

Viruses in aquatic ecosystems: important advancements of the last 20 years and prospects for the future in the field of microbial oceanography and limnology

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(Received 16 December 2009; final version received 12 February 2010)

Over the last two decades, viruses in aquatic systems have been observed to modify, influence and control aquatic systems. Since the determination decades ago that viruses were abundant in aquatic ecosystems, researchers have demonstrated that viruses are pervasive and dynamic across the expanse and depth of all aquatic systems as well as at the water-sediment interface. There have been a wide range of methodological advancements during this time. To date, aquatic viruses have been suggested to play vital roles in global and small-scale biogeochemical cycling, community structure, algal bloom termination, gene transfer, and evolution of aquatic organisms. Even in harsh and difficult to study environments, aquatic and benthic viruses have been demonstrated to be major players in carbon cycling and recycling of nutrients from organic material. Taxonomic and metagenomic research has shown us that there are major globally-distributed groups, but that their genomes are filled with sequence information that has no similarity to sequences in existing bioinformatic databases. And while the field of viral ecology has expanded exponentially since the late 1980s, there is much that we do not yet understand about virus-mediated processes in aquatic systems. Important near-term steps include the combination of advanced metagenomic techniques with studies of function and population control, standardization of methodological approaches to facilitate global data acquisition without concern over methods-based artefacts, understanding of viral life strategies and their triggers, and the role of viruses in the transformation of organic matter. The purpose of this manuscript is to bring the reader a review of the recent advances in aquatic viral ecology in light of new areas of research, applications of viral ecology to real-world problems, and refinement of models of viral interactions on a range of scales.

Keywords: aquatic; viruses; bacteria; phytoplankton; abundances; mortality; diversity; models; biogeochemistry; phage therapy

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Introduction

Viruses are pervasive components of aquatic ecosystems, ranging typically from 10^4 to 10^8 ml^{-1} with an average of 10^7 virus-like particles ml^{-1} , and are generally an order of magnitude more abundant than bacteria [1]. An estimated 10^{31} virus-like particles has been proposed to exist at a given time in aquatic ecosystems, mainly composed of phages (i.e. viruses that infect prokaryotes), and on average more than ca. 10^{28} viruses are estimated to be produced daily [2].

As recently stated by Middelboe et al. [3], most studies of viruses have been carried out in marine ecosystems (Figure 1). However there have been increases in the level of effort to examine the roles of viruses in freshwater systems [4] and also to compare both systems [4,5]. Other research has been conducted in groundwater, high salinity ponds, hyperthermal vents and other acidic geothermal environments characterized by low pH and/or high temperature. Across these extreme environments, virus-like particles have revealed a diversity of complex morphotypes and functions but will not be considered further in this review. For the reader interested in such (extreme) systems, we encourage the reading of Le Romancer et al. [6], Porter et al. [7], Prangishvili and co-workers [8,9], Rachel et al. [10], and Rice et al. [11] to cite only a few.

The importance of viruses in aquatic systems has been suggested for decades (Table 1). However, it was not until the late 1980s that interest in viral ecology was revitalized when observations using transmission electronic microscopy suggested that viruses were much more abundant than previously thought. Three early articles revealed unambiguously high abundances of viruses found in aquatic environments (i.e. [12–14]). After the 1990s, the potential biogeochemical and ecological roles of aquatic viruses in microbial food webs

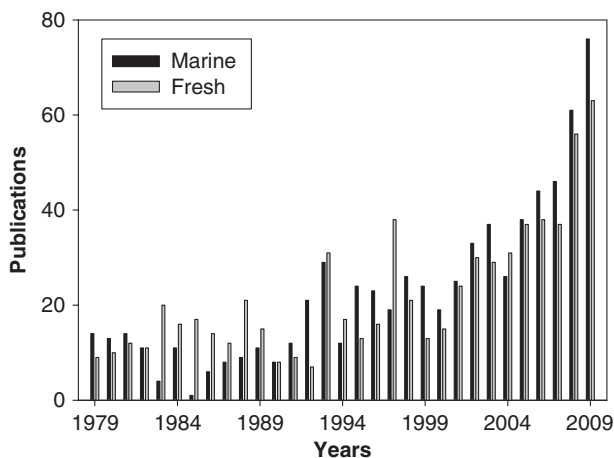


Figure 1. Trends in the number of publications for the last 30 years (1979–2009) dealing with viruses in either freshwater or marine ecosystems. Search was only performed on article title and based on all available databases from ISI web of knowledge. Key words used were viral, virus(es), viroplankton, phage(s), bacteriophages(s), associated to either sea, ocean, marine or lake, river, freshwater, limetic and other variants of these terms. Approximately 750 hits were obtained for both systems. For marine ecosystems, subject areas such as environmental sciences, marine biology and ecology dominated over infectious diseases, biochemistry, molecular biology, genetics, etc., while infectious diseases were predominant for freshwaters. See also Middelboe et al. (2008) for another hit process dealing with the evolution of publications and number of citations between 1987 and 2007.

Table 1. A students primer: classic early papers on viruses in aquatic systems.

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and ecosystems was featured in the literature. The areas explored virus effects on cell growth and photosynthesis efficiency, virus-mediated microbial mortality, roles of viruses in bloom initiation and termination, nutrient cycling, control of community and population structure, cycling of organic matter and microbial carbon transfer, genetic exchange, and host diversity, fitness, and resistance. During the last two decades, viruses have been recognised as an important biological compartment in the functioning of aquatic ecosystems and one could read that viruses are ‘partners’ in pelagic food webs, play important roles in ‘killing the winner’ [15–17], are the ‘new players in the game’ [18], the ‘major players’ in global ecosystems [2]. They ‘rule the waves’ [19], and the world [20], and they ‘manipulate the marine environment’ [21]. Finally, they are ‘the key to survival in the seas for microorganisms’ [22].

For the last 10 years, many excellent reviews have been written on aquatic viruses, mainly about the important roles of bacteriophages, cyanophages and algal viruses (e.g. [1,21,23–35]). At least three special issues in scientific journals have been published in the last 4 years: *Journal of the Marine Biological Association of United Kingdom* (2006, volume 86 issue 3), *Freshwater Biology* (2008, volume 53 issue 6) and *Environmental Microbiology* (2009, volume 11 issue 11). As well, the first freely available e-book on methods in virus ecology, published by the American Society for Limnology and Oceanography, is due out in 2010. Writing a new and innovative review on aquatic viruses can thus be viewed as a difficult task. However, the launch of *Advances in Oceanography and Limnology* represented a fantastic opportunity to highlight some advancements in the field of virus

oceanography and limnology focusing on under explored areas and new research fields that have not been fully reviewed to date.

Viral abundance and dynamics

Direct observation and counting of viruses has been possible since the middle of the twentieth century (e.g. [12]), but it was not until the end of the twentieth century, that epifluorescence microscopy and flow cytometry were widely used methods for viral enumeration [36,37]. Using these techniques, reliable, quick and direct estimations of aquatic viruses (mainly dsDNA viruses) became possible. By contrast, titer determination by plaque assay, combined with the most-probable-number method ('viable' counts determining the ability of viruses to lyse a cultivable host), has been routinely used in water analysis but rarely in virus ecology, since such culture-based methods are inefficient for enumerating natural virus assemblages and strongly underestimate viroplankton abundance. As a result of much global work on aquatic viruses, viral abundance of between $<10^4$ and $>10^8$ particles ml^{-1} has been recorded in aquatic ecosystems, with higher values generally being recorded in lakes [4]. In general, there is only one to two orders of magnitude variation in virus abundance among systems (10^6 – 10^8 viruses ml^{-1}) compared to the three orders of magnitude often observed for the planktonic biomass. A mean value of 10^7 virus ml^{-1} is equivalent to about 10^{30} viruses in aquatic ecosystems, or roughly 90% of the total abundance of planktonic particles [2,38], but it corresponds to $<10\%$ of the carbon biomass because of the small size of viruses.

Viruses are also as abundant in sediments as in the water column, with 10^7 – 10^{10} particles per gram of dry sediment [39]. Data on the virobenthos are still scarce with contradictory results regarding the degree of infectivity and lytic processes in such environments [39]. Briefly a titer of 10^9 virus-like particles ml^{-1} is the rule; such abundance decreases dramatically with depth (within a few cm); the sediment is likely to constitute an important viral reservoir (up to 0.5 and 28.7×10^{28} viruses in lake and marine surface sediments, respectively); viruses can remain infective for a long period of time.

In general, viral abundance, which is a dynamic balance between production and decay processes, increases with ecosystem productivity. Thus, concentrations generally decrease from marine coasts to the open ocean and from surface to deep waters (from 10^8 to $<10^6$ ml^{-1} typically). This is also true for freshwater ecosystems where eutrophied ponds generally own more viral particles than oligotrophic lakes [4,40]. High concentrations (typically 10^8 ml^{-1}) are also found in surface microlayers or in/on suspended particles. However, it is noteworthy that in some cases, both in the marine field (near hydrothermal vents for instance) and in freshwater lakes (in monomictic lakes for instance), viral abundance can be significantly higher in deep waters than in surface [41–43], where such particles are generally the only bacterial predators. By contrast, relatively low viral numbers have been found in high-mountain or some polar lakes [44,45]. As reviewed by Weinbauer [25], there is only little difference in terms of viral production between marine and freshwater systems, with values varying between 10^5 and 10^8 viruses $\text{ml}^{-1} \text{ day}^{-1}$, i.e. turnover times of 0.05–30 days.

Like other aquatic microorganisms, the abundance of viruses does not remain constant over time, but tends to fluctuate over all the time scales investigated (from minute to year), notably with the seasons, with peak concentrations recorded during the spring-summer periods in the surface waters, and a subsequent decline occurring during the

autumn(fall)-winter periods. In spring, when photoperiod and water temperature increase, higher photosynthetic activity and enhanced exudation of algal dissolved organic carbon are recorded [46], resulting in bacterial production increases that promote viral proliferation [47]. The beginning of autumn/fall, with the strong mixing of the surface water, is also often characterized by high viral concentrations [1,48–50]. The seasonal changes in viral concentrations seem to be closely correlated with those of their hosts (bacteria, phytoplankton [51–53]. This correlation is hardly surprising since viruses, which are obligate parasites of cells, need a host if they are to multiply. This host is only encountered as a result of chance events, in a manner similar to the diffusion of particles in the surrounding environment. As bacteria and phytoplankton are the most abundant organisms in aquatic ecosystems (the prokaryotes, including both eubacteria and archaea, constitute up to 90% of the total biological carbon found in aquatic ecosystems), it is easy to understand why close relationships exist between viruses and bacteria. Strong relationships have also been observed between viruses and phytoplankters, often quantified using the proxy measurements of chlorophyll *a* [40]. However, other factors can also intervene, and contribute to changes in the viral abundance in aquatic systems [5]. Based on available data, it appears that viral abundance is relatively low in winter and increase is recorded at key periods (early spring, mid summer, early autumn) but seasonal patterns during the rest of the year are quite variable. Although viral abundance fluctuates with the seasons, it is important to note here that the time scale of observation is severely constrained by the monitoring of microbial communities, which are usually sampled between once a week and once a month, whereas the turnover times of these communities are very short – a few hours to a few days. Working over short time-scales does indeed clearly reveal the extent to which these particles are highly dynamic (10 minutes can be long enough for their concentration to increase four-fold [15]; however there have been few studies that have focused on this short time-scale variability [47,49,54–56], because they are time and energy consuming. We should also note that what is true at the temporal level is also true at the spatial level, and high variability can be recorded over a distance of a few centimeters [57], or even on a smaller scale when considering microzones and aggregates [58–60].

