

Ecology of prokaryotic viruses

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Abstract

The finding that total viral abundance is higher than total prokaryotic abundance and that a significant fraction of the prokaryotic community is infected with phages in aquatic systems has stimulated research on the ecology of prokaryotic viruses and their role in ecosystems. This review treats the ecology of prokaryotic viruses ('phages') in marine, freshwater and soil systems from a 'virus point of view'. The abundance of viruses varies strongly in different environments and is related to bacterial abundance or activity suggesting that the majority of the viruses found in the environment are typically phages. Data on phage diversity are sparse but indicate that phages are extremely diverse in natural systems. Lytic phages are predators of prokaryotes, whereas lysogenic and chronic infections represent a parasitic interaction. Some forms of lysogeny might be described best as mutualism. The little existing ecological data on phage populations indicate a large variety of environmental niches and survival strategies. The host cell is the main resource for phages and the resource quality, i.e., the metabolic state of the host cell, is a critical factor in all steps of the phage life cycle. Virus-induced mortality of prokaryotes varies strongly on a temporal and spatial scale and shows that phages can be important predators of bacterioplankton. This mortality and the release of cell lysis products into the environment can strongly influence microbial food web processes and biogeochemical cycles. Phages can also affect host diversity, e.g., by 'killing the winner' and keeping in check competitively dominant species or populations. Moreover, they mediate gene transfer between prokaryotes, but this remains largely unknown in the environment. Genomics or proteomics are providing us now with powerful tools in phage ecology, but final testing will have to be performed in the environment.

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Keywords: Virus; Phage; Prokaryote; Bacteria; Diversity

Abbreviations: BP, bacterial production; Chla, Chlorophyll *a*; CPS, cyanophage specific; DCM, deep chlorophyll maximum; DGGE, denaturing gradient gel electrophoresis; DMS, dimethylsulfide; DMSF, dimethylsulfonylpropionate; DOM, dissolved organic matter; EFM, epifluorescence microscopy; EPS, exopolysaccharides produced by bacteria often resulting in a capsule around cells; FCM, flow cytometry; FIC, frequency of infected cells; FLV, fluorescently labeled viruses; FVIC, frequency of visibly infected cells; GPCA, great plate count anomaly; HNF, heterotrophic nanoflagellates; LRVI, loss rates of viral infectivity; LRVP, loss rates of viral particles; NER, nucleotide excision repair; MOI, multiplicity of infection; MPN, most probable number; PAR, photosynthetic active radiation; PER, photoenzymatic repair; PFGE, pulsed-field gel electrophoresis; PFU, plaque forming unit; PHS, phage–host system; POM, particulate organic matter; RFLP, restriction fragment length polymorphism; R–M, restriction–modification (an antiphage defense mechanism); SSCP, single-strand conformation polymorphism; TEM, transmission electron microscope; TGGE, temperature gradient gel electrophoresis; T-RFLP, terminal restriction fragment length polymorphism; VBR, viruses to bacteria ratio; VDA, virus dilution approach; VIC, visibly infected cell; VMP, viral mortality of prokaryotes; VPR, viral production rates; WSA, whole seawater approach.

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1. Introduction

1.1. Historical sketch

Bacterial viruses were discovered twice, by Twort in 1915 and by d’Herelle in 1917 [1]. D’Herelle coined the name ‘bacteriophages’ for these infectious agents lysing bacteria, which literally means ‘eaters of bacteria’. Research on (bacterio)phage played a central role in deciphering molecular principles of life such as the finding that DNA is the hereditary molecule and led to the development of an entirely new science, molecular biology [1]. Right after their discovery, phages were also used in an early form of biotechnology to fight bacterial

pathogens [2]. In Western Europe and the United States, this phage therapy was abandoned due to ambiguous results and the discovery of antibiotics. In some Eastern European countries and the former Soviet Union, phage therapy was continued and recently a review was published on this topic [3]. The threat of resistance to antibiotics has revived the interest in phage therapy [4].

Although it was frequently stated in the older literature that phages should significantly impact microbial food webs, their role was not appreciated appropriately. As early as 1968, Wiebe and Liston [5] suggested that phages could exert an influence on “...clonal populations of bacteria in limiting the numbers, types, and duration of active population growth and ultimately,

through possible genetic exchange mechanisms (e.g., transduction), on the biochemical capabilities of microorganisms". They continued "this latter phenomenon is of considerable potential importance because of the prominent role of bacteria as mineralizing and chemical transforming agents within the environment". This opinion on the role of phages was obviously not shared by fellow researchers, since it took ca. 10 years before Torrella and Morita [6] reported in 1979 for the first time on high virus numbers ($>10^4 \text{ ml}^{-1}$) in a natural environment by using transmission electron microscopy (TEM). These authors also pointed out that they had most likely seriously underestimated viral abundance, since most viruses should have passed the filter they used to collect the viruses. In 1980, 10^5 – 10^7 viruses ml^{-1} were reported from sewage by using ultracentrifugation and TEM [7]. Unfortunately, these findings were not included in the concepts of the microbial food web, which started to emerge from theoretical considerations and novel techniques [8,9]. It took another decade, until it was confirmed that viral numbers exceed those of bacteria and that phage infection causes a significant mortality of prokaryotes in aquatic systems [10–14]. These findings have boosted research on the ecology of natural virus communities and their impact on microbial food web processes and biogeochemical cycles.

1.2. Scope of review

Viral ecology is the study of the interactions of viruses with other organisms and the environment. The aim of this paper is to critically review our knowledge of the ecology of viruses infecting prokaryotes (subsequently called phages) in large ecosystems such as limnetic, marine and soil systems. More specifically, it attempts to apply ecological concepts to viruses and describe the ecology of these acellular life forms, which influence cellular organisms and biosphere processes stronger than previously thought. Ecology is here seen strictly from a 'virocentric' point of view [15]. This review focuses on prokaryotic viruses but also uses information from other microorganisms such as viruses infecting phytoplankton. As there are recent and excellent reviews on cyanophages [16,17], i.e., viruses infecting cyanobacteria, this group of viruses will not be dealt with in particular, although data from cyanophage studies will be used, when information from phages infecting heterotrophic bacteria is sparse. Also, this review aims at understanding viral ecology and thus concentrates on in situ studies and viral communities (including experiments with natural communities), however, numerous examples from phage–host systems (PHSs) are presented as well. Reviews on viruses of prokaryotes are restricted to certain groups or ecosystems [16–21] and reviews on viruses in pelagic systems are available [22–34]. There are excellent reviews on phages (including

non-indigenous ones) before the times viral total counts being were used (e.g. [35–39]).

This review is divided into five main sections. In Section 1, methods for estimating viral parameters (mostly at the community level) are critically reviewed. Section 2 deals with types, distribution, abundance and diversity of viruses. In Section 3, factors influencing phage growth and survival are discussed, whereas in Section 4, phage life cycles and the population dynamics of phages as well as their interaction with the environment are described. Section 5 deals with the role of phages in the environment such as, for example, their influence on food web processes, biogeochemical cycles, gene transfer and prokaryotic diversity. These sections are introduced by some basic concepts used throughout the review (Section 1) and followed by an outlook on potential future research (Section 6).

1.3. What is a phage?

Viruses are a group of biological entities with a genome consisting either of DNA or RNA and encapsulated in a protein coat (capsid). The species concept has been applied to viruses and a viral species is defined as "...a polythetic class of viruses that constitutes a replicating lineage and occupies a particular ecological niche" [40]. Also, a new way to classify phages has been proposed based on complete sequences of 105 viral genomes [41]. Recently, a new biological domain beside the 'cellular' domains *Bacteria*, *Archaea* and *Eukarya* was proposed, the *Akamara* (ακαμαρα; Greek for 'without chamber' or "without void"), i.e., acellular infectious agents possessing nucleic acid genomes [42]. A possible organizational structure of this domain was suggested with two kingdoms, and several phyla and classes. A tentative taxonomic affiliation of the cyanomyovirus AS-1 [16] is shown in Fig. 1. Note that in this classification it is assumed that the order *Caudovirales*, i.e., the tailed phages, are a monophyletic group [43–45], and that *Cyanostylovirus* is a valid virus genus. Thus, the Cyanophage AS-1 virus can be classified as belonging to the genus *Cyanostylovirus*, the family *Myoviridae*, the order *Caudovirales*, the class Double-strand DNA viruses, the phylum *Deoxyribovira*, the kingdom *Euviria* and the domain *Akamara*. This concept offers a system with the potential for unifying cellular and acellular life forms. Recent evidence suggests that viral DNA is at the origin of eukaryotic replication proteins [46] and "...thus connect the universal tree of life via a viral linkage" [47]. However, it remains to be shown, whether or not the four domains can be combined in a single phylogenetic tree and to which extent the proposed taxonomy of the *Akamara* is a phylogenetic system. Reconsidering viral taxonomy, it has also been suggested that dsDNA, ssDNA, dsRNA and ssRNA represent domains, which probably evolved independently, and groups such as

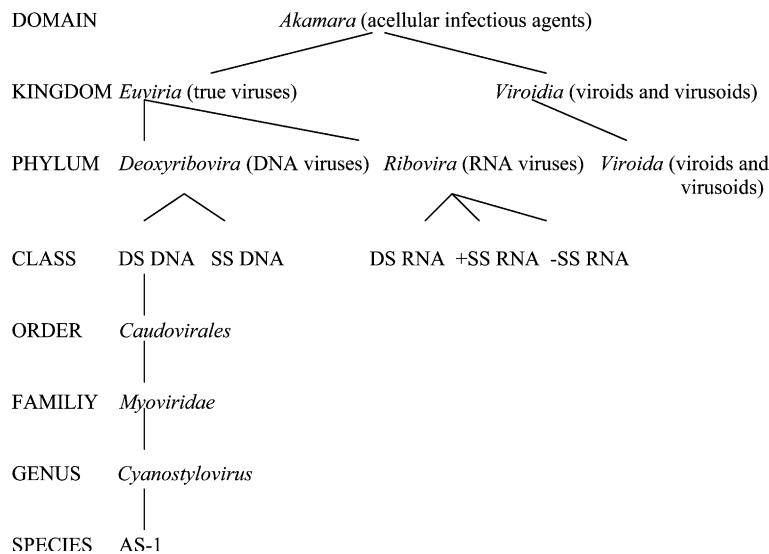


Fig. 1. Potential taxonomic affiliation of the Cyanophage S-1 to the proposed new domain *Akamara* as proposed in [42]. –SS RNA, negative-sense single-stranded RNA genome; +SS RNA, positive-sense, single-stranded RNA genome; DS RNA, double-stranded RNA genome; SS DNA, single-stranded DNA genome; DS DNA, double-stranded DNA genome. Note that the International Committee on Taxonomy of Viruses covers only taxonomic levels from species through order.

‘tailed phages’ or ‘filamentous phages’ represent divisions with only little genetic exchange across division boundaries [48]. In this system, there are most likely no hierarchical groups such as families for phages, but due to gene transfer there are reticulate groups of viruses or ‘modi’, which share a particular genetic module or phenotypic character. In this reticulate, multidimensional phylogeny, phages would then be assigned to different but overlapping sets of modi.

1.4. The phage life cycle

Several steps during the life cycle of a prokaryotic virus can be distinguished that are common to all viruses: adsorption, separation of nucleic acids from protein coat, expression and replication of the nucleic acids, virion assembly, release and transmission [1]. Phage adsorption occurs in two steps. The first step of adsorption to a defined cell surface structure (after initial contact) is reversible and may represent a possibility of the phage to ‘decide’ against infection. During the second step, an irreversible binding between a phage structure (e.g., tail fibers) and the receptor is accomplished. After adsorption, the cell wall is made penetrable (e.g., by special phage enzymes in the tail or capsid) and the nucleic acid is transported into the cell, whereas the capsid remains outside the cell. Following injection, the genetic material is either integrated into the host genome or stays in the cytoplasm. For all different types of viruses, there is a stage where the phage genome exists within the host but outside the host genome. In this stage, gene expression, genome replication and morphogenesis occurs, i.e., the formation of the genomes and the capsids (and tails) and the packing of

the genomes into the capsids. For a specific group of viruses, which are significant in the environments discussed in this review, namely the tailed phages, the morphogenesis is so similar that a general assembly pathway was suggested [43]. The latent period is the phase of an infection, where there are no extracellular or ‘free’ phages detectable and describes the phage life cycle from adsorption to the cell lysis. The phase of the latent period before capsids and genomes are assembled into mature phages is called the eclipse period. The rise period is characterized by the release of mature phages into the environment due to cell lysis and the detection of free phages (virions). The number of virions released per cell into the extracellular environment is called the burst size. In the tailed phages, cell lysis is accomplished by a dual lysis system consisting of peptidoglycan hydrolases (endolysins), which attack the murein part of the cell wall, and holins, which damage the plasma membrane and allow the endolysin to break down the peptidoglycan [43]. Phages can also be released by budding or extrusion. The extracellular stage ends with the ‘death’ of a phage or a new infection.

Viruses show several life cycles: lytic, lysogenic, pseudolysogenic and chronic infections (Fig. 2) [36]. In the lytic cycle, the (lytic or virulent) phage redirects the host metabolism towards the production of new phages, which are released during the lysis of the cell. In the lysogenic cycle, the genome of the (temperate or lysogenic) phage typically remains in the host in a dormant stage (prophage) and replicates along with the host, until the lytic cycle is induced. A ‘lysogenic decision’, whether or not to establish a prophage state is made by the temperate phage after infection [36]. A chronic infection occurs, when a cell is infected and phage progeny is constantly

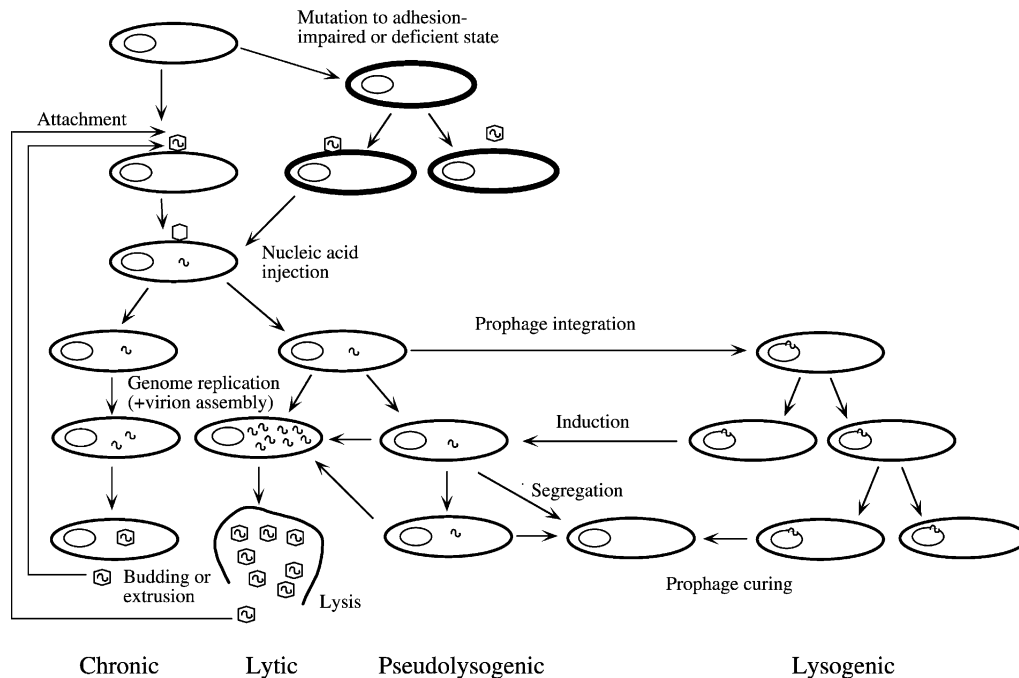


Fig. 2. Types of viral life cycles. The model is adopted from [128] with some modifications and expanded by chronic infection.

released from the host cell by budding or extrusion without lysing it. In persistent infections (pseudolysogeny, phage-carrier state) phages multiply in a fraction of the population. Pseudolysogeny and carrier-state are widely used as synonyms, however, the carrier state is more strictly used for bacteria with a plasmid-like prophage [36]. Mechanisms for establishing the carrier state include a reduced success rate of infection conferred by limited available receptors or by enzymatic loss of receptors or superinfection immunity of a temperate phage, which mutates at a fixed frequency to lytic phages, thus allowing for the simultaneous presence of phages and bacteria in a culture [49]. This suggests that pseudolysogeny is a common name for different phage life cycles. Overall, such classifications are probably a simplification of the diversity of phage life cycles [37].

1.5. Species diversity

Diversity consists of three parameters: species richness, species evenness and species difference. Richness refers to the number of species, evenness means the significance of single species in terms of abundance, biomass or activity, and difference is the taxonomic relatedness of species in a system.

It is possible to estimate the general species richness of viruses based on an educated guess. Most host species (including parasitic ones) have more than one specific virus and the viral host range is often quite narrow. Thus, it is reasonable to assume that the species richness of viruses is at least as high as that of cellular life forms. It has been argued that "...bacteria await biologists as

the black hole of taxonomy" [50]. This fits even better to viruses and we are just beginning to develop the tools for investigating all three aspects of viral diversity.

Typically only a small fraction (often less than 1%) of the prokaryotes from an aquatic or soil environment grow on agar plates (sometimes called Great-Plate-Count-Anomaly, GPCA) [51]. This has hampered the study of prokaryotic diversity (as well as assessing total prokaryotic abundance in a sample) and as prokaryotic isolates are needed to isolate phages, this has also restricted the research on viral diversity. It has even been argued that viral lysis might be one of the reasons for the low plating efficiency of bacteria [52].

With the advent of ribosomal RNA as phylogenetic clock, a tremendous leap forward has been made in studying the diversity and phylogeny of life forms [53]. Three domains of (cellular) life are now distinguished and the former *Prokaryota* are now divided into the domains *Bacteria* and *Archaea*. An array of culture-independent techniques is available to tackle prokaryotic diversity and circumvent the GPCA [54,55]. As discussed below, such methods are now available to start tackling viral diversity as well.

2. Methods in the community ecology of prokaryotic viruses

2.1. Enumeration and concentration of viruses

Typically, two different ways have been used to enumerate viruses: the indirect, 'viable' counts (here used

within quotation marks to distinguish infective viruses from truly viable prokaryotes) and the direct total counts. For a detailed description of methods, see [56–60]. ‘Viable’ counts are obtained as plaque-forming units (PFU) on a lawn of host cells on an agar plate (plaque assay) or in liquid medium as most-probable-number (MPN) assays. Since this requires the isolation of a host either on an agar plate or in liquid culture, and phages only infect a small range of host species (see below), viable counts only represent a small fraction of the total counts. Direct counts are frequently 100–1000-fold higher than PFU counts [32]. This might be termed the ‘Great-Plaque-Count-Anomaly’ and represents a special case of the GPCA.

Transmission electron microscope was used to visualize phages and characterize their morphology, particularly after the negative staining technique was introduced in 1959 [61,62]. The first evidence of high viral abundances was also obtained by using TEM [6,63]. Before total counts of viruses can be obtained, they have to be concentrated (except for flow cytometry, FCM). Typically this is done either by ultracentrifugation onto TEM grids or by filtration onto 0.02- μm pore-size filters. Direct total viral counts can be determined using three different methods, by using TEM and uranylacetate positive staining (Fig. 3), epifluorescence microscopy (EFM) (Fig. 4) and FCM [10,12–14,64]. For EFM and FCM counting viruses are stained with fluorochromes such as DAPI [10,14,65–67], YOPRO-1 [68,69], SYBRGreen I [64,70] or SYBRGold [71]. TEM counts of viruses are typically lower than EFM counts [65,68,70,72,73], whereas FCM counts of viruses are at least as high as those determined by EFM [64,71,74]. FCM as well as digital image analysis based EFM [71,75] allows for a rapid assessment of total viral abundance. There is a tendency that EFM is becoming

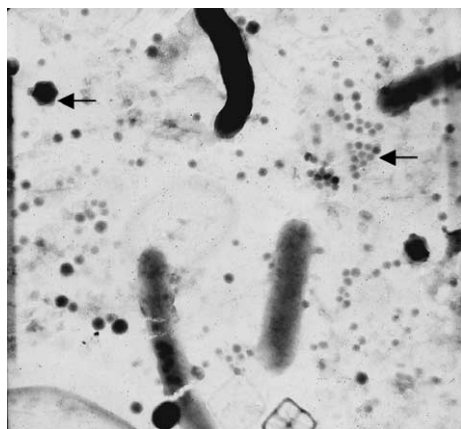


Fig. 3. Transmission electron micrographs showing viruses and bacteria in a water sample from the northern Adriatic Sea. Viruses and bacteria were stained with uranylacetate. Arrows point to viruses of different size. The large virus in the upper right corner has a head diameter of ca.150 nm.

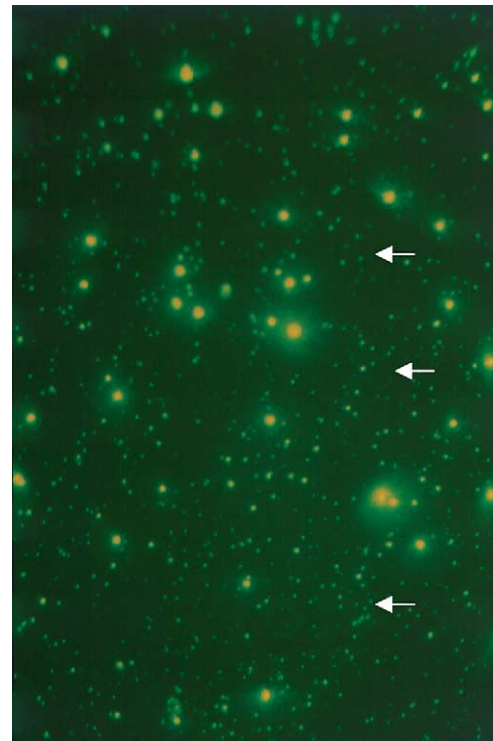


Fig. 4. Epifluorescence microscopy picture showing viruses and bacteria in a water sample from the Bay of Villefranche (France). Viruses and bacteria were stained with SYBRGreen I. Arrow heads point to particles, which are considered viruses based on size and staining intensity.

more frequently used than TEM for estimating total viral abundance. This is probably due to the rapidity of the EFM method, the possible application during field work such as onboard a ship and lower costs combined with the through-put of a larger set of samples, which allows for the collection of more data including those for increasing statistical accuracy [21,30]. In Table 1 some advantages and disadvantages of methods used for ‘viable’ and direct counts of phages are listed. Using the fluorochrome methods, data on the distribution of viral abundance across systems and data from experiments will increase rapidly in the years to come.

