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REVIEW

Viral burst size of heterotrophic prokaryotes in aquatic systems

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Viral burst size (BS), i.e. the number of viruses released during cell lysis, is a critical parameter for assessing the ecological and biogeochemical role of viruses in aquatic systems. Burst size is typically estimated by enumerating the viral particles in bacteria using transmission electron microscopy. Here, we review the average BS reported for different aquatic systems, present several hypotheses on the control of the BS and evaluate whether there are relationships between BS and bacterial activity parameters across systems. Based on reports from a variety of different aquatic environments, we calculated a mean BS of 24 and 34 for marine and freshwater environments, respectively. Generally, the BS increased with the trophic status of the environment and with the percentage of infected cells in marine populations. When diel dynamics were investigated or averages from large-scale environments were used, BS was positively related to bacterial production but no trend was detectable across systems. The across systems' finding that BS was significantly related to the frequency of infected cells (FIC) could be due to co-infection or superinfection. At any given site, BS seems to be influenced by a number of factors such as the size of the host cell and the viruses, the metabolic activity of the host and phage and host diversity. Thus, based on the available data collected over the past two decades on a variety of aquatic systems, some relations between BS and bacterial variables were detectable.

The number of viral progeny released during lysis of cells (burst size, BS) is a critical parameter in viral ecology. The number of offspring produced and the recruitment are not only important parameters regulating population dynamics of cellular organisms but also for the population dynamics and epidemics of viruses. Viral community ecology has experienced a boost in the late 1980s when it was shown that total viral abundance was typically higher than prokaryotic abundance (Bergh et al., 1989) and that a significant fraction of hetero- and autotrophic micro-organisms were infected by viruses (Proctor & Fuhrman, 1990; Suttle et al., 1990). The BS estimates are used when viral production rates are converted to lysis rates of prokaryotes in order to determine virus-induced mortality of prokaryotic plankton. Also, for assessing the frequency of infected cells (FIC) and the frequency of lysogenic cells (FLC), BS estimates are required. Thus, the determination of BS is a prerequisite for assessing the importance of virus-induced mortality and consequently, for quantifying the overall role of viruses via the viral shunt pathway in the cycling of dissolved organic matter in aquatic food webs (Fuhrman, 1999).

In isolated phage–host systems, BS is estimated by one-step growth curves (Jiang et al., 1998). However, this method cannot be used for natural communities. Instead, transmission electron microscopy (TEM) is used to count mature phages inside cells. This is either done in whole cell (WC) approaches (Heldal & Bratbak, 1991; Weinbauer et al., 1993) or in thin sections (TS) (Proctor & Fuhrman, 1990). Independent of the method used, a wide range of BS values has been reported in the literature indicating that BS may vary among phage and host species. The overall metabolic activity of the host, its abundance and other environmental parameters such as substrate composition and availability might also influence the BS. An optimal phage life strategy has to find a balance between the size of the progeny and the timing of lysis for the most successful viral spread.

Here, we review estimates of phage BS of prokaryotic communities in different environments and relate BS to microbial activity parameters in an attempt to determine whether general relationships between the BS and microbial parameters exist.

Minimum and maximum burst size and methodological problems

To obtain estimates on the variability of BS in individual samples, two values can be used (Weinbauer & Suttle, 1996). The first is the minimum BS (minBS), i.e. the number of phages in all visibly infected cells (Figure 1). This minBS should provide a conservative estimate of BS since in some cells, the number of phages might still increase before the cell is actually lysed. The second is the maximum BS (maxBS), i.e. the number of phages in only those host cells which are completely or almost completely filled with phages. This likely represents an overestimation of the actual BS since some cells might lyse before the cell

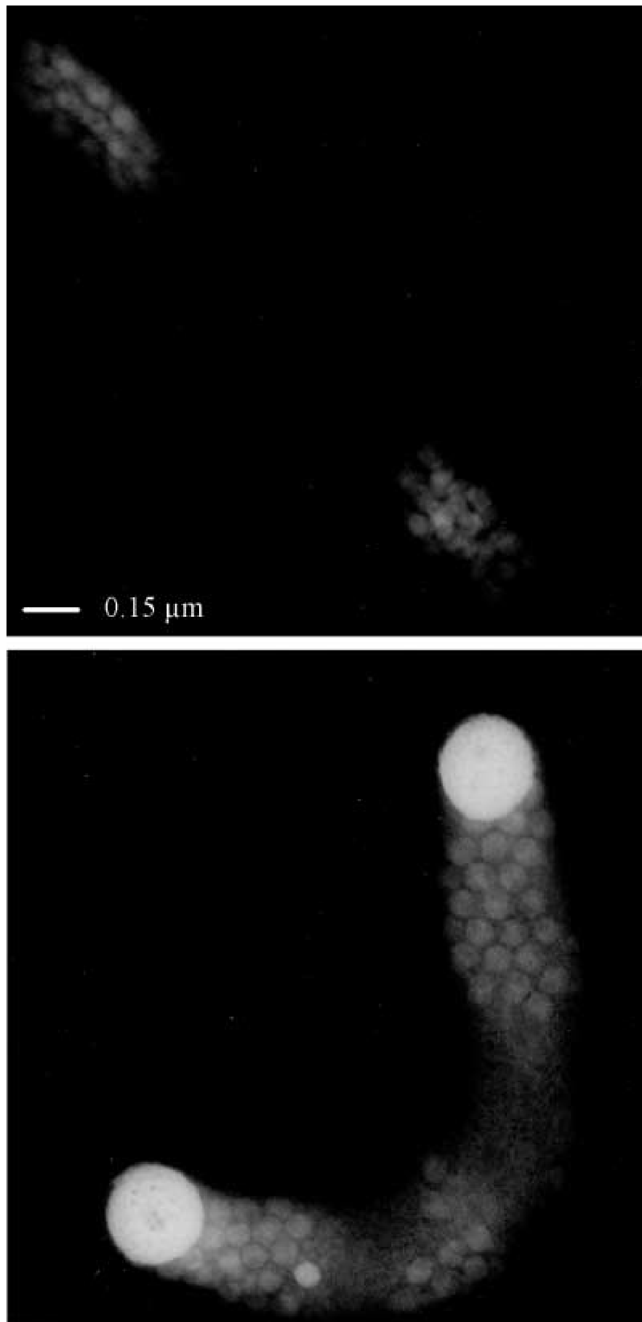

Figure 1. Transmission electron micrographs of infected cells showing examples of minimum burst size.

is completely filled with phages. Thus, while the minBS might highly fluctuate for the same phylotype or strain, maxBS is constrained by the cell size.