The predominance of viral abundance over that of bacteria (or prokaryotes) can easily be observed and is typically defined using the ratio between viruses and bacteria (or prokaryotes, VPR), often used as a descriptor of the relationship between viruses and potential hosts. The VPR generally ranges from 3 to 100 (with a mean value of about 25); the highest values often being recorded in lakes [50] and indicative of viruses actively infecting microorganisms that also must be metabolically active. VPR values are thus logically higher for more nutrient-rich, productive environments. This observation suggests that bacterioplankton host populations produce greater numbers of viruses under environmental conditions favouring fast growth and high productivity. These increases in virioplankton production under nutrient-replete conditions are also due, in part, to higher infection rates and larger burst sizes, two biological parameters which can strongly influence VPR [1]. In the sediment, the VPR remains often very low (ranging from 0.03 to >10) [25]. Values <1 have been rarely reported [61] and low viral abundances in such situations might be explained by important loss (decay) rates because of high temperature, UV radiation, digestion by extracellular enzymes, adsorption on particles, virivory by small nanoflagellates, etc. [25], some processes still poorly investigated.

Viral life cycles

Viruses have three possible development cycles (i.e. the lytic cycle, the lysogenic or temperate cycle, and the chronic cycle), all beginning by the adsorption or fixation of the virus on its specific host. The most common cycles, and the ones most often studied, are lytic and lysogenic infections. During the lytic cycle, after infection viruses use the metabolism of the host cell to multiply. The newly formed viruses (or virions) are then released into the surrounding medium when the host cell bursts, and then potentially infect new hosts. In contrast, lysogenic (or temperate) infection involves integration of the viral genome into the host cell genome, and subsequent replication concomitant with host doubling. The integrated 'prophage' remains inside the cell in a dormant state until the host cell is stressed, and induction occurs (i.e. the lytic cycle is triggered). The resulting host carrying the virus burden is then referred to as the lysogen. Reference has also sometimes been made to a 'chronic infection', during which the newly-formed viruses apparently leave the host cell by a budding process or by the extrusion of filaments, without rupturing the cell as in lysis. While this has found to be less important in aquatic systems, the area remains poorly studied [45,50].

Estimates of the fraction of lysogenic bacteria in aquatic systems have often been determined indirectly by adding a chemical induction agent such as mitomycin C (generally used at a final concentration of $1\text{ }\mu\text{g ml}^{-1}$) to a water sample, and comparing numbers of total virus particles produced over time to a control treatment, although other stressors such as UV or temperature for instance have also been mentioned. Antibiotic treatment stresses the host cell and causes induction of the lytic cycle, and when burst size is known or assumed, one can estimate the prevalence of lysogeny in the sample. Lysogeny is extremely common among cultivated bacteria. It has been estimated that 60–70% of the bacterial genomes sequenced to date contain prophages [22]. Early work with natural populations suggested firstly that a relatively low proportion of marine bacteria were lysogens. Up to now, it has been shown that marine bacterial isolates contain a high proportion (43%) of inducible prophage-like particles [62,63]. Research of this type has revealed considerable variability among and within different aquatic systems, with the proportion of lysogenic bacteria ranging from 0.07% to over 90% [64,65]. Beside methodological constraints, many different explanations for this variability have been put forward, notably involving environmental factors such as physico-chemical (pH, solar irradiation, trophic status) and biological parameters (activity and abundance of the host community) [25], which either induce lysogeny or on the contrary, induce the switch from a lysogenic to a lytic cycle. However, none of these hypotheses is unanimously accepted. As typical results, lysogeny has been found to be higher during winter months in a variety of environments, in low productivity ecosystems or again in the sediments or in near surface waters in summer [1,25]. These results may indicate that lysogeny is favoured in waters of low host abundance and productivity, as a survival strategy for viruses during episodes of low energy sources or unfavourable conditions in general (i.e. protection from proteolytic digestion, grazing to some degree, UV inactivation).

Thus, the advantages of lysogeny from the perspective of a phage are obvious. The initiation of a lysogenic cycle would appear to be an adaptation strategy that enables the viral community to persist during difficult times, even when there are insufficient host cells to permit reproduction by new infection and immediate viral lysis. However, this refuge reason alone cannot explain the preference for a lysogenic cycle. The prophage may also confer new properties on the host cells, such as some degree of immunity towards new viral

infections or superinfection by homologous phages, increased reproduction fitness or the acquisition of new functions encoded by the prophage genome [25]. The abundance of lysogenic cells could affect microbial diversity due to acquisition of immunity against viral attack but also because of metabolic, morphologic or immunogenic properties acquisition thanks to the prophage integration into the bacterial genome. Typical examples are the toxicity acquired by *Vibrio cholera*, the toxin gene being carried by the prophage [66], or again the higher reproductive facilities of some *E. coli* strains [25].

Citing Paul [22]: 'Perhaps lysogenic repressors serve to "throttle down" marine bacterial metabolism, conserving energy by shutting down unnecessary metabolic pathways and ensuring survival under unfavorable conditions. That is, prophages repress not only their own lytic genes but unnecessary and wasteful host metabolic process genes. Such metabolic economization enables the host (and the prophage) to survive disfavorable conditions until environmental clues alert the cell that conditions of growth have changed. Thus, prophages are not solely dangerous molecular time bombs that can bring about cell mortality, but also serve as a key to bacterial survival in unfavourable situations'.

Most of the work on lysogeny has been performed on pelagic bacteria. However, it is noteworthy that lysogeny has also been described in algae and cyanobacteria. There are only a few data on lysogenic bacteria in sediments assessed using Mitomycin C and many results concluded to only a small proportion of lysogen prokaryotes, from undetectable to <15% (reviewed in Danovaro et al. [39]). Bacterial genomes generally lacking prophages fall into two groups, the archaea and intracellular pathogens [22].

The relative importance of these replication mechanisms (lytic/lysogenic) in the aquatic environment has still not been defined, and remains the subject of speculation; however, it would appear that lytic cycles tend to predominate in most ecosystems. This could be because the number of virions produced during a lytic cycle depends on the rate at which the viral gene multiplies to form several copies in a single host cell, whereas the number of phages produced during a lysogenic cycle depends entirely on the rate of growth of the host cell. However, once the virions have been released they are exposed to a higher loss rate notably due to their exposure to irradiation or enzymatic hydrolysis, and so their productivity will depend to a large extent on the probability to encounter a host.

It is obvious that the factors and mechanisms implicated in the competition between the lytic and lysogenic cycle remain somewhat obscure, partially because we lack intensive studies of aquatic lysogens. Further investigation is needed, in particular about the effects of environmental conditions or perturbations on natural populations of lysogens (see also below the *modelisation of virus-mediated interactions* part). It is important to clarify and understand the reasons for the two lifestyles, because the predominance of one or other of the two cycles has major implications for carbon and nutrient cycling, and for genetic exchange in aquatic environments.

The morphology, morphometry and phenotype of viroplankton

Historically, the first observations of viral diversity (in terms of size and shape) were made using electron microscopy. About 10,000 viral species have been identified and described to date. There have been many reviews on the morphology of phages isolated from aquatic systems [67,68], and they have revealed that particles of phage-type viruses predominate. These are viruses that usually infect bacteria, and consist of an isometric capsid (head), the size of which can easily be determined using transmission electronic microscopy (TEM).

Most aquatic viruses are between 30 and 60 nm in size. Averaged sizes range indeed from 51 to 64 nm and 51 to 89 nm in marine/estuarine and freshwater systems, respectively [69]. However, there are some exceptions. For instance, in the backwater of the Danube River, 84% of viruses have been found to have capsids measuring more than 60 nm [70], and in Lake Superior (USA) over 50% of virus have capsids measuring less than 30 nm. Giant viruses (from 200 to >700 nm) have also been discovered in eutrophic reservoirs [71], Antarctic lakes [72], alpine lakes [45,73], and in the digestive vacuole of *Phaeodarian radiolarians* [74]. These giant viruses are currently the topic of many ongoing research projects, especially after the discovery of Mimivirus-like viruses [75,76], (<http://www.giantviruses.org>).

We now know that 96% of the phages isolated from Eubacteria are tailed phages (phylogenetically belonging to the order of *Caudovirales*). Their quantitative importance varies between 8 and 43% of the total viral community [69]. The species that constitute this order contain double-stranded DNA, and they are divided into three families on the basis of their tail length: *Siphoviridae* (with a long, flexible and non-contractile tail), *Myoviridae* (with a contractile tail of medium length), and *Podoviridae* (with a very short tail). Myoviruses, most of which are lytic with a wide spectrum of hosts, appear to be dominant in cyano- and bacteriophages [67,77]. This is due to the fact that they constitute the most important proportion of isolated phages in marine ecosystems. However, this remains to be confirmed, since the number of studies performed is so far still too small to justify a convincing generalization, and because such a scheme might not be true in freshwaters. Indeed, Siphoviridae could be the most highly represented tailed viruses in aquatic system. It is noteworthy that extreme environments (not under the scope of this review) are the refuge of a large variety of bizarre forms including filamentous, bottle shaped, and lemon-shaped, for example (see literature by Prangishvili for instance). Many observations have revealed spatio-temporal or host-related differences in the distribution frequency of viruses related to capsid size in the viroplanktonic population [43,78,79]. However, this has not been confirmed in every case [70].

In addition to observing natural samples, studying the morphological diversity of viruses can require isolation of phages, a good knowledge of their specific host, and the ability to culture them in the laboratory [69]. However, so far it is only possible to culture a very small proportion of prokaryotes, which severely restricts our ability to isolate viruses, making it difficult to produce a reliable estimate of viral diversity in aquatic ecosystems solely on the basis of morphometric and morphological data. Furthermore, over many growth cycles virus-host systems can change, with bacterial cells developing resistance to viral infection. During the last 10 years, molecular methods have emerged that now offer a tremendous new approach to the problem.

Virioplankton genomics

Historically, it has been difficult to assess aquatic viral genetic diversity, notably because (i) most viruses are not good candidates for cultivation and, (ii) viruses do not have universally conserved genes like the ribosomal DNA genes in other organisms.

A study of genetic fingerprints, using the method known as Polymerase Chain Reaction – Denaturing Gradient Gel Electrophoresis (PCR-DGGE), suggests that the diversity of viruses is large, and that many phages are widely distributed in the environment, even in different biomes [80,81]. Marked differences between the genetic

fingerprints found in the genotypes of the viral community, recorded just a few meters apart, have been observed in some studies [82]. The use of techniques of this type has also revealed that the viruses include small RNA viruses [83] and microalgal viruses (known as phycodnaviruses), and that several dozen different genotypes can exist in a single sample of a few milliliters or liters. However, it should be noted that the PCR method can only detect a fraction of the diversity present, because it is based on the use of primers that are specific to certain groups of viruses. Because PCR bias remains an issue, quantitative analysis is not possible.

Pulsed Field Gel Electrophoresis (PFGE) is another method of genetic fingerprinting that can be used to determine viral diversity, however in this case whole viral genomes are used after preliminary concentration of the water sample. Thus, for a given sample, the number of bands obtained is proportional to the sizes of the dominant genomes. According to the literature, viral genomes range in size between 10 and 850 kb in marine environments, and between 12 and 661 kb in freshwater [69], with an average virus size in aquatic systems of around 50 kb, and with most of them being below 80 kb [35]. A major drawback of this method is that it is unable to distinguish between different viral genotypes that have genomes of the same size and as a result it likely under-estimates viral diversity. Furthermore, this method can only detect double-stranded DNA viruses, and this has the effect of underestimating aquatic viral diversity, which also may include a large number of RNA viruses [84,85]. Despite the important limitation of PFGE with relatively low resolution, potentially causing different viral species of similar genome size to be erroneously regarded as the same virus, PFGE is regarded as the least imperfect method to compare viral assemblage composition [53,86,87], and can give results comparable to morphological analyses of viruses by TEM [88].

