake were infected with bacteriophages or carried adherent phage particles on their surfaces. In marine environments, frequency of visibly infected bacteria (FVIB) and virus production rates were reported to decrease with decreasing trophic status in the Adriatic Sea (Weinbauer et al. 1993). Apparently consistent with that trend, Guixa-Boixareu et al. (1999) found that visibly infected bacteria were below their detection limit in the oligotrophic western Mediterranean (i.e. <0.3%). From these data, it might be inferred that viruses have a minor impact on bacteria in oligotrophic areas of the ocean.

On the basis of some other data and simple calculations, however, we reasoned that this might not hold true in general. Typical concentrations of bacteria and viruses in oligotrophic waters are 3 to 6×10^8 cells l^{-1} (Cho & Azam 1988) and 6×10^9 viruses l^{-1} (Guixa-Boixareu et al. 1999). Using turnover times in oligotrophic waters of 2 to 10 d for bacteria (Steward et al. 1992, Kirchman et al. 1993) and 30 d for viruses (Steward et al. 1992), we might reasonably expect bacterial and viral productivity of 3 to 30×10^7 cells l^{-1} d^{-1} and 2×10^8 viruses l^{-1} d^{-1}. Following the reverse of viral productivity calculations (Noble & Steward 2001) and assuming a burst size of 50 (Heldal & Bratbak 1991), the implied mortality due to viral lysis would be 1.3 to 13.3% of bacterial production. Back-calculating, using the equations of Binder (1999), results in estimates of FVIB ranging from 0.2 to 1.6%. The assumed burst size clearly has a direct impact on these estimates. The commonly assumed burst size of 50 may be too high for oligotrophic waters, since estimates of burst size for such areas have tended to be lower (10 to 28; Wilhelm et al. 1998, Weinbauer & Suttle 1999). If we instead assume a burst size of 20, the calculated mortality increases to 3.3 to 33.3% and the estimated FVIB to 0.5 to 3.4%. These estimates cover a wide range, but make the point that FVIB should be easily measurable in oligotrophic environments except under the most extreme conditions.

Although the data are very few, there is at least some indication from previous work that our conclusion derived from these equations is plausible. In the Sargasso Sea, a sample from 25 m depth in which bacteria were concentrated by ultrafiltration and thin sectioned for transmission electron microscopy (TEM) observation, FVIB was 0.9% (Proctor & Fuhrman 1990). In an oligotrophic station in California waters, Noble & Fuhrman (2000) found virus production to be 3.7×10^9 viruses l^{-1} d^{-1} using the fluorescently labeled viruses (FLV) tracer approach. Bacterial production at the same site was 2.8×10^8 cells l^{-1} d^{-1} (Noble & Fuhrman 2000). Assuming a burst size of 50 and following the same calculations as above, these data imply an FVIB of 2.9%.

In this study, our main goals were to examine whether FVIB was in fact measurable in the open waters of the East Sea during summertime and under oligotrophic conditions and, if so, to estimate the relative contributions of viral lysis and grazing to bacterial mortality in this environment.

MATERIALS AND METHODS

Study area and sample collection. The East Sea (Fig. 1) is located off the east coast of Korea. The East Sea is suggested to be a miniature ocean whose physical oceanographic characteristics are common to open oceans (Hahm & Kim 2001). Recent estimation of sinking carbon flux showed that open waters of the East Sea are oligotrophic in summer (Hong et al. 2001). Samplings were made at 3 stations (Stns 1, 2 and 3; depth range, 1000 to 1800 m; Fig. 1). Stn 1 was occupied at 14:00 h on August 15, and Stns 2 and 3 at 14:00 and 12:00 h on August 16, 2001, respectively. Samples were collected from depths of 10, 30, 50 and 70 (or 75) m with 10 l Niskin bottles mounted on a CTD rosette.

Bacterial and viral abundance. Samples for measurements of bacterial abundance (BA) and viral abundance (VA) were fixed with 0.02 µm filtered, borate-buffered formalin (final conc. of 2%), and were filtered through 0.02 µm pore size Anodisc filters (Whatman).