Each of these methods has benefits and pitfalls. The TEM approach seems to underestimate viral abundance due to technical problems such as uneven collection, uneven staining, washing off of viruses, low detection limit as well as the lack of recognition of non-typical viruses [65,68,70,72,73]. For EFM and FCM there is, e.g., the problem that not every fluorescent dot might be a virus but DNA bound to colloids. Similar problems have been reported for direct counts of bacteria using EFM [76]. Also, large viruses can be confused with bacteria. This has been reported as a problem for counting viruses [77]. However, assuming that viruses are ca. 10 times as abundant as bacteria (see below) and that ca. 10% are large viruses (which likely presents an overestimation; Table 2), then the potential bias would

Table 1
Some advantages and disadvantages of methods to enumerate viruses

Technique	Advantages	Disadvantages
Plaque forming unit assay	Good detection limit	Strong underestimation of total abundance
Most probably number assay	Infective 'viable' counts Potential for isolation of phages Potential for morphological and biochemical characterization	Time consuming Need for host isolation
Transmission electron microscopy method	Total counts Rough morphological characterization and sizing of phages	Slight underestimation of total abundance Need for expensive equipment Field application impossible No information on infectivity Particulate matter can obscure counts No detection of unusual viruses Time consuming Low precision and low detection limit No information on infectivity
Epifluorescence microscopy method	Total counts No detection limit for environmental samples Relatively rapid Field implication possible	No information on morphology No distinction between viruses and DNA bound colloids Distinction between phages and bacteria based on size and staining intensity
Flow cytometry approach	High precision Total counts No detection limit for environmental samples Rapid Field implication possible High precision Distinction between several genome size classes	No information on infectivity No information on morphology No distinction between viruses and DNA bound colloids Distinction between phages and bacteria based on fluorescence intensity (genome size)

be only 10% for viral counts but 100% for bacterial counts. In this case, counting particles at an overlapping size range using EFM is likely rather a problem for bacteria than for viruses. An advantage of the TEM method is the possibility of sizing the capsids and assessing morphological structures such as tail types. A shortcoming for all total count methods is that they do not provide information on the infectivity of viruses.

Frequently, it is necessary to concentrate viruses from environmental samples, not only for counting but also for isolation or for obtaining libraries of viruses, which can be used for isolation of viruses and for genetic fingerprinting of viral communities [56,78]. Methods used to concentrate viruses are filter adsorption-elution, flocculation and ultracentrifugation [62]. Ultrafiltration technology is another valuable tool in studying viral communities [79]. Some of the first approaches to estimate total abundance of viruses used ultrafiltration to concentrate viruses for counting [11,13,14], however, due to the losses of viruses during the concentration step, these methods have been abandoned. Microbial cells are removed from water samples by filtration through 0.2- μ m pore-size filters and concentrates of the viral community are obtained by ultrafiltration meth-

odology optimized to avoid losses of infectivity during the procedure [66,80,81]. The two fractions obtained in this approach are the retentate (virus concentrate) and the permeate (virus-free water). Cartridges with 30 and 100 kDa cut-off efficiently remove the virus-size fraction. In numerous studies this method has been used to obtain virus-free water and viral concentrates for experiments to test the effect of a varying viral abundance on food web processes (see below).

2.2. Burst size

In PHSs, burst size is estimated by 'one-step growth curve experiments', also called 'single-burst experiments' [82]. Most data from the environment are from a TEM approach studying visibly infected cells (VIC) (Fig. 5) [83–100]. Such studies estimated the burst size of individual cells but also tried to assess the average burst size of all VIC in a sample for studies at the community level. It has been argued that the average burst size from all infected cells in a sample represents a minimum estimate, since the number of viruses within a cell might still increase [88]. If only cells, which are completely filled with viruses, are used for calculating burst size, this

Table 2
Size distribution of free and intracellular virus particles in marine and freshwater systems^a

Site ^b	n ^c	Virus head size classes ^d					Average ^e	References
		<30 nm	30–60 nm	60–80 nm	80–100 nm	>100 nm		
Marine								
<i>Offshore</i>								
North Atlantic	1	0	69	28	1	2	59	[12]
Southern California Bight	24	12	63	22	<1	<1	56	[159]
Alboran Sea	?		73	27			NA	[497]
<i>Coastal/shelf</i>								
Chesapeake Bay	1	0	41	25	25	10	64	[12]
Chesapeake Bay	60	5	65	32			NA	[168]
Gulf of Bothnia	13	20	64	17	14	3	55	[159]
Northern Adriatic	35	5	63	20	8	5	59	[86]
Northern Adriatic (IC)			72	28			62	[85]
Norwegian fjords	3	0	47	26	19	7	64	[12]
Southern California Bight	8	15	45	36	4	1	58	[159]
Oyster pond	6		76 (≤64 nm)	16 (64–105 nm)		8 (>105 nm)	NA	[163]
Limnetic								
Danube backwater	2		16	84			NA	[94]
Danube	12		74	21 (60–90 nm)	5 (>90 nm)		NA	[95]
Lake Constance	19		>50	<50			NA	[97]
Lake Constance (IC)	9		>50	<50			NA	[97]
Plußsee	1	0	65	17	14	3	62	[12]
Plußsee-oxic	5						68	[152]
Plußsee-thermocline	1						84	[152]
Plußsee-anoxic	1						89	[152]
Lake Superior	?	53	45	3 (60–110 nm)			NA	[153]

^aData on size distribution of viruses in sea-ice and various lakes presented by [160,161] because of non-compatible size classes. Data are percentages of total.

^bIC, intracellular, i.e., phages within cells.

^cNumber of samples investigated. Note that for the Danube backwater, values are calculated from averages of 2 years and those for the from averages of 3 years.

^dIn the study by Wommack et al. [168], viruses >60 nm were combined, and in the Danube, Lake Constance and intracellular phages in the Northern Adriatic Sea viruses <60 and >60 nm were combined.

^eValues were calculated from the size distribution data by assuming median values for each size class and by excluding viruses from the size classes <30 and >100 nm, in order to allow for a comparison between studies. In the study of Demuth et al. [152] the size range of viruses was 41–117 nm in oxic water, 53–106 nm in the thermocline layer and 48–117 nm in anoxic water. NA, not applicable.

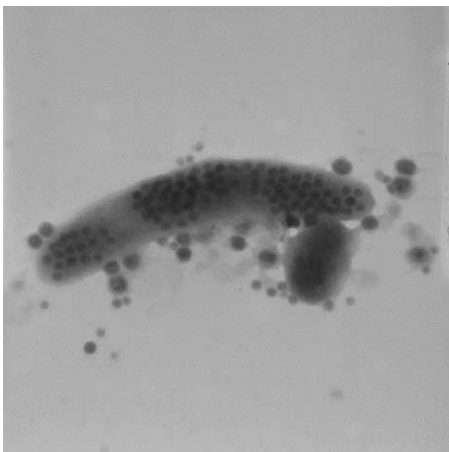


Fig. 5. Transmission electron micrograph of a bacterial cell visibly infected with phages. The sample was collected in the Rimov Reservoir (Czech Republic). Viruses inside the cell have a head diameter of ca. 35 nm.

might be a maximum estimate, since some cells might lyse before they are completely filled with viruses. This minimum and maximum burst size estimates can be used as a potential range of average burst sizes in an environment. Such information is, for example, needed, when viral production data are used to estimate virus-induced losses of bacterioplankton production (see below). Among the potential problems with this approach are disruption of VIC during sample preparation, VIC which are not detected, e.g., due to pigmentation, and the difficulty to count phages in cells with high burst sizes, where the chance is high that phages laying on top of each other cannot be distinguished [86,89,101–103]. Streptomycin treatment and TEM studies have also been used to estimate burst size [83,84]. Streptomycin causes ‘lysis from without’, i.e., it destroys cell integrity, which liberates viruses and makes it easier to count phages. Burst sizes determined by this approach are only

rough estimates as well, since not all VIC might be lysed by streptomycin and since streptomycin might prematurely stop phage production. Burst size was also estimated in experimental approaches, where bacteria were added to seawater containing either the entire virus community or to seawater containing a numerically reduced viral community [104]. Assuming that the difference of bacterial abundance between the two treatments is due to viral lysis and using the net production rates of viruses, the burst size can be calculated. Since viral decay is not taken into account by this approach, the data should be regarded as a conservative estimation of the burst size. Also, this approach assumes that the difference of bacterial abundance between treatments is only due to viruses, and that the non-infected community grows at the same rate in both treatments.

2.3. Infection, viral proliferation and host mortality

The development of methods to estimate viral proliferation and virus-induced mortality in bacterioplankton communities was paramount for assessing the role of phages for the dynamics of food webs and biogeochemical cycles in aquatic ecosystems (see below, Section 6.2). Indeed, such development stood at the cradle of phage community ecology. The lack of such methods has, for example, hampered research on the role of viruses for phytoplankton mortality. This need may also explain why many different methods have been developed to estimate virus-induced mortality of prokaryotes. However, even for prokaryotes, none of these methods has evolved to a state of a standard method [105].

Recently, various methods for estimating viral proliferation were summarized and discussed [105]. Using this compilation and including the methodological progress made since, six methods for estimating viral proliferation at the community level can be distinguished and are briefly summarized as follows (for a more detailed discussion of some of these methods see [32,105]):

1. Quantifying net changes of viral abundance over time [106]. This approach has the disadvantage that only net changes can be observed.
2. Measuring rates of viral decay [84]. This approach assumes that in steady state, viral decay has to be balanced by production. Viral production is stopped in this assay by poisoning host cells with cyanide and the rates of particle decay are measured in natural viral communities. Decay of infectivity was assessed by adding isolated bacterio- and cyanophages as tracers and quantifying loss rates as PFU or MPN [107]. Studies with several phages investigated in parallel incubations (see [108]) indicate that losses in infectivity can vary strongly between isolates. As a consequence, the extrapolation to total communities of findings obtained with single isolates has to be considered with caution.
3. Estimating viral DNA synthesis rates by radiolabeling [109]. Samples are spiked with [^3H]thymidine or [^{32}P]orthophosphate, viruses are size separated from bacteria and radiolabel is quantified in virus-size fraction after nuclease digestion. The incorporated label is converted into viral abundance by using conversion factors estimated for isolates and natural communities [102,110–112], which unfortunately vary strongly between studies. Also, some viruses might be lost during separation from cells.
4. Calculating expected release rates from estimated rates of bacterial lysis and an assumed burst size [86,111]. Viral infection frequencies and burst size estimates can be obtained from, e.g., TEM studies [13,84,86] and a virus-dilution approach [92]. This approach depends on reliable bacterial production estimates.
5. Measuring tracer dilution rates using fluorescently labeled viruses (FLV) as tracer [113]. Viral tracers are made by staining concentrates of the natural viral community with SYBRGreen I. From the decay of FLV and net changes of the non-labeled community, production rates can be calculated.
6. Quantifying the increase of viral abundance over time using a virus dilution (virus reduction) approach, which avoids new infection by reducing viral numbers [92,114].

Several methods have been published to determine the percentage of bacteria in a stage of lytic infection. The first approaches were based on TEM and using thin-sectioning to determine the fraction of cells with mature virus particles [13]. The FVIC has to be multiplied by a factor correcting for the fact that mature phages are only visible after the eclipse period. Initially, a conversion factor of 10 was used based on data from a marine PHS [115]. Later, Proctor et al. [116] performed a careful study with three PHS and determined conversion factors in the range of 3.7–7.14. Using whole cell inspection by TEM and a virus reduction approach, Weinbauer et al. [92] reported conversion factors estimated from natural communities in the range of 4.34–10.78 (average, 7.11). Binder [117] suggested to use a non-linear relationship between FVIC and FIC. Bratbak and co-workers [83,84] used streptomycin to cause ‘lysis from without’ in order to make whole cell inspection for viral infection easier after ultracentrifugation of samples onto TEM grids. This method showed that FVIC values can occasionally be as high as 40% [28]. Using the conversion factors from PHS or natural communities, this would indicate that the entire bacterial community was in a non-reversible stage of lytic viral infection. Since streptomycin is an inducing agent [118], lysogenic bacteria might have been induced in these experiments.

In addition, Bratbak and co-workers [119] reported on the occurrence of significant viral production within minutes after samples had been collected. This might have been due to forcing the lytic pathway in pseudolysogenically infected bacteria, e.g., due to confining communities in a bottle. Such a mechanism might have caused a synchronized lysis [119] and thus extremely high values of FVIC reported in a few samples. The whole cell approach has also been applied without the use of streptomycin [86]. Another approach to estimate FIC is the virus dilution technique, where the number of viruses produced is divided by the burst size yielding an estimation of the percentage of cells in a non-reversible stage of lytic infection [92].

Virus-induced mortality of prokaryotes can be measured using FIC data and models. The initial model assumed that in steady state a cell divides into two and one of these daughter cells dies. Something that kills 50% of the cells is thus responsible for 100% of the mortality. Consequently, FIC has to be multiplied by two to yield mortality [116]. This factor-of-two rule was later replaced by a more refined model [117]. For all the methods listed above to quantify viral proliferation rates, the impact of phages on bacteria can be estimated by dividing the viral production by the burst size yielding a lysis rate of bacteria. This can be expressed as percentage of removal of bacterial standing stock or bacterial production. Another approach has been proposed to quantify the disappearance of bacterial DNA in the absence of protists, which graze on bacteria [102]. This approach was initially developed to quantify total bacterial mortality [120]. It is based on the idea that bacteria take up the added tritiated thymidine label and the loss of labeled DNA in the bacterial size fractions is considered as indicative of bacterial mortality. Virus-induced mortality of bacteria is obtained by removing the protists due to size fractionation [102].

Only a few studies have tried to compare several methods to estimate virus-induced mortality of bacteria and assess the benefits and pitfalls. Fuhrman and Noble [102] have compared the disappearance of bacterial DNA, the radiotracer method and the thin-sectioning TEM approach. Steward et al. [111] have compared the radiotracer with the whole cell TEM method. Both studies showed that different methods resulted in similar estimates of virus-induced mortality. Using decay rates of particles Fischer and Velimirov [95] found an average virus-induced bacterial mortality of 56% compared to 63% based on the whole cell TEM method. It was also suggested that the whole cell TEM method underestimates mortality compared to the approach using thin-sectioning [89,102], the decay of viral particles [121], thymidine incorporation [102] or by manipulating viral abundance [98]. Overall, there is a need for comparing different methods and to test the assumptions on which these methods are based.

Other approaches have manipulated viral (and bacterial) abundance and assessed the effect of varying encounter rates between viruses and bacteria on viral production [98,104,122,123]. Typically, the impact of phages on bacteria was assessed by adding natural bacterioplankton communities to water samples with a strongly reduced viral abundance and an unchanged viral abundance, or by adding viral concentrates to water samples and compare them to non-amended samples, and quantifying the differences of bacterial abundance between the two treatments. A drawback of such studies is the uncertainty to link treatment effects to viral activity. DOM in the virus-size fraction might also contain organic nutrients or bioactive material other than viruses. Microwaving or autoclaving removes these bioactive agents, however, the effects of such experiments also differ from treatments where no virus concentrate was added [67,124] indicating that nutrients able to stimulate bacterial growth are present in the virus concentrate.

2.4. Lysogeny

Percent lysogeny is the percentage of cells in the prokaryotic community containing an inducible viral genome. Measuring this parameter can for example answer questions such as whether or not there are environments favoring the lysogenic life cycle. Two approaches have been developed to estimate the percentage of lysogenic cells in the entire prokaryotic community. Weinbauer and Suttle [88,91] added concentrates of the natural bacterial community to virus-free seawater typically obtained from the same water sample from which the community was retrieved. This procedure resulted in a reduction of viral abundance (virus dilution or virus reduction approach), which also reduced the encounter rate between viruses and potential hosts and by that new infection. This treatment served as control. Additional replicates were treated to induce lysogens, e.g., with UV-C light or by adding mitomycin C. Paul and co-workers [125–127] did not manipulate viral abundance but used whole seawater samples as control and replicates were treated for prophage induction. Prophage induction was defined either as difference in viral abundance between treated and control samples, or as the decrease in bacterial abundance in the treated sample. The number of lysogenic bacteria and thus percent lysogeny was estimated by dividing the number of viruses produced due to prophage induction by the burst size. The whole seawater approach has the problem that the inducing agent can also affect the growth and abundance of non-lysogenic bacteria and by that viral production, thus potentially affecting the validity of the controls. In the virus reduction approach the manipulation of bacteria may affect bacterial growth and thus viral production. For a

more detailed discussion of the two methods consult Paul and Jiang [128]. A third method based on DNA probes has the potential to estimate free viruses and prophage abundance in the environment [129] but so far this has not applied to assess lysogeny at the community level.

2.5. Species diversity

A comparison of the size of isolated phages and viral communities showed that isolated phages were larger than their marine counterparts thus reinforcing the idea that isolated phages are not representative for natural communities [20]. Only ca. 5000 phages infecting prokaryotes have been described by electron microscopy and negative staining of phage isolates (Bacteriophage Names 2000. H-W Ackermann & ST Abedon. <http://www.phage.org/names.htm>). This limits our knowledge on viral species diversity.

Viral taxonomy uses parameters such as nucleic acid and virion characteristics, antigenic properties and host range for species affiliation [36]. These parameters can only be obtained upon isolation of viruses. Genetic diversity of phage isolates was studied using RFLP and DNA–DNA hybridization. However, the number of culturable phages is limited because of GPCA. Molecular tools such as ribosomal RNA gene sequencing can now be used to study the diversity of prokaryotic communities. Unfortunately, there is no common molecule for viruses that could be used as molecular clock such as ribosomal RNA genes for cellular organisms [44]. However, conserved DNA sequences for particular genes have been found for several viral groups as, e.g., in the DNA polymerase gene for *Phycodnaviridae* (algal viruses) [130–132] or the g20 capsid protein gene for cyanophages [133,134], and specific primers have been developed. These primers can be used for identifying and genetically fingerprinting uncultured viral groups in the environment. Using appropriate primers, a fragment of the target molecule is amplified by PCR and techniques such as clone libraries or DGGE [135] allow for a separation of a heterogeneous mixture of target molecules as clones in a clone library or bands in a DGGE gel. Amplification products from viruses can be identified by direct sequencing or – in case of clones – by RFLP [78,136]. Other techniques such as temperature gradient gel electrophoresis [137], single-strand conformation polymorphism [138] or terminal restriction fragment length polymorphism [139] have been used in studies of bacterial species richness and may also be applied to viral communities. The different clones or bands detected with these methods correspond to different genotypes. The number of different clones in a clone library and the number of bands or peaks in a fingerprint technique gives a rough measure of genotype (or ‘species’) richness of the group of viruses targeted by

the selected primers. Since PCR is heterogeneous in a mixture of target molecules, i.e., different sequences are often amplified at different rates (e.g. [140]), a band or peak that is intense in a fingerprint, or a clone which is abundant in a clone library, does not necessarily correspond to an abundant sequence in situ. Thus, as for prokaryotes, PCR-based methods for viral community structure analyses will probably yield some information on species richness and, as bands and clones can be sequenced, on the taxonomic relatedness of the target group, whereas information on species evenness can be hardly extracted from PCR based community fingerprints. Technological progress such as real-time or quantitative PCR [141,142] might circumvent this problem, but has not yet proven to be applicable for communities or target groups. Also, more work is needed to assess the relationship between sequence differences of target molecules and actual differences between viral species.

Attempts have been made to obtain PCR-independent genetic fingerprints from natural viral communities by using PFGE [143–149]. In this whole-genome fingerprinting approach viruses are separated from cells, concentrated, e.g., by ultracentrifugation or ultrafiltration, and the viral DNA is extracted from the capsids. Viral genomes from the community are separated by size on an agarose gel and the number of bands on the gel is a conservative estimation of the number of (dominant) viral species as indicated by the genome size. The intensity of a genome size class can be converted into viral numbers present in this genome size [143]. Such a conversion would allow for estimating evenness. However, the resolution of PFGE is no well tested for virus-size genomes. Moreover, different viral species can have the same genome size and thus, the data on diversity from such approaches have to be taken with caution. Wommack et al. [146] separated viral communities by PFGE and used hybridization with nucleic acid probes specific for phage strains or for a group of viruses with similar genome sizes thus allowing for the detection of the temporal and spatial dynamics of specific viral populations. Excising bands from a PFGE gel and using PCR-based methods could be combined to increase the resolution of studies on viral diversity.

PFGE and genetic markers are useful tools for identifying novel viral sequences and tracking their biogeography and relationship with other biological, chemical and physical parameters. However, it might be impossible to estimate species difference between groups such as DNA phages and RNA phages. Even within monophyletic groups such as the tailed phages, gene exchange might prevent phylogenetic comparison of phage species based on sequence comparison of single genes [48]. Despite these gloomy prospects, the possibility remains that there are conserved genes, which can be used as genetic markers to study viral diversity.

3. Characterization, distribution and diversity of prokaryotic viruses in the environment

3.1. Characterization

3.1.1. Morphological and size characteristics

A large variety of morphological features among viral species has been described [36,150]. A typical phage has a head and a tail hold together by a connector, however, cubic, spindle, lemon-shaped, filamentous or pleomorphic viruses are also known. Facultative structures such as head appendages, collar and tail fibers or spikes are numerous and diverse. The capsid ('head') diameter and the genome size of isolates vary over more than one order of magnitude. Several reviews have described the morphological features of phages isolated from natural systems (e.g. [20,30,151,152]).

For natural viral communities the size distribution has been described in various systems (Table 2). Most of these viruses have a roughly isometric head and thus head size can be easily determined. The majority of viruses are usually found in the size range of 30–60 nm, exceptions are a backwater system of the Danube where 84% of the viruses had capsids larger than 60 nm [94], and Lake Superior where >50% of the viruses were smaller than 30 nm [153]. Giant viruses [154] with head diameters ranging from 200 to >700 nm have been reported from a variety of freshwater and marine environments [77,112,155–157]. The largest virus-like particles with a head diameter of up to 750 nm were found in the food vacuole of *Phaeodarian radiolarians* [158]. The average head diameter in viral communities ranged from 55 to 64 nm in marine systems. Average viral head diameter was only reported from one freshwater system, the Plußsee [152]. The average head diameter was 62–68 nm in the oxic surface layer, 84 nm in the thermocline layer and 89 nm in the anoxic layer of this eutrophic lake.

Head size distribution of viruses can vary with time and space [106,159–163], however, not all of the studies have shown such changes [86,94,95,101]. Heterotrophic nanoflagellates are a major type of predators of prokaryotes in aquatic systems and their grazing is size-selective, i.e., they graze preferentially on a particular size class [164,165]. As HNF can also ingest and digest viruses [166], they might remove preferentially large viruses [167]. Thus, it is tempting to assume that a reduced grazing pressure can cause a shift towards larger capsids. Interestingly, such an increase of head diameter was observed at the transition from oxic to anoxic waters in the Plußsee [152] coinciding with an increase of viral infection and a decrease of grazing pressure on bacterioplankton in anoxic waters [89]. In the Northern Adriatic Sea the head size did not vary along a trophic gradient or between months, however, it was larger in 1992 than in 1991 along with a higher HNF abundance in 1991 compared to 1992 [86].