Metagenomic approaches, based on sequencing the genomes of an entire viral community present in a sample, has made it possible to provide further information, leading to the discovery of the incredible diversity of viruses in aquatic systems. In some cases, several thousand different viral genotypes have been discovered in a few liters or grams of sediment (the estimation, using mathematical modeling based on overlapping sequences, was between 400 and 7000 genotypes in 200 l of coastal seawater and 10^6 genotypes in 1 kg of sediment!), and 30 to 90% of these viral sequences had no homologue in existing genetic databases [89,90]. Angly et al. [91] offered the first large-picture view of the diversity and distribution of marine viruses using whole-community genome sequencing (i.e. pyrosequencing). Using 184 water samples originating from 68 sites (in the Sargasso Sea, the Gulf of Mexico, the British Columbia coastal waters, and the Arctic Ocean) collected over 3000 m in depth and a 10-year period, these authors revealed that >91% of the viral sequences obtained were unknown, including many new cyanophages and ssDNA viruses. They could also propose that up to 129,000 different genotypes could be present in the upwelling waters area of the British Columbia coast. This study illuminated our degree of ignorance regarding viral diversity. It is likely that the global viral richness is huge, reaching a few hundred thousands species [92,93]. Breitbart et al. [92] also showed that metagenomic analysis of viral communities from coastal ecosystems revealed that the most abundant viruses represented only 2–3% of the total viral abundance, suggesting that viral diversity is enormous but dominance is rather low. More recently, Williamson et al. [94] demonstrated that viral sequences represented ca. 3% of total proteins in the 0.1–0.8- μ m planktonic fraction. At least, it is noteworthy that among the approximately 550 phage genomes currently available in the public domain, <5% are marine phage [95]. This method has clearly revealed the incredible

potential viral diversity, and shown that this is considerably greater than that of the prokaryotes. However, it remains relatively onerous and difficult to use for the purpose of determining the spatio-temporal variability of viral diversity, and still tends to be expensive for large numbers of sample analyses [96].

An alternative method has recently been applied to aquatic virus studies called: RAPD-PCR (Randomly Amplified Polymorphic DNA-PCR) [97] that can offset the drawbacks of the various methods mentioned above. This appears to be a convenient and effective method suitable for routine use in high-resolution studies of viral diversity in various different environments, as long as one is able to concentrate a large volume of water and thereby isolate relatively large concentrations of viral DNA. For instance, in the Chesapeake Bay, this method has detected very high viral diversity, and above all shown that the viral assemblages varies more over time than over distance [97]. Once again this method is specific to DNA viruses.

Viruses are likely to represent the largest reservoir of genetic diversity on Earth, referred to as the virosphere [2]. We know today that the three life domains (i.e. Bacteria, Archaea and Eukarya) have their own associated viruses. The mechanisms that control viral diversity, and how it changes, are still poorly understood. Some studies have apparently revealed co-variation between prokaryotic diversity and viral diversity [52,86], whereas others have not [98,99]. Accordingly, changes in viral communities have also been observed in association with changes in phytoplankton communities [100,101]. The transfer of genes between phages and their hosts during the transduction process, and the transfer of genes between co-infecting phages, are probably both factors that play an important role in viral evolution and diversification (see below). It has been suggested that bacteria that have co-infecting phages act as 'phage factories', producing large quantities of mosaic phages that increase viral diversity [102]. Furthermore, biological factors, and other parameters such as the influence of solar irradiation (typically UV), the aggregation of particulate organic matter, and temperature can define ecological niches that could have a considerable effect in controlling viral diversity [25].

Human pathogenic viruses, mostly stemming from human waste become part of the viroplankton once released into estuarine and coastal marine waters and riverine and lake freshwater [103]. Research by Culley et al. [83] showed that marine virus communities contained sequence information that placed them into a divergent group of picorna-like viruses. The *Picornaviridae* family includes major human pathogens such as poliovirus, rhinovirus (the causative agent of the common cold), and hepatitis A and B. Interestingly, the information derived from Culley et al. [83], indicated isolation of a lytic pathogen of the important harmful algal species, *Heterosigma akashiwo*. Given the sequence similarity observed among samples from a wide range of environments, picorna-like viruses are likely to be globally important. They could be an example of a virus-type that could be pursued for aquatic virus therapy (see below). Furthermore, and possibly important on global scales, it has been suggested that knowledge surrounding these ancient RNA viruses might contribute simultaneously to revealing the clues of genetic systems and unraveling the origins of globally destructive viral disease caused by RNA viruses [104].

Virus-induced microbial mortality

Viruses play a crucial role in the operation of the microbial trophic network and in biogeochemical cycles. A range of methods and approaches, including estimating the

frequency of visibly infected cells (FVIC), measuring the loss of viral particles over time, measuring the decline in viral infectivity, and determining rates of virus production divided by estimates of burst size have been used to calculate the bacterial mortality attributable to viral infection. These processes are difficult to measure accurately, and newly developed methods may improve our ability to estimate the importance of viruses in mortality in the future. Estimating rates of production and virus-mediated mortality is very important for incorporating viral processes in functional models of aquatic systems, especially those to examine carbon flux.

The frequency of visibly infected cells (FVIC) is the most commonly-used approach. It is estimated directly by observing a thin cell section using TEM [49,105–107]. However, since a mature or intracellular phage are only visible inside a host cell at the end of the lytic cycle, the FVIC is then converted into the frequency of cells that really is infected (FIC: Frequency of Infected Cells) [14,42] using theoretical conversion factors or algorithms derived from laboratory cultures [107,108]. The first model estimating the frequency of mortality due to viral lysis (FMVL) from the FIC is that of Proctor and Fuhrman (1990) [14], the main hypotheses of which are that at steady state, (i) one of the two daughter cells resulting from the division of the mother cell dies as a result of viral lysis (i.e. if viruses kill 50% of cells, they are in fact responsible for 100% of the mortality observed), (ii) the latent period is equal to the generation time, and (iii) infected cells are not grazed. Thus, the bacterial mortality due to viral lysis was estimated by multiplying the FIC by two. This rule, known as that of the ‘factor of 2’, was subsequently revised using a more rigorous model by Binder [108]. This method is still used today, and has shown that viruses destroy 10–20% of the daily production of bacteria, and up to 50% of the biomass. It remains that a minimum number of virus-like particles must be observed inside the cells (around 5) and that such a method is difficult to apply to sediment samples. Transmission electron microscopy is also very useful for looking through cells and counting mature phages inside whole cells [109,110] or in thin sections [14].

Determination of the number of viral particles released when an infected cell bursts, commonly known as the burst size (BS) or viral load, is an important parameter in evaluating the link between viral production *in situ* and the rate of lysis of the host cell [70,111,112]. The BS is usually estimated on the basis of TEM observations of viral particles in the host cells or can be estimated by causing lysis of cells and conducting counts of viruses released via epifluorescence microscopy [36]. Wommack and Colwell [1] and then Parada et al. [113] have summarized the various values of BS reported in the literature, and have proposed that bacterioplankton have a mean BS of 24 and 34 for marine and freshwater environments, respectively. Higher values are found for photo-synthetic organisms (between 400 and 500 and sometimes higher, i.e. a few thousands), probably linked to the volume of the cells, which enable them to contain more viruses. The morphology, size, and activity of the host cells, phage and host diversity and the trophic status of the system are factors that can explain the wide range of BS values reported in the literature. These values also seem to reflect a difference between seawater and freshwater, with higher concentrations of viruses and hosts being found in freshwater [4].

The rate of replication of viruses is also a good estimator of the viral contribution to the bacterial mortality, and this is described as a measurement of viral production [114–117]. Various different approaches have been proposed in the literature to determine viral production; the decay rate of viruses after poisoning the host cells with potassium cyanide [109], the rate of synthesis of viral DNA in the presence of a radioactive precursor [118], the use of inert, fluorescently labeled viruses as tracers [119,120], and finally ‘the dilution

method' [117]. There does not seem to be large scale differences between viral production in freshwater and marine environments, which typically ranges from 10^8 to 10^{11} virus $l^{-1} day^{-1}$ [4], corresponding to turnover times ranging from 0.09 to 3.5 days [25].

It was recently proposed that the modified dilution approach, initially presented by Evans et al. [121] and mostly applied to viral-induced phytoplankton (including cyanobacteria) mortality [98,99,121,122] could also be applied to infer bacterioplankton mortality through cell lysis [97,98,123,124]. Briefly, it consists of mixing a natural sample with grazers and/or virus-free water in order to obtain a linear range of dilutions of the whole water (i.e. 20, 40, 70 and 100% typically). Assuming that the dilution reduces the encountering probability between prey and grazers/viruses, bacterial growth rates obtained from abundance variations from 24 to 48 h are expected to increase with dilution. A linear and statistically robust regression can be used to infer the loss rate and thus the calculation of the mortality rate due to viral lysis [123].

Even though these various methods are subject to possible bias from many sources (from the fact that theoretical conversion factors and algorithms are used that are usually derived from laboratory cultures, and from the hypotheses on which these approaches are based), we now realize that viruses are commonly responsible for the loss of 10–20% of bacterial production in aquatic systems [1]. It has also been shown that virus-mediated mortality (bottom-up control) can match grazer-mediated mortality (top-down control [125]), and that different environments can present virus-mediated mortality ranging from zero to 100% [126]. For instance, VIBM for some anoxic lakes, solar slattern and polar lakes i.e. freshwater environments are among the highest reported so far. However, even though numerous hypotheses have been advanced to explain the factors responsible for these differences (the activity of the host, the trophic status, the synergistic/antagonistic relationships with the grazers, the level of anoxia, UV irradiation and other environmental parameters) their relative importance remains to be determined (e.g. [30,116,127,128]).

Despite this information, there is still no standard approach to estimate and compare the mortality in prokaryotic and eukaryotic communities that is attributable to viruses in different aquatic systems. Each of the approaches suffers from its own limitations and problems preventing the accurate integration of viral processes into global models of biogeochemical cycling.

The role of viruses in biogeochemical cycles

The importance of viruses in marine and freshwater systems is commonly suggested to be tightly linked to their ability to impart mortality on their host organisms. As agents of mortality, phages influence microbial community composition, biogeochemical cycling of nutrients, elements, and both the flux and character of carbon (both organic and inorganic) in marine surface waters [32,129]. The inclusion of phages in models of marine food webs is widely accepted, as it is known that upwards of 25% of the photosynthetically fixed carbon in these environments is directed to dissolved organic matter (DOM) pool by the 'viral shunt' [24]. The importance of this process is that it removes carbon from the classical grazing food web and diverts it to the microbial loop.

Viruses effectively redistribute nutrient elements from large biological particles (i.e. bacteria, algae) back into biologically inactive (dead) particulate and dissolved pools of organic compounds. These compounds are not, however, biologically inert. Many compounds contain macro- and micronutrients (e.g. P, N, Fe) that can be rapidly recycled

back into the food web (e.g. [130,131]). Several efforts to model the role of viruses in carbon cycles have been made over the last two decades [2,24,125,132]. In most cases, these efforts have suggested that viruses cause the release of ~20–30% (and up to >50%) of the daily carbon production back into the water column by cell lysis. Direct measurements of this process are difficult, however, as they are confounded by the activity of other members of the microbial community.

Studies of nutrient release from model heterotrophic bacteria, cyanobacteria and a coastal phytoplankter (*Aureococcus anophagefferens*) have measured the uptake of the nutrient elements to artificial communities. In lysing the pelagophyte *Aureococcus anophagefferens*, Gobler et al. [130] demonstrated that elements are partitioned differentially between the particulate and dissolved size classes (operationally distinguished in that study as materials that pass through (dissolved) or are retained on 0.2- μ m filters). Surprisingly only C and Se moved to the dissolved phase, while N, P and Fe were retained as particulate materials throughout their early observations. This in part is attributable to the populations of heterotrophic bacteria that sprung up during the lysis of the algal populations.