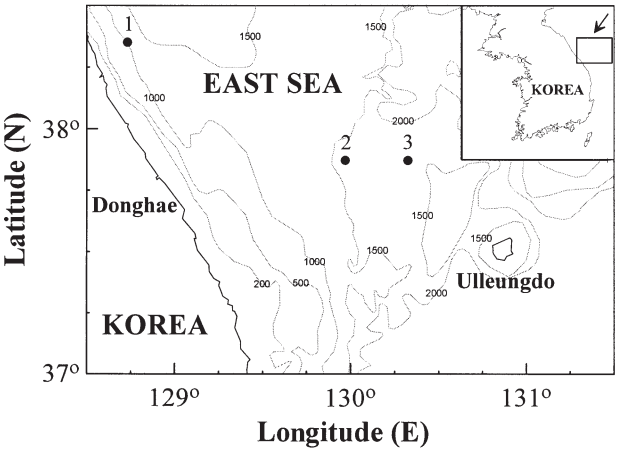

Fig. 1. Study area and sampling stations in the East Sea. Depth contour lines are shown in meters

Filters were then laid on a drop of 100 µl of diluted SYBR Green I (final dilution, 2.5×10^3-fold; Noble & Fuhrman 1998) for 15 min in the dark. Bacteria and viruses were counted in duplicate samples by using a blue filter set on an epifluorescence microscope (Olympus model BX60) at ×1250. Bacteria were distinguished from viruses on the basis of their relative size and brightness.

Bacterial production. Bacterial production was measured by the ^{14}C-leucine (Leu) incorporation method (Simon & Azam 1989). For each sample, quadruplicate 10 ml subsamples of seawater were dispensed to sterile polypropylene tubes and ^{14}C-Leu (specific activity = 315 mCi mmol^{-1}) was added to each tube at 10 nM (final conc.). One of the 4 replicates was immediately killed with formalin (final conc. of 2%) to serve as a blank. The samples were incubated in the dark at *in situ* water temperature for ca. 1.5 h. Leucine incorporation was converted to cell production using a conversion factor of 0.18×10^{18} cells mol^{-1} Leu incorporated (Ducklow et al. 1992, Cho et al. 2000).

Viral infections and mortality of bacteria. Samples for TEM observation were preserved with electron microscopy-grade glutaraldehyde (final conc. of 2%), and stored in sterile polypropylene centrifuge tubes at 4°C. Bacteria were harvested directly onto Fomvar-coated, 400-mesh electron-microscope grids that were made evenly hydrophilic by floating on a drop of 1% poly-L-lysine for 1 min (Suttle 1993). Grids were stained for 20 s with 0.5% uranyl acetate followed by 3 sequential rinses with 0.02 µm filtered Milli-Q® water. For half of the samples, duplicate grids were prepared to measure standard deviations (coefficient of variation: 4.9 to 27.6%). Infected cells were enumerated on a JEOL 2000 EXII TEM at an accelerating voltage of 100 keV. For each sample, 500 to 600 cells were examined by TEM at 30000 to 50000× magnification, and scored as infected if they contained 5 or more intracellular VLP. Bacteria were classified into 4 groups based on morphotypes (i.e. rods, cocci, curved shapes and spirillae). BA, FVIB and burst size were measured for each morphotype by TEM observation. The effect of centrifugation speed on FVIB was tested by centrifuging samples (10 and 70 or 75 m depths at each station) at 2 different speeds (i.e. $100000 \times g$ vs $30000 \times g$) for 30 min at 20°C. Bacterial mortality due to viral lysis was estimated according to Binder (1999) assuming that infected and uninfected bacteria were grazed at the same rate.

Viral production. With the assumptions that the latent period of viruses is approximately equal to the generation time of bacteria (Proctor et al. 1993, Guixa-Boixareu et al. 1996) and that infected and uninfected bacteria are grazed equally, viral production was estimated by multiplying together burst size, the fraction of mortality due to viral lysis and bacterial production (Noble & Steward 2001). Burst size was estimated as the average number of VLP observed in all visibly infected cells. Thus, viral production in this study could be a minimum estimate (Weinbauer et al. 2002).

Heterotrophic nanoflagellate (HNF) grazing. Samples for measurements of HNF abundance were fixed immediately with alkaline Lugol solution (final conc. of 0.5%) and borate-buffered formalin (final conc. of 3%). Primulin-stained HNF collected on 0.4 µm polycarbonate filters (25 mm diameter) were enumerated with UV excitation using an epifluorescence microscope (Caron 1983). HNF grazing rates were estimated as the product of *in situ* BA, *in situ* HNF abundance and the mean clearance rate (2.4 ± 0.5 nl HNF^{-1} h^{-1}, n = 2) obtained at stations where Secchi depth was >25 m in the euphotic zone of the East Sea in summer (Cho et al. 2000).