Studies on the morphology of viral communities were often restricted to grouping viruses into size classes. Some studies have also tried to estimate the proportion of tailed viruses and reported either on a predominance of tailed phages or a lack of numerical dominance of this type of viruses [6,12,13,65,106,159,168]. Most of these TEM studies have used ultracentrifugation for preparation and positive staining to investigate viruses. During ultracentrifugation, the tails might be dislocated from the capsid and positive staining is not an optimum procedure for detecting fine structures such as the small tails of *Podoviridae* [30]. There is only one study from a natural viral community avoiding these problems [152]. By letting viruses adsorb to TEM grids without centrifugation and using negative staining, Demuth et al. [152] found that only one virus of the viral community in the Plußsee was a non-tailed phage. However, no test was performed in this study to check, whether or not tailed phages adsorb preferentially to the used filters. The author is not aware of TEM studies on the morphology of viral communities from soil or sediments.

The finding that 96% of all isolated phages from prokaryotes have a tail [169] suggests that the majority of viruses such as those found in the Plußsee belong to the monophyletic group of tailed phages (order *Caudovirales*). The origin of tailed phages occurred before the separation of life into the three domains *Bacteria*, *Archaea* and *Eukarya*, and tailed phages are probably at least 3.5–3.7 billion years old [43]. 'True' tailed phages have been reported for *Bacteria* and *Archaea*, whereas their presence for *Eukarya* remains doubtful [43]. Species belonging to the order *Caudovirales* have a double-stranded DNA as genetic material and are divided into three families according to their tail (as well as biochemical and molecular) characteristics [43,44]: Phages with a long flexible tail (*Siphoviridae*), phages with a contractile tail (*Myoviridae*) and phages with a very short tail (*Podoviridae*). Myoviruses, for example, constitute the majority of isolated cyanophages [170,171] and the majority of marine phage isolates [20,30]. Also, it has to be pointed out that doubt can be raised, whether tailed phages can be divided into genetically coherent groups, or whether they represent an indivisible continuum [48].

Proctor [30] reported on a single filamentous viral isolate from marine systems. In limnetic systems, filamentous viruses have been found at the community level in two mountain lakes as remote as the Tyrolean Alps and the Central Pyrenees [96,157]. In an alpine lake, the filamentous viruses contributed between 7% and 100% to total viral abundance [96]. In the study of Demuth et al. [152] no filamentous virus were detected indicating that also in limnetic systems, filamentous viruses might be rare and restricted to particular habitats such as high mountain lakes.

Typically, free viruses in aquatic systems have no membranes, although there is at least one report on

viruses with membranes in a study of viral communities from an ultra-oligotrophic lake [172]. However, phages with a membrane are not easily detected by using TEM and positive staining. Only one phage isolates with a membrane is known for marine systems [20,30].

3.1.2. Nucleic acid characteristics

The majority of aquatic phages seem to be DNA viruses. Only one RNA phage isolate is known from marine systems and this phage has a lipid outer coat [20,30]. One study has compared DAPI total counts of viruses with YOPRO-1 total counts of viral communities using epifluorescence microscopy [72]. DAPI stains only DNA, whereas YOPRO-1 stains DNA and RNA. The finding from the Gulf of Mexico that the DAPI counts averaged 86% of the YOPRO-1 counts suggests that the portion of RNA viruses is small, and the faster fading and lower fluorescence of DAPI may have even resulted in an underestimation of the estimates of DNA virus abundance. Others have found that DAPI counts were considerably lower than YOPRO-1 counts [73,121]. However, because of the fast fading and low fluorescence of DAPI compared to YOPRO-1, it is difficult to assess whether the low estimates of DNA virus abundance in these studies are real or a result of an artifact.

3.1.3. Host characteristics

A quantitative assessment of the abundance of viruses infecting different phylogenetic groups of hosts such as prokaryotes or unicellular eukaryotes, or functional groups such as photoautotrophs or bacterial heterotrophs has not yet been performed in natural systems. The generally good correlation between bacterial and viral numbers reported for marine systems has been used to argue that most viruses come from lysis of prokaryotes, whereas the good correlation with Chlorophyll-*a* in Canadian lakes and the lack of it with bacterial abundance has been used to suggest that in lake water a significant portion of viruses might be associated to algae and cyanobacteria [161]. For a more detailed discussion of the correlations between viral abundance and with bacteria and Chl *a* (and other parameters) see the review of Wommack and Colwell [32]. On the one hand, the finding that in a marine and a freshwater environment the free viral community and the phages within bacterial cells had the same size distribution [85,97] suggests that bacteria are a predominant source of viruses. On the other hand, some reports on high titers of ca. 10^5 ml⁻¹ or more of viruses infecting cyanobacteria or the eukaryotic picoplankton species *Micromonas pusilla* in coastal waters point to a significant proportion of viruses infecting the autotrophic plankton community [171,173–175]. Using a metagenomics approach of coastal virus communities, it was shown that 75–90% of the virus hits to GenBank entries

were most similar to phages suggesting that prokaryotic viruses dominated in these systems [176].

It is more and more appreciated that the domain *Archaea* is not restricted to extreme environments and more ubiquitous than previously thought. For example, in deep marine waters *Archaea* are as abundant as *Bacteria* and may comprise ca. 1/3 of the prokaryotes in the world ocean [177]. Thus, phages infecting *Archaea* might be more common than previously assumed, however, our knowledge on archaeal viruses is sparse [18]. For pelagic environments, this is due to the fact that no archaeal isolate is available from this environment, which could be used to isolate phages. Viruses infecting *Archaea* have been detected in high salinity environments such as brines [178], fermented fish sauce [179] or solar salterns [101]. Spindle-shaped viral types resembling archaeal viruses were also reported from the Dead Sea [180].

3.2. Abundance and biogeography

Total viral abundance across aquatic systems typically varies between lower than 10^4 and higher than 10^8 ml⁻¹ [32]. In this reference a detailed compilation of viral abundance in marine and freshwater systems can be found. The variability of viral abundance is larger than the typical range of corresponding bacterial numbers indicating that viral abundance is less tightly controlled than bacterial abundance.

3.2.1. Pelagic systems

In general, viral abundance increases with the productivity of the system. For example, in marine systems, it is lowest in the deep sea (10^4 – 10^5 ml⁻¹), intermediate in offshore surface water (10^5 – 10^6 ml⁻¹) and highest in coastal environments (10^6 – 10^7 ml⁻¹) [19]. It has been reported that viral abundance decreases along transects from coastal to offshore waters (e.g. [159,181]). Viral abundance in estuaries or very productive lakes can be as high as 10^8 ml⁻¹. The highest viral number of 9.6×10^8 viruses ml⁻¹ found in an aquatic environment (except sediments) was reported from a cyanobacterial mat [68]. Viral abundance is typically higher in freshwater than in marine systems [161]. Viral abundance in the surface microlayer, i.e., the first 20 μ m, of Lake Superior (USA), was ca. 2–15 times higher than in 20 m depth [153]. A highly variable enrichment factor of total viral abundance in the surface microlayer compared to underlying water (20 cm) was found in the Mediterranean Sea and in Kings Bay (Spitzbergen, Norway) [182].

Viral abundance along depth profiles from oxic surface waters to anoxic deep waters has been studied in limnetic and marine waters. In the eutrophic Plußsee viral abundance was significantly higher in the anoxic than in the oxic water layer, but it was highest in the O₂/H₂S interface [89]. In the Baltic Sea, viral abundance

decreased with depth in the oxic layer and increased again in the anoxic layer reaching levels similar to that at the surface [182]. In the Chesapeake Bay, viral abundance was not affected by bottom anoxia [168] and in the Cariaco Trench off Venezuela, viral abundance was not clearly associated to the presence of oxygen [183]. However, peaks of viral abundance occurred in the O_2/H_2S interface of this environment [184]. Discontinuities in the water column such as fronts or pycnoclines are often associated with changes in viral abundance. Such discontinuities can be seen as ecotones, i.e., transition zones between two systems, typically with higher abundance and diversity of organisms than in the individual systems. Known examples for viruses are the pycnocline layer, which is separating the surface mixed layer from deeper water in the pelagic systems (e.g. [100,159,185,186]), fronts [114,168], or chemoclines from oxic to anoxic waters [89,183,187,188].

Numerous studies document the temporal variability of total viral abundance at the scale of months, weeks, days and hours [12,86,94–97,99,100,106,114,119,153,156,160,163,168,173,185,189]. Bacterial abundance and production is highest during and after the demise of a phytoplankton bloom, which is most probably the reason for the high viral abundance following a phytoplankton bloom (although a significant contribution of algal viruses cannot be excluded); such a dynamics occurs at the scale of days and weeks (e.g. [97,106,185,190]). During a diurnal study following a drifting buoy in the Adriatic Sea, oscillations of total bacterial and viral abundance fluctuated in a predator–prey type of oscillation [185]. Even at the scale of 10–20 min, dramatic changes of viral abundance by a factor of 2–4 have been reported indicating that host cell lysis might have been synchronized [119]. Data on temporal changes of viral abundance belong to the most convincing evidence of the dynamic nature of viruses in natural systems. It has been estimated that there are 3.5×10^{29} viruses in the world ocean [191].

3.2.2. Sediments, soils, sea ice and particles

The occurrence of phages in marine and limnetic sediments is well documented. However, data on total abundance are rare. In freshwater sediments, viral abundance ranged from 0.65 to 2.90×10^9 g^{-1} , compared to 0.03 – 11.71×10^9 g^{-1} in marine systems [192]. Viral abundance typically decreases with sediment depth and the abundance of benthic viruses is 10–1000 times higher than that in the overlying water column [111,193–199]. Suttle [17] studied the distribution of cyanophages infecting *Synechococcus* in offshore sediments (45 km from shore, ca. 50 m water depth) in the absence of light and thus in the absence of hosts which could be infected. He concluded, based on sedimentation rate estimates, that the age of the cyanophages found in 30 cm sediment depth was at least 100 years suggesting that sediments

could be a reservoir for viruses. Preservation of viruses infecting the toxic algae *Heterosigma akashiwo* in sediments has also been suggested [200]. Thus, sediments might also be a reservoir for pelagic phages infecting heterotrophic hosts.

In sea ice, viral abundance was in the range of 9.0×10^6 – 1.3×10^8 ml^{-1} [160] and is thus ca. 10–100 times higher than in surrounding water [160]. A high viral abundance of 5.6 – 8.7×10^{10} cm^{-3} was also found in algal flocs (marine snow) formed during phytoplankton blooms suggesting an enrichment of viruses compared to surrounding waters [201]. Other particles such as inorganic colloids may be colonized in a quite different way. Viruses are usually negatively charged in natural environments and colloids such as clay are negatively charged as well [202]. These repulsive electrostatic forces are, however, weaker than the attractive van der Waals forces resulting in the adsorption of phages to colloids [202].

To my best knowledge, there is only one report on total viral abundance in soil [203]. Total abundance in soil and rhizosphere varied between 0.7 and 2.7×10^7 g^{-1} . Spiking of soils with a known phage titer to test recovery indicated a strong underestimation of total abundance. After correction total viral abundances was 1.5×10^8 g^{-1} [203]. It has been suggested that phages might be the most numerous ‘genetic objects’ in soil [39], however, the contrasting view has been also put forward, namely that the heterogeneous distribution of bacteria in soils might result in a reduced viral transmission and infection [38,39,204]. The comparatively low viral abundance in soils [203] seem to favor the latter view.

3.2.3. Viruses to bacteria ratio

VBR has indicated the numerical predominance of viruses over bacteria and has been used to infer the relationship between viruses and bacteria [32]. VBR can be as high as 100. In marine pelagic waters the VBR is typically 5–10 and even in the deep sea the VBR is high [21,205]. Low VBRs of 0.01–1.2 have been documented from organic matter collected in sediment traps, however, these low values might be due to strong viral decay during storage [184]. In lake snow, VBRs varied between 0.3 and 8.5 with an average of 4.7 [206]. In sea ice VBR can be as high as 72 [160], whereas variable VBRs ranging from 0.11 to 71 have been reported for marine sediments [111,192,193,195–197] indicating that some marine sediments are depleted with respect to viruses. For lake water and a river, a sediment VBR of 1–4 [194] and 0.7–1.2 [207], respectively, has been reported. In anoxic waters, VBR can be high as well [89,168,184,188]. Lower VBR values were reported for anoxic than for oxic lake sediments [194]. VBR varies at temporal scales and sometimes increases with productivity of systems, although there is no general trend. In limnetic systems,

VBR is typically higher than in marine pelagic systems [161]. It was suggested that the increased dependence of freshwater bacteria on allochthonous material and the higher relative contribution of cyanobacteria is the reason for these differences. Using data from the literature, Maranger and Bird [161] reported on an average VBR of 10 in marine and 20 in freshwater pelagic systems. VBR in soils is low averaging 0.04 for the few investigated samples [203].

3.2.4. Biogeography

Data on the geographical distribution of viral species other than those infecting humans, causing disease of livestock or pets or interfering with human nutrition are relatively rare. Cottrell and Suttle [208] report on a worldwide distribution of a virus infecting the picophytoplankton *M. pusilla*. Wide-spread occurrence of specific bacteriophages [209–213] and cyanophages [170,214] was also reported. However, there are also reports on a geographically restricted distribution of phages in the ocean [210,215]. Due to the lack of information on the biogeography of viral species, little is known on their affiliation as ubiquitous or endemic species. The use of approaches such as DGGE and sequencing will certainly increase our knowledge on viral biogeography in the years to come. In addition to PFUs and MPNs, molecular techniques such as real-time PCR may now allow the identification and enumeration of individual viral species [141]. Other methods, which may turn out useful in ecological research of viruses are specific probes for the detection of single virions of single species, such as, for example, by monoclonal antibodies [216], DNA analysis or enzyme-based detection of phages in situ [217,218], specific primers for investigations of specific viral groups [78,133], or probes to detect genomes within cells [129,219–221].

3.3. The infectivity paradox

Studies with isolated PHS have shown that once a steady state is established, hosts are (sometimes orders of magnitude) more abundant than bacteriophages [37]. Yet, in the environment, viral abundance exceeds bacterial abundance typically by 10–20-fold [32]. One explanation for this paradox could be that most of the viruses in natural systems are not infectious. However, a study combining data on decay rates of infectivity and particles, and DNA damage and repair in viruses indicates that more than 50% of the viruses in a natural community are infectious [222]. Moreover, for marine viral and bacterial communities, Middelboe and Lyck [104] conservatively estimated that between 25% and 80% (average, 45%; calculated only for incubations with entire viral communities) of the phages were infective in batch culture experiments. Also, abundances of infective marine cyanophages are frequently 10-fold higher than

their cyanobacterial hosts [16]. The infectivity paradox becomes even more obvious considering the high titers of infective phages that are found in surface waters in the presence of the destructive action of sunlight on viral infectivity (see below) [107,173,223].

3.4. Diversity, dominance and controlling mechanisms

3.4.1. Diversity of communities or groups

Methods are available to tackle the problem of viral diversity in the environment. However, assessing viral diversity in natural systems is still a difficult task and depends in the used approach. Typically, between 7 and 35 bands can be distinguished by applying PFGE to individual samples collected from marine systems [145–149,186,224–226]. This number also serves as a conservative estimate of abundant viral types in an investigated community (Table 3). The genome distribution of viruses from a variety of marine systems was multi(bi)modal with major peaks between 31–36 and 58–63 kb and an average of ca. 50 kb [147]. Riemann and Middelboe [186] found two dominating size classes between 33–48 and 50–60 kb along the Skagerrak–Kattegat plume front. The genome size as determined by PFGE ranged from 12 to 630 kb in marine systems and from 10 to 850 kb in the rumen of sheep (Table 3). It has to be pointed out that not all ‘genomes’ were tested for their viral identity. It is often assumed that the large genomes belong to algal rather than to prokaryotic viruses based on the finding of large viruses infecting algae [227]. However, there are also bacteriophages with extremely large genomes such as phage G infecting *Bacillus megaterium* with a genome size of 725 kb, “. . .the largest known viral genome in the viral world. . .” [43]. Hence, more direct evidence is necessary to confirm that large viral genomes generally originated from algal viruses. The relative abundance of single genome sizes was at maximum ca. 15%, which may indicate that at times the viral community in marine systems could be moderately dominated by a single viral species. No data are available on the genome size distribution of viruses from limnetic or terrestrial systems.

The PFGE approach has allowed for detecting a variable viral community structure in the rumen between individual sheep as well as diurnal and day-to-day changes within single sheep specimen [144]. Also, in combination with hybridization techniques, a seasonal as well as spatial variability of viral diversity could be detected in the Chesapeake Bay [145,146]. Mesocosm studies simulating phytoplankton blooms have revealed that the viral community composition is dynamic and closely linked to changes of the algal and bacterioplankton community dynamics [224–226]. Others have shown that the variability of viral community composition during a phytoplankton bloom was low, but different water adjacent masses also differed with respect to

Table 3
Distribution of viral genome size and estimates of numbers of viral types in the environment

Environment	Genome size (kb)		No. of bands (per fingerprint)		Reference
	Mean	Range	Mean	Range	
Sheep rumen phages		10–850	11		[143]
Sheep and goat rumen phages		1–>500			[144]
Chesapeake Bay phages		2–324	11	7–16	[145]
N. Pacific Coast, Scripps pier				8–16	[147]
N. Pacific Coast, Monterey Bay			≥ 35		[149]
Average from various marine environments	50	26–350		8–35	[147]
Marine mesocosms, Norwegian fjord		19–415	10		[225]
Marine mesocosms, Norwegian fjord		19–485	20		[224]
Skagerrak-Kattegat front				14–29	[186]
Mesocosm, Danish Fjord		25–630	13	5–21	[226]

The number of bands per fingerprint gives a conservative estimate of the number of viral types. All fingerprints were obtained by pulsed field gel electrophoresis indicating that only linear ds viruses were analyzed.

their viral community [186]. Data from a transect along a plume front with marked physico-chemical changes suggest that the viral community patterns cannot be explained by simple mixing of the single water masses [186].

Cyanophage diversity can be studied with cyanophage specific primers targeted against a region of the capsid assembly protein gene (gp20). Using non-degenerate CPS primers and DGGE, it was shown that cyanophage diversity varied along a South–North transect in the Atlantic, although some common bands suggest that some cyanophages have an ubiquitous distribution [133,134]. A diverse and variable cyanophage community composition was found along depth profiles. Changes in genotype richness were associated with water stratification, nutrient concentrations and cyanobacterial abundance. Overall, between 2 and 10 distinct bands were observed on DGGE gels. Using other CPS primers, which amplify a larger fragment of gp20 of ca. 592 bp, and clone libraries and sequencing, Zhong and co-workers [214] investigated the phylogenetic diversity of marine cyanophage isolates and natural virus communities. The primers were targeted against cyanomyoviruses, the most frequently isolated group of cyanophages, (note that there is some uncertainty with respect to the specificity of these primers). One hundred and fourteen gp20 sequence homologs were found in estuarine waters, open ocean waters and in the deep chlorophyll maximum layer. Nine clusters were identified and six of these did not contain any known sequences from isolated cyanophages. Three clusters were specific for the estuary and four clusters were specific for the open ocean, one of these four being only found in the DCM layer, indicating that there are cyanophages specific for certain environments. In the surface samples of the Skidaway Estuary, 29 different gp20 sequences were found, compared to 23–26 sequences in the DCM samples and 13–15 different sequences in offshore surface sea, suggesting a potential increase of diversity with host abundance.

Attempts have been made by Suttle and co-workers [78,136,228] to study viral diversity of uncultured algal viruses using sequence analyses, clone libraries and DGGE. Using algal specific primers targeted against the *pol* gene encoding for DNA polymerase, cloning and sequencing, new algal virus sequences were obtained from samples from the Gulf of Mexico [131,132]. These sequences clustered within the *MpV* group and one sequence was also within the family *Phycodnaviridae* (algal viruses) but outside known groups thus representing even a new lineage [132].

A metagenomics approach was recently applied to viral communities from two coastal ecosystems [176]. In this first study on the community genome of viruses, estimates indicate that there were between 374 and 7,114 viral types present. The most abundant viruses comprised 2–3% of the total abundance and the Shannon index was 7.56 and 7.99 in the two environments. This suggests that viral diversity is enormous and that dominance is rather low.

3.4.2. Diversity of isolated phages

Diversity of viruses was also studied using phages infecting a single bacterial host species. Early studies [210] have indicated high phage diversity in the Atlantic. In the North Sea, 13 viral species from the order *Caudovirales* were isolated and described. These species infected bacterial isolates that showed a more than 99% sequence similarity of their 16S rRNA gene with cells belonging to the genus *Pseudoalteromonas* [229]. Such a sequence similarity indicates that the hosts belonged to a single species. Thus, there would be at least 13 species of phages from a single environment infecting a single species, a fact that supports the idea of a high viral diversity. With the host *Serratia liquefaciens* CP6 isolated from the sugar beet phytosphere, six different phages were detected, which belong to all three families of the order *Caudovirales* [230]. Paul and co-workers [19,213] have investigated the genetic diversity of phages of the

cosmopolitan bacterium *Vibrio parahaemolyticus*. During a seasonal study of the observed seven genetically different groups of phages one was consistently dominant (71% of total isolates) and the others ranged from 1% to 16% thus supporting the idea that dominance of species is a phenomenon also occurring with phages, at least when phages infecting one host type are considered. This somewhat contradicts findings of low viral dominance using the metagenomics approach [176]. Sequencing a 500-bp DNA fragment and comparing sequence similarity indicated that diversity on a temporal scale can be as high as diversity on a spatial scale. A similar conclusion, i.e., that phages from distant locations can be genetically similar while viruses from the same environment can be quite different, was also derived from studies with cyanophages [16,134,231] as well as *MpV* viruses infecting the *M. pusilla* [208]. Finally, a study on cyanophage isolates reports that genetically related cyanophages can be widely distributed without showing a geographical segregation [214]. These studies also show that our knowledge on viral diversity is increasing rapidly.

3.4.3. Mechanisms controlling diversity

Less progress has been made on the mechanisms controlling viral diversity and these mechanisms remain indeed largely unknown. Certainly, the availability and quality of host resources is a significant, maybe even the main factor influencing this diversity. Moreover, removal of viruses by grazing has been documented [166]. Grazing rates of heterotrophic nanoflagellates on viruses depend on the type of viruses and on the grazer type and are highest at high viral abundances [166]. These authors also report on marine choanoflagellates specialized in grazing on the virus-size fraction of seawater, grazing rates were low. However, grazing can only have an influence on the diversity of viruses, when not all viral types are grazed with the same rate. An example would be grazing on large or giant viruses, which could be significant enough to influence the diversity of these viruses. An indirect influence can be assumed by the fact that grazing influences bacterial growth and community structure [232–239] and by the fact that grazing can stimulate viral infection of bacteria [93]. Gene exchange between phages and hosts during transduction and gene exchange between co-infecting phages is probably a significant factor driving phage diversity at an evolutionary scale [240,241]. Indeed, it was suggested that bacteria with several co-infecting phages function as ‘phage factory’ and produce a variety of chimeric or mosaic phages, which increases viral diversity [242]. Since the genetic exchange is probably higher among lysogenic than among lytic phages, the diversity of lysogenic phages should be higher [243]. Hence, the type of viral life cycle could have a direct influence on viral diversity in the sense that lysogenic phages might dom-

inate the species richness. In addition to the biological factors discussed above, a variety of physicochemical factors such as adsorption to particles, UV light or temperature (see below, Section 4.1) may define the niches of phages and thus influence viral diversity, however, data are not yet available at the community level.