In a subsequent study, Poorvin et al. [131] worked to dissect this process using model bacterial and cyanobacterial populations. In their study Fe was released from heterotrophic bacteria during lysis into a continuum of size classes. While most of the Fe (~75%) stayed in the particulate phase upon lysis with a virus, this amount released to the dissolved phase was 250% of the amount 'leaked' out from actively growing cells. This and the above work of Gobler suggest that important nutrient elements may be tied up in lysates and not instantaneously available for assimilation. In longer term observations (28 days), Gobler and colleagues demonstrated that the majority (80%) of this material eventually moves into the dissolved phase. Within the dissolved size classes a continuum of different molecules are seen: Poorvin et al. [131] demonstrated that Fe was enriched in the smallest (<3 kDa) size class but depleted in the 3–30 and >30 kDa size classes in lysates relative to virus-free cultures of heterotrophic bacteria. The opposite was however true for virus-exposed and virus-free cultures of cyanobacteria. Given the stark contrasts in physiology between heterotrophic and phototrophic prokaryotes, these results imply that many intact cellular structures and components must make up the 'lysates', suggesting that their fates could ultimately be very different in mixed oceanic communities.

Beyond the sheer quantities of material released by virus activity, we have very little insight concerning the biological availability of these materials and the time course over which they can be consumed. In studies of nutrient release, Poorvin and colleagues demonstrated that Fe in bacteria lysates was actually assimilated more quickly and effectively than in standard microbial growth media [131]. Further, they demonstrated that viruses could release sufficient biologically available, organically-complexed Fe to meet all of the requirements of the eukaryotic phytoplankton in the Peruvian upwelling region. As such this work and follow-up studies have demonstrated that viruses influence the size fractionation of Fe and the rate of assimilation of virus-released Fe relative to other Fe species [131,133].

While we now have a better (although not complete) appreciation of the role of viruses in the regeneration of nutrient elements, we remain almost completely ignorant to (and have almost no data for) the role of viruses in the regeneration of organic carbon and subsequently the partition of this carbon by size (e.g. dissolved vs. particulate) and bioavailability (labile vs. semi-labile vs. recalcitrant). Surprisingly little work has been conducted to attempt direct measures of the fate of carbon and cell contents released by

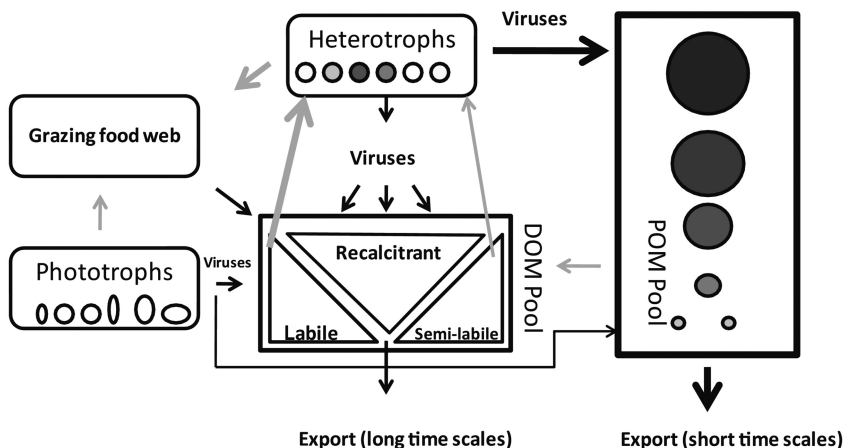


Figure 2. A revised look at the fate of carbon released by the lysis of cells due to virus infection. Viruses release carbon from heterotrophs and phototrophs into the particulate organic matter pool (represented here as a continuum of different sized particles) and into the DOM pool. The rate of re-entry of materials from the DOM pool back to the food web can be used to define fractions within this pool. Current research efforts are addressing the partitioning of DOM into the labile, semi-labile and recalcitrant pools. Efforts are needed to dissect the host pools into different functional groups (i.e. species of bacteria and algae) as previous work has shown that for Fe the size fractionation of materials in the lysate can vary depending on the virus-host system in question.

virus-mediate cell lysis [134–137]. With radiolabelled virus material, Noble and Fuhrman [138] demonstrated rapid uptake of viral lysis products in P-limited, oligotrophic Villefranche Bay. In host-virus model systems, uptake of amino acids, and use of organic substrates produced through viral infection have been shown to be rapidly utilized by other marine bacteria. These fates are highly complex (Figure 2). However, the dynamics of these processes are relatively unstudied in aquatic systems, including any quantitative understanding of shifts of organic matter into refractory pools through virus-mediated mortality.

Perhaps one area that remains the most overlooked is how the availability of nutrient elements influences virus productions and their interactions with hosts. While studies looking at perturbations of trophic status commonly have not included the virus component, recent work in both marine [139,140] and freshwater [141] systems suggests that a tight coupling of nutrient availability, primary production and microbial growth and the rate of the lytic cycle and production of viruses exists. Understanding how this coupling occurs and its strength (as well as plasticity) is a significant need for future researchers to consider: changes in global climate and nutrient cycling are likely to directly affect this process and as such influence how viruses work to influence microbial processes throughout aquatic environments.

Role of viruses in microbial community composition

Since the high abundance of viruses in aquatic environments was clearly revealed [13], reference has been made to their potential control of the structure of microbial

communities (see [1,26]). Numerous studies based on the principle of the fractionation of microbial communities (i.e. their separation on the basis of cell size), and by using the dilution or concentration of viral particles within samples, have made it possible to corroborate this key role of viruses [42,87,126]. During these studies, prokaryotic community structure is generally accompanied by changes in viral infection patterns indicating direct and indirect impacts of viral lysis on microbial communities.

Viruses can influence the genetic diversity of prokaryotes via several different pathways [26]. They can affect the composition of the bacterial community in the manner of the '*Killing the Winner*' model [17] by constraining the abundance of the most abundant bacterial groups, which are also thought to be the most competitive for the substrate resources. The *killing the winner hypothesis* is a food web model that assumes trade-offs between competition strategists and defence strategists, when the dominant bacteria (the winner) for nutrient acquisition are controlled (killed) by the viruses. Thus, due to density-dependent interactions [142], bacterial groups with higher growth rates may lead to higher abundances of their specific viruses [143], resulting in higher per-capita viral-induced mortality rate. The consequence of this control on populations with higher competitive ability for nutrients is to maintain the species diversity within these communities, since the minority groups, often described as being less competitive [54,144], can make use of the remaining resources to grow (possibly benefiting from the lysis of the cells of the dominant bacteria). In fact, bacterial abundance is determined by the balance between competitive ability and sensitivity to viruses [143]. Differently said, trade-offs mentioned above result in coexistence. It is noteworthy however that it has also been shown that some minority bacterial groups may be susceptible to viral lysis, thus suggesting that their low abundance may in fact be linked to the effects of viruses [145]. This is another aspect of the *killing the winner* model where the most dominant hosts would be the least susceptible to viral lysis while the rare marine bacterial groups would be the most susceptible.

Viruses can also have an impact on bacterial diversity via the transfer of genes between organisms [1,26]. This impact can occur directly (by transformation) and/or indirectly (by transduction). Transformation consists of the assimilation and incorporation of free extracellular DNA by a prokaryotic cell. This type of genetic transfer may be stimulated by the lytic action of viruses. In marine systems, Jiang and Paul [146] have estimated that between 17 and 30% of the dissolved DNA results from viral lysis, suggesting that the viral lytic action could be a major source of dissolved DNA in the environment. However, even though numerous studies have tried to measure the DNA released during the viral lysis of bacterial and algal cells, there is no data available about the real contribution of this released DNA to gene transfer. What does emerge clearly is that extracellular dissolved DNA constitutes an important reservoir of genetic information, and that viral and cellular DNA released during viral lysis considerably enhances the available pool [147]. Transduction can have two forms. One is described as generalized and the other as specialized. In both cases, the transfer of genes from an "old" host cell to a "new" one occurs. During generalized transduction, part of the genetic material of the host is packaged by a virus (lytic or lysogenic) and transferred into a new host when it is infected. In the case of 'specialized' transduction, a specific host DNA sequence may be excised when the temperate (lysogenic) phage moves back into the lytic phase (this process is known as induction), and this DNA sequence can then be transferred to a new host during a subsequent infection. For many years, the transfer of genes via transduction was thought to be of negligible importance compared to transformation. However, when the importance of the lysogenic cycle was discovered, and viruses began to be viewed as a

reservoir of genes protected by their capsids against degradation by nucleases, unlike transformation, transduction could be seen to be an important, if not the most important, gene transfer mechanism. Rohwer and Vega Thurber [21] estimated that as many as 10^{24} genes are moved by transduction from virus to host each year in the world's oceans.

Also, the development of new metagenomic sequencing techniques has brought the study of horizontal gene transfer (HGT) to a new level, revealing a considerable proportion of genes unknown or related to potential hosts [148]. Some significant studies carried out in aquatic environments have been done in lacustrine systems by Miller and colleagues [149] using microcosms and the bacterial species *Pseudomonas aeruginosa*. The quantitative importance of these processes in nature is still largely unknown. Some very convincing and striking examples of horizontal transfer have been obtained as a result of the work of Chiura [150], who observed the transfer of amino acids from prototrophic to auxotrophic strains of *E. coli* during viral production in five cultures of marine bacteria. Waldor and Mekalanos [66] also showed that cholera is caused by a lysogen of *Vibrio cholerae*, and that the toxin is encoded by the genome of the prophage and not by the genome of the host. Another very striking example is the demonstration that viruses contain photosynthetic genes, notably the key components of the photosynthetic machinery (proteins D1 and D2) are present in cyanobacteria, and are encoded in genes of viral origin (*psbA* and *psbD*) [151]. The majority of sequenced cyanophages carry this gene and clear evidences have been provided that these viruses need the D1 protein for successful infection and replication [152–155]. The expression of such viral genes coding for the photosynthetic function would explain why cyanobacteria are so performant in a variety of environments. All these examples show that some particular characteristics of the host cells have been acquired as a result of the horizontal transfer of genes via viral infection. So far, we are only aware of a single study that has estimated the rate of transfer of genes in natural marine bacterial communities, and this was carried out using a simple model. This study revealed the full importance of this random process in regulating the diversity, diversification and adaptative evolution of aquatic microbial communities, with as many as 1.3×10^{14} transduction events possibly occurring per year, in this case in Tampa Bay [156]. Fuhrman [23] also proposed an exercise to infer global HGT frequency in the ocean. He calculated that such a process could occur about 10^6 times per day, considering 10^6 bacteria ml^{-1} dividing once a day in the euphotic zone, a volume of about $3.6 \times 10^7 \text{ km}^3$ for the top 100 m layer of the ocean, and considering a very low value for such an event, i.e. a probability of 10^{-20} . More recently, Ogunseitan [157] reviewed that these genetic exchanges occur at frequencies that vary widely, from 10^{-2} to 10^{-10} transductants per recipient cell, depending on the influence of the environment on the outcome of phage-host encounters.

In a study quantifying lysogenic and lytic infections in bacterial communities, in surface water and bottom water, carried out by Weinbauer et al. [158], it was found that 35% of these bacteria contained a functional viral genome. This would correspond, after taking into account the total prokaryotic abundance, to 4×10^{28} bacterial cells worldwide containing and carrying a functional viral genome. This clearly indicates that viruses are of considerable importance in the transfer of genes in aquatic systems.

It is important to point out that a change in the species composition of the bacterial community following viral lysis could result from a simple change in composition and/or concentration of the DOM. In other words, the release of DOM and of inorganic nutrients during viral lysis (viruses themselves are operationally considered DOM due to their size)

may provide a specific niche permitting the rapid growth of certain bacterial communities [127,159].

It is clear that viruses are not only agents of mortality and/or stimulants of the growth of uninfected populations, but are also very important vectors in the maintenance of diversity of microbial communities [25], and drivers of the genetic evolution of aquatic microorganisms.

Modelling of virus-mediated interactions

To understand the fundamental role(s) of viruses (mainly bacteriophages) in population, community, food webs, and biogeochemical cycling, researchers have developed several distinct lines of mathematical models. Here, we review the advancements in theoretical studies focusing on (1) population dynamics of a single pair of bacteria and phage, (2) community and food web dynamics with multiple pairs of bacteria and phages, and (3) biogeochemical effects of viruses especially on carbon cycling (Figure 3).