Other analyses. Depth profiles of water temperature and salinity were measured with a CTD. Transparency of the water was measured with a Secchi disk and the depth of the euphotic zone was determined by multiplying Secchi depth by 2.7. Regression analysis, analysis of variance (ANOVA) and *t*-tests were carried out using SPSS for Windows (Version. 8.0, 1997).

RESULTS

Hydrography

In August 2001, the temperature was 23.7 to 25.7°C in the surface layer and decreased to 3.1 to 11.7°C near the bottom of the euphotic zone (Fig. 2). Salinity in the surface was 33.4 to 33.9 psu, but increased to 34.0 to 34.3 psu near the bottom of the euphotic zone. The euphotic zone was stratified and the mixed layer extended to 25 to 30 m depth (Fig. 2). Secchi depth was 26, 30 and 25 m for Stns 1, 2 and 3, respectively.

VA, BA and VA:BA ratios (VBR)

Depth profiles of VA and BA showed similar patterns that varied among the 3 stations. Pronounced subsurface peaks of VA (9.2×10^9 viruses l^{-1}) and BA (2.4×10^9 cells l^{-1}) were found at 30 m depth at Stn 1. VA and BA tended to decrease with depth at Stn 2 and were nearly homogenous at Stn 3 (Fig. 2). VA ranged from 0.6 to 9.2×10^9 viruses l^{-1}, BA from 0.3 to 2.4×10^9 cells l^{-1}, and VBR from 0.6 to 7.2 and was generally higher at Stn 2 (Fig. 2). Rod- and curve-shaped bacteria each made up more than 31% of the total community, another 20% were cocci and roughly 3% were spirillae (Table 1).