4. Growth and survival of phages in the environment

4.1. Ecological niche: environmental conditions and resources

A niche is not the location or habitat of a population, but can be seen as a multidimensional space or hypervolume and every *dimension* represents an important environmental factor such as ecological *conditions* or *resources* determining the survival of a population [244]. This *fundamental* niche can be restricted by interactions such as competition and predation to the actual or *realized* niche. For example, a phage may display a large temperature tolerance (a dimension of the fundamental niche) but only occur at a rather narrow temperature range (a dimension of the realized niche). Comprehensive data on the fundamental niche of viral species from marine, freshwater or soil environments are not available, however, fragmentary information on niche dimensions was obtained by studying isolated phage–hosts systems [35] and from in situ observations [32]. Comprehensive studies targeting the realized niche are missing altogether, although some niche dimensions are known for phages. An assessment of niche dimensions, the niche width, can be used to classify organisms as specialists or generalists. Although the niche dimensions of phages can vary with respect to many conditions and resources, the typically rather narrow host range may suggest that phages are often specialists.

Among the environmental conditions and resources for phages are water, temperature, hydrostatic pressure, radiation, the ionic environment, oxygen, pH, organic matter and host availability (see [39,204,245,246]). For instance, water is the medium for viral transmission in aqueous environments, whereas the ability to deal with the problem of desiccation might be crucial for phages in soil. Soil moisture varies strongly and thus should affect phages by influencing their hosts, but it likely does not directly affect phages [39]. Temperature is another important environmental parameter and phages can be found from ‘freezing to boiling waters’ such as sea ice and hot springs. Although marine phage isolates vary with respect to heat tolerance [20], they are generally more sensitive to heat than phages from other environments [247]. Wiebe and Liston [5] reported that a bacteriophage, which was isolated from a marine sediment sample collected in a depth of 825 m, produced only plaques below 23 °C, whereas the host could grow at

temperatures up to 33 °C. Since soil temperature varies strongly with season, depth and maybe also locally with micro-habitats, it might be a significant dimension in the ecological niche of soil phages. The varying temperature tolerance of phages (see, e.g. [20,204,245,246]) supports the view of temperature as important niche dimension.

Another physical condition is light, which can stimulate adsorption to hosts and this phenomenon might be due to charge neutralization at the cell surface or by changes in the ionic environment near the cell [16]. As discussed below (Section 4.5), the UV-B portion of sunlight can cause damage to viral DNA, whereas UV-A light can be used for repairing DNA damage. Decay rates (in sunlight and dark), DNA damage and repair capacity can differ strongly among phages even amongst those isolated from the same environment [32,34]. This suggests again a niche differentiation between phages living in a seemingly homogeneous environment such as surface waters.

Physical forces such as wind, waves and currents probably affect phages only in their environmental distribution, e.g., by transferring them from the liquid to the gaseous phase. Also, phages are too small to sink in water or to be directly affected by turbulence. For aquatic viruses, the term virioplankton (from virion) or viroplankton (from virus) meaning drifting viruses is thus appropriate. Phages may also spread by attachment to mobile organisms or when attached to sinking particles. In the atmosphere, phages can be transported by wind forces or by flying organisms such as insects and birds. Atmospheric transmission might not only be a distribution pathway for soil phages, but also for phages in surface waters. A comparatively novel transmission pathway is in the ballast water of ships [248]. Although the chances are low for non-indigenous phages to find a new host, a host might travel together with its phage in the ballast waters and might flourish in the new environment and thus allow for the spread of the introduced phages.

Ecological niche conditions depending on chemical parameters are known as well. The ionic environment can affect phages from freshwater, marine and soil at all stages of the life cycle such as the adsorption, replication, lytic activity and survival [204,245,246]. For example, divalent cations such as Mg^{+2} or $Mg^{+2} + Ca^{+2}$ frequently seem to have a positive effect on phages, whereas the effect of Na^{+} is quite variable. Such data suggest a strong effect of the ionic environment on the definition of the ecological niche of phages. pH is comparatively stable in marine environments, but can vary strongly in limnetic systems from alkaline (e.g., in salt lakes) to acidic (e.g., in particular lakes or fens). In soils the pH can also vary strongly. pH can affect adsorption of phages in freshwater [245], whereas marine bacteriophages are typically only affected by pH values deviating from that of seawater, although some isolated

bacteriophages remained stable for months over a wide range of pH values [20]. The tolerance of many cyanophages against a broad range of pH values suggests that changes in pH do not affect their distribution in freshwater [16].

The microenvironments and environmental gradients at scales that matter for prokaryotes (submicron to micron scale) are largely unknown [249]. This holds also true for phages. The microenvironment of a phage can change dramatically during its life history, from within the genome to within the cytoplasm or outside a cell. In all these different environmental conditions, a successful phage has to survive, for which special adaptations such as the stability of capsid proteins are required. For phages, hosts are islands or habitable patches in the environment.

Phages have no own metabolism. Instead, they ‘borrow’ or ‘enslave’ the metabolism of their hosts, and use the host as resource of energy and matter. For a significant part of the latent period, infected heterotrophic bacteria can continue with their ecological role as transformers and oxidizers of organic matter. For example, it has been shown that infected bacterial isolates incorporate thymidine until cell lysis occurs [250]. Also, in unicellular cyanobacteria, photosynthesis is not affected by viral infection until lysis is initiated [16]. The incorporated matter and energy is used to synthesize viral macromolecules and even host material can be ‘consumed’ for the formation of viral particles. For example, a significant portion of the host DNA is used to synthesize viral DNA [251]. Thus, the host cell with its nutrient content, nutrient uptake system, general metabolism and molecular machinery serves as bioreactor or factory for the production of phages.

4.2. Host physiology and nutrients

The nutritional or metabolic status of the host is critical for viral infection and proliferation by affecting adsorption, replication, lytic activity and survival of the phage [39,204,245,246]. Thus, one may ask whether or not a phage requires a living or metabolically active cell for adsorption and proliferation. Numerous culture studies have shown that maximum proliferation rates and yield of phages are observed at optimum growth conditions of the host [37]. The finding that host generation times often influence phage latent periods [101,116,250] and that a low nutrient availability often results in increased latent periods and in a reduced burst size [116,250,252] suggest that phage proliferation strongly depends on host metabolism. In stationary phase, phage maturation may proceed, however, cell lysis can be stopped (e.g. [250,253]). It has also been shown that starvation (even as long as five years) does not prevent infection and viral production in all host species [252,254] and even recently killed cells may still

be exploited to produce viral progeny [255], although in both cases cell lysis is delayed and burst size reduced. Moreover, variable effects of starving hosts on phage production have been reported for several marine PHS [256,257]. Studies on viral replication in cyanobacteria indicate a strong dependence of cyanophage production on light and nutrient availability, however, ecologically relevant research is rare [16]. Darkness delays lysis and decreases or even stops phage production.

Limitation by light or by elements such as carbon, nitrogen, phosphorus, sulfur or iron can only indirectly influence phage proliferation by controlling the host metabolism. Thus, it is important to know how nutrient limitation of a cell affects phage proliferation. It has been reported that nitrate and phosphate depletion reduce the burst size and viral titers [258]. In this study, P depletion did not affect adsorption rates of cyanophage S-PM2, however, a large fraction of cells with adsorbed phages did not produce viral progeny. It has also been shown that the addition of inorganic nutrients increases phage infection or numbers [258,259]. Adding nutrients increased proliferation rates of ϕ R2f phages infecting suspended host cells (*Pseudomonas fluorescens* R2f) as well as cells attached to alginate beads [260]. Stimulation of viral production by addition of inorganic nutrients has also been shown for natural communities [199]. In mesocosms amended with either sucrose, inorganic nitrogen or phosphorus, or with a combination of sucrose and inorganic nitrogen and phosphorus, viral abundance was highest in the treatment receiving all nutrients. However, FVIC was highest in mesocosms receiving only sucrose [261], indicating a stimulating but complex influence of organic and inorganic nutrients on viral production and infection. It was shown for a virus infecting the coccolithophorid *Emiliania huxleyi* that P limitation decreased the proliferation rate and the stronger effect of phosphate over nitrate was attributed to the higher nucleic acid to protein ratio in viruses [262]. This appears logical, since viral gene expression requires phosphorus for synthesizing RNA. Similar effects of phosphorus limitation on phytoplankton growth and viral proliferation were reported [263]. Whole genome sequencing of two marine phages, roseophage SIO1 and the cyanophage P60, suggested that they acquired DNA metabolism genes from the host, maybe as a consequence of P limitation [243,264]. The SIO1 genome also encodes for a host-born protein induced in cells during P limitation [264] and phages can use host DNA [251].

A major quest in microbial ecology is now to assess the metabolic status of prokaryotic cells in situ, and to find out how 'healthy' cells are and how well they are doing (for a brief introduction on the problem and on methods see, e.g. [265]). The available data indicate that a variable and at times significant fraction of the prokaryotic community in marine, limnetic and soil systems

is either dead or not growing well. Future research has to show whether or not these cells are still a resource for phages and how significant their contribution is for phage production and survival.

4.3. Latent period and burst size

Latent period and burst size together with the number of infected cells determine the proliferation rate of phages. The length of the latent period depends on the phage species, the physiological condition and type of host, the composition of the medium and on temperature [36]. Different phages infecting the same host can have different latent periods [39,204,245,246], however, on average the latent period of phages infecting heterotrophic prokaryotes seems to equal the generation time of the host, at least at generation times typically occurring in the environment [101,116,250]. Also, when pooled data from various studies were used, viral and bacterial turnover rates were positively related in marine systems [100]. At elevated temperatures latent periods tend to shorten [266], which might be due to a stimulation of host growth. Superinfection of an infected cell with the same phage strain results in a longer latent period. This lysis inhibition is accompanied by an increased burst size [267].

Burst size estimates of single prokaryotic cells in situ are extremely variable and range from a few to ca. 500 phages. Wommack and Colwell [32] summarized data from literature and calculated an average burst size of 24 for bacterioplankton. Burst size can increase in more productive systems [86,91]. This may be due to higher nutrient concentrations and thus a more efficient metabolism and higher growth rates of hosts allowing for more viral progeny to be produced per cell. Such data were frequently reported from many different PHSs (e.g. [266,268]). However, burst size also increases with cell size (and cell size increases often with growth rate) indicating that larger cells can contain more phages. It depends on phage size as well, i.e., larger phages may produce less progeny [85,89]. One study showed that infected cells are larger than non-infected cells in natural prokaryotic communities [90]. This was interpreted as size-specific infection of larger cells, which are typically also more active and thus infected preferentially [90], however, the possibility remains that infected cells can grow to a larger size than the non-infected counterparts. One report from natural communities showed that burst size changes correlated significantly with temperature [94], a phenomenon well known from PHSs [266,268]. Some changes of burst size with season have been reported [86,94] and burst sizes can also differ between bacterial morphotypes [85]. Since burst size differs between species [35], the species composition of hosts should have an effect on average burst size in a community as well. Because protist grazing can change the

size spectrum and growth rates of bacterioplankton, protists might indirectly influence burst sizes. However, in dialysis bag incubations in a reservoir, the removal of protist grazers changed the size distribution and production rates of bacterioplankton, whereas burst size was only minimally affected [93].

Burst sizes of isolated PHSs are consistently higher than those found in the environment [20]. One of the reasons could be that cells in cultures are larger than their counterparts in the environment, for example, due to higher growth rates in nutrient-rich medium. An additional explanation could be that isolates growing on agar plates are most likely adapted to high nutrient concentrations, which could favor high burst sizes. Since isolated bacteria are typically not representative for bacterial communities due to the GPCA a species effect might also be involved.

4.4. Viral survival, mortality and antiphage defense

4.4.1. Viral survival and mortality

All organisms found in the environment (including phages) can be considered as survivors. Defining the conditions allowing for this survival is an important topic in ecology. One way to look at viral survivors is to study the mechanisms and rates of mortality or loss of infectivity of phages, since there are more data available on these aspects than on proper survivorship. These mortality rates are often given as decay rates, i.e., destruction rates of phages. Also, decay rates serve as an indication of production rates necessary to secure viral survival. There is a long history of studies on the loss of infectivity of phages in limnetic, marine and soil systems [35,269]. These studies intended to investigate the mechanisms of viral destruction for enteroviruses or coliphages as tracers for environmental pollution or to determine the most effective way to remove viral contamination [270]. The finding of high viral numbers in aquatic environments also stimulated the research on the mechanisms destroying phages in their natural environment.

The damaging effect of high-energy radiation (UVC and X-ray) on viral infectivity has long been known and numerous laboratory studies were performed with human viruses and phages infecting enterobacteria. Coliphages were used as biological UV dosimeters in aquatic systems by assessing either sunlight-induced losses of infectivity [271–273] or formation of photoproducts [274]. But it is only recently that phages isolated from aquatic environments were used to study the effect of sunlight on infectivity [23,107,173,222,223,275–279].

Decay rates of infectivity reported in the literature vary from not detectable to $80\% \text{ h}^{-1}$ for phages compared to particle decay rates of $0.2\text{--}54\% \text{ h}^{-1}$ for viroplankton [32]. It has also been suggested to express type-specific mortality rates of phages as half lives, and,

e.g., in soil, half-lives of phages infecting streptomycetes ranged from 1.19 to 18.9 days [39]. In a study conducted to identify the major mechanisms that cause a loss of phage infectivity, solar radiation was recognized as the most important single factor in coastal surface waters of the Gulf of Mexico exposed to high doses of radiation [107]. Decay rates of infectivity of bacteriophages in surface waters are higher than decay rates of viral particles suggesting that a portion of viruses in sunlit surface waters may be non-infective [108,223,275,278]. These studies typically did not include the effect of light-dependent repair on restoring infectivity to phages (see below) and thus the decay rates estimated in sunlight and the fraction of inactivated phages in the environment might have been overestimated. The UV-B portion of sunlight is probably the most important wavelength range responsible for the loss of viral particles and infectivity [107,277,278]. However, there is experimental evidence that UV-A and/or visible light can also contribute significantly to the destruction of phage infectivity [107,277,279]. These studies also suggest that the relative effect of UV-B, UV-A and photosynthetic active radiation might differ between phage species. Moreover, studies with several phages investigated in parallel incubations show that the effect of sunlight on infectivity can vary strongly between different phages [108].

In addition to sunlight effects (or in the absence of it), there are also other significant sources of viral mortality. It has been reported that autoclaving inactivated the factors destroying viral infectivity in dark incubations (e.g. [277,280,281]) suggesting a strong biological influence on the decay mechanisms. Surprisingly, the bacterial fraction of seawater does not seem to destroy viruses at high rates [107], although bacteria as a part of the heat-labile particulate material show virucidal effects (e.g. [282,283]). Removing cells by filtration reduced the dark decay rates of phages by only 20% [277]. In the absence of sunlight, microparticles (potentially heat-labile particles larger than $1.0 \mu\text{m}$) [107] and heat-labile colloidal DOM [277] seem to be the most important factor resulting in loss of viral infectivity in aquatic systems. However, it has also been shown that inorganic and organic colloids and particles can increase the survival of the viruses [260,270,283–285]. In soil, desiccation and adsorption to soil colloids could be major mechanisms causing viral mortality [39,204]. Decay of viral particles and infectivity was also determined after poisoning of samples with cyanide, thus representing an indicator of viral losses due to non-biological mechanisms [107]. Using this approach, it has been shown that decay rates of viroplankton differed between size classes of viral particles [84,94].

In addition to these major mechanisms of viral decay, other factors have been identified that cause viral mortality. Temperature affects viral mortality [35]. The decay rates of cyanophage communities increased be-

tween 4 and 25 °C suggesting a negative effect of increasing temperature for viral survival [279]. The negative effects of elevated temperatures on phage infectivity might be linked to temperature dependent biological activity. Protists ingest viruses, but it is very likely that grazing is not a significant factor for the loss of viruses [286], maybe with the exception of large viruses that might be ingested preferentially [167]. Murray [167] also speculated that the exudates of healthy phytoplankton are a means to attract bacteria and by that flagellates. Thus, bacteria-induced and flagellate-induced mortality of viruses might help to reduce viral infection of algae and keep them 'healthy'. However, there is no hard data to support this hypothesis. Overall, the finding that single loss factors have a variable influence on different phages suggests an effect of decay on niche differentiation.

4.4.2. Antiphage defense

A phage has to overcome several obstacles during its lifetime before it can infect a cell and reproduce, i.e. access its resource. The term (anti)phage defense is used to describe the mechanisms of a potential host cells to prevent viral infection. The economical impact of viral infection of microbes used in biotechnological processes has resulted in an enormous amount of studies on host antiphage defense mechanisms as potential ways to fight viral infection (e.g. [287–290]). Typically four defense mechanisms are distinguished based on their point of action in the phage life cycle: Adsorption inhibition, DNA injection blocking, restriction-modification and abortive infection.

Adsorption inhibition involves strategies to prevent a phage to attach to the cell surface such as the lack of receptor expression, an alteration of a receptor and a masking or shielding of receptors. The cellular exopolysaccharide and surface layer (S-layer) proteins are among the potential factors blocking attachment. EPS and biofilms may be a barrier against infection [291–294], however, although this might slow down infection rates, EPS or biofilms cannot always prevent infection [295,296]. In contrast, EPS and capsules might 'invite' viral infections. It has been shown that a significant amount of marine bacteria has a capsule and it seems that these cells are the most active ones [297–301]. As phages are proliferating best in the most active cells (see above), it could be that the EPS or capsule is rather a recognition and attachment site for phages to identify an active host cell than an effective way to reduce infection. Phages have to penetrate the exopolymeric matrix and find the specific attachment sites on the cell wall of a host. Thus, phages must be able to identify the host by components of the EPS and then be able to identify the cell wall as site to introduce the genetic material into the cell. For example, phages use specific proteins to attach to the polysaccharide capsule of *Escherichia coli* as well

as to degrade the capsule [302,303]. Although not metabolically active and not motile, phages can penetrate the capsule or biofilms by the use of enzymes [304–306], which are located in the tail or capsid, by depolymerizing this potential barrier. Making a channel through the capsule by enzymatic digestion is another way to access the host cell. In marine snow particles, i.e., organic aggregates in the water column, viral infection rates of prokaryotes were roughly as high as those found in ambient water [307] suggesting that this organic matrix did not prevent infection of cells. A different way of adsorption inhibition would be host proteases destroying incoming phages. This may not strictly be considered as adsorption inhibition, although its effect would be the same.

DNA injection blocking can involve changes in the cell permeability such as resistance to phage lysis. A temperature dependence of DNA injection blocking, potentially due to altered membrane fluidity, has been documented as well as the involvement of cell membrane proteins. Unfortunately, information on DNA injection blocking as antiphage defense is relatively rare [288].

Restriction–modification consists of a methylase that modifies bases of the host DNA by methylating them at the restriction sites and rendering them non-accessible to restriction endonucleases. Thus, a foreign (non-methylated) DNA, such as phage DNA, can be detected and hydrolyzed. Phages have responded to the danger of their DNA being recognized and destroyed by restriction enzymes by adopting strategies such as elimination of restriction sites, base modification, proteins inhibiting endonucleases and acquisition of methylase genes. It has also been questioned that R–M was solely developed as a phage defense mechanism [37].

Abortive infections suppress phage development by interfering with processes that occur after the DNA was successfully injected into the cell. This includes processes such as replication, transcription, translation, phage assembly and infectivity of phage progeny. Abortive infection results in the destruction of the cell before phages can replicate. This 'suicidal sacrifice' [288] protects cells of the same population from infection by phages. From an evolutionary perspective the development of suicide genes becomes then rational.

The host-mediated defense mechanisms appear to be serially ordered and may act in a coordinated way at consecutive stages of the phage life cycle to reduce or prevent phage proliferation. Antiphage defense is important for understanding the ecology of phages in the same way as defense mechanisms of all types of prey are significant for all types of predators, since they decrease the accessibility of the prey. Defense mechanisms against grazing by protists have been studied in some detail for prokaryotes [237,238]. Unfortunately, there is still very little information on the actual role and significance of phage defense in situ.

4.5. DNA damage and repair

4.5.1. DNA damage

The UV portions of sunlight or mutagenic substances are potential mechanisms causing DNA damage in viruses. As in bacterio- and phytoplankton [308], cyclobutane pyrimidine dimers are the dominant type of photodamage in the DNA of viroplankton. Cyclobutane pyrimidine dimers were ca. an order of magnitude more abundant than pyrimidine–pyrimidone (6–4) photo-products in the DNA of natural viral communities collected in situ, and bacteriophage and cyanophage isolates deployed along depth profiles [309]. Cyclobutane pyrimidine dimer and pyrimidine–pyrimidone (6–4) photo-product concentrations were significantly higher in the bacteriophage PWH3a-P1 than in the cyanophage SYN-M3 in parallel deployments in the mixed layer [309], and consequently the decay rates were lower in the cyanophage [278,279]. Nothing is known on other types of photodamage in nucleic acids of viruses in situ or on potentially deleterious effects of sunlight on capsid proteins of phages. However, the finding that phages which were irradiated with UV light between 300 and 400 nm lost the ability to adsorb to host cells [310] indicates the likelihood of deleterious effects of sunlight on capsid or tail proteins.

Resistance mechanisms against DNA damage are known for phages and viroplankton. For example, in surface incubations, photodamage formation was less pronounced in the DNA of natural virus communities than in the DNA of isolates [309]. Studies with bacterio- and cyanophages indicate that phages exposed to sunlight develop resistance against loss of infectivity [277,279]. One of the mechanisms to confer resistance is to increase the G + C content of the DNA [311]. Since the protein coat of phages is only a few nanometers thick, it is unlikely that the capsid contributes to the protection against damage.

Photodamage in viral DNA accumulates during the solar day, decreases with depth and is higher in more pristine offshore waters than in more turbid coastal waters. However, when the thermocline is shallow, significant photodamage can also occur in more turbid coastal waters [309]. Strong mixing can reduce photodamage formation by reducing the residence time of phages in the upper water layer where harmful UV doses are highest [222,309]. These data support the finding derived from models that understanding mixing processes and thermocline formation is paramount for understanding the survival of phage in sunlight [312]. Upwelling, i.e., transport of deeper water to the surface, may also have an influence on residence time and DNA damage accumulation in natural viral communities [313].