Estimating the BS by TEM is time consuming and difficult (Proctor, 1997). For BS determination, whole cells (BS-WC) or thin sections (BS-TS) are used. For both methods, it might be difficult to decide whether the detected structure is a phage. This is particularly true if only a few virus particles are detected. This problem might be more pronounced for BS-WC than for BS-TS. A potential problem if using the BS-TS approach is that the abundance of viruses present in a thin section has to be extrapolated to the entire cell volume. Although it is possible to reconstruct the cell volume using consecutive layers of thin sections and thus obtain more accurate BS estimates, this is time consuming and not practicable for routine measurements. For the BS-WC approach, pigmentation of cells and a high detritus content of the sample can make detection of visibly infected cells and hence, estimates of BS difficult. Pre-filtration through, for example 1.0 μm filters, can reduce this problem. Spinning down viruses and bacteria on TEM grids can also make the detection of visibly infected cells (VIC) difficult. To circumvent this problem, bacteria can be collected at low centrifugation speed, thus avoiding the sedimentation of a significant amount of viruses. When centrifugation speed and time are kept at $\sim$20,000g and 20 min, only a small number of viruses is co-collected and host cells also seem to be flattened out somewhat, thus facilitating the detection of VIC. If phages are on top of each other in VIC, they might be counted as one. This becomes more problematic with increasing cell size. Changing the plane of focus in the TEM helps to distinguish between single phage particles but this cannot completely solve the problem.

Another TEM approach used to estimate BS is the treatment of samples with streptomycin, which induces lysis, i.e. cell walls are destroyed ('lysis from without') (Heldal & Bratbak, 1991), thereby facilitating the enumeration of phages. The drawback of this method is the need to incubate the samples for a certain period of time and the fact that it is sometimes difficult to distinguish between phages originating from this lysis event and phages collected on a thin organic aggregate occasionally present in the sample as well.

MinBS and maxBS might correlate well with each other as shown for samples collected in the Gulf of Mexico and Lake Plussee (Figure 2) (Weinbauer & Suttle, 1996; Weinbauer & Höfle, 1998b; M.G. Weinbauer, unpublished data). The slope of the regression analysis of all data ($P<0.0001$) suggests that maxBS is on average 41% higher than minBS (Figure 2). However, under specific conditions, the maxBS might be twice as high as the minBS (Fischer & Velimirov, 2002) or, as found for the North Sea (unpublished data), smaller than the minBS (Table 1). The latter was due to a high percentage of small

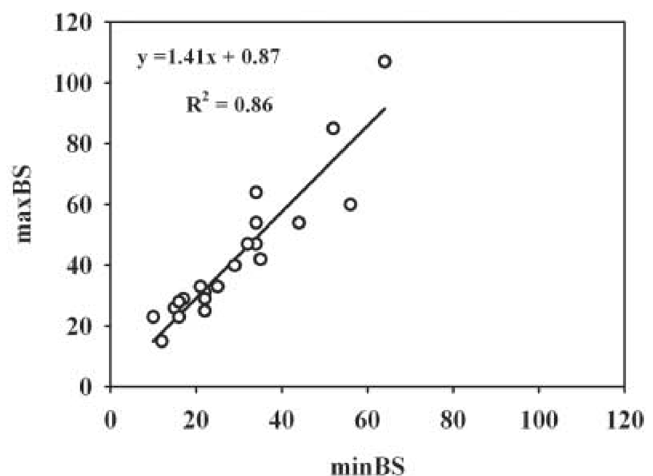

Figure 2. Relationship between maximum burst size (maxBS) and minimum burst size (minBS) estimated from the number of virus-like particles within visibly infected cells from all the data given in Table 1.

Table 1. *Minimum burst size (minBS) and maximum burst size (maxBS) in different environments based on* in situ *observations.*

Environment	Location	minBS	maxBS	Method	Comments	Reference
Coastal shelf	Massay Bay, Korea	17	ND	WC	data extracted from graph	(Choi et al., 2003)
	Gulf of Mexico	29	48	WC		(Wilhelm et al., 1998a; Weinbauer et al, 1999)
		24	35	WC		(Weinbauer & Suttle, 1996)
	Raunefjorden, Norway	50	ND	LFW		(Heldal & Bratbak, 1991)
	North Sea	23	ND	WC		(Winter et al., 2004)
	North Sea	27	ND	WC		(Winter et al., 2005)
	Northern Adriatic Sea	ND	22	WC	measured along a trophic gradient	(Weinbauer et al., 1993)
	Baltic Sea	ND	93–94 (93)	WC	anoxic waters	(Weinbauer et al., 2003a)
		ND	26	WC	oxic waters	(Weinbauer et al., 2003a)
Average		**28**	**33 (45)**			
Offshore	East Sea, Korea	14	ND	WC		(Hwang & Cho, 2002)
	Gulf of Mexico	13	22	WC		(Wilhelm et al., 1998a; Weinbauer et al., 1999)
	Mediterranean Sea	ND	20	WC		(Weinbauer et al., 2003a)
Deep sea	Mediterranean Sea	ND	31–36 (33)	WC		(Weinbauer et al., 2003a)
Average		**13**	**21 (25)**			
Freshwater						
Oligotrophic	Lake Pavin, France	26	ND	WC		(Bettarel et al., 2004)
	Lake Gossenköllesee, Austria	4–45 (24)	ND	WC		(Pina et al., 1998)
		13	ND	WC		(Hofer & Sommaruga, 2001)
	Gäddtjärn, Sweden	6–18 (12)	ND	WC		(Vrede et al., 2003)
	Fisklösen, Sweden	6–21 (13)	ND	WC		(Vrede et al., 2003)
Oligo-mesotrophic	Sep Reservoir, France	8–140 (74)	ND	WC		(Pradeep Ram et al., 2005)
Average		**19**	ND			
Mesotrophic	Bourget, France	11–49 (30)	ND	WC		(Jacquet et al., 2005)
Meso-eutrophic	Rimov Reservoir, Czech Republic	8–47 (27)	ND	WC		(Simek et al., 2001)
		ND	19-40 (29)	WC		(Weinbauer et al., 2003b)
Eutrophic	Alte Donau, Austria	22	42	WC		(Fischer & Velimirov, 2002)
	Lake Aydat, France	30	ND	WC		(Bettarel et al., 2004)
	Lake Plussee, Germany	19–87 (53)	ND	WC		(Bergh et al., 1989; Demuth et al., 1993)
		28	46	WC	oxic and epi-metalimnion	(Weinbauer & Hofle, 1998)
		60	83		anoxic hypo-limnion	
	Danube backwater, Austria	26	ND	WC	estimated burst sizes for different bacteria morphotypes	(Mathias et al., 1995)
	Lake Constance, Germany	70	ND	TS		(Hennes et al., 1995)
Average		**39 (38)**	**57 (50)**			
Others						
Solar saltern	Multi-pond La Trinitat, Spain	22	ND	WC	prokaryotes except square archaea	(Guixa-Boixareu et al., 1996)
Solar saltern	Multi-pond La Trinitat, Spain	203	ND	WC	square archaea	(Guixa-Boixareu et al., 1996)

ND, not determined; WC, whole cell; TS, thin section; LFW, 'lysis from without'. Where the method was not indicated, WC was assumed. Data are given as average from a number of single-cell observations and as median (in parentheses) of a range of individual observations.