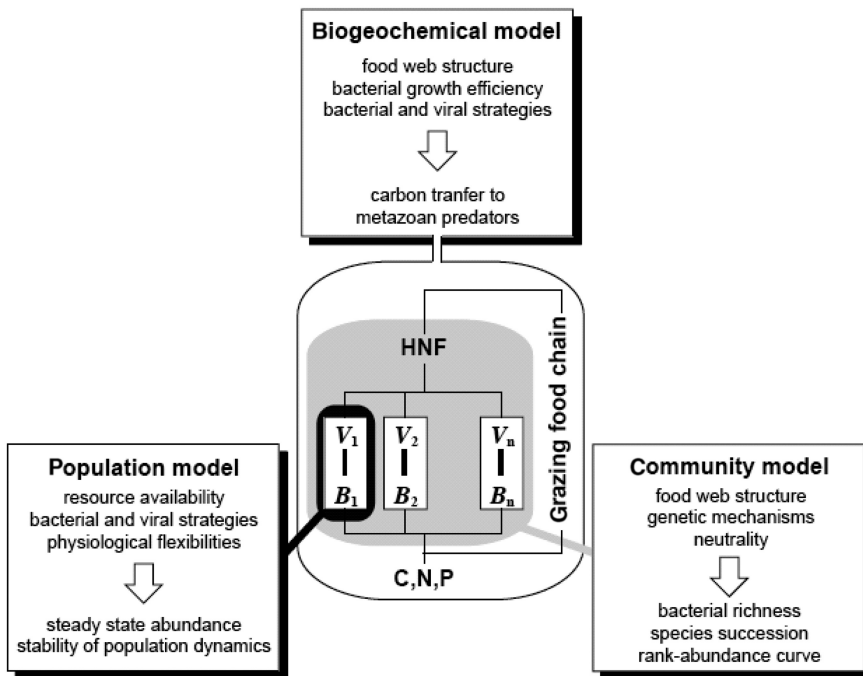


Figure 3. Hierarchical relationships among population, community and biogeochemical models. Population models have been developed to understand how resource availability, bacterial and viral strategies, and physiological flexibilities determine steady state abundance and stability of population dynamics. Community models have been developed to understand how food web structure, genetic mechanisms, and neutrality determine bacterial richness, species succession, and rank-abundance curve. Biogeochemical models have been developed to understand how food web structure, bacterial growth efficiency, and bacterial and viral strategies determine carbon transfer to metazoan predators.

Mathematical models for single pair of bacteria and phage

Levin et al. [160] proposed a theoretical framework for understanding the population dynamics of a single pair of host bacteria and its phage (Figure 3). They developed a simple food chain model based on one resource, two states of one prey (uninfected and infected), and one predator as a representation of the experimental systems of glucose-bacteria-(virulent) bacteriophage. The model predicts an equilibrium between bacteria and phages, confirmed by experimental results. Their model also predicted that enrichment of the system (i.e. the increase in the resource supply) can destabilize the equilibrium and lead to periodic cycles of host-phage populations.

A modified version of Levin's model, with simpler formulation neglecting the infected state of prey [161], predicts that the evolution of resistant types of bacteria stabilizes the total population size (sensitive + resistant) of bacteria whereas it elongates the period of oscillation in viral population. These predictions as well as the stability of the populations were confirmed by the chemostat community dynamics of *E coli* and T4 phage (also see Yoshida et al. [162]). Middelboe [127] developed a similar type of food chain model for marine bacteria-phage systems. The new development of his work was that he incorporated critical viral parameter values such as latent period and burst size into the model. With the estimated parameter values (e.g. $12 < \text{the burst size} < 60$; $1.7 \text{ h} < \text{latent period} < 2.0 \text{ h}$), the model, which can reproduce the short-term ($< 100 \text{ h}$) behaviour of populations of *Pseudoalteromonas* sp. and its virus grown in chemostats, predicted the stable coexistence of sensitive and resistant types of *Pseudoalteromonas* sp. and its virus in oligotrophic marine systems. This study is very important in that a framework was proposed for linking small-scale experiments and large-scale observation via mathematical modelling. Linking scales in studies of aquatic systems is one of the strongest impediments to successful model development.

A simple model for populations with a pair of host and temperate phage predicted a stabilizing effect of lysogeny on populations [163]. They also demonstrated that the fluctuation in nutrient supply and thus non-equilibrium dynamics are keys for the dominance of temperate phages over virulent phages, noting that just an oligotrophic condition is not enough for the dominance of temperate phages (see also Mittler [164] for discussion from evolutionary viewpoint). Physiological flexibility is an important determinant of host-phage population dynamics. With an assumption that viral lysis rate is positively correlated with host cell growth rate, Weitz and Dushnoff [165] demonstrated that viruses cannot invade the host population with high density due to low rates of host growth and viral lysis whereas viruses can invade a host population with low density. It is also predicted that oscillation between normal growth stage and dormant stage at which the viral lysis is suppressed stabilizes the population cycle of host-phage [166].

Mathematical models for multiple pairs of bacteria and phage

Another type of theoretical studies has focused on community dynamics of multiple pairs of bacteria and phage (Figure 3). In microbial food webs, every pair of bacteria and its specific phage is linked to each other in the food web (1) through the interspecific competition among bacteria for common resource(s) such as organic carbon and inorganic nitrogen and phosphorus and/or (2) through the density-regulation on bacteria by the generalist protozoan predators (garzers) such as heterotrophic nanoflagellates (HNFs).

By using idealized food web models, Thingstad and Lignell [17] and Thingstad [143] proposed a very original theory referred to as the '*kill the winner*' hypothesis in which the host-specificity of phages contributes to the maintenance of bacterial coexistence. In this hypothesis, viruses with high host specificity act as the mechanism that more frequently 'kill' hosts with higher growth rate (the 'winner' of the competition for nutrients) with a density-dependent manner, preventing the competitive exclusion. By focusing more on temporal dynamics of bacteria and phage, Middelboe et al. [167] developed a mixed food web model with multiple bacterial groups, each of which consisted of sensitive and resistant types to viral infection. The model behaviour fitted with the short-term (300 h) dynamics of chemostat populations and could be used for long-term (60 days) simulations. The long-term simulations demonstrated (1) the cyclic succession of dominant types of bacteria, (2) the suppression of resistant types in each bacterial species by the protozoan predator, and (3) the succession of dominant bacteria with shorter timescale in the presence of protozoan predator than in the system without protozoan predator.

Other features in complex (and more realistic) interactions among bacteria, phages and protozoan grazers have been also taken into account in theoretical studies. The evidence that viruses within infected host cells are indirectly destroyed via protozoan predation on bacteria has been firstly incorporated into models [108,168]. These models demonstrated that the longer the latent period, the weaker the viral impacts on bacterial mortality because a long latent period leads to higher risk for viruses for being killed by predation on infected host cells. This process is critical to estimate virus-induced bacterial mortality [108] and also affects the number of coexisting bacteria [168]. All the above models assume matching-allele interactions, resulting in one-by-one relationships between host and phage strains. However, for example, in a co-evolutionary system of *E. coli* and phage T7, it is known that mutant phages not only acquire infectivity in new host strains but also keep infectivity from ancestral host strain. In addition, a kind of (modified) gene-for-gene interaction keeps the infectivity from ancestral host highest. With this interaction, the food web model without protozoan predator predicts the negative effect of enrichment on bacterial diversity (see also [168]) whereas there is no such an effect with matching-allele interactions [169]. These results suggest the importance of complex interactions among microbes in regulation population and community dynamics, which also implies the limited generality of model prediction from specific formulations [169]. It is also worth noting that only simple loss processes have been assumed in these models; there would be space in community models to take into account realistic features in loss processes, e.g. loss by adsorption to non-host bacteria [170]. It may affect the consequences of bacteria-phage interactions in the maintenance of diversity.

A new generation of mathematical models has emerged in the age of environmental genomics. Hoffmann et al. [171] tried to explain the power-law relationship between species rank and relative abundance in the viral community, which has been highlighted by environmental genomics in marine systems [92]. Their model is a kind of neutral model (cf. Hubbell [172]). A key assumption is that the population dynamics of all pairs of host-phage in a community follow an identical prey-predator model, which drives the periodic population cycles, i.e. the periodic bloom patterns. Another key assumption is the absence of interactions between each pair of host-phage, following that the occurrence and decline of the bloom are independently determined among different pairs. In other words, time phases of each virus (and bacteria) population cycles in the community are randomly and uniformly distributed. Therefore, the iteration of random sampling of phase-dependent viral density can generate the rank-abundance distribution.

By using a generalized Lotka-Volterra model (GLV model), i.e. that the infection rate on bacteria increases dramatically with viral abundance (proportional to B and V^2 : BV^2), they found that the rank-abundance pattern from the GLV model fits well to the power law rank-abundance relationship. Conceptual advances in this theoretical work are (1) the introduction of neutral hypothesis into host-phage systems and (2) the introduction of new functional forms of local interaction between host and phage from an emergent pattern at the community level.

Mathematical models for biogeochemical roles of viruses

The impact of viral lysis on carbon cycling has been evaluated by steady-state models in which influxes to and outfluxes from each component in ecosystems are assumed to be balanced (Figure 3). Murray and Eldridge [173] constructed a model in which the microbial loop (DOM- $\rightarrow$ bacteria) is linked to the macrozooplankton ($>200\mu\text{m}$) via the microbial food chain. In this model, the viral shunt converts a fraction of the bacterial production to DOM. They demonstrated that the negative impact of the viral shunt on macrozooplankton production (5–10% reduction) is greater in an oligotrophic regime. Macrozooplankton rely more on the microbial food chain and the trophic pathway from phytoplankton is longer than in the mesotrophic regime where macrozooplankton can directly utilize primary production from large phytoplankton. A similar estimation, i.e. a 7% reduction in the production of macrozooplankton due to the virus shunt, was obtained in Fuhrman's model [23]. This model assumed that viruses induce 50% of bacterial mortality and 7% of phytoplankton mortality, and that macrozooplankton is able to utilize primary production directly from phytoplankton and indirectly from the microbial food chain. These predicted low impacts of viral lysis come from low transfer efficiency of DOM to macrozooplankton via the microbial food chain due to its long chain (DOM $\rightarrow$ bacteria $\rightarrow$ flagellates $\rightarrow$ ciliates $\rightarrow$ crustacean zooplankton) [23,173]. Contrary to the relatively high bacterial growth efficiencies used (BGE, 40–50%) in these models, Anderson and Ducklow [174] assumed much lower BGE (9–28%) in their steady-state model. They estimated that the contribution of lysis-originated carbon to bacterial production is very low (less than 20%). Due to assumed low BGE (14%), doubling viral lysis from 25 to 50% does not substantially affect bacterial production and thus zooplankton production. An important lesson from these models is that the impacts of viral lysis on the production of higher trophic levels (zooplankton and fishes) would be highly sensitive to bacterial growth efficiency and food web structures. If the average food chain length from bacteria to crustacean zooplankton is shorter due to omnivorous features in microbial food webs, the contribution of the microbial loop to zooplankton production and thus negative impact of the viral shunt will be larger.

It is also worth noting that BGE and the viral shunt are not always independent because the values of BP would be underestimated unless virus-free samples are used in experiments. Experimental results [175] and a simple steady state model analysis [176] suggest that the viral shunt negatively affects BGE values. Although there is not enough data to conclude this, it would be safe to use high BGE values when the viral component is included in models. Otherwise, the roles of the microbial loop would be underestimated as a link from DOM to the grazing food chain.