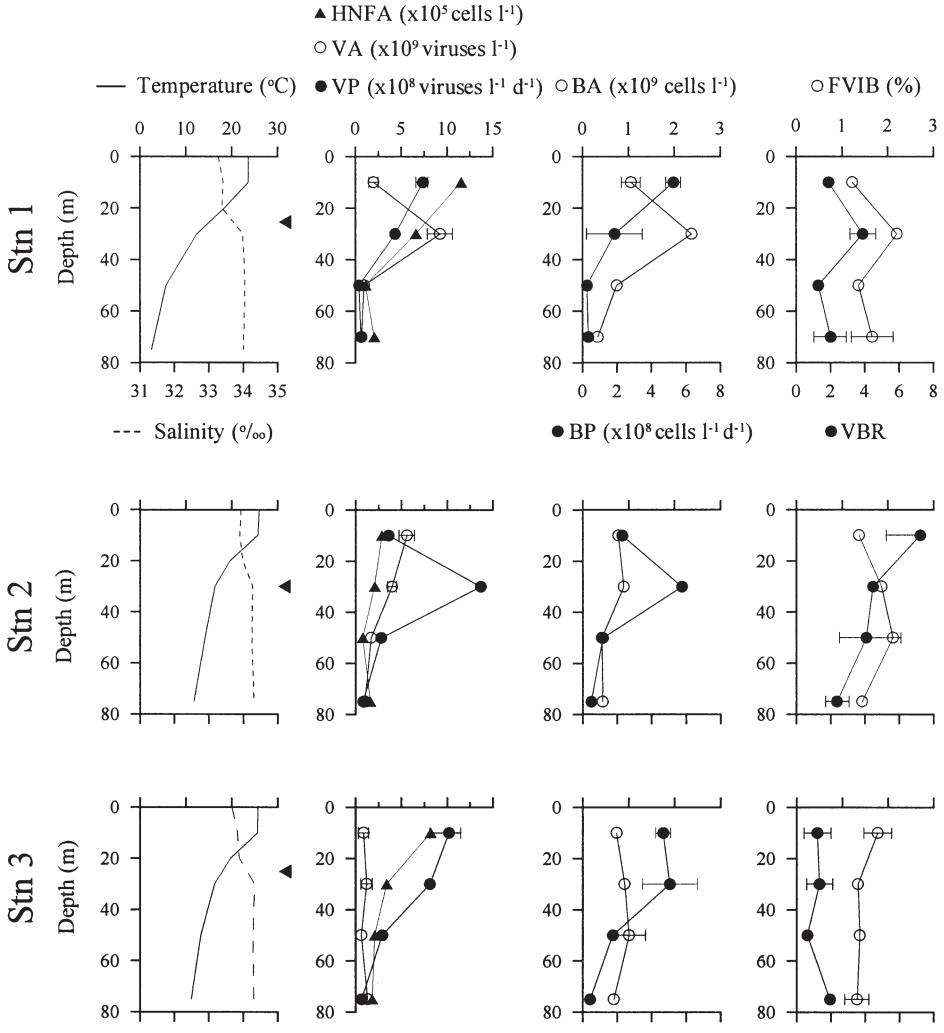

Fig. 2. Depth profiles of salinity and temperature, heterotrophic nanoflagellate abundance (HNFA), viral abundance (VA) and viral production (VP), bacterial abundance (BA) and bacterial production (BP), and frequency of visibly infected bacteria (FVIB) and viral:bacterial abundance ratios (VBR) at the study site. (◀) Secchi depth at each station. When bars are not shown, 1 SD is less than size of symbol

FVIB and burst size

FVIB of the entire bacterial community ranged from 1.2 to 2.2% (Table 1, Fig. 2). FVIB in various bacterial morphotypes ranged from 0.4 to 6.3%, except for spirillae (not detected; Table 1). The mean FVIB was significantly higher in coccoid bacteria than in those that were rod- or curve-shaped (ANOVA, $p < 0.001$; data not shown). FVIB of samples prepared using the higher centrifugation speed showed somewhat decreased values (up to 22%) compared to those centrifuged at the lower speed in 5 out of 6 tested samples, but the differences were not statistically significant (*t*-test, $p > 0.05$; data not shown). Burst sizes in the entire bacterial community ranged from 12 to 15; in the 3 individual morphotypes for which there were data, it ranged from 8 to 21 (Table 1). There was no significant difference in mean burst sizes among those 3 bacterial morphotypes (ANOVA, $p > 0.28$; data not shown).

Production of viruses and bacteria

Viral production ranged from 0.4 to 13.7×10^8 viruses l^{-1} d^{-1} (Fig. 2). Variations in viral production with depth followed a pattern similar to that seen for bacterial production. Bacterial production varied within a range of 0.3 to 5.8×10^8 cells l^{-1} d^{-1} (Fig. 2). Turnover times of viruses (VTT) ranged from 0.9 to 23.9 d and those for bacteria (BTT) ranged from 1.5 to 28.8 d.

Bacterial mortality

Using an average conversion factor of 5.42 (Proctor et al. 1993) and models of Binder (1999), estimated bacterial mortality due to viral lysis corresponded to 9.8 to 19.2% of bacterial production (Table 2). HNF abundance ranged from 0.7 to 8.2×10^5 cells l^{-1} (Fig. 2). Calculated grazing rates ranged from 0.05 to 1.5×10^8 cells l^{-1} d^{-1}, being much wider than a range reported in the oligotrophic phase in the East Sea (0.4 to 0.5×10^8 cells l^{-1} d^{-1}; Cho et al. 2000). Our range of grazing rates corresponded to the range from 1.6 to 32.8% of bacterial production (i.e. bacterial mortality due to HNF grazing; Table 2). The ratio of bacterial mortality due to viruses versus HNF varied from 0.6 to 11.2 during the investigation, indicating that mortality of bacteria in the oligotrophic waters of the East Sea may sometimes be dominated by viruses. Overall, HNF grazing and viral lysis caused comparable losses of bacteria, 9.5 ± 8.4 and 13.1 ± 3.2%, respectively (Table 2).