Table 2. *Mean values and number of samples shown in parentheses for compiled data of burst size (BS), frequency of visibly infected cells (FVIC), growth rate (GR) and bacterial production (BP).*

Environment	Status	BS	FVIC (%)	GR (d^{-1})	BP ($\mu g\ C\ l^{-1}\ d^{-1}$)
MARINE	Oligotrophic	19.8 (36)	1.01 (36)	0.43 (36)	7.19 (36)
	Eutrophic	24.9 (90)	1.82 (26)	0.60 (90)	27.4 (90)
FRESHWATER	Oligotrophic	27.6 (9)	2.23 (9)	—	60 (9)
	Eutrophic	39.8 (28)	2.21 (22)	1.10 (18)	83.4 (21)

ND, not determined. Growth rate was obtained only for a subset of data. Bacterial production estimates are derived from leucine or thymidine incorporation measurements converted into carbon units using the factors given in the individual papers.

cells completely filled with a low number of phages. Overall, minBS might provide conservative estimates of viral production calculated from FIC and FLC, whereas maxBS might provide more conservative estimates of FIC and FLC. When virus production is calculated from decay rates or from the increase of viral abundance in virus dilution approaches, virus-induced mortality is inferred by dividing viral production by BS. Thus, a maxBS results in a conservative estimate of virus-induced mortality based on viral production measurements.

The batch culture approach to determine the BS uses the reduction of viral abundance while the natural bacterial assemblage ideally remains unaltered. The difference in bacterial abundance evolving during the course of the incubation between the treatments with bacteria and viruses present in their natural abundance and bacteria incubated in the presence of greatly reduced viral abundance is assumed to be the result of viral lysis. The BS can be estimated from the observed net production of viruses over the incubation period. As the decay of viruses during incubation is not taken into account, it should be regarded as a minBS estimate (Middelboe & Lyck, 2002). Mei & Danovaro (2004) and Bongiorni et al. (2005) used this approach, however, they also estimated the fraction of active bacteria.

Burst size hypotheses

Several hypotheses explaining the variability in BS have been published or can be inferred from published results. These hypotheses are mainly based on studies using isolated phage–host systems. The hypotheses listed below are not mutually exclusive.

Size and morphology of cells and phages

Individual cells of natural prokaryotic communities differ in size and morphotypes and studies with isolates have shown that an intraspecific morphological plasticity occurs as well. Phage size and morphology does not only vary between species but a moderate plasticity such as elongated capsids is also possible for single phage isolates. Thus, the taxonomy of phages as well as hosts likely influences the BS. It has been shown for bacterioplankton that BS increases with cell size and that larger phages have a smaller burst size (Weinbauer & Peduzzi, 1994; Weinbauer & Höfle, 1998b). Therefore, the amount of phages that can be packed into an individual cell depends on the cell size as well as on the size of the phage.

However, the concentration of intracellular components such as holins also depends on cell dimensions. Since these enzymes regulate the lysis time and thus, control BS (Hadas et al., 1997) other spatial constraints than packing capacity for phages might also be important. A main factor determining the size of bacteria is the number of ribosomes which is generally related to the growth rate. In rapidly growing cells, RNA comprises about 35% of the cell mass while it is only about 15% in slow-growing cells (Knoll et al., 1999). It has been demonstrated that the elongation rate of ribosomes and the number of ribosomes strongly influence viral growth. Viral growth also depends on cell volume by affecting the concentrations of interacting molecular species. For example, an increase in cell volume results in a decrease in the concentration of capsid proteins leading to a reduction in the capsid assembly rate. This increase in cell volume might also affect the phage concentration in the cell. Nevertheless, a direct relation between the BS and cell volume was not always found (You et al., 2002). The rate of phage production, however, was found to be proportional to the amount of the protein synthesizing system at the time of infection (Hadas et al., 1997). As the BS is also a function of the rate of phage synthesis, an increased rate of phage production at a high cellular content of the protein synthesizing system results in a larger BS.

In contrast to the results obtained for the northern Adriatic Sea where BS varied widely among different morphotypes (Weinbauer & Peduzzi, 1994), such differences were not detected in the East Sea (Hwang & Cho, 2002). Size could also play an additional role. Being small might not only be a strategy of bacteria to reduce encounter rates with phages but infected small cells might also produce less phage progeny and thus, reduce the spreading of phage infection among a specific host population.

Physiological control

Fast growing cells should provide more resources for phage formation hence, accelerating cell lysis and increasing BS (Zachary, 1976; Kokjohn et al., 1991; Proctor et al., 1993; Kadavy et al., 2000; Middelboe, 2000; Young et al., 2000). Although an increasing bacterial activity translated to larger BS of bacterioplankton in different surveys (Wilhelm et al., 1998b; Hwang & Cho, 2002a; Bettarel et al., 2004), no general relation between BS and bacterial production across