All of the above models assume a fixed value for the relative contribution of viral lysis to total bacterial mortality. However, as it is discussed in the above two sections, many

factors such as the presence of resistant hosts, varying host range, lytic/lysogenic replication, and complex interactions among viruses and protozoan predators are predicted to influence the impact of viruses on bacterial mortality and the partitioning of bacterial production between viruses and grazers. For example, the food web model developed by Miki et al. [177] predicts that the evolution of resistant host and the prevalence of lysogeny mitigate the negative impact of the viral shunt on carbon transfer to protozoan predators.

Next steps for theoretical studies

There is much space for the improvement of models, especially for understanding the global biogeochemical effects and importance of viruses. Most of the models for evaluation of the viral shunt assume that elements fluxes are balanced at steady state. Such models are useful to explain spatio-temporal variations in the impact of viral shunt, which can be understood as the steady states are realized with different parameter values [173,174], but this is not realistic. When population dynamics cannot immediately track environmental changes, as is the case in disrupted ecosystems, it is necessary to explicitly formulate non-equilibrium dynamics for understanding shorter time-scale dynamics of microbial food webs, e.g. in response to environmental changes such as phytoplankton blooms and cascading effects of global climate change.

For this purpose, flexible features in host-phage interactions should be incorporated into models. For example, it is important to relax the assumption of fixed rate for switching between different states of hosts [166] or those of viruses [163]. The flexible switch between lysogenic and lytic cycles depending on environmental cues, which has been well studied in mathematical models for *E. coli* and λ phage (see Weitz et al. [178]), can be incorporated into biogeochemical models. Another example would be the incorporation of flexibility in viral traits such as infection rate (host recognition ability), latent period, and burst size, in response to changes in host cell growth rate (e.g. Middelboe [127]). It is likely that rapid evolution of hosts and phages are partly responsible for these flexibilities. For example, theoretical models [179,180] and evolutionary experiments [181] predict that shorter latent periods are selected with the trade-off between latent period and burst size with high host density and resource availability.

It is also necessary to evaluate the impact of patchiness of microbial distribution [182] in mm to cm scales on the bulk rates of biogeochemical processes. It has been demonstrated that heterotrophic bacteria and viruses show distinct spatial distribution [57]. If the patchiness is spatio-temporally stable and thus affects the bulk rates, it is necessary to find a conversion factor to link the degree of patchiness to the bulk rates, since it is difficult to incorporate microscale dynamics explicitly into large-scale biogeochemical modelling.

Aquatic phage therapy

For the last few decades, interest in the use of (bacterio)phages to control bacterial populations has evolved from medical applications into the fields of agriculture, food industry, and wastewater treatment systems as an alternative method of treating bacterial diseases. The history of phage therapy is well documented [183,184]. Phages were

discovered to be anti-bacterial agents (1917) and implemented (1919) as soon as they were discovered, with varying success. However, antibiotics were discovered in 1928 and used for decades as they were widely popular because of their broad spectrum, ease of bulk manufacturing, shelf stability and ease of use. Hence, development of phage therapy was largely abandoned in Western countries, but continued throughout 1940s in the former Soviet Union for treating bacterial infections, with widespread use including the soldiers in the Red Army. Much of the literature (because it is written in the Russian or Georgian languages) was unavailable for many years to the Western world [184]. We know that treatments now used in hospitals either to replace antibiotics or used in conjunction with them can help to combat bacterial diseases [185], and that viruses are being used in ready-to-eat preparations, cheese-making, and agriculture to prevent and/or cure bacterial infections. What is less well known, however, is that phage therapy also seems to be promising as a way of treating bacterial infections in aquatic systems, typically in the field of aquaculture, and might offers an alternative to antibiotics for these systems [186–189].

The idea to use viruses or phages to control aquatic pathogenic bacteria or harmful algae is not new. In the 1960s, researchers who worked on isolation and characterization of cyanophages pointed out that it would be useful to be able to control (harmful) algal blooms (HABs) with viruses. Thus, beginning with Safferman and Morris [190], researchers speculated on the potential importance of cyanophages as controlling agents against cyanobacterial blooms based upon the results of a few experiments. Typical experiments were conducted in small ponds or enclosures where cyanobacterial biomass reduction was demonstrated when cyanophages were present/added [191–194]. However, when cyanophages were declared unlikely to be useful as biological control agents because of technical problems and feasibility, and unknown ecosystem cascading effects, such ecological investigations largely ceased [29]. Among key issues, there was obviously the fact that aquatic environments are generally large and open (e.g. enclosed systems would obviously be a better target) and that lysogenic hosts could gain new phenotypic characteristics, including superinfection immunity, resistance, antigenic changes, enterotoxin production and virulence factors, because of virus-mediated gene transfer. Moreover, it became apparent that resistant strains, selected for by the varying activity of the viruses, would arise quickly within populations [4]. It still remains a goal though to use viruses to control HABs, but this will require a refined understanding of the biochemical processes associated with infection and lysis. Such efforts have been suggested for bloom-forming species such as the toxic dinoflagellate *Heterosigma akashiwo* [195].

Since phages are naturally occurring, there may be substantially fewer problems involved in obtaining regulatory approval for their use in preventing and treating bacterial infections in aquatic animals. Phages are ubiquitous in soils, sediments, and aquatic environments, providing an enormous supply of candidates that can potentially be used to attack a wide range of bacterial infections. The specificity of phages in targeting only a single pathogenic bacterium could present a significant advantage over the use of conventional antibiotics, which are much more general in their suppression of bacterial growth and as such can damage beneficial host and environmentally-important bacteria, and stimulate the development of antibiotic-resistant strains of bacteria in aquatic environments. It is noteworthy that important information documenting the use of phages in experimental trials has been lost in lab reports and some non peer-reviewed literature may be difficult to access (see below).

While using citation and reference databases such as the ISI Web of Science and with key words like virus(es)/phage(s)/bacteriophages(s), therapy/biocontrol/bioremediation

and aquatic/aquaculture, only a few citations can be found, and they are mainly related to fish-farming. This is not surprising when considering that the aquaculture industry represents ~30% of the seafood used for human consumption, including millions of tons of fishes (and also crustaceans and mollusks) with a net value of billions of Euros (or USD). In parallel, it has been reported worldwide that aquaculture operations show increasing susceptibility to disease, and phage therapy has been considered for a few years as an alternate to antibiotics, and a supposedly eco-friendly approach to fish and shrimp health management.

There are examples dealing with the use, or attempted use, of phage therapy for preventing and controlling bacterial infections in aquaculture (farming) with subsequent diseases in fishes (trout, salmon, yellowtail, ayu), crustaceans (lobsters, shrimps), mollusks (oyster) and also corals. Phages have been demonstrated to be very active against many different pathogenic bacteria (such as *Flavobacter*, *Pseudomonas*, *Vibrio*, *Aeromonas*, *Lactococcus*, *Piscirickettsia* to name a few) supplanting or replacing the use of antibiotics widely used in aquaculture operations such as oxytetracycline, fluroquinolone, florfenicol, sulfadimethoxine-ormetoprim (Table 2).

Scientists have examined various fishes and crustaceans to determine whether phage-based therapy could be used to treat bacterial parasitic infections. Phages of the bacterial pathogens that affect fish, such as *Aeromonas salmonicida*, *A. hydrophila* and *Yersinia ruckeri*, have been reported, but the potential use of phages to control bacterial infections in fish has not been investigated. From Paterson et al. [196], likely the earliest published record of the application of bacteriophages to treat bacterial fish pathogens, to Imbeault et al. [197], a dozen of papers can be found, for instance, related to the application of bacteriophages to *Aeromonas salmonicida* fish pathogens. However, it is still unclear from these publications whether the host range of the *A. salmonicida* phages extends to any of the various atypical *A. salmonicida* subspecies.

Wu and Chao [198] investigated the effect on *Edwardsiella tarda* of the Φ ET-1 phage, isolated from a pond in Taiwan. In *in vitro* experiments, this phage was shown to be capable of producing lysis in 25 out of 27 strains of *E. tarda* (i.e. >90%) and reduced the total number of bacteria to less than 0.1% of the starting concentration within 8 h (with an initial concentration of 1.2×10^{12} bacteria mL^{-1} and a MOI of 0.08). During this time period, the number of plaque-forming units (pfu) of the bacteriophage increased from 10^8 to 10^9 pfu mL^{-1} . This was the first demonstration of biological control of pathogenic bacteria by phages in infected farmed fish.

However, it was not until the late twentieth and early twenty-first century with the works of Nakai and Park and colleagues [186,199,200], that the first applications of phage therapy in fishes were published. In their various experiments, these authors showed that the oral or parental administration of phages (typically using phage impregnated food pellets) not only allowed the yellowtail fish (*Selora quinqueradiata*) infected with *Lactococcus garvieae* to recover, compared to untreated fishes, but also that the phage treatment could prevent subsequent infection by other bacteria. They could use bacteriophages to treat infections of ayu (*Plecoglossus altivelis*) and this did not harm the normal gut microbiota. More interestingly, in a field trial, when ayu were fed phage-impregnated food, Park and Nakai [200] also showed that neither phage-resistance nor phage-neutralizing antibodies were detected and that the daily mortality of treated fish decreased regularly.

Such a reduction in mortality rates was corroborated by Imbeault et al. [197] in a study of phage therapy in the treatment of furunculosis in salmon, caused by the pathogen

Table 2. Examples of phage therapy applied to pathogenic bacteria infecting fishes or shrimps (modified from Almeida et al. [189]).

Treated organism	Bacterial pathogen	Phage type	Phage application	Effects or results	References
Brook trout <i>Salvelinus fontinalis</i>	<i>Aeromonas salmonicida</i> HER 1107	HER 110	Addition of phage stock suspension (10^9 pfu/ml) in aquarium water	Delay by several days of the onset of furunculosis	[197]
Yellowtail <i>Seloria quinqueradiata</i> and Ayu <i>Plecoglossus altivelis</i>	<i>Lactococcus garvieae</i> formerly <i>Enterococcus seriolicida</i>	Siphoviridae isolated from diseased fish and sea water in fish culture cages	Oral administration of phage-impregnated feed or intraperitoneal injection	Protection or cure effect with increase of the survival rate	[186]
Yellowtail <i>Seloria quinqueradiata</i>	<i>Lactococcus garvieae</i> formerly <i>Enterococcus seriolicida</i>	Siphoviridae isolated from diseased fish and sea water in fish culture cages	Oral administration of phage-impregnated feed or intraperitoneal injection	Protection or cure effect with increase of the survival rate	[268]
Ayu <i>Plecoglossus altivelis</i>	<i>Pseudomonas plecoglossicida</i>	Podoviridae (PPPpW4) and Myoviridae (PPpW3)	Oral administration of phage-impregnated feed (10^7 pfu/fish)	Reduction of infection and increased fish survival	[200]
Ayu <i>Plecoglossus altivelis</i>	<i>Pseudomonas plecoglossicida</i>	Podoviridae and Myoviridae isolated from diseased Ayu and the rearing pond water	Oral administration of phage-impregnated feed	Protection against experimental infection	[199]
Atlantic salmon <i>Salmo solar</i>	<i>Aeromonas salmonicida</i>	<i>A. salmonicida</i> phages O, R and B	Injection ($>10^8$ pfu/fish), oral administration ($>10^5$ pfu/g) and bath ($>10^4$ pfu/ml)	No protection, lower rate mortality but similar absolute mortality	[201]
Shrimp larvae <i>Penaeus monodon</i>	<i>Vibrio harveyi</i>	Siphoviridae isolated from oyster tissue and shrimp hatchery water	Amendment of water in hatchery tanks with phage suspension (10^6 pfu/ml)	Improvement of larval survival	[188]
Shrimp larvae <i>Penaeus monodon</i>	<i>Vibrio harveyi</i>	Siphoviridae	<i>In vitro</i> amendment with phage suspension (10^9 pfu/ml)	Improvement of larval survival	[269]

Aeromonas salmonicida. However, during a comparison of orally-administered phage treatment (1.88×10^5 pfu g⁻¹ fish⁻¹ day⁻¹ for 30 days) and antibiotic treatment (10 mg kg⁻¹ bw⁻¹ day⁻¹) [201] in Atlantic salmon infected with furunculosis, the results obtained showed that the antibiotic treatment was significantly more effective than the phages. These authors suggested that it would thus be difficult to control furunculosis in the Atlantic salmon by simply using bacteriophages.