Table 1. Bacterial abundance (BA), frequency of visibly infected bacteria (FVIB) and burst size among the 4 morphotypes. Mean values are calculated for all samples investigated (n = 30). Ranges are in parentheses. nd: not detected

Bacterial morphotypes	BA (% of total)	FVIB (%)	Burst size (viruses)
Rods	38.7 (31.7–46.0)	1.2 (0.4–2.3)	14 (10–21)
Cocci	21.5 (12.6–27.4)	3.0 (1.5–6.3)	14 (8–19)
Curved shapes	36.9 (30.8–45.6)	1.0 (0.4–1.7)	15 (12–21)
Spirillae	2.9 (1.7–4.7)	nd	nd
Total bacteria	100	1.6 (1.2–2.2)	14 (12–15)

DISCUSSION

The most significant observation of this study was that visibly infected bacteria could be detected in all of the examined samples from the open waters in the East Sea. Several results strongly supported the fact that the study sites were oligotrophic during the study. First, Secchi depth ranged from 25 to 30 m in the East Sea in summer and seawater with Secchi depth greater than 16 m is regarded as oligotrophic (Morita 1997). Second, the observed chlorophyll *a* (chl *a*) concentrations during the cruise varied from 0.09 to 0.35 µg chl *a* l^{-1} (B. C. Oh pers. comm.). This is within the range of ca. 0.01 to 1.5 µg chl *a* l^{-1} found in the oligotrophic Mediterranean where FVIB was below detection limits (Guixa-Boixareu et al. 1999). Third, sinking carbon flux estimates in the East Sea in summer (<30 mg C m^{-2} d^{-1}; Hong et al. 2001) were typical of oligotrophic open oceans. Thus, the microbial communities observed in summer were exposed to oligotrophic conditions and those in the mixed layer (with a BTT of 2 to 3 d)

Table 2. Estimates of bacterial mortality due to viral lysis and heterotrophic nanoflagellate (HNF) grazing, and mortality ratios of viral lysis to HNF grazing (Virus:HNF) during the study in the oligotrophic East Sea in 2001. Model A is calculated from the factor of 2 rule (= frequency of infected bacteria × 2; Proctor & Fuhrman 1990) and Model B from the models of Binder (1999; see 'Materials and methods'). Ranges in parentheses were obtained using a minimum (3.7) and a maximum (7.14) conversion factor (Proctor et al. 1993). SD: standard deviation

Stn	Depth (m)	Bacterial mortality (%) due to Viral lysis: Model A	Viral lysis: Model B	HNF grazing[a]	Summed bacterial mortality (%)[b]	Virus[b]:HNF
1	10	13.3 (9.1–16.3)	9.8 (6.9–13.9)	8.9	18.7	1.1
	30	23.9 (16.3–31.4)	19.2 (12.8–26.9)	32.8	51.9	0.6
	50	14.8 (10.1–19.5)	11.0 (7.7–15.6)	12.3	23.3	0.9
	70	18.0 (12.3–23.8)	13.8 (9.5–19.5)	8.0	21.8	1.7
2	10	14.8 (10.1–19.5)	11.0 (7.7–15.6)	5.7	16.7	1.9
	30	20.2 (13.8–26.6)	15.7 (10.7–22.1)	1.9	17.6	8.3
	50	22.9 (15.6–30.1)	18.2 (12.2–25.6)	1.6	19.8	11.2
	75	15.6 (10.6–20.5)	11.7 (8.1–16.5)	8.1	19.8	1.4
3	10	19.2 (13.1–25.3)	14.9 (10.2–20.9)	7.5	22.4	2.0
	30	14.5 (9.9–19.1)	10.8 (7.5–15.3)	3.5	14.3	3.0
	50	15.0 (10.2–19.7)	11.1 (7.8–15.8)	7.1	18.2	1.6
	75	14.3 (9.8–18.8)	10.6 (7.4–15.0)	16.6	27.2	0.6
	Average	17.2	13.1	9.5	22.6	_
	±SD of total	3.6	3.2	8.4	9.8	_

[a]Calculated based on HNF abundance shown in Fig. 2 and the clearance rate of 2.4 nl HNF^{-1} h^{-1} (Cho et al. 2000)
[b]Viral mortality of bacteria based on Model B

Table 3. Chlorophyll *a* (chl *a*) concentration and burst size in various marine environments. Burst size was determined by transmission electron microscopy. nd: no data

