Viruses in freshwater ecosystems: an introduction to the exploration of viruses in new aquatic habitats

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SUMMARY

1. Viruses have become widely recognized as the most abundant biological entities and important players in aquatic environments, and this realization has profoundly changed our conceptual understanding of the functioning and regulation of aquatic ecosystems in the last two decades. However, most of this research has focussed on marine viruses, especially in pelagic environments.
2. Here we introduce a special issue of *Freshwater Biology* dealing with viruses in freshwater ecosystems. It represents the first attempt to summarize progress in freshwater viral ecology made by diverse research groups and to direct attention of viral ecologists towards fresh waters.
3. Six review-type articles and ten original research papers cover a wide range of aspects of freshwater viral ecology. This includes reports on the distribution of freshwater viral communities in contrasting habitats (e.g. sediments, wetlands, littoral zone, open waters), on different roles of viruses in freshwater ecosystems (e.g. mortality rates of bacteria and phytoplankton, transduction, influence on bacterial diversity and organic matter), and on different types of viruses (bacteriophages, cyanophages, algal viruses, and a fish-pathogenic virus).
4. Collectively the series of papers presented in this special issue indicates that freshwater environments cover great habitat diversity and that the significance of some of the mechanisms controlling viral dynamics and impacts may differ between freshwater and marine habitats.

Keywords: ecology, fresh water, review, virus

Introduction

The discovery of large abundances of viruses in pelagic marine environments (Bergh *et al*., 1989; Proctor & Fuhrman, 1990) opened a new research area, which has been expanding rapidly over the past 15 years. We now know that viruses are important players in the aquatic environment, and this realization has changed our conceptual understanding of the function and regulation of aquatic ecosystems, from microscale microbial processes to global biogeochemical cycling (e.g. Wommack & Colwell, 2000; Weinbauer, 2004; Suttle, 2007). Research in aquatic viral ecology was initiated mainly by marine bacterioplankton ecologists, who started to pursue this, at the time, new line of research in the early 1990s. A few years later, a number of second-generation marine viral ecologists expanded the field, and now a number of research groups world-wide are investigating diverse aspects of viral ecology. Research focussing

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on marine viruses now contributes significantly to the body of scientific literature that constitutes marine microbial ecology. A search in the ISI Web of Science for papers in marine viral ecology reveals rapid growth of the pertinent scientific literature. Publication rates have increased from 1–2 papers year^{-1} in the late 1980s to 20–30 year^{-1} in the early 1990s and >100 papers year^{-1} since 2004, concrete evidence of the increasing effort dedicated to marine viral ecology over the past decade (Fig. 1a). Similarly, the cumulative citations of papers in marine viral ecology have increased exponentially over the same period, reaching >3500 citations of marine viral ecology papers in 2006 (Fig. 1b).

While research efforts in aquatic viral ecology during the early years mainly focussed on the marine pelagic environment, it took only a few more years before other aquatic habitats such as lakes, rivers, and both marine and freshwater sediments, as well as soils, were approached from a viral ecology perspective. With a few years delay, research in freshwater viral ecology followed the same path as marine studies; it has increased significantly over the past 4–5 years, producing approximately 30 papers and >400 citations year^{-1} since 2005 (Fig. 2). Freshwater viral ecology clearly is a rapidly expanding research field.

This special issue represents the first initiative to compile the input of many research groups and to direct the attention of viral ecologists towards fresh waters. This effort serves to (1) provide an overview of the research foci and currently available information in freshwater viral ecology and (2) to initiate a discussion about the potential differences and similarities in the role of viruses in freshwater and marine ecosystems. While several reviews on marine viral