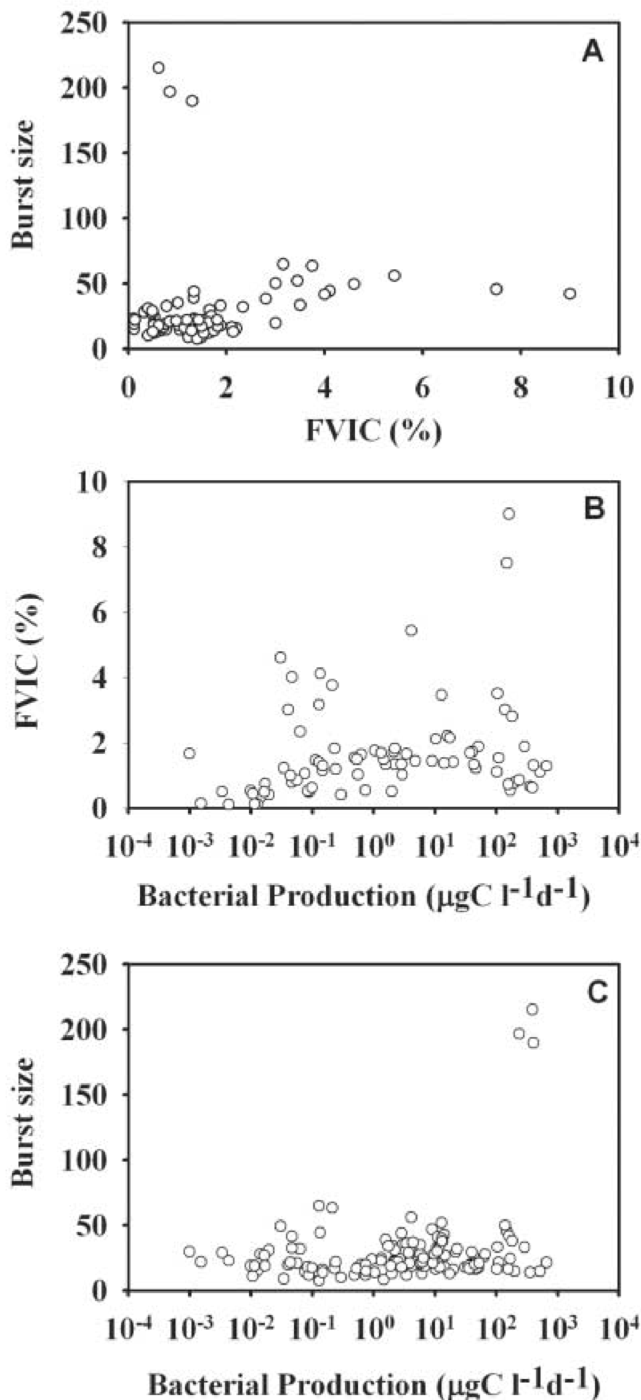

Figure 3. Relationship between (A) burst size and frequency of visible infected cells (FVIC); (B) frequency of visible infected cells and bacterial production; and (C) burst size and bacterial production. The burst size data are given in Table 1, the frequency of infected cells and bacterial production are derived from the papers cited in Table 1.

systems was detectable for the data set given in Table 1. Metabolic activity also influences cell size and morphology (Simek et al., 2001) thereby, linking BS indirectly to metabolic activity.

Habitat control

Cells living in favourable environments should produce more phage progeny than in non-favourable environments. For example, the BS of facultative anaerobes is lower in anoxic than in oxic environments (Moebus, 1996a,b). Thus, the number of infected cells and the viral progeny should increase with enhanced bacterial production, temperature and along trophic gradients as found in different studies (Weinbauer et al., 1993; Mathias et al., 1995; Wilhelm et al., 1998b; Hwang & Cho, 2002a; Bettarel et al., 2004). Poor environmental conditions along with low host quality prolong the latent period of lytic phages (Zachary, 1976; Kokjohn et al., 1991; Proctor et al., 1993; Middelboe, 2000; Young et al., 2000). In phytoplankton, the BS was found to be reduced by nutrient limitation (phosphorus and nitrogen) (Wilson et al., 1996) and by light availability (Bratbak et al., 1998). Habitat control is therefore the net outcome of different controlling mechanisms and might explain variations in BS when habitats change, e.g. with respect to eutrophication. The hypothesis that the BS is small at high productivity levels is not supported by any study.

Latent period control

It has been argued that the control of the latent period is a strategy for optimizing BS (Abedon et al., 2001; Wang & Chen, 2004). An extension of the latent period will increase the BS since more phages can be assembled. This strategy can be useful in a non- or slow-growing population, when a prolongation of the latent period and an increase in the BS will ensure viral spread when more favourable conditions become available (Doermann, 1948; Wommack & Colwell, 2000). Following this reasoning, a phage will have a shorter latent period and a smaller BS when generation times are short or when host abundance is high. Some studies suggest that temperate phages have a long latent period and a high BS, favoured by multiplicity of infection (Wilson & Mann, 1997) and low host abundance (Echols, 1971, 1972; Steward & Levin, 1984), whereas lytic phages exhibit a short latent period and a low BS. This concept can be extended to decay rates, i.e. BS should be high when decay rates are high as well.

Superinfection and lysis inhibition

The host can be infected by a single phage (usually at low multiplicity of infection), co-infected or superinfected (at high multiplicity of infection) (Miralles et al., 2001). In co-infection, hosts harbour infections by two or more phage types at the same time. As co-infection selects for within host competitive ability (Turner & Chao, 1998), the latent period and the BS increase for both phages, although they can have different latent periods (Brussow et al., 2004). The phage already residing in a co-infected host has a numeric advantage at the moment of infection by another phage and thus can produce more progeny (Delbrück, 1945; Nowak & May, 1994). Superinfection, i.e. re-infection with a homologous phage, might cause a delay in cell lysis, a phenomenon known as lysis inhibition (Doermann, 1948; Bode, 1967) and is thus responsible for the extension of the latent period and consequently, for an increase in BS (Abedon, 1999).

Phage control

There is evidence that growth of infected and non-infected cells in a bacterial population is similar and infected cells remain metabolically active until cell lysis