4.5.2. Repair

Damaged phages can survive as long as they are able to inject their genome into the host, and the DNA

damage can be repaired within the host. Two basic types of repair are known: photoenzymatic or light-dependent repair (photoreactivation) and nucleotide excision or dark repair [308,314]. Dark repair consists of a complex system (SOS network) and requires ATP, whereas the enzyme photolyase is activated due to the photon energy of light in the range of ca. 365 and 445 nm and removes cyclobutane pyrimidine dimers [314].

Repair of damaged phages by host cells has been studied for a long time [315,316] and the process of photoreactivation was first demonstrated for phages [317,318]. Viruses typically exploit the repair system of the host, however, viruses can carry the genes for their own repair system, i.e., the *denV* gene encoding for the endonuclease V [319,320]. The sequence similarity of the *denV* gene from very distantly related viruses infecting prokaryotes (T4 phage infecting *E. coli*) and eukaryotes (viruses infecting the *Chlorophyceae* genus *Chlorella*) may indicate that a virus-specific repair system is more frequent than previously thought. Recently, a *recA*-independent, UVA- and sunlight-inducible DNA repair system was described for the phage UNL-1 infecting *Pseudomonas aeruginosa* [321]. These findings indicate that the contribution of viral genes to repair is more important than previously anticipated.

Studies on the relative significance of photorepair and dark repair of damaged phages are rare. It was demonstrated in experiments with artificial light (254 nm to damage phages and 365 nm to induce PER) for the T4 and the λ phage that PER is roughly as efficient as dark repair [322]. The dominance of PER over NER was also suggested in a study with isolated marine vibriophages [311]. In the Gulf of Mexico, experiments were performed using sunlight and natural viral and bacterial communities to assess the significance of PER in situ [276]. At oligotrophic offshore stations between 21% and 26% of the phage community that could not be repaired by dark repair regained infectivity by photoreactivation; at coastal stations this percentage ranged from 41% to 52%.

The importance of PER was also shown using natural viral communities damaged by sunlight in the Gulf of Mexico and *Vibrio natriegens* strain PWH3a as host [276]. In general, the repair capacity varies between phages [222]. Since PER is ATP-independent [323], whereas dark repair requires ATP, PER might be an important energy-saving repair mechanism in nutrient-limited systems. As photoreactivating wavelengths penetrate deeper than damaging wavelengths, mixing of damaged phages below a critical depth should allow for PER in the absence of further damage [222]. Based on loss rates of infectivity and viral particles, it was estimated that damage must be repaired to between 39% and 78% of the phages in which infectivity was lost [278]. No distinction between dark and light-dependent repair was made by this approach. Dark repair of phages has

not been investigated in the environment, but given the ubiquitous taxonomic distribution of the excision repair, it is likely that the SOS network contributes significantly to restoring infectivity.

Damaging wavelengths can decrease nucleic acid and protein synthesis rate and enzymatic activity of cells [324]. This may also reduce their capacity to repair phages. Factors such as nutrient limitation or starving of host cells might significantly affect in situ repair rates [325]. However, such studies have not been performed yet. Moreover, the significance of sources of DNA damage other than sunlight such as mutagenic substances for viral infectivity in situ is not known. Overall, light-dependent and dark repair mechanisms are an important means for partially counterbalancing losses of phage infectivity due to DNA damage. Repair may also be an explanation for the sunlight-infectivity paradox [276].

4.6. Phage proliferation and host density

Despite the uncertainties associated with the methods to estimate phage production and turnover, it is possible to detect differences between contrasting environments such as open ocean and coastal/shelf waters. Turnover rates of the entire viral community range from 0.38 to 30 days in oceanic surface environments and from 0.036 to 12.8 days in coastal and shelf environments (Table 4). Calculating average values from single studies (when only ranges were given the average was calculated from the range of turnover times) were 6.1 and 1.6 days in oceanic and coastal/shelf environments, respectively. Data compiled in Table 4 also suggest that viral production is on average higher in the oceanic than in the coastal/shelf province, ranging from non-detectable to 14×10^9 viruses $\text{ml}^{-1} \text{d}^{-1}$, and from 12×10^9 to 230×10^9 viruses $\text{ml}^{-1} \text{d}^{-1}$, respectively. This concurs with differences in prokaryotic and general productivity in these environments. In limnetic systems, viral turn-

over time ranged from 0.09 to 3.5 days with an average of 0.8 days, and viral production ranged from 0.16×10^9 to 50.4×10^9 viruses $\text{ml}^{-1} \text{d}^{-1}$.

A mathematical model based on diffusive transport theory has been developed to estimate the contact rate between particles such as viruses and bacteria in aquatic systems [326]. Other more simple models have been used as well [12,171]. Suttle and Chan [223] have shown for a cyanophage that the calculated contact rates match measured adsorption rates in cultures. Fischer and Velimirov [95] included temperature and a relationship between swimming speed and cell size in this model. Recent methodological progress allows for quantifying the fraction of motile bacterioplankton by detecting their flagellae [327], which might increase the precision of contact rate estimates. A general conclusion of such models is that contact rates increase at higher bacterial abundance. Among the potential reasons for the increased viral production at higher productivity levels are the higher contact rates and subsequent higher infections rates of hosts, higher per cell activity, larger cells and a higher abundance of potential hosts.

Several studies suggested that prophage induction is probably not a significant contribution to total viral production [28,88,91,92,122,188]. However, other studies opposed this view arguing that prophage induction could be even responsible for the majority of viruses found in marine systems [126,127] and it is possible that mass induction is occasionally important. It was also suggested that phage production from pseudolysogenic infections could contribute significantly to viral production [328–330]. Currently, we cannot estimate the relative significance of lytic phage production and prophage induction for total phage production.

It has been argued that there is a host density threshold for phage infection and replication, and that phage replication increases with host density. For example, Wiggins and Alexander [280] reported on thresholds of ca. 10^4 cells ml^{-1} , concluding that this

Table 4
Potential interactions between a phage and a prokaryotic species

Species		Type of interaction	Examples
Phage	Prokaryote		
0	0	Neutralism	Typical interaction?
–	–	Competition	Phage competes with grazers or predatory bacteria for prey
+	+	Mutualism	Some phage conversions
+	–	Predator–prey	Lytic and lysogenic and chronic infection
–	+	Predator–prey	Phage as nutrient source for cell (decoy receptor) Cell digests its predator/parasite Abortive infection
0	+	Commensalism	Viral lysis products taken up a cell
+	0	Commensalism	Transport of a phage on the surface of a non-specific cell ?
0	–	Amensalism	Phage lysozymes killing or damaging a non-host cell
–	0	Amensalism	Ecto-enzyme destroying (non-specific) phage

0, no effect; + positive effect; – negative effect.

might be a reason for low viral numbers in the environment. In contrast, Kokjohn and co-workers [252] reported that attachment and replication were not impaired by host-densities (10^2 cells ml^{-1}) found in the environment. Host density thresholds were also found for cyanophages infecting *Synechococcus* cells along a transect in the Gulf of Mexico [173] and predicted by a modeling approach [331]. Moreover, the existence of thresholds was inferred from studies with natural communities of viruses and prokaryotes [85,110,122], although in one study no threshold was found [159]. Such thresholds might occur when the destruction rate of phages is higher than the rates at which they can find a suitable host.

4.7. Host range

Typically, it is assumed that the host range of phages is narrow, i.e., that they do not “trespass generic boundaries” [36]. This has been inferred from studies of the more than 4000 bacteriophage isolates described at that time and was also reported for isolated marine bacteriophages [20]. For example, phages were isolated from *V. parahaemolyticus* that did not infect all strains of this species and no other *Vibrio* species [332,333]. However, a broad host range or polyvalence has been also demonstrated, e.g., for cyanophages, which belong to the most thoroughly investigated group of phages. These studies are very likely representative for their group, since cyanobacteria can be relatively easily isolated and thus isolation of cyanophages is not so strongly biased by GPCA. A broad host range was reported for cyanophages infecting different genera of freshwater and marine cyanobacteria. A note of caution has to be added because this may also be the result of problems with the taxonomic affiliation of cyanobacteria [16,17]. Cyanophages infecting phycoerythrin-containing *Synechococcus* strains seem to have a broader host range than cyanophages infecting phycocyanin-containing *Synechococcus* strains [170,171]. Such host range differences may be related to foraging strategies for obtaining resources in the form of a suitable host. Wichels and co-workers [229] noted for bacteriophages that *Myoviridae* seem to exhibit a broad host range, and there is some data from cyanophages supporting this hypothesis [171,174]. In general, the host range of cyanophages seems to be variable ranging from strain specificity for some cyanophages to broad host ranges overlapping with those of other cyanophage species [16,17]. It should be noted that the broadness of a host range is not well defined and depends on the availability of isolated prokaryotes, which is in turn affected by GPCA. One study reports on a larger host range of phages isolated from the open ocean than from coastal waters [334]. This may be seen as an adaptation of phages to the lower bacterial abundance in offshore than

coastal waters. However, presently there is no data available on the host abundance for specific phages and on the population or species identification of the hosts in situ. Only such data will allow for assessing the environmental conditions favoring a broad versus a narrow host range.

The central role of the receptor structure for determining the host range has been shown for broad-host range vibriophage KVP40 [335,336]. Also, the presence of more than one specific tail fiber protein may allow phages to increase their host range, since these proteins are used by the phage to identify a host and attach to it [302,303]. The occurrence of similar conserved regions of tail fiber genes in different viral families suggests that host range is probably wider than previously assumed [337]. It has also been argued that the host specificity concept arises from methodological artifacts of bacteriophage isolation [338]. The authors demonstrated that in the presence of several phages the typical enrichment performed during phage isolation selects for a single type of phage, namely those with the highest affinity for the host. A broad host range is also suggested in the study of Chiura [339] showing that marine phages were able to transduce enterobacteria. Such data underline the problems of the GPCA and call for studying natural communities or developing novel isolation strategies. If the host range for some phages is indeed larger than previously assumed, they might be better described as generalists than as specialists.

4.8. Resistance

Resistance, as seen from the phage perspective, is the problem of reduced or lack of accessibility of prey, i.e., resources. Resistance of a lysogen against co-infection can be conferred either by repressor molecules produced by the prophages, or by changing the antigenic properties. For example, ϵ -prophages of *Salmonella* encode enzymes that modify polysaccharides of the cell envelope and render the lysogens resistant to infection by other phages of the same type [340,341]. Suttle [16] showed by using a phage typing approach that a cyanophage attached to only 10% of its host cells, but those where attachment was observed were covered with phages on the entire cell surface. This indicates an example of resistance due to the lack of receptor expression. Expression of receptors during a short period of the life cycle would minimize the number and exposure time of receptors [16] and could explain why some bacteria only get infected in specific stages of their life cycle (see below, Section 5.1). Moreover, the nutrient source can determine the receptor density at the cell surface [37]. Also, receptors might be altered and resistance could be conferred by a masking or shielding of receptors. Receptors can be transporters, porins, lipopolysaccharides or filaments and pili, however, the

receptors responsible for adsorption *in situ* remain unknown.

The high viral abundance in ecosystems is puzzling, since it is well known from studies with pure cultures that bacteria can rapidly develop resistance against infection [37]. Thus, resistance is a key problem of the infectivity paradox. A potential solution to this problem is that the development of phage resistance has physiological costs [342,343]. For example, due to the modification of a nutrient uptake receptor, which also serves as attachment site for phages, bacterial strains may become resistant against infection but at the same time less competitive for acquiring this specific nutrient. An indication that resistant strains are doing not so well as sensitive strains is the frequently reported finding that growth rate and yield of resistant strains are lower than that of sensitive strains (e.g. [37,250,342,344]). The resistant strain can survive only because the more competitive but phage-susceptible strains are controlled by lytic infection. Once the phage numbers decrease due to the lack of susceptible hosts, the surviving competitive dominants may become abundant again. While this model assumes mutation and selection (hereditary resistance), adaptation at the physiological level is also possible, where the host actively changes the number, structure or accessibility of viral receptors on the cell surface (non-hereditary resistance).

It has also been speculated that resistance might not be pervasive among marine prokaryotes [26]. Instead, bacteria may have developed efficient mechanism to stop viral activity at an intracellular stage as part of the antiphage defense or might even invite incompatible phages by decoy receptors to attach and inject the genetic material. Viral macromolecules are a potentially rich source of nutrients (P, N or organic carbon) in oligotrophic waters. Fuhrman [26] has pointed out that even if phages would get occasionally through the antiphage defense system and kill the host cell, the bacterial line could still benefit from phage lysis products. The finding that the contact success of viruses is higher in oligotrophic than in eutrophic environments and resistance against cyanophage infection decreases from coastal to offshore habitats [173,278,279] would support this hypothesis, since acquiring nutrients is more critical in oligotrophic waters. Carbon limitation can result in the expression of a multitude of high-affinity transporters in *E. coli* [345]. Such transporters might also serve as receptors for phages. The need to express nutrient receptors in nutrient-limited systems may allow phages to exploit their hosts efficiently. Thus, a trade-off between the chance to get infected and the chance to acquire nutrients might explain the high levels of infection found in natural bacterial communities.

Also, there is some evidence that viral infection causes a net increase of bacterial production at the community level (see below). Thus, viral infection might be

beneficial for bacteria and suggests that bacterial populations might have to ‘sacrifice’ a portion of their cells to gain this advantage. In such a situation a sort of group selection would be required to explain why resistance is not developed in the presence of the possibility of ‘cheating’ [26]. However, it is also possible that cheating is no option if there is an absolute need to express nutrient receptors for ensuring bacterial survival.

A potential solution for the resistance paradox (as well as explaining the co-existence of phages and hosts) was provided recently by Middelboe et al. [346]. Using phage–host systems they showed that phages had only a temporal effect on bacterial abundance and on the dynamics of specific bacterial populations due to the development of resistant populations dominating the system. They developed a model describing the interaction between phages and bacteria by including the generation and selection of resistant cells. Interestingly, when the effect of a grazer was introduced in this model, it predicted that grazing by protists allowed for a recovery of sensitive cells, which in turn sustained viral infection. Data from dialysis bag incubations with natural communities, where the presence of grazers stimulated viral infection of bacteria [93], are compatible with this idea. This underlines the importance of considering natural situations in the quest to understand the ecology of phages.

5. Life cycles and strategies of phages in the environment

5.1. Interactions of prokaryotic viruses with other species and co-existence with hosts

5.1.1. Interactions

Interactions between organisms or species can be neutral, adverse (antagonistic) or beneficial (synergistic). Frequently, the interactions between two species or organisms are described by pairs such as $+/+$ meaning mutualism or $+/-$ meaning predation or parasitism. In Tables 5 and 6, potential interactions between phages and cells and between phages themselves, respectively, are summarized. Neutralism, i.e., no interaction between a phage and another (cellular or acellular) species (0/0) might be the rule, but is actually hard to prove. Indeed, one might also argue that neutralism is rare, since indirect effects can usually be anticipated.

A specific phage may compete with other phages, parasites and predators for hosts. For example, a phage may compete with another phage, a predatory bacterium such as *Bdellovibrio* and a heterotrophic flagellate for the same prey. The major form of competition between phages is that for hosts, i.e., resources. Competition between phages is not well studied, but obviously important. For example, when *E. coli* is co-infected with

Table 5
Potential interactions between two phage species

Species		Type of interaction	Examples
Phage 1	Phage 2		
0	0	Neutralism	Typical interaction?
–	–	Competition	Phages with same host
+	+	Mutualism	Co-infection with gene exchange
+	–	Predator–prey	Satellite-helper phage
0	+	Commensalism	Viral lysis products increasing host abundance for another phage
0	–	Amensalism	Phage lysozymes removing receptors from a host for another phage

0, no effect; +, positive effect; –, negative effect.

the λ phage and a lytic coliphage, the lytic activity of λ is suppressed [347,348]. The phage $\phi 6$ infecting *P. phaseolicola* has developed mechanisms to reduce co-infection as a competitive strategy [349]. Also, one of the rare in situ studies on this topic suggests competition between the lytic phage $\phi CP6-4$ and a temperate phage $\phi CP6-1$ infecting *S. liquefaciens* CP6RS in the rhizosphere [350]. Intraspecies competition between phages is also known. An example are prophages that change the cell wall moieties of the host rendering these resistant against homologous (genetically related) phages [38]. Moreover, lysozyme liberation during cell lysis and sweeping of receptors might be a weapon against competing phages [38]. The most mundane example are repressor molecules produced by prophages, which prevent the proliferation of related phages upon infection.

Mutualistic or even symbiotic interactions between phages and hosts may exist as well. For example, phage conversion may result in an increased fitness of the host. This was shown for various phages infecting *E. coli* [351–353]. An increased fitness of the host should also result in higher replication and thus survival rates of the phage genome and this might be considered a mutualistic interaction. Establishing a lysogenic interaction always confers immunity to the lysogenic cell against superinfection with the same or related phage types [36]. Interactions of the (+/+) type might also exist between phages. Co-infection of cells with two or more phages is not always adverse, but occasionally also beneficial due to the exchange of genetic material, a process that can occasionally contribute to phage diversity and thus enhance the chance to adapt to the environment [354]. Acquiring tail fibers to increase the host range [337] might be such a case. Beneficial effects of viruses on prokaryotes at the community level seem possible as well (see below) [26].

Predator–prey type and parasitic interactions (+/–) between phage and cellular species, considering “...viruses as predators of the plankton...” [31], have been documented in some detail [32,37]. However, phages can also be prey for heterotrophic nanoflagellates and a resource of carbon, phosphorus or nitrogen [166]. Grazing of nanoflagellates on phage-infected cells is another case of phages being prey. Lytic phages infecting unicellular

prokaryotes are perhaps better described as predators than parasites, since they do not make prokaryotes ‘sick’ but lyse them, whereas chronic infection is in accordance with the idea of parasitism. An example of a (+/–) type interaction between phages are satellite phages that parasitize on a predator-type helper phage. The genome of these satellite phages is so small that they lack capsid genes. Hence, they are using the genes from a co-infecting phage, the helper phage, to form their capsid [355]. Assuming that the helper phage has a disadvantage from this interaction, since more of its progeny could be formed without the satellite phage, this interaction is a parasitic one between two phages. Most studies have investigated phages as predators or parasites. This might reflect the predominant ecological role of these life forms, however, the focus of research was certainly not on mutualism.

Another type of interaction is where one species is not affected but the other phage is either adversely (0/–, amensalism) or favorably affected (0/+, commensalism). Almost nothing is known on amensalistic or commensalistic interactions between phages and prokaryotes or between phages and phages. A few potential examples for amensalism and commensalisms are listed in Tables 5 and 6. Even more complex interactions can be expected in nature, where more than just two species interact. Overall, a description of prokaryotic viruses as parasites of unicellular organisms seems to be an oversimplification regarding the sophisticated and diverse interactions with other cellular and acellular organisms.

5.1.2. Co-existence and co-evolution

The topic of interactions between phages and hosts is tightly linked to the problem of co-existence and co-evolution between the two. Resistance is also involved (see above, Section 4.8), however, the focus is here on co-existence. Schrag and Mittler [344] list four hypotheses for the coexistence of a virulent phage and its host: (1) the numerical refuge hypothesis [343], (2) the physiological refuge hypothesis, (3) the endless arms race hypothesis and (4) the spatial refuge hypothesis. Evidence for thresholds of host abundance necessary for sustaining phages and thus for the *numerical refuge hypothesis* has been presented, however, there is also

Table 6

Frequency of visibly infected cells, frequency of infected cells, virus-induced mortality of prokaryote, viral production and viral turnover in various marine and freshwater environments

Location [reference]	Method	FVIC (%)	FIC (%)	VMP (%)	Viral production (10 ⁶ ml ⁻¹ d ⁻¹)	Turnover time (days)
Marine						
<i>Offshore</i>						
Sargasso Sea [13]	TEM	0.9	6.2	7.0		
Caribbean Sea [13]	TEM	2.8–3.9	16.1–24.3	25.7–39.3		
Gulf Stream [13]	TEM	4.3	26.4	44.7		
Bering/Chucki Sea [111]	TEM	0.2–3.3	1.4–21.0	1.4–31.6	0.02–14 (1.9)	
Bering/Chucki Sea [111]	TEM				0.39–14.0	0.38–9.8
Bering/Chucki Sea [111]	VPR			1.9–12 (7.9)	0.51–4.2 (1.8)	1.2–15
NW Mediterranean Sea [92]	TEM	1.3–1.5	8.8–10.4			
NW Mediterranean Sea [92]	VDA		9.4–9.5			
Southern California Bight [110]	VPR			0.97–29	0–2.8 (0.82)	8.9–30 (14.5)
Gulf of Mexico [173]	LRVI					0.48
Gulf of Mexico [278]	LRVI			6.3–12.2 of BP		
Offshore California Waters [113]	FLV			26–66		2.2
North Water Polynia [98]	TEM	1.1–2.7		4–19 (6–28% of BP)	0.09–0.16 d ⁻¹	4–8
East Sea [100]	TEM	1.2–2.2		9.8–19.2 (13.1) of BP	0.04–1.37	0.9–23.9
Mediterranean Sea [188]						
Surface	TEM		8.5–12.0 (10.5)	10.9–16.3 (13.9)		
Mesopelagic	TEM		0.9–6.0 (3.5)	1.0–7.6 (4.2)		
Deep	TEM		0.7–7.0 (3.2)	0.8–8.9 (4.0)		
<i>Coastal/shelf</i>						
Long Island Sound, USA [13]	TEM	4.1	25.3	41.9		
Southern California Bight, USA [110]	VPR			1.3–740	12–230 (60.2)	0.57–3.9 (2.0)
Santa Monica pier, USA [102]	VPR			29–67 d ⁻¹	24.7–42.7 (32.8)	0.46–0.6
Santa Monica pier, USA [102]	TEM	3.3	21.0	31.6		
Northern Adriatic Sea [86]						
Mesotrophic	TEM	0.9–1.0		7.0–64.3	0.3–2.9	
Eutrophic	TEM	1.9–2.7		3.5–24	1.7–17.0	
Northern Adriatic Sea [87]	TEM	0–4.2	0–25.9	0–43.3		
NW Mediterranean Sea [92]	TEM	0.7–1.9	4.5–12.6	4.9–16.0		
NW Mediterranean Sea [92]	VDA		4.7–17.3			
Baltic Sea [92]	TEM	1.5–1.8	10.0–11.7	12.1–14.6		
Baltic Sea [92]	VDA		8.3–14.4			
Baltic Sea [188]						
Oxic	TEM		7.3–16.6 (12.9)	8.5–24.2 (15.3)		
Interface	TEM		3.8–21.3 (10.6)	4.1–15.4 (14.7)		
Anoxic	TEM		8.2–32.7 (22.5)	9.8–71.4 (42.0)		
North Sea [92]	TEM	0.8–5.0	5.5–29.8	6.0–54.8		
North Sea [92]	VDA		6.2–43.4			
Raunefjorden, Norway [84]	LRVP		8–13	48–576 d ⁻¹		0.08
Aarhus Bay, Denmark [83]	LRVP		12–29	1728 d ⁻¹		0.14
Gulf of Mexico, USA [107]	LRVI		4–13			2.4
Tampa Bay, USA [156]	LPVI			10–53 d ⁻¹		
Northern Adriatic Sea (5 m) [185]	LRVI			39–212 d ⁻¹		
Northern Adriatic Sea (22 m) [185]	LRVI			19–157 d ⁻¹		
Gulf of Mexico, USA [173]	LRVI			2–7% d ⁻¹		8.3
Gulf of Mexico, USA [279]	LRVI			1–8 d ⁻¹		1.3–2
Gulf of Mexico [278]	LRVI			7.2–34.0 of BP		
Two Harbors, USA [113]	FLV			29–125		1.3–1.4
San Pedro Channel, USA [113]	FLV			30–119		0.68–2.2