Location	Environmental characteristics	Chl *a* ($\mu g\ l^{-1}$)	Burst size	Source
Raunefjorden, Norway	Coastal	nd	10–300	Heldal & Bratbak (1991)
Santa Monica Bay, USA	Coastal	nd	20	Fuhrman & Noble (1995)
Northern Adriatic Sea	Mesotrophic	0.85–1.44	16–20	Weinbauer et al. (1993)
	Eutrophic	2.13–2.75	30–32	
Gulf of Mexico, USA	Oligotrophic	nd	10–23	Wilhelm et al. (1998)
	Mesotrophic	nd	29–64	
Gulf of Mexico, USA	Oligotrophic	0.2–0.3	17–54	Weinbauer & Suttle (1996)
	Coastal	0.9–2.9	15–33	
Gulf of Mexico, USA	Offshore	0.04–0.08	15–28	Weinbauer & Suttle (1999)
	Coastal	0.14–0.36	33–64	
Mediterranean Sea	Offshore	nd	18–27	Weinbauer et al. (2002)
Masan Bay, Korea	Eutrophic	0.3–20.9	14–46	Unpubl. data
East Sea, Korea	Oligotrophic	0.09–0.35	12–15	This study

would have been adapting to these conditions for many generations.

Our observed values of FVIB (1.2 to 2.2%) and 1 estimate from the Sargasso Sea (0.9%; Proctor & Fuhrman 1990) are much greater than 0.3% that was a maximum estimate in oligotrophic Mediterranean waters (Guixa-Boixareu et al. 1999). We checked if our measurable FVIB was due to our low centrifugation speed ($30\,000 \times g$ for 30 min) for collecting bacteria on grids compared to high speed ($100\,000 \times g$ for 30 min) used by Guixa-Boixareu et al. (1999). We could not find significant differences between the 2 centrifugation speeds. Thus, it seems that the high centrifugation speed used by Guixa-Boixareu et al. (1999) might not be the cause of the undetectable FVIB in their samples.

Our observation of FVIB in oligotrophic seawaters is, perhaps, not surprising because even starved bacteria are known to be infected by bacteriophages and subject to lysis (Schrader et al. 1997, Kadavy et al. 2000). This raises the question as to why viral infection rates are so low in the Mediterranean. Middelboe (2000) recently demonstrated that the growth condition of the host bacterium is a central parameter for understanding the roles of viruses in bacteria mortality. Depth and time averaged bacterial specific activities of Leu incorporation in the Mediterranean (1.7 to 4.3×10^{-20} mol Leu $cell^{-1}\ h^{-1}$; Gasol et al. 1998) were lower than those in the East Sea (4.5 to 9.6×10^{-20} mol Leu $cell^{-1}\ h^{-1}$; data not shown), suggesting that the differences in FVIB for these 2 regions might be explained by different metabolic activities of the bacteria. Possibly, greater availability of carbon and energy to bacteria stimulated higher virus production (Tuomi et al. 1995) in the East Sea compared to in the Mediterranean. The presence of different FVIB in the 2 environments might also be due to the presence of different virus-host systems. For instance, it has been shown that UV-damaged bacteriophages can be reactivated by repair mechanisms of host cells (Weinbauer et al. 1997, Kadavy et al. 2000), but this process varies among host-virus systems (Schrader et al. 1997, Kadavy et al. 2000). Further, different microbial communities could vary in their relative repair capabilities and thus influence the propagation of viruses.

Burst size (i.e. the number of viruses released during cell lysis) is an important parameter for estimating viral production and virus-mediated mortality of bacteria based on virus decay (Heldal & Bratbak 1991), radiolabeled tracer (Steward et al. 1992) and FLV approaches (Noble & Fuhrman 2000). Estimated burst sizes for marine bacteriophages have ranged widely from 6 to ca. 300 (Wommack & Colwell 2000). Burst size estimates ranging from 8 to 21 in the present study were quite similar to those observed in the oligotrophic Gulf of Mexico (Table 3), and much lower than the generally used burst size of 50 (Heldal & Bratbak 1991, Suttle & Chen 1992, Steward et al. 1996). Since burst sizes are related to the growth rate and growth phase of host bacteria (Webb et al. 1982, Schrader et al. 1997, Middelboe 2000), it seems reasonable to expect that viral infections in oligotrophic areas would result in smaller burst sizes than bacteria in more eutrophic conditions. Thus, our estimated burst size of 15 to 20 might be more appropriate to estimate virus-induced bacterial mortality or viral production in oligotrophic marine environments. In our samples, there was no significant difference in burst sizes among different bacterial groups. This is in contrast to results from mesotrophic and eutrophic regions of the northern Adriatic Sea where burst sizes varied widely among different morphotypes (Weinbauer & Peduzzi 1994).