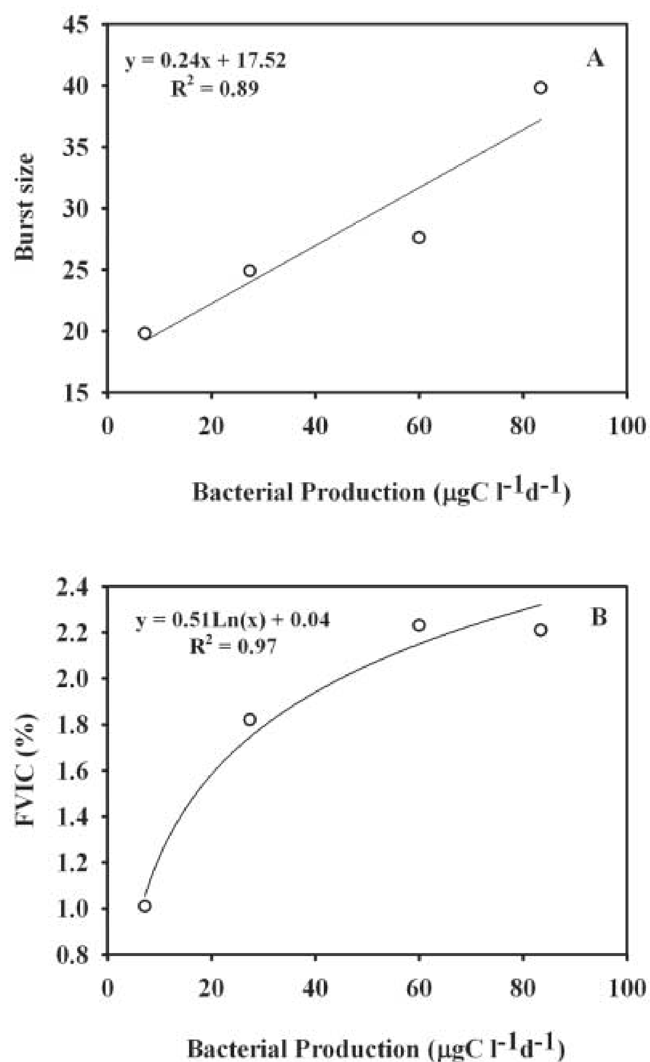

Figure 4. Saturation-type relationships from averaged data shown in Table 2 between (A) bacterial production and burst size; and (B) bacterial production and frequency of visible infected cells.

(Middelboe, 2000). This has also been observed for the photosynthetic activity in cyanobacteria (Suttle & Chan, 1994). It is possible that phages control this activity. For example, photosynthesis of infected cyanobacteria is prolonged by phage photosynthesis genes preventing a shut-off of photosynthetic activity. This might be considered a strategy to increase BS as well (Mann et al., 2003).

Burst size in different environments

Estimates of BS for single prokaryotic cells using TEM range from a minimum of 3–5, usually considered the detection limit of BS, to a maximum of ~500 found in long filamentous cells such as reported for an anoxic part of a eutrophic lake (Weinbauer & Höfle, 1998). A large BS in the range of 100 to 380 (Guixa-Boixareu et al., 1996) was also reported for the square-shaped archaeon *Haloquadratum walsbyi* (Bolhuis et al., 2004) growing in solar salterns. The BS in isolated phage–host systems is typically much larger than under *in situ* conditions (Børsheim, 1993).

Burst size for bacterioplankton ranges from 6 to 300 in marine systems and from 4 to 140 in freshwater systems. In Table 1, BS estimates for prokaryotic communities from a variety of aquatic systems are compiled. Average minBS was 28 in coastal and 13 in offshore environments and the corresponding values are 33 and 21 for maxBS (Table 1). In oligotrophic freshwater systems, only minBS estimates are available, with a mean BS of 19. MinBS and maxBS are higher in eutrophic waters and average 39 and 57, respectively. The same trend was found when the median of minBS and maxBS was calculated from studies where average values are not given. In addition, several studies from the Bering and Chukchi Seas, the Gulf of Mexico, the Mediterranean Sea and freshwater environments indicate that BS in oxic waters increases towards more eutrophic conditions or with bacterial production (see Table 1 for references). This supports the intuitively reasonable assumption that in nutrient poor systems, the number of phage progeny per cell is small. The grand average of minBS was higher in freshwater (37) than in marine habitats (24).

The BS of benthic bacteria differs from that of pelagic bacteria probably because of the differences in cell size (Fischer et al., 2003; Peduzzi & Schiemer, 2004; Bongiorni et al., 2005). In the anoxic waters of lake Plussee, a high BS was reported for large cells (Weinbauer & Höfle, 1998). The BS estimates in the Baltic Sea were significantly higher in the anoxic than in oxygenated surface waters (Weinbauer et al., 2003a). Visual inspection showed that cells in anoxic systems were much larger than in oxic and suboxic waters (unpublished data). Interestingly, anoxic environments are characterized by low grazing rates. Flagellate grazers often consume large active cells and cause a shift towards smaller cells. Thus, grazing might influence BS by influencing cell size. Another effect of grazing is that cells can develop grazing resistant morphologies such as filaments. This might cause an increase in BS, however, it has been shown that large grazing resistant cells in oxic habitats are often infected at lower frequencies (Weinbauer & Höfle, 1998b; Simek et al., 2001) and thus, might only insignificantly affect BS estimates.

Burst size estimated from non-TEM methods ranged from ~15 to 110 (Middelboe & Lyck, 2002; Mei & Danovaro, 2004; Bongiorni et al., 2005). These values are similar to TEM data. However, a direct comparison between methods has not been performed so far.

Relation between burst size, viral infection and bacterial production

In the following, BS is discussed in relation to viral infection and bacterial production. For studies where all these parameters were measured in parallel, BS estimates ranged from 7.5 to 215, thus covering the typical range of BS estimates. The frequency of visible infected cells (FVIC) ranged from 0.1 to 9% and bacterial production (BP) from 9.9×10^{-4} μg C l^{-1} d^{-1} to 661.3 μg C l^{-1} d^{-1}. The BS as well as BP were significantly different ($P=<0.001$, as determined by Kruskal–Wallis analysis of variance, Dunn's test) between different environments and trophic levels. However, FVIC was only significantly different ($P=<0.001$) when oligotrophic and eutrophic marine environments were compared. The BS and BP

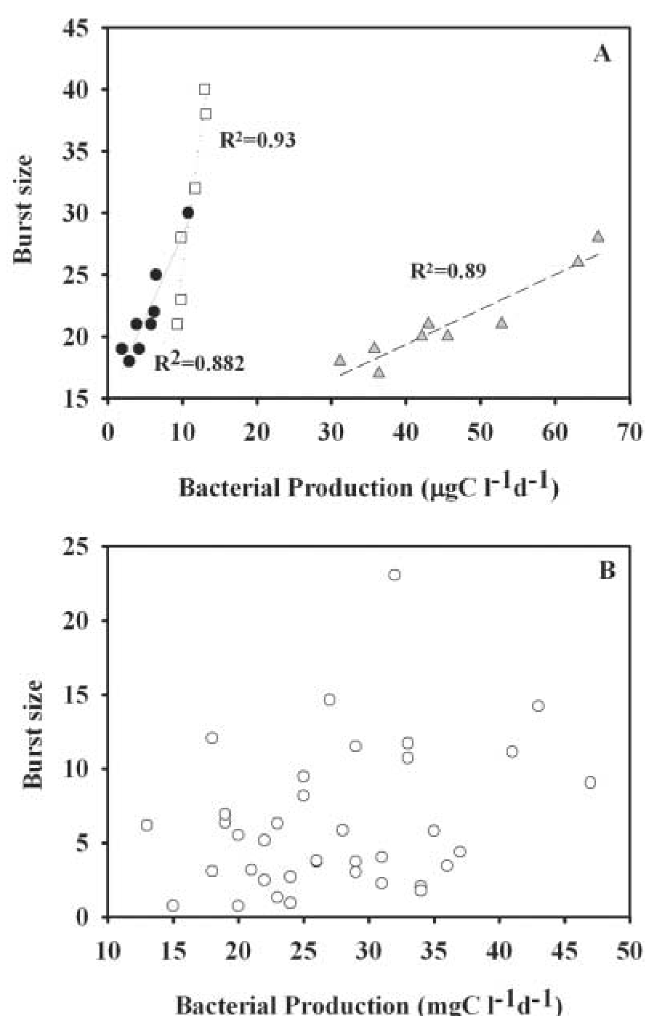

Figure 5. Bacterial production vs burst size. (A) Diel cycles in the North Sea during 2001 and 2002; (B) pooled data from several cruises in the North Sea. Cruises took place in the years 2000 and 2001. Data were used in Winter et al. (2004, 2005) but not shown in the presented form.