Table 6 (continued)

Location [reference]	Method	FVIC (%)	FIC (%)	VMP (%)	Viral production (10 ⁶ ml ⁻¹ d ⁻¹)	Turnover time (days)
Playa del Rey Jetty (5–60 m), USA [113]	FLV			24–59		1.1
Strait of Georgia (tidally mixed), Canada [114]	VDA			1102–3288 (2306) d ⁻¹		0.036–0.119 (0.084)
Strait of Georgia (non-mixed), Canada [114]	VDA			444–643 (548) d ⁻¹		0.139–0.196 (0.159)
Strait of Georgia [384]	Bulk change			27		
Ria de Aveiro estuary, marine [123]	VDA			30–41 (36)		
Ria de Aveiro estuary, brackish [123]	VDA			49–74 (61)		
<i>Others</i>						
Sinking particles (Pacific) [307]	TEM	0.7–3.7	4.9–32.3	5.3–36.6		
Solar saltern [101]	TEM	0–3.8	0–23.5	0–36.6		
<i>Limnetic</i>						
Lake Erie, USA [307]	TEM	1.6	11.0	13.5		
Gössenkollesee, Austria [96]	TEM	0.9–2.3	6.2–15.1	7.0–20.2		
Lake Constance, Germany [97]	TEM	0–1.7	0–11.4	0–14.1		
Plußsee, Germany [89]	TEM	1.5–3.3	10.3–21.0	12.5–31.6		
Plußsee, Germany [89]	TEM					
Oxic		0.7–2.6 (1.6)	4.9–16.7 (11.0)	5.4–23.1 (14.0)	0.16–1.40 (0.92)	
Transition layer		1.8–4.3 (3.0)	12.1–26.5 (19.0)	15.2–45.0 (28.55)	3.0–15.5 (11.1)	
Anoxic		3.5–9.0 (6.3)	22.3–45.6 (34.7)	34.6–128.2 (79.8)	4.2–7.8 (6.2)	
Danube backwater, Austria [94]	TEM	1–4	6.9–24.8	7.8–40.6		
Danube backwater, Austria [96]	TEM	2.8–9	18.1–45.7	25.7–128.8		
Reservoir, Czech Republic [93]	TEM	2	13.3	17.1		
Lake Hoare, Antarctica [112]	VPR				49.0	0.3
Lake Kalandsvannet [84]	LRVP		2–16	48–576% d ⁻¹	0.44 h ⁻¹	0.09
Danube backwater, Austria [94]	LRVP			15.2–30.1 (20.1)	10.1–31.2 (17.5)	1.4–3.5 (2.0)
Danube backwater, Austria [96]	LRVP			42–88 (56)	12.0–50.4 (29.3)	0.5–2.2 (0.9)
Lake Pavin [498]						
5m	TEM	0.5–1.2 (0.8)		3.5–10.3 (6.4) of BP		
10 m	TEM	0.7–3.5 (1.7)		6.0–33.7 (15.6) of BP		

Data are given as range (average).

studies questioning thresholds at densities occurring in the environment (see above, Section 4.7). The *physiological refuge hypothesis* assumes that resistance is transitory and not hereditary. An example for a physiological refuge is the lack of infection of *Halobacterium* at high salt concentrations [178,356]. Physiological refuges can also be due to starvation of bacteria, depletion of a factor in the environment required for phage attachment, bacterial clumping or enzymatic removal of receptors [37]. Other examples are bacteria, which can only be infected by phages during certain life stages. Such a dependence of viral infection on the developmental stage of hosts has been reported for marine systems (see [31] and literature therein). For example, phages infect a stalked bacterium (*Caulobacter* sp.) and a gliding bacterium (*Cytophaga johnsonae*) only during

their motile phase. Another example are phage isolates from *Bdellovibrio* that did infect this cell parasite only when it was attached to its host cell. It has also been suggested that some cyanobacteria do not express receptors during their entire life cycle and by that reduce the chance of viral infection [16]. The *endless arms race hypothesis* assumes that there are endless cycles of bacterial defenses and phage counter defenses. However, the finding that bacterial mutants can acquire resistance to a phage that can no longer develop new host-range mutants, suggests that the co-evolutionary potential of phages is less than that of the bacterial hosts [357,358]. The structural constraints for phage adsorption (receptor availability) seem to be more severe than the physiological constraints for resource allocation by bacteria. Nevertheless, the possibility for this hypothesis remains

because resistance might not be as widespread in nature as previously assumed (see above, Section 4.8). In a study with coliphages, the *spatial refuge hypothesis* was found to be the most likely hypothesis for explaining the long-term persistence of phage and bacteria [344]. A spatial refuge can, for example, be the glass wall of an experimental container where the sensitive bacteria attach, but are not infected. Spatial refuges in the environment could be attachment to particles. Indeed, it has been argued that spatial heterogeneity is the principle mechanism of bacteria in soil to escape their phage predators [359]. Vice versa, some phages adsorb to colloids together with their hosts, and, hence, adsorption could be a way for phages to find their hosts. In this case, adsorption to colloids would provide no refuge for phages.

Considering interactions between phages, competition can result in an endless arms race, niche differentiation, or competitive exclusion [354]. Niche differentiation and endless arms race would result in a coexistence of phage species. Spatially separated phages infecting the same host might also represent a niche differentiation. An example would be two phages, one adsorbing to organic particles, and the other remaining in the ambient water, while the host species lives on particles as well as in ambient water. Also, an overlapping but not identical host range could result in niche differentiation.

It has been argued that lysogeny has evolved from lytic phages by co-adaptation as a mean to stabilize the interactions between phages and hosts [32,37]. Such an interaction would allow for the survival of both. In this context, pseudolysogeny and maybe even chronic infection could be seen as an intermediate evolutionary step. However, Lederberg [360] pointed out when discussing pathogen–host interactions that, “... we are dealing with a very complicated co-evolutionary process, involving merger, union, bifurcation and reemergence of new species. . .”. For example, it is well known from PHSs that temperate phages can mutate back to lytic ones [37]. Also, we do not know for certain whether or not lytic infection is the evolutionary older life strategy. For example, the ‘moron-accretion hypothesis’ for the origins of phages [361] does not seem to favor one of the two life cycles as strategies of ‘proto-viruses’. Moreover, environmental variables and interactions with other species such as flagellates might be critical in structuring the co-existence and co-evolution of phages and hosts.

5.2. Population dynamics and metapopulations

Considering lytic phages rather as predators than as parasites, predator–prey type oscillations between phages and hosts can be anticipated. Such oscillations have been reported from chemostat studies and models

[37,346]. Wommack and co-workers [146] presented a conceptual model on the dynamics of single populations of phages and their hosts based on predator–prey type interactions applicable to natural communities. In this model the abundance of the single phage and host species changes periodically, whereas total viral and bacterial abundance in the system remains constant. A relatively constant viral and bacterial abundance over time scales of days to weeks has been shown in systems or seasons when the systems is supposed to be in a kind of steady state [32]. In support of this model, it was found using hybridization and PFGE that individual viral populations varied strongly on a temporal and spatial scale [145,146]. Also, the in situ bacterial populations in a lake as determined by polyclonal antibodies fluctuated in a way [261] that is compatible with prey oscillations in the presence of lytic phages. Again, in another investigation using monoclonal antibodies, it was shown that the natural viroplankton affected the growth of two bacterial populations within the bacterioplankton community in a eutrophic lake [362]. However, it is not possible to assess host dynamics using genetic fingerprints of bacterioplankton, since it is not known how 16S rRNA gene analysis is related to host diversity. In a mesocosm study simulating a phytoplankton bloom, viral genome sizes appeared or vanished at the scale of days [224–226], thus supporting the model of Wommack and co-workers [146]. With respect to soil systems the population dynamics of various phages infecting bacteria has been studied in sugar beet rhizosphere [350,363]. Differences in temporal distribution patterns suggest that phages were adapted to different temporal niches in the environment. The abundance of phage ϕ CP6-4 increased after an increase of host abundance, which is in accordance with predator–prey-type oscillations.

Overall, the population dynamics of phages in situ has been only poorly studied. A major reason for this is the lack of methods to study the interaction between phages and their hosts. Phage-typing [364] might be a solution for this problem. Hennes and Suttle [189] have presented a new application of this approach, which was originally used to identify bacterial isolates. They labeled isolated phages with a dye (fluorescently labeled virus probes) and inoculated them in excess into water samples. The labeled phages adsorbed to the hosts and this allowed to distinguish them from non-host cells [189]. In parallel a conventional plaque assay can be used to quantify phages infecting the host and thus it is possible to study the population dynamics of a specific host. This requires that additional tests are performed to check whether all PFUs are from the same phage. They also performed an experiment to test the effect of indigenous phages on bacterial populations. Adding a host bacterium (*V. natriegens* PWH3a) to microcosms resulted in an increased proliferation of the indigenous

bacteriophages infecting this host, finally causing the collapse of the added host bacterial population. Considering that phages are used for more than 70 years to for bacterial identification [364], it is surprising that phage typing was not used before for studying phage population dynamics and phage–prey interactions, although this is a central topic of viral ecology.

Phage–host interactions were studied with PHSs to test the theory of predator–prey interactions and provide data for mathematical models [37]. However, PHS studies are far from the complexity of nature with respect to the diversity of organisms and interactions taking place. Only with such data can we tackle and understand the population dynamics of infection in the environment. In situ approaches such as phage typing may be a means to accomplish this goal.

The ecological concept of metapopulation dynamics assumes that a metapopulation consists of local aggregates of subpopulations. Exchange of individuals between these subpopulations is reduced, however, significant enough to influence local dynamics. Soil is not homogenous and characterized by many microenvironments. For example, typically aerobic soils can contain anaerobic sites with anaerobic bacteria [39]. Recent evidence shows that also aquatic habitats are more heterogeneous than previously assumed with a variety of particles ranging from colloids of various size to larger visible particles called marine or lake snow [365–368]. The larger particles can also contain anaerobic sites [369,370]. Such a variety of microhabitats may allow for a high prokaryotic diversity and consequently phage diversity and may also sustain a high variety of functions and interactions with hosts. The community structure of attached bacteria differs from that detected in ambient water in freshwater and marine systems, however, there are also phylotypes existing in the form of free-living and attached cells [371–373]. Such ‘hot spots’ of microbial activity [249] may also structure viral populations into smaller aggregates of subpopulations and fulfill the requirements of a metapopulation. However, so far a metapopulation concept has not been applied to viruses but it might prove useful for understanding phage population dynamics [354].

5.3. Evidence and distribution of phage life cycles in the environment

5.3.1. Chronic infection

There is a single report on a potential chronic infection studied in situ at the community level [96]. In an alpine lake, bacteria containing filamentous viruses were detected by TEM. Chronic infection assumes that phages are released from host cells without destroying it. However, there is some doubt based on the size of the filamentous viruses in this system, that the phages are actually released without causing cell death [96].

5.3.2. Lytic infection

Lytic phages have been frequently isolated from marine, limnetic and soil ecosystems [35,37]. In presumably the most comprehensive effort to screen marine bacterial isolates, 65% of the co-isolated phages from the Atlantic Ocean were lytic [210,211]. For *P. aeruginosa*, it has been reported that for ca. 80% of the bacterial strains isolated from a river sediment a virulent phage was found [374].

At the community level, methods are available to detect bacteria in a lytic stage of infection. However, it is not possible to distinguish between lytic, pseudolysogenic or lysogenic infection. FIC ranged from 1.4% to 26.4% in oceanic waters and from not detectable to 43.4% in coastal/shelf waters (Table 4), but average values were similar (13% vs 15%, respectively). Phage-induced mortality of bacteria was 58% in coastal waters and 25% in offshore waters. Inconsistency in the trends between FIC and phage-induced mortality of bacteria might be due to the different methods and data sets used. In the only study from deep marine waters [188], FIC and virus-induced mortality of prokaryotes was lower than average values reported for these parameters in coastal and offshore waters. In limnetic systems, FIC ranged from non-detectable to 45.6% (Table 4). Several studies have shown that FIC can increase with bacterial abundance or production or – more general – with productivity of systems [86,98,110,111,113,123,188,375]. This suggests that the higher bacterial numbers and their higher activity in more productive systems also support higher viral infection frequencies. Fuhrman [21] has argued that higher host abundance could explain this observation. Host abundance consists of total prokaryotic abundance, species diversity and host range, however, we are only able to determine total but not host bacterial abundance. Although data on bacterial diversity based on 16S rRNA gene sequences accumulate, this does not solve the problem because differences in 16S rRNA gene sequences do not yield information on host diversity. Hence, there is not enough data available to explain the relationship between FIC and system productivity.

5.3.3. Pseudolysogeny

Pseudolysogeny is a somewhat enigmatic phage life cycle. It occurs in the domains *Bacteria* and *Archaea*. Moebus has described pseudolysogeny of the marine phages H3/1 and H2 [376–378] and suggested that it might be widespread and significant in situ. Others have come to the same conclusion based on studies with freshwater *P. aeruginosa* phage UT1 and with *Listonella pelagia* phage ϕ HSIC [328–330]. For example, one study found that ca. 45% of phages isolated with *P. aeruginosa* as host were pseudolysogenic [281]. Persistent infections have been investigated in some detail for the archaeal genus *Halobacterium* [178,179,356,379,380].

Some studies with natural viral communities indicate that phage production is due to lytic and not pseudolysogenic (or lysogenic) phages [92,122]. However, there is also evidence from natural communities that is compatible with the idea of pseudolysogeny [330]: (1) An approximate steady state between phages and hosts is often found in the environment and this is a definition of pseudolysogeny. (2) The relationship between phages and bacteria may be the result of a mixture of resistant and sensitive host cells or a mixture of lytic and temperate phages, which also serves as a criterion for pseudolysogeny.

5.3.4. Lysogeny

Ackermann and DuBow [36] reported that 47% of all isolated bacteria were lysogenic. Similar figures were shown in other studies from marine and estuarine bacterial isolates, where ca. 40% were lysogenized [156,381], whereas Moebus and Nattkemper [209] found that only ca. 10% of the isolated marine phages were temperate. The problem of the GPCA makes an inference from PHS studies to the significance of lysogeny (and other viral life cycles) in natural communities difficult.

Among the inducing agents found in natural systems (including such originating from anthropogenic sources) are sunlight, hydrogen peroxide, temperature changes, polyaromatic hydrocarbons, Bunker C fuel oil, trichloroethylene, polychlorinated biphenyls mixtures, Arochlor 1248, pesticide mixtures and sunscreen products [91,126]. Mitomycin C and UVC radiation (254 nm) are the most powerful agents used to induce the lytic cycle in lysogenized bacterial isolates [36]. Data from several studies indicate that mitomycin C is often a better inducing agent for natural bacterial communities than UV-C radiation [88,125,153]. Schrader and co-workers [325] reported that broadband solar radiation is a weak inducing agent. Nevertheless they were able to isolate some lysogens that were inducible. Wilcox and Fuhrman [122] and Jiang and Paul [125] did not find evidence that solar radiation induced lysogens in natural bacterial communities, whereas in another study solar radiation caused prophage induction in 86% of the samples [91]. Induction of prophages by solar radiation was also suggested for freshwater communities [382]. The relationship between lysogeny and temperature is also unclear. Cochrane and Paul [127] conducted a seasonal study on lysogeny in Tampa Bay (Gulf of Mexico, USA) and detected prophage induction only at temperatures >19 °C. In contrast, Weinbauer and Suttle [88,91] found prophage induction within a temperature range of 14 and 29 °C in the Gulf of Mexico, and the percentage of lysogenized bacteria was not correlated with temperature. Moreover, data from the Baltic Sea and from deep waters in the Mediterranean Sea show that lysogeny can be higher than 50% at temperatures <15 °C [188].

Although several studies have been conducted to elucidate the role of lysogeny in natural bacterial communities [88,91,125–127,153], the relationship between lysogeny and the productivity of habitats remains unknown. There are studies indicating that the percentage of lysogens in natural bacterial communities is higher in pristine offshore waters than in more turbid coastal waters [91,125], and the percentage of lysogenized isolates was higher in offshore than in coastal waters [156,381]. Also, evidence for highest occurrence of lysogeny was found in environments with lowest bacterial and primary production [383], and an across-systems-study conducted in estuarine, offshore surface and deep waters suggest that percent lysogeny is negatively related to total prokaryotic abundance [188]. However, a recent study from a Canadian fjord suggests that also in pristine coastal waters lysogeny can be as high as 80% [384], and Jiang and Paul [125] showed that percentage of lysogens was highest in estuarine waters. Overall, lysogeny in natural communities as determined by prophage induction due to mitomycin C ranged from not detectable to almost 100% (Table 7). The finding that environmentally relevant pollutants can be more effective inducing agents than mitomycin C [126] and that mitomycin C does not induce all lysogens [36] indicates that we might underestimate the significance of lysogeny by using this inducing agent. One has also to consider that lysogeny estimates based on mitomycin C induction might be low when factors such as high temperature, salinity, or mutagens had already induced a significant portion of the bacterioplankton community in a sample [127]. There is only one study on lysogeny in freshwater reporting on lysogeny values of 0.1–7.4% [153].

Lysogeny has been long known for freshwater cyanophages [16], whereas it has been documented only comparatively recent for marine cyanophages infecting unicellular (*Phormidium persicinum*) [385] and colonial cyanobacteria (*Trichodesmuim*) [386]. Lysogeny was also detected in marine cyanobacteria at the community level using mitomycin C as inducing agent [384]. McDaniel and co-workers [387] observed that prophage induction in *Synechococcus* was negatively related with cyanophage abundance and exhibited a pronounced seasonal signal with highest values typically detected in late winter. Lysogenic PHSs have been isolated from soil as well [204], however, no data are available on lysogeny at the community level.

5.4. Phage life cycles and life strategies

5.4.1. Pseudolysogeny

Persistent infections are known from studies with the archaeal genus *Halobacterium* thriving in high salinity ponds and brines [178,179,356,379,380]. At high NaCl concentrations, which are conditions favorable for host growth, the archaeal viruses do not, or only at a very

Table 7
Lysogeny in natural prokaryotic communities

Location	Method	Lysogeny (%)	% samples with detectable prophage induction	Remarks	Reference
Marine					
<i>Offshore</i>					
Various	WSA	2.5–7 (4.5)	27	BS = 30	[125]
Gulf of Mexico	VDA	1.5–11.4	100		[91]
Mediterranean Sea	VDA	9.2–21.9 (14.6)	100		[92]
Mediterranean Sea					[188]
Surface	WSA	10.9–13.1 (12.2)	67		
Mesopelagic	WSA	15.5–38.8 (21.2)	80		
Deep	WSA	58.3–84.3 (73.2)	100		
<i>Coastal</i>					
Various	WSA	1.5–3.5 (2.8)	60	BS = 30	[125]
Gulf of Mexico	VRA	0.8–2.2	100		[91]
Gulf of Mexico	VRA	0.1–4.4	100		[88]
Strait of Georgia	WSA	80		BS = 50	[384]
Mediterranean Sea	VDA	5.2–24.1 (11.8)	100		[92]
<i>Estuarine</i>					
Tampa Bay, Florida	WSA	0–37.3 (6.9)	52.2	Seasonal, BS = 30	[127]
Various	WSA	2–38 (13)	80	Where BS = 30	[125]
Baltic Sea					[188]
Oxic	WSA	0.7–18.2 (7.4)	83		
Interface	WSA	4.4–78.5 (29.6)	100		
Anoxic	WSA	1.8–5.2 (3.7)	73		
<i>Others</i>					
Various	WSA	0–121 (16.8)	56	Estuarine to oligotrophic, BS = 30	[126]
Limnetic					
Lake Superior	WSA	0.1–7.4	100	Surface microlayer and subsurface water	[153]

Lysogeny was identified as difference of viral abundance between controls and inducing agent. Lysogeny is given as range (average). Average values were calculated from samples with detectable prophage induction.

reduced rate, produce viral progeny. Such conditions may represent a refuge for the host against mortality due to viral lysis. When salinity drops, as, e.g., as a consequence of rainfall, the persistent phages enter the lytic cycle and reproduce. This can be seen as a strategy to produce progeny when the conditions are still favorable and before the decreased salinity impairs the growth of the host. Phages have a larger tolerance against salinity changes than hosts allowing them to survive at low salinity until new hosts colonize the environment. Wild-types of this phage show a low virulence, which is due to a low adsorption rate [379]. This is considered an adaptation to high salinity ponds where turbulence is high and adsorption to non-target particles may be a significant factor for phage mortality.

It was suggested that pseudolysogeny represents a state of phage–host interaction where the phage cannot enter the lytic or the lysogenic cycle because of nutrient depletion [328,329]. Relieving this starvation condition by adding nutrients forces the phage genome to enter either the lytic cycle or the lysogenic cycle. Data using

natural communities where the growth of the host was stimulated due to the dilution of the host density suggest that enhanced growth did not increase the frequency of lysogenic cells in the total bacterial community or stimulate viral production [88,92,122]. Also, Moebus [256,257] did not find evidence for the hypothesis that mature phages in starving cells are released, when nutrients become available. In a detailed study, Moebus [376–378] reported for the phage H24 that it establishes a lysogenic interaction at low nutrient concentrations, whereas at high growth rates mutations resulted in pseudolysogeny. Thus, different life cycles and strategies seem to be summarized by the term pseudolysogeny, and their role in the environment remains largely unknown.