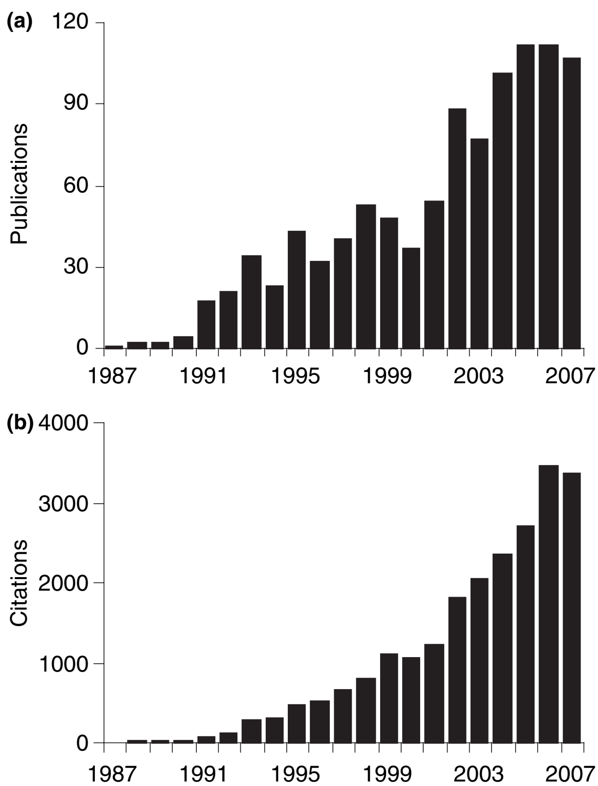

Fig. 1 Trends in (a) the number of publications and (b) the number of citations to these publications in marine viral ecology based on a search in the ISI Web of Science in November 2007. Key words used were virus, phage, marine, ocean, ecology and variants of these terms. The hits were subsequently screened and the publication list narrowed down manually to the pertinent papers.

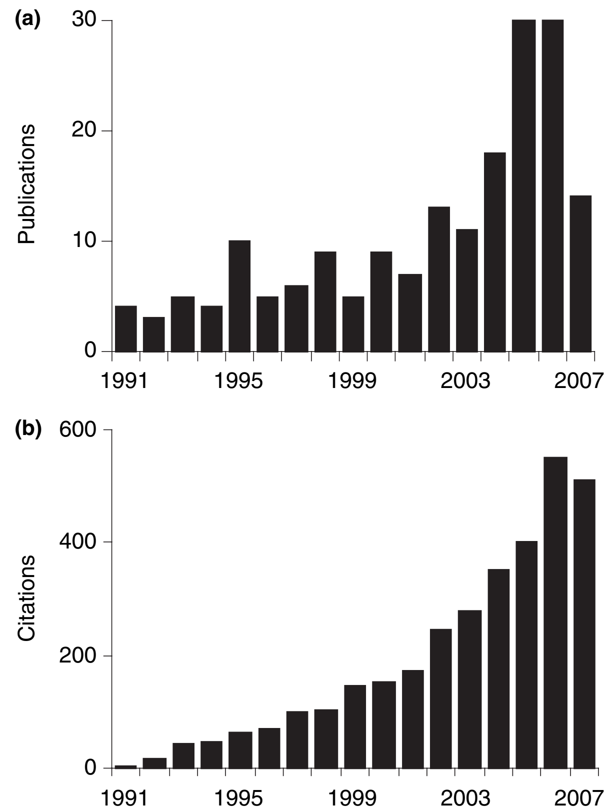

Fig. 2 Trends in (a) the number of publications and (b) the number of citations to these publications in freshwater viral ecology based on a search in the ISI Web of Science in November 2007. Key words used were virus, phage, freshwater, lake, river, ecology and variants of these terms. The hits were subsequently screened and the publication list narrowed down manually to the pertinent papers.

ecology have been published over the last decade (e.g. Fuhrman, 1999; Wilhelm & Suttle, 1999; Wommack & Colwell, 2000; Mann, 2003; Weinbauer, 2004; Suttle, 2005, 2007), including a recent special issue in the *Journal of the Marine Biological Association of the United Kingdom* (Raven, 2006), an overview of viruses in freshwater systems has not previously been attempted.

The set of papers presented in this special issue aims to diminish this imbalance. It consists of a mixed collection of review-type articles (6) and original research papers (10). The special issue thus summarizes to some extent the current knowledge in the field and provides a basis for further focus and for pointing towards future areas where increased effort appears to be particularly worthwhile. The presented set of papers covers a wide range of aspects of freshwater viral ecology, including viral distribution and community composition in different freshwater habitats (e.g. sediments, wetlands, littoral zone, open waters), and roles of viruses as mortality agents of bacteria and phytoplankton, mediators of genetic exchange, and driving forces of bacterial diversity and organic matter dynamics. The papers fall into several major categories, which broadly reflect the current focus areas in freshwater viral ecology.