More recently, Ronnest et al. [202] isolated and characterized 22 phages infecting the bacterial pathogen *Flavobacterium psychrophylum*, which is implicated in various diseases in Danish farmed rainbow trout. Eighteen of the 22 phages were strain-specific, and displayed varying degrees of infectivity. The high lytic potential, very short latent period, and high specificity of the phages isolated from this pathogen look very promising. This provides a justification for carrying out an in-depth exploration of the use of bacteriophages to treat disease in farmed fish.

The potential application of phage therapy in the rearing of shrimps has also been investigated [188,203]. Some bacteria, such as *Vibrio harveyi*, can affect the health and development of the shrimp larvae, and even kill them. Due to the prohibition of antibiotic use in shrimp farming, it is essential to find other means to control pathogenic bacteria. The microcosm study by Karunasagar et al. [188] using *Penaeus monodon* larvae infected by *V. harveyi* showed that a phage treatment with 2×10^6 pfu ml⁻¹ allowed 85% of the larvae to survive, compared to only 10% survival for those that received the control treatment without phages. Crothers-Stomps et al. [203] reported the isolation of lytic bacteriophages against *Vibrio harveyi* and also discussed the possibility of applying phage therapy.

Other examples can be obtained from mollusks [204,205]. The bacterium *Vibrio vulnificus* is related to *Vibrio cholera*. *Vibrio* spp. occur naturally in estuarine, brackish and oceanic waters and have been demonstrated to prefer to attach to phytoplankton, protozoan grazers, and copepods in seawater. When oysters filter-feed on the planktonic organisms, pathogenic *Vibrio* spp. become concentrated in their tissue. People can come into contact with the highly pathogenic *Vibrio vulnificus* by eating raw oysters or by waterborne contact of an open wound when *V. vulnificus* is present in high enough concentrations [206]. People who suffer from liver damage are particularly at risk for infection likely due to increase iron stores in their blood, and *V. vulnificus* diseases is the leading cause of food-borne illness in many places [207] in the United States. Cervený et al. [205] isolated phages that prey naturally on the *Vibrio* from oysters, grew the phages in the laboratory, and then injected solutions containing concentrated amounts of the virus into the tail veins of mice infected with *Vibrio*. They found that the phages cured the mice even well after they had begun experiencing symptoms of the disease. Such a result led to the idea that one could try to submerge the harvested oysters in vats of phage-treated water, allowing them to filter in the phage and kill off the *Vibrio vulnificus* before the oysters reach the market and the consumer. More recently it has been proposed that oysters undergo post-harvesting processing during harvest in the summer months via heat or pressure treatment to reduce concentrations of pathogenic *Vibrios* (FDA 2009), but with so much protest that the FDA requirement was later lifted. This illuminates the need for more proactive treatment, possibly including phage therapy for reduction of the dangerous pathogenic *Vibrio* spp. for shellfish.

Phage therapy may also provide an effective way to treat some infectious diseases in corals [208]. For example, in the study carried out by Efrony et al. [209], bacteriophages were isolated from two types of bacterial pathogens: (1) *Vibrio coralliilyticus*, which causes

tissue lysis in *Pocillopora damicornis*, and (2) *Thalassomonas loyaeana*, which produces bleaching in *Favia fava*. Using these phages in controlled aquaria, the authors observed that these infections could be controlled by their specific viruses. The phage attached to the pathogen in the water and was carried to the surface of the coral, where it multiplied as a result of bacterial lysis. The phage then remained associated with the coral, thus helping to protect it against other subsequent infections.

Studies to date have demonstrated that specific phages do have the potential capacity to produce both a significant reduction in the impact of bacterial pathogens, and a beneficial impact on the survival of the resource they are intended to protect. The main idea is that ‘my enemy’s enemy is my friend’ [210]. However, the effectiveness of phage therapy to fight bacteria depends on various parameters: the purity of the phage preparation, the use of lytic-phase only viruses or phages, the width of the spectrum of infection, the mechanism and speed of development of bacterial resistance, the highly diverse phenotypes and genetic diversity of both parasite and pathogen, and specificity of the phage (in contrast to antibiotics, phages infect only the target bacteria). The response time and relatively low cost make future treatment of many bacterial diseases possible. A better understanding of the levels of adsorption, host specificity, and the lytic potential is now more pressing than ever, since if bacteriophages are to be used successfully, this will have to involve a group of phages (described as a cocktail) with a fairly wide combined spectrum of action in order to cover all the phenotypes of typical bacterial pathogens associated with a given disease. Note, similar to medicine applications, it is likely that the best solution might be an optimized combination of antibiotics and phages [184].

Citing Munn [211] who wrote in a part of his review dealing with marine viruses as biological control agents: ‘*although the idea of viruses as biocontrol agents is an appealing prospect, the diversity and complexities of life in the sea must be considered carefully; more knowledge and great caution is needed before we attempt to apply processes similar to those used in agricultural biocontrol on land (Secord 2003)*’ [212].

Under-explored topics and future prospects

As outlined in this review, a wealth of information has accumulated during the last 20 years of virus ecology and related studies. The previously underemphasized role of viruses in aquatic environments has changed. It is now known that viruses can have a critical influence on microbial food webs and biogeochemical cycles [30,213]. Whereas the field has expanded rapidly, leading to an exponential increase in high quality scientific literature (e.g. [3], Figure 1), the specific areas of research are clustered around ‘mainstream’ issues. Therefore, despite intensive research activities, other deserving areas of investigation have been relatively ignored. Though by no means exhaustive, this section aims to identify important, less broadly considered areas for future research (Figure 4). As mentioned earlier, there are obvious differences between marine and freshwater environments. Even when considering only freshwater, rivers and other flowing waters are strongly underrepresented in the field of virus ecology. In addition, habitats like sediments or even smaller entities like suspended particles are poorly studied. As was once stated, ‘there is no arena so small nor so large that it does not have an ecology’ (‘arena’ in this case used as the space in which ecological processes take place [214]).

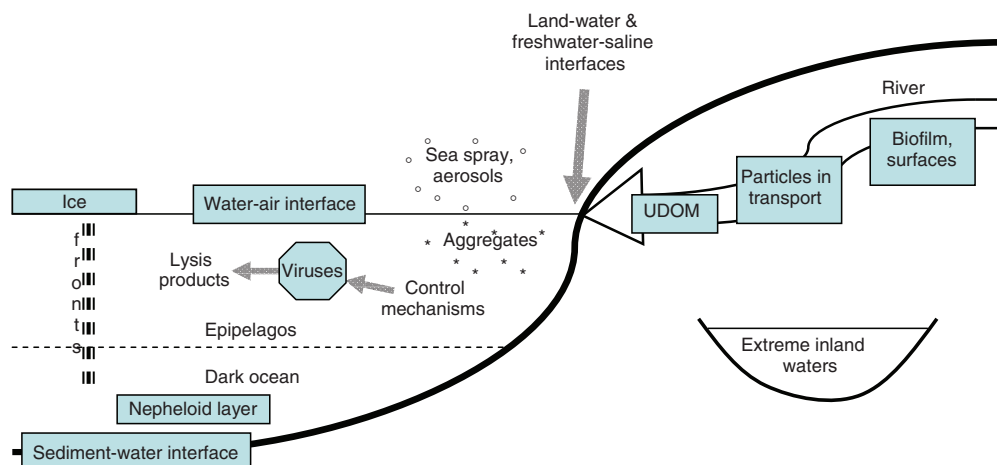


Figure 4. Schematic representation of some understudied environmental aspects in virus ecology of the ocean, rivers and inland waters. The controlling mechanisms on viruses and the lysis products are of interest also in inland water systems. UDOM: utilizable (young) dissolved organic matter linked to viral activity may be of particular importance in riverine waters with typically high load of recalcitrant carbon.

Marine vs. Freshwater

An excellent comparison of knowledge between marine and freshwater viroplankton has been recently provided by Wilhelm and Matteson [4]. Therefore, our aim is to continue further on points presented in that work. A main conclusion was that both environments share many commonalities in relation to virus activity, but some salient differences exist. There are indications that viral abundance tends to be higher in freshwater compared to marine environments with numbers as high as 10^8 ml^{-1} in estuaries and very productive lakes, and maximum values of up to $2 \times 10^9 \text{ ml}^{-1}$ in Mono Lake, California [4,213,215]; however, exceptions are reported [5]. This observation may be based on the generally higher biological productivity of most inland waters. The observed variability in viral abundance is greater in freshwater than in most marine environments. This makes sense considering the more pronounced effect of seasonal cycles in many lakes and rivers [50]. Generally, the linkage between seasonal variability and virus abundance is still not well documented, and many questions remain unresolved in both types of ecosystems, thus calling for further long-term studies. Bacterial burst size appears to be generally larger in freshwater (e.g. 20–40 for freshwater vs. 20–25 for marine environments; [4,213] and also the VPR [50]. In some groups, viruses in inland waters may be genetically distinct from their marine counterparts [216,217]. Regarding genetic diversity of viruses, freshwater systems are by far less well-studied compared to marine environments. Interestingly, there have been several comparisons of virus- vs. grazing-induced bacterial mortality in freshwater [4,49,123,218]. However, the triggers responsible for the different patterns of mortality in prokaryotes are still studied only in a rudimentary fashion in both aquatic environments. Comprehensive knowledge on abundance, production and host mortality in marine vs. freshwater systems, is essential to understand potential differences in carbon flux or in remobilization of growth-limiting nutrients (e.g. P in many freshwaters vs. other nutrients in marine systems) bound in host cells.

Flowing waters

Surprisingly, in river and inland flowing waters, virus ecology is underrepresented or even largely ignored, i.e. ecological studies on viruses in flowing water ecosystems are strikingly scarce. Some relevant studies are listed in Peduzzi and Luef [213,219], very recent work is specifically examining river-related environmental properties, like flow regime and particle load, or seasonal effects [4,219–221]. However, in very recent time the interest among limnologists and microbial ecologists in this field is growing. Flowing waters and their floodplains are particularly interesting since pronounced environmental heterogeneity implies differences in virus-related processes on narrow spatial and temporal scales (for example $0.3\text{--}11 \times 10^7 \text{ ml}^{-1}$ viruses in a river-floodplain system of the Danube; [219,220]). Moreover, river systems are often particle-rich environments, thus exhibiting heterogeneity even on very small spatial scales with up to $5.4 \times 10^9 \text{ viruses cm}^{-3}$ (see below). It may be of particular interest for example investigating whether disturbances by flooding influences viral-induced mortality of prokaryotes, or whether viral lysis contributes significantly to a pool of rapidly cycling carbon in an environment with typically high proportions of aged and recalcitrant carbon. So far, almost nothing is known about the significance of viral activity on the carbon cycle in riverine systems. Finally, considering all types of inland waters (lakes, rivers, wetlands) information on viral dynamics could be used to develop an amended understanding and a better management of freshwater resources.