5.4.2. Lysogeny and lytic infection

Lysogeny is thought to be of advantage when the host abundance, i.e., the food resource, is below that necessary to sustain lytic infection [388–390], or when the destruction rate of free phages is too high to allow for lytic replication [37]. Both explanations assume that the

rate of successful encounters between phage and hosts is too low to sustain lytic phage production. Consequently, lysogeny should predominate at low encounter rates with specific hosts and lytic infection should predominate at high host abundance. In pristine offshore waters, solar radiation penetrates deeper into the water column than in more turbid coastal stations and thus destroys the infectivity of free phages more rapidly [278]. Therefore, a higher percentage of lysogeny in offshore than in coastal waters [91] would be in agreement with the hypothesis of destruction of infectivity. Alternatively, the host abundance hypothesis might hold true. Proctor [31] has suggested that bacteria in oligotrophic environments need to produce as many nutrient receptors as possible to maximize nutrient uptake. Indeed, “considering the central role of carbon/energy sources for both maintenance and growth, it seems logical to presume that, at the low concentrations of carbon compounds found in highly oligotrophic environments, a successful growth strategy for a competitive heterotroph would be to take up as many as possible of the different available carbon/energy sources at the same time” [391]. Since this also increases the chance of viral infection, there might be an evolutionary pressure on bacteria to invite or force lytic phages to become temperate. For bacteria this would not only reduce losses due to lysis, but they could also benefit from phage conversion.

Among the benefits of lysogeny for prophages are that they do not have to find a host, can exploit the repair system of the host (without major delay between damage and repair) and are generally protected in the host cell [38]. Other benefits of lysogeny are that the phage genes can confer immunity to superinfection by homologous phages to the host and increase the general fitness of the host by phage conversion, which in turn should increase the survival rate of the prophage. Lysozymes released during viral lysis of lysogens can attack bacteria sensitive to the lysozymes as well as phages. Also, sweeping of receptors might reduce proliferation of competing phages. Thus, prophage induction and lysozyme release could be a potential weapon for temperate phages and lysogenic bacteria [38]. However, lysogeny means also a metabolic burden, since the host has to direct resources to the replication of the phage DNA and the expression of prophage genes.

The reports and reasoning on the benefits of lysogeny are contradictory. It has been reported that lysogeny is beneficial to lysogens via phage conversion [351–353], that they have lower survival rate [392,393], that they can co-survive with their non-lysogenic counterparts [350], and that their abundance does not differ from nonlysogens [39]. Also, it has been argued that a high host density destabilizes the interaction between a lytic phage and its host [394]. Assuming that this is true, development of lysogeny would stabilize the interaction

and support the survival of the phage line [39]. In this case lysogeny might also be beneficial in high-host density environments.

The patchy distribution of bacteria in soil and unfavorable conditions for free phages such as local drops in pH could favor lysogenic interactions with hosts. However, as phages can bind strongly to clay and phage infectivity can persist for at least several months [38,204], the lytic life strategy might be more important in soil than previously assumed.

The ‘lysogenic decision’ depends on multiplicity of infection (MOI), nutritional status, or growth rate. For example, low phosphate concentrations might trigger lysogenization of *Synechococcus* cells [258]. A link between MOI and nutritional status on lysogenization is likely, because the latter can influence the former [395]. Moreover, a high MOI, high intracellular cyclic AMP concentrations (characterizing starved cells) and elevated concentrations of divalent cations have been shown to stimulate lysogenization in PHSs [281,395–397]. In a model developed for the λ phage [396], the lytic pathway prevails in rapidly growing cells, but the lysogenic pathways is favored in starving cells, i.e., in cells where the host physiology does not allow for fast and high-yield phage production. The starved bacteria surviving are then attacked by many phages produced during the lytic pathway and this high MOI results in lysogenization of cells. Lenski [37] has pointed out that the distinction between temperate and lytic phages is somewhat artificial and that environmental conditions might exist (or even prevail), which favor the lytic pathway in infections with temperate phages. Schrader and co-workers [325] argued that the ability of phages to grow, establish lysogeny or being induced varies strongly in isolated PHSs under environmentally relevant conditions, which may preclude the application of a single model to the environment.

Prophages may enter the lytic cycle when the host is in an ‘unhealthy’ state such as suffering from DNA damage. This mechanism has been described in detail for the λ phage [396]. One of the most curious cases of ‘abandoning the sinking ship’ is that of lysogenic bacteria induced in the food vacuole of ciliates [398]. A portion of the produced phages is egested together with non-digestible cell wall fragments of bacteria. For an amoeba grazing on filamentous cyanobacteria, it was reported that infective cyanophages were released from the food vacuole [399]. Viruses were also found in the food vacuole of radiolarians [158] and amoeba [400], however, it is not clear whether they originate from ingested prey. As discussed above for the λ phage, it is also known that rapid growth and relieving nutrient limitation can cause prophage induction [396]. The strategy behind this behavior might be to produce a lot of progeny as long as the hosts grow well (‘feast’) in order to find new host cells and then enter the lysogenic

pathway to survive a starving phase of bacteria, which might occur, when the resources have been consumed to completion. Although the available data do not suggest that this is a significant process in marine viral communities [28,88,122,381], more communities from different environments have to be investigated before this problem can be settled.

It is not a priori evident, why lysogens should be discriminated against in environments allowing for rapid viral production due to high host abundance and activity. The number of genomes of a prophage population depends on the replication rate of the host resulting in one additional viral genome per cell division, whereas lysis results in multiple genome copies determined by the burst size [38]. This may result in a competitive disadvantage of prophages. Thus, in more productive environments allowing for fast viral proliferation, lytic phages may out-compete lysogenic phages by higher replication rates [188]. Such a situation might be more complex in systems with a strong seasonal change in host abundance, which might allow for a dominance of lysogeny vs. lytic infection in different seasons thus allowing for co-existence of the two types of phages. Data on the seasonal successions between a temperate and lytic infecting the same host would be in accordance with this idea [350].

Analyses of lysogeny and lytic infection at the community level deal (among others) with the question, whether there are environments favoring one of the two life strategies. For a more detailed understanding of the factors controlling lytic and lysogenic infections, we need to know the number of (dominant) lytic and lysogenic PHSs in a system and the abundance and dynamics of the phages and hosts including parameters such as the host range. Also, we need more information on the factors that determine the lysogenic decision and prophage induction and on the competition between lysogenic and lytic phages in the environment. Tackling this question poses a challenge for viral ecologists in the years to come.

5.4.3. Latent period optimization

Controlling the latent period might be a strategy of phages to increase reproduction success [401,402]. For example, an increased latent period allows for the formation of more viral progeny, i.e., a higher burst size. This can be seen as a strategy to maximize resource exploitation. Also a delay in cell lysis might be an ecological strategy of a lytic phage to survive in a non-growing host-population [32]. For example, lysis inhibition results in a delay of the latent period and in an increase of the burst size as well [267]. Progeny might be released when conditions are more favorable for host growth and thus new infection. Vice versa, a short latent period may be a strategy at high host densities to quickly infect new hosts [401]. It has been argued that a short

latent period and consequently a low burst size should prevail in productive, high host density environments and that prophages might be considered as very long latent period phages established at very low host density [401]. Not only host quantity, but also host quality, i.e., the metabolic status of host cells, may play a role [402]. Multiplicity of infection may also cause lysogeny and thus it might be linked to latent time optimization [395]. This model is also in agreement with the idea of Steward and Levin [388–390] that lysogeny is a survival strategy at low host densities. In situ data from rhizosphere systems and characterization of isolated rhizosphere phages in the laboratory showed that the temperate phage ϕ CP6-1 had a long latent period and a high burst size, whereas the lytic phage ϕ CP6-4 showed a short latent period and a low burst size [230,350,363]. Thus, these data on two phages infecting the same host are also in agreement with latent period optimization. If only one of the phages released during cell lysis lives long enough to infect a new cell, the viral lineage will survive [23,26]. Thus, the burst size could be an indication for the survival capacity of free phages, i.e., a large burst size would indicate a low survival rate of the virions.

5.4.4. Life strategies

Attempts have been made to affiliate prokaryotes according to their life strategies such as the oligotrophy/copiotrophy dichotomy, grouping into autochthonous and zymogenous prokaryotes [403,404] or the r/K selection continuum [405]. Although these types of life strategies are not interchangeable, such concepts might be seen in a more general way as an evolutionary trade-off under conditions of resource abundance and scarcity [406,407]. Applying this concept to phages, lytic infection can be seen as adaptation to resource abundance and lysogeny or chronic infection as adaptation to resource scarcity. Assuming that pseudolysogeny is due to lytic or temperate phages, which cannot proceed in their life cycle because of energy limitation in the cell [328,329], this life strategy could be an adaptation to the metabolic status of the host cells. It has also been suggested that pseudolysogeny might be a life strategy allowing phages to quickly react to changes in the environment and that it might be an evolutionary transition step towards lysogeny [32]. Also, it is possible that the life strategy of the host has an influence on the strategy traits of the phages. Other factors such as resistance against damage or repair capacity should also be considered in such concepts and this actually leads beyond the abundance vs. scarcity of resource concept. Data such as infection efficiency, carrying capacity, burst size, intrinsic rate of increase and virion survival are parameters that – if obtained from studies relevant for in situ conditions – may allow for an ecological classification of phages according to their life strategies

and will reveal, whether or not these the life cycles might have evolved (and are still evolving) as ecological strategies.

6. Role of prokaryotic viruses in the environment

6.1. Interactions with food web processes and biogeochemical cycles

The amount of carbon in the oceanic dissolved organic carbon (DOC) pool equals approximately that in atmospheric carbon dioxide [408]. DOC is predominantly consumed by bacteria and bacterial production averages ca. 50% of the primary production. The composition, sources and degradation of DOC has been a long standing focus for research in biogeochemistry and microbial ecology, since DOC dynamics may impact regional and global carbon cycling, which contributes to controlling atmospheric carbon dioxide. Not even 30% of the components of DOC have been identified [409] and the sources of DOC are still not entirely resolved. For example, it is still controversial, whether the majority of DOC originates directly from phytoplankton or from prokaryotic cells. Recent findings indicate that organic matter derived from bacteria could be a more significant source of the DOC pool than previously thought. There is increasing evidence that capsular polysaccharides, cell wall components and membrane derived bacterial proteins (e.g., porins) are important and rather refractory constituents of the marine DOC pool [299,367,409–413]. As viral lysis converts cells into DOM, phages might play a role in the formation of

DOM. The food web structure shows distinct differences between terrestrial and aquatic systems [414]. Since to date virtually all information on the interaction of viral communities with food web processes and biogeochemical cycles has been gathered from aquatic systems, only aquatic viruses are discussed in the following. However, some of the principles may also hold true for soils.

The concept of the aquatic food web and its link to carbon cycling has changed considerably during the last decades. The classical (or grazing) food chain model is the idea that the food web is a chain starting with phytoplankton followed by a series of grazers increasing in size (Fig. 6). The realization of the significance of microbes in the ocean [8,9] has led to a more complex understanding of the food web. Prokaryotes take up DOM, which originates predominantly from primary producers as leachates and which would be otherwise lost from the food web. Prokaryotes are consumed by small grazers such as flagellates and ciliates, which are in turn consumed by larger grazers, and by that organic matter enters again the grazing food chain. This flow of organic carbon from DOM via prokaryotes and small eukaryotes back to the grazing food chain is called ‘microbial loop’ [8,9]. The finding that viruses infect all components of the aquatic food web has resulted in an even more complex concept of aquatic food webs. Primary production is due to eukaryotic phytoplankton and cyanobacteria, and thus algal viruses are discussed briefly to obtain a more complete picture on viral activity in food webs as well as on carbon and nutrient cycling. One of the open questions is to which extent viral lysis of cells contributes to the DOC composition

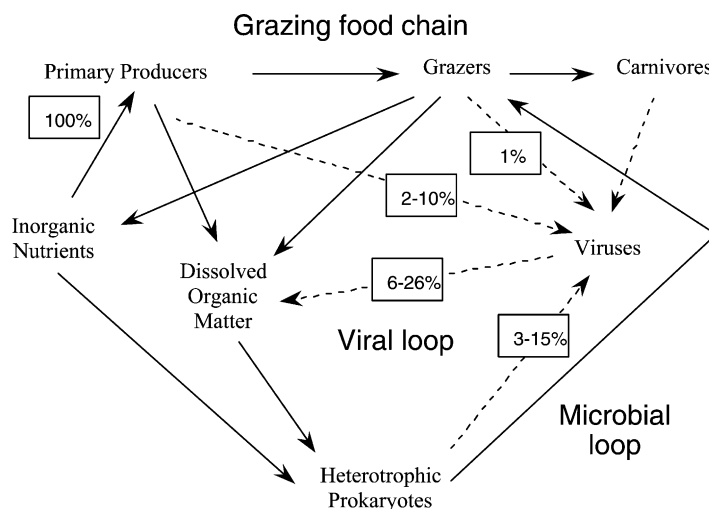


Fig. 6. Pelagic food chain model and virus-mediated carbon flow. The dotted lines point to virus-mediated pathways. The model is adopted from [26] and slightly modified. Values are from [34] on a steady model on the influence of viruses in marine carbon cycles. All values are in terms of the flux of carbon fixed by primary producers (100%). Only data for viruses are shown. The data indicate that between 6% and 26% of the carbon fixed by primary producers enters the DOC pool via virus-induced lysis at different trophic levels.

and turnover. The following two sections deal with the effect of viruses on food web processes and biogeochemical cycles, however, this separation is somewhat artificial as some concepts are discussed in both sections.

6.1.1. Viruses and food web processes

The role of viruses in structuring the food web was assessed in studies, where the abundance of the natural community was manipulated. Enriching the natural community with viruses (viral concentrates) or removing the virus-size fraction was used to experimentally test the effect of viruses on prokaryotic and phytoplankton abundance and production. Manipulating viral abundance caused a change of bacterial abundance and viral infection of bacteria [13,67,97,124,155,415], enzymatic activity [124], bacterial production and respiration [104,416], concentration and composition of DOM and POM [155,201,415], the dynamics of flagellates [155, 417], as well as Chl *a* concentrations, primary production and photosynthesis [14,198,201,418,419]. Typically, the addition of viral concentrates resulted in a decrease of bacterial abundance by ca. 20–40% compared to the control [32], however, a short-term stimulation of bacterial abundance was also documented [374,415]. Overall, these data suggest that viral lysis induces changes in the relative importance of functional groups in the food web.

Phages and heterotrophic nanoflagellates are the two main predators of prokaryotes in aquatic systems. Their role in bacterial cell death has important consequences for the food web (as discussed below in more detail). Viral infection of bacteria is on average as significant as grazing for bacterial mortality [102], however, the relative importance may vary with space and time and with environmental characteristics [87,89,93,101,102,111,121, 123,261,375,420]. Models suggest that bacterial abundance and diversity should be controlled by predators such as phages in high-productivity systems and by competition in low-productivity systems [421]. This is in agreement with the hypothesis that viral mortality of bacteria increases with host abundance and system productivity. Estimates of virus-induced mortality of bacteria from anoxic environments are among the highest reported in the literature [89]. This is probably due to the fact that anoxic grazers are not abundant and their grazing rates are low [422]. Also, in a solar saltern, viral lysis rates of bacteria were highest when grazers were not present due to the high salt concentrations [101]. Such environments may still somewhat resemble a prokaryotic world before the evolutionary appearance of eukaryotic grazers, when phages were the main predators of bacteria. Guixa-Boixereu et al. [375] suggested that low water temperatures such as those prevailing in Antarctic marine waters might reduce HNF activity and thus favor viral infection of bacterioplankton. In general, viral infection is a source of mortality and unfulfilled carrying capacity of host populations.

FIC and lysis determined at the community level might not only depend on the productivity of a system, but could be a sequence of catastrophic events such as prophage induction or loosing a refuge, which may result in a chaotic distribution of lysis events and a non-predictive influence on bacterial mortality. Diurnal variations of FIC have been documented [99] and synchronized cell lysis was assumed to explain dramatic fluctuations of total viral abundance at the time scale of minutes to hours [119]. Such findings may blur potential relationships between FIC and environmental characteristics and indicate that the effects of viral lysis on food web processes and biogeochemical cycles might vary strongly and in an unpredictable way in some systems.

One of the implications derived from Fig. 6 is that viral lysis of cells at all trophic levels converts biomass into DOM and thus elements such as C, N, P, S and Fe should remain longer in the euphotic zone (reviewed in [23,26,27,32,34]). Biomass and production is lost from the grazing food chain and becomes available to prokaryotes, thus representing a ‘short circuit’ or semi-closed trophic loop [13,25,26,83,106]. Consequently, viral infection should enhance prokaryotic respiration and production and by that the transformation of organic matter and regeneration of nutrients. At the same time, viruses should reduce the production and respiration of protists and larger zooplankton, since prokaryotic cells which are lysed cannot be taken up by protists. There is some support for this at the bacterioplankton level from modeling and from experimental studies [26,124,423]. Fuhrman [25] presented a model in which he predicted the role of viruses on abundances and processes in the food web in a (theoretical) system with no viral infection of prokaryotes and a system, where bacterial mortality was equally distributed between phages and protists. In the presence of phages, bacterial respiration and production were elevated by 27%, whereas bacterial grazing by protist and zooplankton production were reduced by 37% and 7%, respectively. Later this model was expanded by adding 7% virus-induced loss of phytoplankton production and 3% loss of viral production due grazing of protists on viruses [26]. The net effect was essentially the same. Modeling the effect of viral lysis of bacterioplankton on mesozooplankton productivity, Murray and Eldridge [424] showed that this effect was stronger in an oligotrophic than in a mesotrophic situation indicating that the strongest influence of phages will be found in systems, where prokaryote-mediated recycling of DOM predominates. The finding that average phage-induced mortality of prokaryotes is 25% in oceanic and 58% in coastal waters suggests that the strongest impact of viral lysis on bacterioplankton should occur in coastal systems. Protists consume viruses, and this represents an alternative pathway of carbon transfer, although the transfer rates are low [107,166,286].

6.1.2. Viruses and biogeochemical cycles

Viruses represent a significant biotic carbon pool in the ocean [34], which represents 5% of the carbon of bacteria and is 25-fold higher than the carbon pool of protists [425]. Due to the high protein and DNA content, viruses may also contribute significantly to the biotic nitrogen and phosphorus pool. For example, during one lytic cycle, a phage requires an amount of DNA, which roughly equals a bacterial genome [426]. However, due to their small size, viruses are more important contributors to elemental cycles than to elemental pools. It has been estimated that between 6% and 26% of the organic carbon produced by photosynthesis finally ends up in the DOC pool due to viral lysis of cells (viral shunt) (Fig. 7) [34]. Thus, viral lysis products might be a significant source of DOC. A detailed analysis of the chemical nature, molecular size and bioreactivity of viral lysis products of bacteria is not available for natural communities. Average C:N:P ratios of lysis products and cells should be roughly similar and only affected by

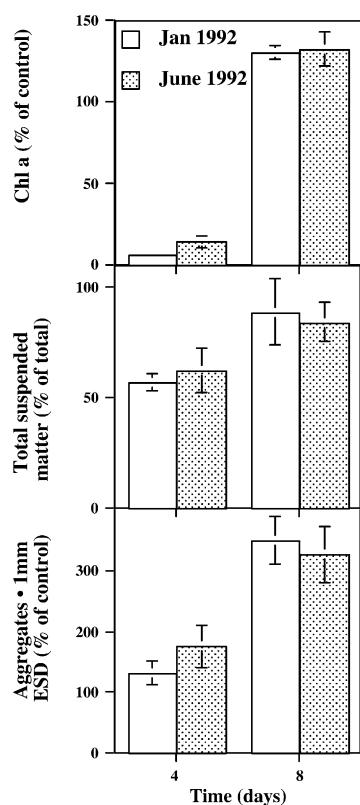


Fig. 7. Effect of viral concentrates on particle size, suspended organic matter and Chlorophyll *a* concentrations. Water samples were collected in the Northern Adriatic Sea. Water samples were filled with and without adding viral concentrates into 10-liter plastic tanks and placed on a rolling table and kept under artificial light to initiate a phytoplankton bloom. The experimental approach was similar to that described in [201]. Data are expressed as percentage compared to the control treatment, i.e., a value of more than 100% indicates a stimulation due to viruses. Data and error bars are means and ranges, respectively, of duplicate incubations.

the compositional changes occurring during infection of a cell. Cell content should be rich in N and P (e.g., proteins and nucleic acids) resulting probably in low C:N and C:P ratios of the labile material of the cell content, whereas carbon is preferentially bound in cell wall structures [34,124]. Thus, phage-induced transfer of cell wall fragments into the dissolved fraction may be significant for the formation of recalcitrant DOC. Studies with PHSs suggested that release of phage progeny results rather in a disintegration of cells than in the formation of ghost cells [427]. This could increase the accessibility of lysis products to bacteria.

Since bacteria need additional carbon for respiration, uptake of viral lysis products of cells (particularly the cell content) by prokaryotes should result in a net release of inorganic nitrogen and phosphorus [34]. Viral lysis products including phages turn over relatively rapidly, especially in oligotrophic, P-limited environments [124]. This supports the view that viral lysis is a significant pathway for phosphate recycling [423,428, 429]. Thus, the recycling of DOM by the viral shunt may even sustain the availability of inorganic nutrients and by that primary production. Lysis products are available to bacteria at the expense of a reduced growth efficiency [104,423] and could at times be the dominant source for meeting bacterial carbon demand [191]. This should enhance prokaryote-mediated CO₂ production. The release of dissolved organic nutrients due to cells lysis has also been documented for phytoplankton species [262,430,431]. The DOM released during lysis may stimulate the non-infected bacterial populations [25,250,423]. However, as infected bacteria also take up organic matter (see above), they might also use viral lysis products to synthesize new viral progeny. Moreover, viral lysis removes competitors for resources, which should increase the production of non-infected populations.

In addition to the grazing food chain, the microbial loop and the viral shunt, the sinking of organic aggregates (marine or lake snow) influences productivity and organic carbon flux. Sinking organic aggregates are a means to remove organic carbon from the euphotic layer by transporting it into the interior of the ocean (biological pump) [249]. Sinking organic aggregates have different origins such as phytoplankton or zooplankton. Marine and lake snow is colonized by bacteria, which differ in their community composition from bacteria from the ambient water [371–373]. Bacteria attached to aggregates produce more extracellular enzymes than their free-living counterparts and this results in the disintegration and dissolution of aggregates [432,433]. However, bacteria can also be involved in the formation of organic aggregates [434]. Overall, prokaryotes play a pivotal role in the formation, transformation and degradation of marine and lake snow and thus aggregate-mediated carbon flow (for a recent review, see [206]).

Processes such as viral infection, which influence bacterial (and phytoplankton) activity, may also affect aggregate formation and degradation and thus the biological pump. Various potential pathways of how phages might influence carbon flow of organic aggregates have been suggested [307]. For example, release of 'sticky' lysis products of bacteria or phytoplankton may result in an increased rate of aggregate formation, whereas the enzymes released during cell lysis might contribute to aggregate degradation [307]. Experimental support comes from the finding that phage lysis product can coagulate to form larger colloids [435]. Also, adding virus concentrates to seawater samples during the formation of a phytoplankton bloom under laboratory conditions increased the size and stability of algal-derived organic aggregates maybe due to sticky lysis products [201]. Two further experiments were performed with water from the same environment, the northern Adriatic Sea, but were collected at different times of the year. The data corroborate the results of the previous study (Fig. 7) that algal flocs ≥ 1 mm became up to ca. 3-fold more abundant when virus concentrates were added. Although viruses themselves do not affect light scattering [436], the changed size distribution of particles due to viral activity could affect the light climate. Depending on the type of particles such as derived from phytoplankton or zooplankton, a changed particle size distribution may either increase the retention time of particulate organic matter in the euphotic zone or increase sinking rates and by that affect the biological pump [26]. Thin sectioning and TEM inspection of marine snow particles suggested that a significant amount of bacteria is infected [307] and that viral abundance can be high [155]. Methodological progress is needed to study the role of viruses within aggregates. For example, laser scanning microscopy seems to allow for precise and relatively rapid quantifying viral abundance on organic aggregates.