were higher in more eutrophic environments. For FVIC, this pattern was also found in marine environments, whereas in freshwaters, FVIC showed the same average of 2.2% in oligotrophic and eutrophic environments. In addition, FVIC was higher in freshwater than in marine waters. This could indicate differences between freshwater and marine systems. Such differences are well documented. For example, virus and bacterial abundance and VBR are often higher in freshwater than in marine systems (Maranger & Bird, 1995) and growth rate and bacterial production are also often higher (Table 2). Mean BS, FVIC and bacterial production increase along trophic gradients in marine as well as in freshwater habitats (Weinbauer et al., 1993; Steward et al., 1996; Wilhelm et al., 1998a; Bettarel et al., 2004).

An across systems analysis (using all data available) showed that BS and FVIC were not related to bacterial production, although FVIC was low at low bacterial production (Figure 3). When average data for environments shown in Table 2 were used, BS increased significantly with bacterial production ($P<0.01$) (Figure 4A) and FVIC increased significantly with BP ($P<0.01$) in a type of saturation curve (Figure 4B). Such a saturation-type relationship between FVIC and BP has been shown for tropical lakes (Peduzzi & Schiemer, 2004).

As for the across system analysis, BS was not related to BP in a survey in the North Sea (Figure 5). However, when investigating these parameters during three diel cycles following a drifting buoy, BS increased with BP for each diel cycles ($P<0.005$) (Winter et al., 2004) (Figure 5). Nevertheless, the relationship between BS and BP differed strongly among the diel cycles. This suggests, on the one hand, a strong link between viral progeny production and bacterial growth (physiological control hypothesis) and explains, on the other hand, why the across systems analysis failed to show trends. These data strongly support the idea that BP is a major factor influencing BS as also indicated by the analysis when average values for BS and BP were used for the different systems (see above). More specifically, these data also support the idea that phage lyse their host when they grow fast, since this increases BS and thus the number of offspring. However, the data clearly show that other parameters play a significant role as well, since the relationship between BS and BP varied between cruises (Winter et al., 2004). Phage and cell size and diversity could be responsible for that. 16S rRNA gene based genetic fingerprints showed a strong difference in the bacterial community composition between the three diel cycles (C. Winter, unpublished data). Thus, assuming some host specificity, this could mean that the specific relationships between BS and BP found were shaped by a different phage–host system being dominant.

The only parameter exhibiting a significant relationship ($R^2=0.626$; $N=79$, $P<0.0001$) with BS across systems was FVIC when three outliers from salt ponds (Guixa-Boixareu et al., 1996) with an extreme BS of ~200 were excluded (Figure 3A). Burst size increased until a FVIC of ~3% was reached and remained then relatively constant. Higher infection frequencies and BS are often also related to the trophic level of the system (Table 2). Thus, the high infection frequencies could increase the chance of superinfection and co-infection, which can result in an increase in BS.

Concluding remarks

In this review, we provide average estimates of BS for coastal and offshore marine waters and oligotrophic and eutrophic freshwater systems. In addition, we discuss the relationship between minBS and maxBS, which can be used to calculate conservative BS estimates for phage production and the effect of phages on the mortality of bacterioplankton. Data on bacterioplankton show that BS increases with cell size and the trophic status of the system. Burst size is linked to BP at small scales but this link differs between environments probably as a result of phage and host diversity. An across systems' feature seems to be the relationship between BS and FVIC, potentially a result of superinfection. The data show a large variability of BS, however, they also demonstrate that hypotheses on the control of BS can be tackled at the community level.

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REFERENCES

Abedon, S.T., 1999. Bacteriophage T4 resistance to lysis-inhibition collapse. *Genetic Research*, **74**, 1–11.

Abedon, S.T., Herschler, T.D. & Stopar, D., 2001. Bacteriophage latent-period evolution as a response to resource availability. *Applied Environmental Microbiology*, **67**, 4233–4241.

Bergh, O., Borsheim, K.Y., Bratbak, G. & Heldal, M., 1989. High abundance of viruses found in aquatic environments. *Nature, London*, **340**, 467–468.

Bettarel, Y., Sime-Ngando, T., Amblard, C. & Dolan, J., 2004. Viral activity in two contrasting lake ecosystems. *Applied and Environmental Microbiology*, **70**, 2941–2951.

Bode, W., 1967. Lysis inhibition in *Escherichia coli* infected with bacteriophage T4. *Journal of Virology*, **1**, 948–955.

Bolhuis, H., Poele, E.M.T. & Rodriguez-Valera, F., 2004. Isolation and cultivation of Walsby's square archaeon. *Environmental Microbiology*, **6**, 1287–1291.

Bongiorni, L., Magagnini, M., Armeni, M., Noble, R. & Danovaro, R., 2005. Viral production, decay rates, and life strategies along a trophic gradient in the north Adriatic sea. *Applied and Environmental Microbiology*, **71**, 6644–6650.

Børsheim, K.Y., 1993. Native marine bacteriophages. *FEMS Microbiology Ecology*, **102**, 141–159.

Bratbak, G., Jacobsen, A. & Heldal, M., 1998. Viral lysis of *Phaeocystis pouchetii* and bacterial secondary production. *Aquatic Microbial Ecology*, **16**, 11–16.

Brussow, H., Canchaya, C. & Hardt, W.D., 2004. Phages and the evolution of bacterial pathogens: from genomic rearrangements to lysogenic conversion. *Microbiology and Molecular Biology Reviews*, **68**, 560–602.

Choi, D.H., Hwang, C.Y. & Cho, B.C., 2003. Comparison of virus- and bacterivory-induced bacterial mortality in the eutrophic Masan Bay, Korea. *Aquatic Microbial Ecology*, **30**, 117–125.