Dark ocean

In the marine realm it is evident that the so-called ‘dark ocean’ has been poorly studied compared to the sunlit oceanic surface waters. However, the dark ocean is the largest reservoir of ‘active’ dissolved organic carbon (DOC), is the largest habitat on earth, and contains the largest pool of microorganisms – thus also of viruses – in aquatic systems [222]. The limited number of investigations has revealed that in the meso- and bathypelagic ocean, the viral abundance may range between 4×10^4 and even 10^7 ml^{-1} (see review of Aristegui et al. [222]). Some bathypelagic environments (like near hot vents; [41]) or in the Antarctic bottom water [115] display rather high numbers (approx. 10^6 ml^{-1}). High viral abundance is often found when prokaryotic host numbers are high as well [48,222]. A substantial difference in VPR among sediments (typically lower) and water column (higher values) apparently exists [223], although higher variability of this ratio is reported for benthic environments [39,224]. Nevertheless, viral abundance decreases in a typically monotonic fashion (with some depth-specific maxima) from the surface to deeper layers [36,110,222]. The life span of free viruses is suggested to be much longer in deep waters due to different physical settings (e.g. lower average temperature, lack of solar irradiation). This apparently also contributes to the relatively high VPR observed in the deep ocean. Some studies report on lower virus-mediated mortality together with higher frequency of lysogenic cells in deeper waters and a negative correlation of lysogeny to total prokaryotic abundance (see Weinbauer et al. [158]). This can be viewed as a survival strategy for viruses at low host abundance, which also corroborates the substantially longer viral turnover times observed in the deep North Atlantic than typically reported for the epipelagos. This suggests that the deep ocean viral community originates partly from allochthonous sources (e.g. sinking particles), lateral transport and/or significant lysogenic production [115,222,223]. A potential lack of viral control on the deep-water prokaryotic

community would also be of consequence for the oceanic carbon flux, which cannot be accurately modeled without further investigation into this interesting research area.

Freshwater and marine viriobenthos

The virus ecology of sediments is extensively discussed in [39,224]. Benthic viruses are abundant in both marine and freshwater sediments with numbers from 10^7 to 10^{10} g⁻¹ of dry sediment [39]. The scarce data suggest that viral production and decay rates in freshwater sediments could also be high. Estimates of global abundance in the top 1 m of freshwater and marine sediments are 0.5 and 28.7×10^{28} viruses respectively. Rough estimates of production are 0.6 and 34.4×10^{28} viruses day⁻¹, suggesting an average turnover time of 20 h [39]. This supports the evidence that the viriobenthos is of ecological importance in both marine and freshwater ecosystems. However, large numbers of visibly infected cells have not been documented, leaving many open questions. For example, in sediment of a productive freshwater reservoir viruses destroyed only 3% of bacterial production [225]. Interestingly, benthic viruses were found to be important in deep-sea ecosystems. On average 7.7×10^{12} viruses are estimated per m² of oceanic sediment (top 1 cm), with an assumed predominance of *in situ* production rather than a downward flux of viruses associated with settling particles [224]. Further, >99% of the prokaryotic infection appears to be lytic, suggesting that deep-benthic viruses are functionally active. It was estimated that benthic viruses are responsible for the abatement of 80% of the total prokaryotic heterotrophic production in global deep-sea sediments [224]. Thus, viruses may be the main agents of prokaryotic mortality in deep-sea sediments, shunting most of the prokaryotic carbon production into organic detritus. This labile organic material is thought to contribute to some 35% of the total benthic prokaryotic metabolism, increases turnover and promotes the recycling of key elements.

Viriobenthic diversity and community structure are even less studied. There are indications that the viriobenthic assemblages might be highly diverse and apparently are distinct from the viroplankton [39,93,226].

In summary, when comparing marine and freshwater sediments, some differences – like generally higher abundance and probably lower impact on hosts (reported as a paradox) in some freshwater environments – may exist [227,228]. However, there is definitely a general gap in knowledge, and benthic virus ecology needs to be developed further in the future (compare [3,39]). Even though we have some snapshots of information in freshwater and marine sediment environments, it is far from comprehensive. In addition, methods to process sediment samples for viral analysis are time-consuming, and there is much controversy about the best methods to employ to study viral dynamics.

Biofilms

One particular aspect of the viriobenthos (almost completely ignored) is environmental biofilms. Biofilms are formed wherever free-floating cells encounter surfaces and form sessile communities associated with a self-produced hydrated polymeric matrix. Particularly in running waters like streams, biofilms have been recognized to influence biogeochemical processes on a broader scale [229]. Some of the available work was reviewed by Sutherland et al. [230] and synthesized by Weinbauer et al. [223]. Despite not being metabolically active and not motile, viruses are able to penetrate biofilms by using enzymes. This may also

influence the integrity of the biofilm. Coexistence may develop between viruses and host cells, thus, biofilms might be significant reservoirs for bacteriophages or other types of viruses. In an experimental study it was also found that virus-sized particles can be entrapped and concentrated 100-fold within wetland biofilms [231]. Recent research indicates that, particularly in running water environments, biofilms may contribute significantly to ecosystem processes [229]. As living zones of transient storage, biofilms bring hydrodynamic retention and biochemical processes into close spatial proximity, thus influencing biogeochemical processes [232]. Biofilms harbor highly efficient ecological communities that may contribute to the influence that headwater streams have on rivers, estuaries and even oceans through longitudinal linkages of local biogeochemical and hydrodynamic processes [229,232]. The urgent need for studies dealing with natural viral assemblages and their functions in biofilms is therefore evident.

Suspended particles, floating aggregates

A comprehensive review on the viral ecology of inorganic and organic particles of aquatic systems is provided by Weinbauer et al. [223]. Recent methodological progress was made by the use of Confocal Laser Scanning Microscopy to visualize and quantify viruses and their hosts on particles together with the spatial distribution and the structure of floating aggregates [59,60]. Important factors controlling viral abundance are size, age and quality as well as exposure time of viruses to aggregates [116,219,221,223,233]. This results in a range between 10^5 and 10^{11} attached virus particles cm^{-3} or ml^{-1} . In turbid riverine water up to 35% of the viruses were found to be attached to suspended matter [220]. Weinbauer et al. [223] also reported that viral abundance and VPR on particles tend to be higher in marine than in freshwater environments, a feature that might be due to different types of particles, flocculation in marine environments due to ionic character, or to methodological differences.

The scarce data suggest that naturally occurring aggregates are rather viral scavengers or reservoirs than viral factories (see Weinbauer et al. [223]). Removal (via adsorption) of viruses by the presence of suspended particles can stimulate the growth of free-living host cells e.g. by reducing viral lysis [221]. Potential inactivation of viruses due to attachment was suggested for both marine and freshwater aggregates [234,235,236]. But, also protection from decay has been proposed [237], and viral release has been observed in an experimental approach with isolates and artificial particles [238]. Viral lysis of host cells may also affect the formation, fate, size and stability of aggregates, e.g. by changing the DOM field or providing sticky colloidal precursors for aggregate formation [25,239]. Thus, via impacting aggregate formation, viruses potentially also influence carbon transport, either vertically (biological pump of the ocean) or horizontally (watershed and riverine transport). All this would have consequences for host diversity, food web structure and biogeochemical cycles. Particularly rivers are strongly impacted by climate or anthropogenic alterations, which are likely to change the flow regime and particle load. Investigating the effect of viruses on these transport mechanisms will be an important task for assessing the biogeochemical significance of viruses.

To the best of our knowledge, virus diversity on particles has not been studied so far. However, it is suggested that particle-associated viruses differ in their community composition from their free-living counterparts [233]. Furthermore, nothing is known on viral life strategies (lytic vs. lysogenic) in association with floating particles. Whether the presence and nature of particulate matter affect the frequency of lytic vs. lysogenic cycles

need to be investigated. The research on a specific type of particles, black carbon, is in its infancy, however, potential significance in aquatic systems has been proposed by Weinbauer et al. [223] and Cattaneo et al. [240].

For an ecological understanding of virus – particle relationships, more specific data on pools, rates and processes have to be collected, and methodological refinement and progress are needed to accomplish this task. This is particularly important since vertical (ocean, lakes) and horizontal (rivers) transport of particles influence system productivity and organic carbon flux, where virus have the potential to play significant roles. Results can be scaled up to assess the relevance of virus – particle interactions in ecosystem function and biogeochemical cycling.

Transition zones, ecotones

Spatial patchiness in the distribution of viruses is in general not well understood. The scale of patchiness may range from μm to the ecosystem scale. There are some larger-scale studies available on horizontal variations (mostly related to trophic gradients; [53,114,241,242], medium-scale in freshwater [47,220,243] and at the micro-scale [57,59,60]. However, transition zones and ecotones, such as the terrestrial – water interface, freshwater – saline water transition zones, nepheloid layers, tidal and other fronts of water bodies, small-scale sediment – water interface, air – water interface, biofilms, surfaces of living aquatic plants and animals, ... necessitate much closer examination. At many types of interfaces, turbulence may be an important factor that has to be considered as suggested by Malitis and Weinbauer [244]. Thus, transition zones apparently represent a promising field for aquatic microbial ecologists with significant relevance for our understanding of microbially mediated processes even on a larger scale.

Diversity, genomics, metagenomics, proteomics

It should be strongly emphasized that this is a field in its infancy, and surprisingly for freshwater viruses, and to the best of our knowledge, no large metagenomic studies (like for the marine environment by Breitbart et al. [93]) have been conducted so far. New molecular approaches are becoming increasingly important in microbial ecology, and functional genomics, metagenomics and proteomics may assist the scientific community in unraveling the questions still present in the fields of viral ecology and evolution. Development of affordable, high-throughput molecular analyses and improved management of large data sets will illuminate many arenas of virus and aquatic ecology in the future [4,21]. The ultimate ecological test will always have to be performed in the real-world environment [25].

Overview on additional knowledge gaps

To conclude, one will have understood that viruses (especially phages) are very important biological entities in aquatic ecosystems and indeed key players of the game, with an estimated 10^{23} microbes being infected by a phage every second [245]. Without attempting to be exhaustive, we present a list of research needs for the short-term (2–5 years). These are areas with immediate need for further research, so as to bring the aquatic virus research community to the next level of scientific discovery.

- (1) Studies of viral enumeration and dynamics need to be standardized, or at least tested across a multitude of environments. The research community is currently at a crossroads where conflicting information is being presented about similar environments. Sharing of methodological details and inclusion of multiple redundant methods in research will help the global research community to understand how much of the observed variability is methodological in nature.
- (2) The interplay between viruses and eukaryotic grazers as controlling agents of the prokaryotic plankton is an extremely important issue, especially for the development of accurate models of C and nutrient transfer. As pointed out by Miki and Jacquet [128], this is particularly critical since there are many possible interactions between viruses, prokaryotes and small predatory flagellates.
- (3) Data regarding the significance of the two major replication strategies of viruses (lytic vs. lysogenic) are scarce. Most importantly, understanding how environmental conditions drive the selection of one cycle or the other is vital in light of global climate change.
- (4) We still only poorly understand the chemical nature and bioreactivity of lysis products fueling the DOM-pool, the potential role of viral lysozymes in the environment, and the release of dissolved nucleic acids due to host lysis. The biogeochemical relevance of the virus shunt [24] in the various highly diverse aquatic environments (from surface and deep-ocean to flowing waters) warrants further investigation.
- (5) The mechanisms of control on viral activity and proliferation (e.g. defense mechanisms, abiotic environmental parameters like solar irradiation, temperature, salinity, pH, etc.) are still important especially considering the possible changes in times of rapid global climate change.
- (6) Viruses infecting Archaea are poorly studied. Archaea are likely to represent a significant percentage of the prokaryotic community, even in non-extreme conditions [246–248]. Archaeal viruses that are currently known exhibit a wide range of peculiar morphotypes, and most of them are genetically very different from those of other known eubacterial viruses. Functional aspects are largely unknown and given the importance of Archaea, they deserve further attention. This is also true while considering virus-zooplankton relationships for which almost nothing has been published so far.
- (7) Researchers from a range of disciplines must come together to understand more effective ways to translate data from small-scale studies needs to larger spatial and temporal scales to answering regional and global scale questions. A well-established global database funded by multiple collaborative sources could facilitate our understanding of the role of viruses as stabilizing or destabilizing agents for biogeochemical cycles and ecosystems.
- (8) Finally, transduction in aquatic environments is poorly elucidated and may profoundly affect the co-existence of viruses and bacteria.

Acknowledgments

We thank Roberto Danovaro who accepted considering this manuscript for the launch of AIOL, and the excellent work of anonymous reviewers to improve the former version of the manuscript. This study is a contribution to the ANR French project AQUAPHAGE.

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