Other abundant types of particles such as colloids, submicrometer particles and transparent polymer particles have been detected leading to the concept of the organic matter continuum [365–368]. Bacteria can be concentrated in such activity hot spots including the plume of a sinking particle, which results in a patchiness of the distribution of bacteria [249,437]. Such a concentration of host cells should also result in enhanced encounter rates with phages and thus infection of bacteria and an interference with processes producing or dissolving this kind of particles. Recently, it has been suggested that leaking cells due to viral infection might attract motile bacteria [427], which might shorten the residence time of lysis products and stimulate prokaryote-mediated biogeochemical processes. While this has to be proven yet, it is also possible that an optimization of DOM leakage by phages is a strategy to attract potential host cells.

Almost nothing is known on the potential effects of other phage life strategies such as lysogeny on biogeochemical processes. Mass lysis of prokaryotes due to prophage induction in lysogens and release of lysis products might locally result in increases of utilizable DOM sources. Lysogeny might affect the ecological performance and species composition of bacterioplankton (see below, Section 6.2). If this holds true, prokaryotic functions such as DOM oxidation might be also influenced.

Photosynthesis in aquatic systems is not only due to eukaryotic cells. Cyanobacteria can contribute significantly to primary production, and are in many, especially oligotrophic environments such as the open ocean, responsible for 20–80% of the carbon fixation [438,439]. Viral lysis can affect the carbon fixation rates of eukaryotic and prokaryotic primary producers [14,418], suggesting a significant effect of viruses on primary production mediated carbon cycling. Recently, a method has been presented to estimate virus-induced mortality in phytoplankton [440]. This will increase our knowledge on the effect of viruses on phytoplankton growth and allow for comparison of phage effects on prokaryotes. Lysed phytoplankton production is lost to grazers, but as DOM consumption of these products by bacteria should result in a remineralization or inorganic nutrients, lysis might stimulate also phytoplankton growth in situ. Viral lysis has also been implied as mechanism producing dimethylsulfoniopropionate from phytoplankton [441,442], which is, when converted by bacterial activity into dimethylsulfide, a significant greenhouse gas. In conclusion, "... viral activity has the potential effect of helping to maintain higher levels of biomass and productivity in the systems as a whole" [21]. Thus, phages seem to be 'beneficial' for prokaryote (and maybe also for phytoplankton) mediated processes at the community level.

6.1.3. Phage-induced mortality without viral proliferation

There is a variety of ways, how phages can cause mortality of prokaryotes and influence the cycling of matter and energy without viral reproduction. For example, phage ghosts, i.e., phages devoid of DNA, can attach to cells and lyse them, inhibit host metabolism including the synthesis of macromolecules or affect the function of the cell membrane as barrier resulting in a release of small molecules such as ATP [443,444]. Such ghosts can be frequently observed in TEM studies of isolates [443] as well as viral communities [67]. A significant fraction (13–48%) of viruses in the natural viral community might be devoid of DNA and thus represent ghosts [67]. As discussed above, abortive infections as antiphage defense mechanism may cause cell death without phage proliferation. Some bacteriocins are of viral origin and can resemble for instance a complete phage or a phage tail [150]. They are used by *Bacteria*

and *Archaea* for their warfare with other microorganisms [445,446] and some bacteriocins are thought to represent an interaction between phages and a host with an evolutionary outcome beneficial for the host population. Bacteriocins can kill cells that could produce phages and thus reduce the phage attack, e.g., for the population of bacteriocins producing cells [446]. Phage tails and empty heads were found in natural viral communities and were also documented in lysed marine bacterial isolates [152,156,381].

At the end of an infection of a cell by a phage from the order *Caudovirales*, a lytic enzyme system is produced and these lysozymes are set free during cell lysis [36]. This might pose a threat to competing bacteria. It has been reported that phage-borne enzymes, which are liberated from a marine *Vibrio* host during viral lysis are bacteriolytic against a variety of gram-negative and gram-positive bacterial strains [447]. It has also been argued that lysozyme liberation might be a weapon against competing phages by sweeping receptors on host cells [38]. It remains unknown, whether or not such enzymes play a role in the transformation of DOM.

Moebus [210,211] reported that 35% of the isolated phage–host systems from the North Atlantic showed an inhibition reaction, i.e., a phage lysate inhibited the growth of bacteria without phage proliferation. Inhibition reactions were also reported in another study for *V. cholerae* [448]. From such data, it is not clear whether it was phages, bacteriocins or other bioactive material that killed or inhibited bacteria, although viral ghosts show similar activity [443,444]. However, it indicates that such agents could be an important phage-mediated factor for the mortality of prokaryotes. Finally, lysis-from-without can be caused by several phages attaching to a host thereby damaging the permeability barrier of the cell. These mechanisms are poorly studied and not well understood in marine, limnetic and soil environments. Such factors of bacterial mortality linked to phages [449] may explain why estimates of phage-induced and grazing-induced mortality cannot always explain total mortality of bacterioplankton [89,102,111]. Also, they remove potential host cells, which should result in a reduced phage proliferation.

6.2. Interactions of viruses with horizontal gene transfer and prokaryotic diversity

6.2.1. Horizontal gene transfer

The three types of gene transfer known for prokaryotes are conjugation, transformation and transduction. Transduction is the phage-mediated gene transfer between a donor and a recipient host cell followed by phenotypic expression of transferred genetic traits in the progeny of the recipient. Transduction occurs in two entirely different types [36]. During generalized transduction, host genetic material can be packed mistakenly

from the donor into the capsids of temperate or virulent phages and transferred to a recipient host by infection. During specialized transduction a host sequence is excised along with the prophage and transferred to a host. The donor DNA can be incorporated into the host genome or persist as plasmid. By conjugation, gene transfer occurs directly between two host cells and by transformation, uptake of dissolved DNA cause gene transfer.

Transduction rates have not been often determined in natural environments and were mainly investigated as tools for studying bacterial genetics or for the risk assessment of the spread of genes from genetically engineered microorganisms. Most studies from lake water were performed by Miller and co-workers [281, 284,450,451] by using microcosms and *P. aeruginosa* as system. However, transduction was also studied in a number of microcosms obtained from soils and the sea [452–455]. A factor restricting transduction is the typical low host range, allowing the spread of a gene only in a limited population. However, recent data suggest that transduction might be more common and cross the host range barriers at the genus level, i.e., from several non-related marine isolates to the enteric bacterium *E. coli* and *B. subtilis* [339,456]. The transduction rates observed in these studies were 4–7 orders of magnitude higher than those reported from freshwater environments [339]. Since phage-encapsulated DNA is protected against degradation and phages may survive for years in special environments without loss of biological activity, gene transfer by generalized transduction might well be more important than previously thought [457]. Four gene transfer agents resembling a ds DNA tailed phage without the ability for lytic activity have been detected for *Bacteria*, *Archaea* and *Eukarya* [458]. Gene transfer agents resemble ‘constitutive’ generalized transducing phages, but their transducing frequencies seem high. Thus, gene transfer agents may represent a highly efficient, but largely unstudied mechanism of gene transfer.

Transduction is influenced by several factors. For example, transduction seems to occur predominantly when the donor is non-lysogenic and the recipient a lysogen [450,451]. Another factor influencing transduction is the MOI [454,459]. It has been shown that suspended matter increases transduction frequencies [284,285] potentially by bringing hosts and bacteriophages more closely together. Marine and lake snow could act in a similar way. Any other activity hot spot, such as the plume of a sinking particle or a decaying phytoplankton cell, may result in a non-homogeneous distribution of bacteria [249,437] and a transient local accumulation of hosts could increase transduction events. Recently, Sander and Schmieger [460] reported on a host-independent method to detect generalized transducing phages. This approach is based on the detection of 16S

rDNA in genomes of phages concentrated from the environment and this may be the long asked for method to efficiently study transduction in the environment and its ecological relevance for short-term adaptation and population dynamics. Also the influence of viruses on the spread of potentially ‘harmful’ genes such as those responsible for antibiotic resistance could be studied with this technique.

Phage genes may alter the metabolic properties of hosts and this also opens a potential for gene transfer. A spectacular case for such a ‘phage conversion’ is the finding that cholera infection is due to a lysogenic strain of the *V. cholerae* bacterium [461]. Since the toxin is encoded in the genome of the prophage and is not part of the host genome, non-lysogenic cells do not cause cholera. Strictly, every lysogenization can be considered as phage conversion, since two properties, lysogeny and superinfection immunity are always conferred [36]. The term phage conversion is usually applied for other effects on hosts such as on changes in morphology, outer membrane proteins, toxin production, metabolic properties, antibiotic resistance or R–M systems. These effects may become ‘hereditary’ once the prophage loses its ability for entering the lytic cycle. The potential significance of gene transfer mediated by phages is also underlined by the finding that toxin production of bacteria can be associated with lysogeny [462].

As host genetic material is released into the free DNA pool in ecosystems during viral lysis of cells, transformation rates, i.e., the uptake of ‘free’ genetic material by prokaryotes, might also be stimulated by phages. Free DNA has been found in pelagic limnetic and marine habitats and in sediments and soils [463,464]. Dissolved DNA (usually defined as DNA passing a $<0.2\text{-}\mu\text{m}$ pore-size filter) occurs in two forms, as truly soluble and bound to colloids in the virus-size range. In marine systems, Jiang and Paul [465] estimated that ca. 50% of the dissolved DNA is bound and 17–30% can be accounted for by viruses. The percentage of bound or viral dissolved DNA can vary strongly with systems and on a temporal scale [66,86,181,185,465–471]. For a range of studied lakes it was shown that the percentage of soluble DNA increases with productivity [470].

Some studies have tried to estimate the release of host DNA during viral lysis of bacteria and algae into the environment [33,86,185,307,472,473], suggesting that viral lysis might be a significant source of dissolved DNA. Most of these studies have assumed that the entire host DNA is set free as non-viral dissolved DNA. However, it has been shown that a significant fraction of the host DNA can be degraded and synthesized into viral DNA [251]. Thus, we have no good data on the release rates of dissolved DNA from host lysis and on the significance of virus-mediated dissolved DNA release for gene exchange. Moreover, viral lysis may result in the release of a conjugative plasmid that may trans-

form a bacterium and then be transferred by conjugation with transducing potentially able to carry this conjugative plasmid [452]. Consequently, the three modes of gene transfer “. . . may diffuse into grey areas of overlap when describing transfer in natural environments. . .” [452]. Overall, the extracellular DNA can be seen as a reservoir of genetically encoded information, and viral DNA and host DNA released during viral lysis contributes to this pool.

Unfortunately, there is almost no data on phage-mediated gene transfer in the environment, since it is extremely difficult to measure such rates in ecosystems. In the only report trying to estimate gene transfer for a natural marine bacterial community, it was predicted by using a numerical model that up to 1.3×10^{14} transduction events per year could occur in Tampa Bay Estuary [454]. In a study, quantifying lytic infection and lysogeny in natural bacterial communities in surface and deep waters, it was shown that on average 35% of the bacteria contain a functional viral genome [188]. Using average values for total viral infection of 35% in the world ocean and estimates of total prokaryote abundance [474], 4×10^{28} cells in the world ocean would carry a functional viral genome. The large number of infected prokaryotes in the sea as well as the estimated production of 9.3×10^{29} prokaryotic cells per year [474] suggests a high potential of gene transfer mediated by phages. A similar argument has been used by to stress the potential of phage-mediated horizontal gene transfer [26]. Moreover, whole genome sequence data of prokaryotic species have revealed the presence of viral genomes or their fragments in sequences genomes indicating that lateral gene transfer is an important factor for bacterial evolution (e.g. [475,476]). Finally, transduction contributes to the generation of genetic variety in prokaryotic populations [477], which is not only a prerequisite of evolutionary change but also influences the population dynamics of hosts. Transduction can also increase the fitness of recipients and thus allow for their survival [478].

6.2.2. Prokaryotic diversity

Many studies have speculated that viral infection could influence the diversity of bacterio- and phytoplankton [32]. One of the underlying concepts is that phages control competitive dominants once they get abundant [23] and they may even sustain species diversity by ‘killing the winner’ and allowing the losing competitors to co-exist [479,480]. This concept may also help to explain Hutchinson’s paradox of the plankton [481], i.e., the question why is there so many (phyto)plankton species in the presence of only a few limiting nutrients when competition theory predicts only a few species. However, it has also been suggested that Hutchinson’s paradox does not hold true for bacteria, e.g., due to the variety of substrates in the DOM pool as

source of carbon and nutrients [482]. Instead, the question has to be asked “Why is there so few dominating species when simple steady-state models can predict so many?” [480].

The ‘killing the winner’ concept is appealing, however, the experimental data are still sparse. Phage and host populations may oscillate in a more-or less predictable way [204], thus representing a kind of dynamic equilibrium [281]. Such oscillations are in accordance with the concept of ‘killing the winner’ and have been discussed in Section 5.2. For example, the termination of an artificially created bloom of *V. natriegens* PWH3A by the natural viral community was documented [189]. Wommack and Colwell [32] pointed out that induction of lysogenic populations as, e.g., due to sudden changes in the growth rate and subsequent cell lysis may be related to bloom formation and termination of single bacterial populations. It has been reported that bacterial morphotypes show different infection frequencies [85] and that upon addition of viral concentrates only rod shaped bacteria were significantly affected [415], thus suggesting an effect of phages on bacterial diversity. In an attempt to assess the effect of phages on bacterial population dynamics and species richness, a concentrate of the bacterial community was added to virus-free (<100 kDa) and virus-rich seawater (<0.2 mm) [483]. The presence of viruses resulted in a changed community composition as detected by SSCP of the 16S rRNA, thus indicating an effect of viral lysis on species composition. Blooms of bacterial populations and their decay are predicted by the concept of ‘killing the winner’ and their occurrence has been reported in marine and freshwater environments (e.g. [52,187,362,484,485]), however, no attempt was made in these studies to relate the bloom termination to viral lysis.

Suttle [418] reported that the addition of virus concentrates changed the community composition of marine primary producers including cyanobacteria. In another publication the same author [16] cites several studies, suggesting that cyanophages can control cyanobacterial blooms in freshwater. A mass lysis event of cyanobacteria caused a change in the composition of bacteria [486] and these data support the hypothesis that viral lysis indeed influences diversity. The data also allow for an additional or even alternative interpretation how viral lysis may affect the community composition. Release of organic matter due to cell lysis may provide niches for specific species [250] and by that induce changes in species composition.

However, it has also been argued that viral lysis is not a significant factor for controlling the abundance and species diversity of hosts but instead the clonal diversity of heterotrophic bacteria and cyanobacteria is affected [171,346]. Such a diversification of host genomes due to bacteriophages has also been implied for *E. coli* [242]. Thus, one of the ecological roles of phages might be to

modify specific genetic properties of bacteria by exerting a selection pressure for phage resistance [250,346,394,487,488]. Overall, viral lysis may not only be beneficial for bacterial community growth, but also for sustaining prokaryotic species diversity and biodiversity in general.

6.2.3. Phage genomics and ecology

In the Age of Genomics, viruses might be a particularly attractive target due to their small genomes, which allows for relatively rapid and inexpensive assessments of whole genome sequences. Whole genome sequence data have increased our understanding of the modular theory of phage evolution and the concept of mosaicism [44,48,361,489–492]. Genomics has also helped to formulate the moron-accretion hypothesis for the origins of phages [361], however, genomics is here considered for its use to unravel the ecology of phages.

Up to now, more than 100 full genomes of phages have been sequenced [41]. A metagenomics approach of viral communities has, for instance, revealed an astounding diversity of viral types [176]. Paul has recently reviewed marine phage genomics. The data provided by genomics, which are meaningful for the ecology of phages, are discussed here following the presentation of Paul [426]. In summary, phage genomics can be used to classify phages, study the evolution and biogeography of phages and predict some dimensions of the ecological niche of phages. Phage genomics has resulted in a novel way to classify phages [41]. Genomics also enables to determine the evolutionary relationship between phages and help answering questions such as whether or not the oldest phages can be found in the ocean. For example, sequence similarities of the capsid protein gp23 of the marine cyanophage S-PM2 with several marine vibriophages as well as the T4 phage suggest that phages in the marine environment have access to a common gene pool [493]. Sequence information derived from genomes also allows for developing primers of specific phages. This may allow for studying the biogeography and ecology of phages as discussed in this review. Finally, genomics can provide us with clues on some dimensions of the ecological niche of phages. For example, analysis of phage genes indicates that the marine phages seem to have developed mechanisms to deal with phosphorus limitation [243,264], which seems crucial when considering that 50% of the weight of phages consists of DNA. As the small size of phage genes allows for rapid whole genome sequencing, they are relatively easy to understand. This could guide our understanding of the interactions of organisms and the environment as “...a reductionist’s view of phage biology led to significant advances in the field of molecular biology...” and thus “...it is reasonable to expect that a reductionist’s view will prove invaluable in our understanding of complex natural microbial systems.” [426].

7. Concluding remarks

The theory of the dynamics of infectious diseases is the oldest branch of biomathematics and one of the most sophisticated ecological sciences [494]. Plant, medical and veterinary parasitologists have studied parasites relevant for mankind; ecologists basically ignored them. Only recently have researchers started to investigate the ecological consequences of parasitism [494], and viral research experiences a bloom in the field of microbial ecology, since accumulating evidence shows that phages are crucial members in microbial food webs. As phages and phage–host systems turnover rapidly, they may also serve as experimental systems to study evolutionary aspects and test ecological theories. Such research may finally change the perception of viruses as nasty parasites, but one will perhaps consider them as catalysts of information transfer and sustainers of bacteria mediated cycling of energy and matter. Thus, viruses may turn out to be rather benign than malign for ecosystem functioning.

There is a considerable interest in the links between ecosystem stability, functioning and diversity (e.g. [495,496]). As shown in this review, phages have a strong influence on the performance of microbial food webs, diversity and biogeochemical cycles, although many questions are still without answer. For example, it has yet to be shown, whether viruses have a stabilizing or destabilizing effect on ecosystems or geochemical cycles. Assuming that an increased complexity of a network has stabilizing effects by allowing for alternative routes, viruses should result in the former. The methodological progress that has been made in the last 1–2 decades and the technological progress that can be anticipated in the years to come, puts us at the verge to study the influence of viruses on the links between ecosystem stability, functioning and diversity. A refinement of the methods for estimating phage-induced mortality of prokaryotes and studying more of the relevant ecosystems would be a prerequisite.

Another ‘beneficial’ role of viruses could be their use in the biological control of harmful or nuisance blooms of algae and cyanobacteria, introduced species or bacteria with harmful activity. Phage therapy, now discussed again as an alternative to antibiotics, could be expanded to the environment and used in conservation biology. However, before such viral therapies can be applied to ecosystems, the ecology of these viruses and their implications for food web processes have to be studied profoundly, in order to avoid unwanted side effects.

Genomics is now applied in microbial ecology and functional genomics and proteomics are on the verge. This will shed light on the ecology of viruses. In the near future, we will probably have methodologies at hand such as biochips and nucleic acid arrays, which will al-

low for a comparatively rapid deciphering of phage genomes and proteoms from natural communities. The information on the phage genome and proteom will provide an invaluable resource to develop theories on viral ecology and evolution. However, the ultimate ecological test will have to be performed in the environment.

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Glossary

Autotrophs: Here used for organisms synthesizing organic matter from carbon dioxide and oxygen by photosynthesis (photoautotrophs).

Cyanobacteria: Photoautotrophic bacteria.

Decomposer: Organisms decomposing organic matter (heterotrophic bacteria).

Deep chlorophyll maximum: Layer close to the pycnocline with higher Chlorophyll concentrations than in the water layer above or below. Often due to accumulation of sinking phytoplankton and thus often characterized by higher biological activity.

Dissolved organic matter (DOM): Fraction of seawater smaller than bacteria. Typically operationally defined as material passing filters with pore-sizes from 0.2 to 0.45 μm .

Diversity: Diversity of species. Described by species richness (number of species), species evenness (significance, e.g., abundance of species) and species difference (taxonomic relatedness of species).

DNA repair: Restoration of normal base sequence and structure of damaged DNA. Repair only takes place within cells, but viruses can carry their own repair system, which is activated after infection.

Ecological niche: Comprises all environmental *conditions* and *resources* (niche dimensions), which determine the survival of a population.

Gene transfer: Exchange of genetic material between cells by conjugation (through physical link between cells), transformation (uptake of soluble DNA) or transduction (virus mediated gene transfer).

Grazer: Here used for protists feeding on prokaryotes and other protists.

Heterotrophs: Organisms consuming already assimilated organic matter (grazers and heterotrophic bacteria).

Lysogeny, lysogenic phage: Type of phage infection, where the phage genome stays within the host cell (either as plasmid or integrated

into the host genome) in a dormant stage (prophage). Lysogenic phages can enter the lytic cycle, produce phage particles and lyse the host cell by prophage induction.

Lytic phage: Virus, which is not able to establish a lysogenic infection and always kills infected cells.

Microorganisms: Non-taxonomic term used for microscopic organisms. Here used for viruses, prokaryotes, protists and single-celled algae.

Mineralizer: Organisms converting organic matter to inorganic nutrients (heterotrophic bacteria and heterotrophic protists).

Phage: Here used for viruses infecting the domains *Bacteria* and *Archaea*.

Phage ecology: Science of the relationship of phages with other (cellular and acellular) organisms and the environment.

Plankton: Drifting organisms. Divided into virioplankton, bacterioplankton, phytoplankton and zooplankton, respectively, for viruses, heterotrophic prokaryotes, photoautotrophic prokaryotes and eukaryotes, and eukaryotic grazers, respectively.

Primary producers: Here used for organisms assimilating organic carbon from inorganic material (photoautotrophs; primary producers).

Prokaryotes: The domains *Bacteria* and *Archaea*.

Protists: Small, single-celled (photo- and or heterotrophic) eukaryotes, which might not form monophyletic group.

Viruses: Pieces of nucleic acid (DNA or RNA) surrounded in their extracellular form by a protein coat. Viruses are typically not considered alive, since they use the metabolism of hosts for reproduction. Bacteriophages, viruses replicating in a bacterial host cell. Cyanophages, viruses replicating in a cyanobacterial host cell. Algal viruses, viruses replicating in an algal host cell (eukaryotic photoautotrophs).