Delbrück, M., 1945. The burst size distribution in the growth of bacterial viruses (bacteriophages). *Journal of Bacteriology*, **50**, 131–135.

Demuth, J., Neve, H. & Witzel, K.P., 1993. Direct electron microscopy study on the morphological diversity of bacteriophage populations in Lake Plussee. *Applied Environmental Microbiology*, **59**, 3378–3384.

Doermann, A.H., 1948. Lysis and lysis inhibition with *Escherichia coli* bacteriophage. *Journal of Bacteriology*, **55**, 257–276.

Echols, H., 1971. Lysogeny: viral repression and site-specific recombination. *Annual Review of Biochemistry*, **40**, 827–854.

Echols, H., 1972. Developmental pathways for the temperate phage: lysis vs lysogeny. *Annual Review of Genetics*, **6**, 157–190.

Fischer, U.R. & Velimirov, B., 2002. High control of bacterial production by viruses in a eutrophic oxbow lake. *Aquatic Microbial Ecology*, **27**, 1–12.

Fischer, U.R., Wieltschnig, C., Kirschner, A.K.T. & Velimirov, B., 2003. Does virus-induced lysis contribute significantly to bacterial mortality in the oxygenated sediment layer of shallow oxbow lakes? *Applied and Environmental Microbiology*, **69**, 5281–5289.

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

Guixa-Boixareu, N., Paz, J.I.C., Heldal, M., Bratbak, G. & Pedrós-Alió, C., 1996. Viral lysis and bacterivory as prokaryotic loss factors along a salinity gradient. *Aquatic Microbial Ecology*, **11**, 215–227.

Hadas, H., Einav, M., Fishov, I. & Zaritsky, A., 1997. Bacteriophage T4 development depends on the physiology of its host *Escherichia coli*. *Microbiology*, **143**, 179–185.

Heldal, M. & Bratbak, G., 1991. Production and decay of viruses in aquatic environments. *Marine Ecology Progress Series*, **72**, 205–212.

Hennes, K.P., Suttle, C.A. & Chan, A.M., 1995. Fluorescently labeled virus probes show that natural virus populations can control the structure of marine microbial communities. *Applied and Environmental Microbiology*, **61**, 3623–3627.

Hofer, J.S. & Sommaruga, R., 2001. Seasonal dynamics of viruses in an alpine lake: importance of filamentous forms. *Aquatic Microbial Ecology*, **26**, 1–11.

Hwang, C.Y. & Cho, B.C., 2002. Uneven growth and different susceptibility to viruses among bacteria increase estimates of virus production in the East Sea based on TEM observation. *Aquatic Microbial Ecology*, **27**, 211–218.

Hwang, C.Y. & Cho, B.C., 2002a. Virus-infected bacteria in oligotrophic open waters of the East Sea, Korea. *Aquatic Microbial Ecology*, **30**, 1–9.

Jacquet, S., Domaizon, I., Personnic, S., Ram, A.S.P., Hedal, M., Duhamel, S. & Sime-Ngando, T., 2005. Estimates of protozoan- and viral-mediated mortality of bacterioplankton in Lake Bourget (France). *Freshwater Biology*, **50**, 627–645.

Jiang, S.C., Kellogg, C.A. & Paul, J.H., 1998. Characterization of marine temperate phage–host systems isolated from Mamala Bay, Oahu, Hawaii. *Applied and Environmental Microbiology*, **64**, 535–542.

Kadavy, D.R. et al., 2000. Influence of infected cell growth state on bacteriophage reactivation levels. *Applied Environmental Microbiology*, **66**, 5206–5212.

Knoll, A., Osborn, M.J., Baross, J., Berg, H.C., Pace, N.R. & Sogin, M., 1999. *Size limits of very small microorganisms: proceedings of a workshop*. Washington, DC: National Academy Press.

Kokjohn, T.A., Sayler, G.S. & Miller, R.V., 1991. Attachment and replication of *Pseudomonas aeruginosa* bacteriophages under conditions simulating aquatic environments. *Journal of Genetic Microbiology*, **137**, 661–666.

Mann, N.H., Cook, A., Millard, A., Bailey, S. & Clokie, M., 2003. Marine ecosystems: bacterial photosynthesis genes in a virus. *Nature, London*, **424**, 741.

Maranger, R. & Bird, D.F., 1995. Viral abundance in aquatic systems: a comparison between marine and fresh waters. *Marine Ecology Progress Series*, **121**, 217–226.

Mathias, C.B., Kirschner, A.K.T. & Velimirov, B., 1995. Seasonal-variations of virus abundance and viral control of the bacterial production in a backwater system of the Danube river. *Applied and Environmental Microbiology*, **61**, 3734–3740.

Mei, M.L. & Danovaro R., 2004. Virus production and life strategies in aquatic sediments. *Limnology and Oceanography*, **49**, 459–470.

Middelboe, M., 2000. Bacterial growth rate and marine virus–host dynamics. *Microbial Ecology*, **40**, 114–124.

Middelboe, M. & Lyck, P.G., 2002. Regeneration of dissolved organic matter by viral lysis in marine microbial communities. *Aquatic Microbial Ecology*, **27**, 187–194.

Miralles, R., Ferrer, R., Solea, R.V., Moya, A. & Elena, F., 2001. Multiple infection dynamics has pronounced effects on the fitness of RNA viruses. *Journal of Evolutionary Biology*, **14**, 654–662.

Moebus, K., 1996a. Marine bacteriophage reproduction under nutrient-limited growth of host bacteria. I. Investigations with six phages. *Marine Ecology Progress Series*, **144**, 1–12.

Moebus, K., 1996b. Marine bacteriophage reproduction under nutrient-limited growth of host bacteria. II. Investigations with phage–host system [H3:H3/1]. *Marine Ecology Progress Series*, **144**, 13–22.

Nowak, M.A. & May, R.M., 1994. Superinfection and the evolution of parasite virulence. *Proceedings of the Royal Society* B, **255**, 81–89.

Peduzzi, P. & Schiemer, F., 2004. Bacteria and viruses in the water column of tropical freshwater reservoirs. *Environmental Microbiology*, **6**, 707–715.

Pina, S., Creus, A., Gonzalez, A., Girone, R., Felip, M. & Sommaruga, R., 1998. Abundance, morphology and distribution of planktonic virus-like particles in two high-mountain lakes. *Journal of Plankton Research*, **20**, 2413–2421.