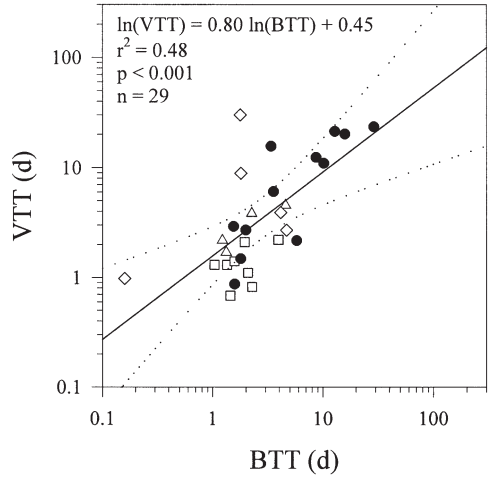

Fig. 3. Relationship between bacterial turnover time (BTT) and viral turnover time (VTT). Symbols represent data of the studies (□: Noble & Fuhrman 2000, △: Wilhelm et al. 1998, ◇: Steward et al. 1992, ●: this study). The solid line represents a regression line of BTT and VTT for all data and the dotted curve the 99% confidence intervals

The observed BTT (e.g. 2 to 29 d) in the East Sea were comparable to those in other oligotrophic marine environments (Cho & Azam 1988, Turley et al. 2000). VTT (0.9 to 23.5 d) in our samples were also comparable to those reported in other oligotrophic environments (1 to 30 d; Steward et al. 1992, Wilhelm et al. 1998, Noble & Fuhrman 2000). A positive correlation was observed between VTT and BTT for the available data from both mesotrophic and oligotrophic samples (Steward et al. 1992, Wilhelm et al. 1998, Noble & Fuhrman 2000, this study; Fig. 3). This type of relationship seems to be reasonable because the impact of phages on bacteria would strongly depend on the metabolic activity of the host (Weinbauer & Peduzzi 1994).

When we compared bacterial mortality due to viral lysis with that due to HNF grazing in the East Sea, viruses and HNF had a comparable impact on bacterial losses. Interestingly, the ratio of bacterial mortality due to viral lysis versus HNF grazing correlated positively with bacterial growth rates ($r^2 = 0.40$, $p < 0.05$, $n = 12$; data not shown), in spite of insignificant correlations between bacterial growth rates and each source of bacterial mortality considered individually. Thus, the relative role of viruses to HNF in bacterial mortality seemed to be partially explained by bacterial growth rates in our study. However, the sum of viral lysis and HNF grazing did not account for more than 52% of the bacterial mortality (Table 2). A similar result was also found in the Bering and Chukchi Seas (Steward et al. 1996) and could be a general phenomenon in open oceans. Additionally, there might be another source of bacterial losses, e.g. bdellovibrios, small ciliates. It is also possible that our methods used in these estimates underestimated bacterial mortality due to viral lysis and HNF. It has been suggested that the FVIB:FIB conversion factor (e.g. 5.42) might be an underestimate for natural communities (Weinbauer et al. 2002) and use of fluorescently labeled bacteria, which are dead and nonmotile, could underestimate HNF clearance rate (González et al. 1993). The magnitude of bacterial mortality due to HNF grazing in this study is directly dependent on the chosen clearance rate. Our choice of mean clearance rate (2.4 ± 0.5 nl HNF^{-1} h^{-1}) was coincidently comparable to that reported in the oligotrophic eastern Mediterranean (2.6 nl HNF^{-1} h^{-1}; Christaki et al. 1999).

In all of our samples, products of VA and BA were larger than 10^{18} l^{-2}, suggesting that lytic infections might be a major viral mode of bacterial death (Wilcox & Fuhrman 1994). However, considering that the percentage of lysogenic bacteria was higher in offshore compared to coastal bacterial communities (Jiang & Paul 1998, Weinbauer & Suttle 1999), lysogens in oligotrophic environments may serve as a source of viruses and contribute significantly to viral production during natural induction events (Jiang & Paul 1998). Further research will be needed to test which mode of viral infection contributes more to bacterial mortality in oligotrophic waters.

In summary, our data showed much higher rates of viral infection than seen previously for an oligotrophic environment. It remains to be determined how the percentage of visibly infected bacteria and accordingly the ecological roles of viruses vary among the diverse oligotrophic environments, and what factor(s) is responsible for such variations.

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