Pradeep Ram, A.S., Boucher, D., Sime-Ngando, T., Debroas, D., Romagoux, J.C., 2005. Phage bacteriolysis, protistan bacterivory potential and bacterial production in a freshwater reservoir: coupling with temperature. *Microbial Ecology*, **50**, 64–72.

Proctor, L. & Fuhrman, J., 1990. Viral mortality of marine bacteria and cyanobacteria. *Nature, London*, **343**, 60–62.

Proctor, L.M., 1997. Advances in the study of marine viruses. *Microscopy Research and Technique*, **37**, 136–161.

Proctor, L.M., Okubo, A. & Fuhrman, J.A., 1993. Calibrating estimates of phage-induced mortality in marine-bacteria—ultrastructural studies of marine bacteriophage development from one-step growth experiments. *Microbial Ecology*, **25**, 161–182.

Simek, K., Pernthaler, J., Weinbauer, M.G., Hornak, K., Dolan, J.R., Nedoma, J., Masin, M. & Amann, R., 2001. Changes in bacterial community composition and dynamics and viral mortality rates associated with enhanced flagellate grazing in a mesoeutrophic reservoir. *Applied and Environmental Microbiology*, **67**, 2723–2733.

Steward, F.M. & Levin, B.R., 1984. The population biology of bacterial viruses: why be temperate. *Theoretical Population Biology*, **26**, 93–117.

Steward, G.F., Smith, D.C. & Azam, F., 1996. Abundance and production of bacteria and viruses in the Bering and Chukchi Seas. *Marine Ecology Progress Series*, **131**, 287–300.

Suttle, C.A. & Chan, A.M., 1994. Dynamics and distribution of cyanophages and their effect on marine *Synechococcus* spp. *Applied and Environmental Microbiology*, **60**, 3167–3174.

Suttle, C.A., Chan, A.M. & Cottrell, M.T., 1990. Infection of phytoplankton by viruses and reduction of primary productivity. *Nature, London*, **347**, 467–469.

Turner, P.E. & Chao, L., 1998. Sex and the evolution of interhost competition in RNA virus F6. *Genetics*, **150**, 523–532.

Vrede, K., Stensdotter, U. & Lindström, E.S., 2003. Viral and bacterioplankton dynamics in two lakes with different humic contents. *Microbial Ecology*, **46**, 406–415.

Wang, K. & Chen, F., 2004. Genetic diversity and population dynamics of cyanophage communities in the Chesapeake Bay. *Aquatic Microbial Ecology*, **34**, 105–116.

Weinbauer, M.G., Brettar, I. & Hofle, M.G., 2003a. Lysogeny and virus-induced mortality of bacterioplankton in surface, deep, and anoxic marine waters. *Limnology and Oceanography*, **48**, 1457–1465.

Weinbauer, M.G., Christaki, U., Nedoma, A. & Simek, K., 2003b. Comparing the effects of resource enrichment and grazing on viral production in a meso-eutrophic reservoir. *Aquatic Microbial Ecology*, **31**, 137–144.

Weinbauer, M.G., Fuks, D. & Peduzzi, P., 1993. Distribution of viruses and dissolved DNA along a coastal trophic gradient in the northern Adriatic Sea. *Applied Environmental Microbiology*, **59**, 4074–4082.

Weinbauer, M.G. & Höfle, M.G., 1998a. Significance of viral lysis and flagellate grazing as factors controlling bacterioplankton production in a eutrophic lake. *Applied Environmental Microbiology*, **64**, 431–438.

Weinbauer, M.G. & Höfle, M.G., 1998b. Size-specific mortality of lake bacterioplankton by natural virus communities. *Aquatic Microbial Ecology*, **15**, 103–113.

Weinbauer, M.G. & Peduzzi, P., 1994. Frequency, size and distribution of bacteriophages in different marine bacterial morphotypes. *Marine Ecology Progress Series*, **108**, 11–20.

Weinbauer, M.G. & Suttle, C.A., 1996. Potential significance of lysogeny to bacteriophage production and bacterial mortality in coastal waters of the Gulf of Mexico. *Applied and Environmental Microbiology*, **62**, 4374–4380.

Weinbauer , M.G. & Suttle, C.A., 1999. Lysogeny and prophage induction in coastal and offshore bacterial communities. *Aquatic Microbial Ecology*, **18**, 217–225.

Weinbauer, M.G., Wilhelm, S.W., Suttle, C.A. & Garza, D.R., 1997. Photoreactivation compensates for UV damage and restores infectivity to natural marine virus communities. *Applied Environmental Microbiology*, **63**, 2200–2205.

Wilhelm, S.W., Weinbauer, M.G., Suttle, C.A. & Jeffrey, W.H., 1998a. The role of sunlight in the removal and repair of viruses in the sea. *Limnology and Oceanography*, **43**, 586–592.

Wilhelm, S.W., Weinbauer, M.G., Suttle, C.A., Pledger, R.J. & Mitchell, D.L., 1998b. Measurements of DNA damage and photoreactivation imply that most viruses in marine surface waters are infective. *Aquatic Microbial Ecology*, **14**, 215–222.

Wilson, W., Carr, N. & Mann, N., 1996. The effect of phosphate status on the kinetics of cyanophage infection in the oceanic cyanobacterium *Synechococcus* sp. *Journal of Phycology*, **32**, 506–516.

Wilson, W. & Mann, N., 1997. Lysogenic and lytic viral production in marine microbial communities. *Aquatic Microbial Ecology*, **13**, 95–100.

Winter, C., Herndl, G.J. & Weinbauer, M.G., 2004. Diel cycles in viral infection of bacterioplankton in the North Sea. *Aquatic Microbial Ecology*, **35**, 207–216.

Winter, C., Smit, A., Szoeke-Denes, T., Herndl, G.J. & Weinbauer, M.G., 2005. Modelling viral impact on bacterioplankton in the North Sea using artificial neural networks. *Environmental Microbiology*, **7**, 881–893.

Wommack, K.E. & Colwell, R.R., 2000. Virioplankton: viruses in aquatic ecosystems. *Microbiology and Molecular Biology Reviews*, **64**, 69–114.

You, L., Suthers, P. & Yin, J., 2002. Effects of *Escherichia coli* physiology on growth of phage T7 *in vivo* and *in silico*. *Journal of Bacteriology*, **184**, 1888–1894.

Young, R., Wang, I.N. & Roof, W.D., 2000. Phages will out: strategies of host cell lysis. *Trends in Microbiology*, **8**, 120–128.

Zachary, A., 1976. Physiology and ecology of bacteriophages of marine bacterium *Beneckea natriegens*: salinity. *Applied and Environmental Microbiology*, **31**, 415–422.

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