Overview of the papers in the special issue

Viral ecology in freshwater versus marine environments

Wilhelm & Matteson (2008), Clasen *et al.* (2008), Danovaro *et al.* (2008) and Jackson & Jackson (2008) together provide a general overview of viral abundance and ecology in pelagic, benthic and wetland ecosystems, and discuss potential differences in the role of viruses between different marine and freshwater habitats. It is important to realize that the difference between freshwater and marine systems is not limited to the concentration of NaCl, but spans a wide range of biological, physical and chemical factors. Moreover, limnetic environments, like the oceans, comprise a diverse collection of habitats, including, for example, water-filled tree holes, small forest streams, hot springs and alpine lakes. Some of the central physiochemical properties of the lake environment which differentiates lakes from most marine ecosystems are the generally larger temporal and spatial gradients of factors such as temperature, pH, salinity and oxygen saturation, higher concentrations of terrestrially derived dissolved organic material (e.g. humic substances), and the physical delimitation of the lake ecosystem, with reduced exchange of genetic material with other environments. However, little is known about how these fundamental differences between marine and freshwater ecosystems affect the composition, production and control mechanisms of viruses in the respective environments. The present special issue sheds some light on these differences.

Clasen *et al.* (2008) report that slope coefficients from plots of viral versus bacterial abundance are significantly higher in lakes than in marine environments and suggest that the mechanisms controlling viral production and loss processes are fundamentally different in the two types of environments. Lymer, Lindström & Vrede (2008a) compared lakes with varying trophic status and humic content and also concluded that important differences exist among and even within lakes in terms of virus-induced bacterial mortality. This may have important implications for the impact of viruses on bacterial mortality and biogeochemical cycling in different environments, perhaps pointing to viruses being a less important mortality factor in lakes than in oceans, as suggested by Clasen *et al.* (2008).

Viruses in the benthos

In sediments, there appear to be ecosystem-specific differences in viral communities with generally higher densities of viruses in freshwater sediments (Danovaro & Serresi, 2000; Middelboe, Glud & Finster, 2003; Duhamel & Jacquet, 2006). Our understanding of benthic viral ecology is, however, rather limited, with many basic questions about the distribution and activity of benthic viruses still unresolved. This is exemplified by the unexplained paradox of high viral abundances but low impact of viruses in some freshwater benthic environments (Bettarel *et al.*, 2006; Filippini *et al.*, 2006) whereas viruses in marine sediments have been shown to multiply at high rates (Danovaro *et al.*, 2008; Middelboe, 2008). As many of the sediment studies have been performed using a wide variety of methods for quantifying viral activity, it is not yet clear to what extent these differences are system-specific or merely a result of different experimental approaches. This illustrates the general lack of understanding of the function and regulation of viruses in sediments, and emphasizes that benthic viral ecology is an area that needs much more attention. The study of Leroy *et al.* (2008) in this issue reveals that phage-homologous sequences in Seine River (France) sediment were most similar to temperate phages infecting Proteobacteria, the predominant bacteria in the investigated sediment. Although the study was rather limited in scope, such a correspondence supports the idea that temperate phages may play an important role in influencing the dynamics of bacterial hosts also in freshwater sediments.

Spatial and temporal variation

Assessing the regulation and dynamics at various spatial and temporal scales is a prerequisite to understanding the ecology of viruses and the impact of factors such as climate change. Thus, the spatial variation and dynamics of viruses within lake habitats are also investigated in this special issue. The papers by Lymer *et al.* (2008b) and Filippini, Buesing & Gessner (2008) provide basic characterizations of temporal and spatial variations in viral abundance and community composition on temporal scales ranging from hours to seasons and on spatial scales extending from littoral-pelagic gradients to between-lake variation. Analyses of the environmental factors potentially controlling viral community composition, abundance and impact suggest that these factors differ between seasons and lakes. The studies thus elaborate on the suggestions by Clasen *et al.* (2008) and contribute to elucidating which mechanisms are important in controlling the loss and production of viruses in lakes.

Viral impacts on host populations

A central prerequisite for understanding the role of viruses in natural environments is our ability to quantify their impact on bacterial and phytoplankton host population dynamics, *i.e.* to obtain solid data on virus-induced bacterial and algal mortality. As is clear from several papers in this issue (Danovaro *et al.*, 2008; Jackson & Jackson, 2008; Lymer *et al.*, 2008a; Tijdens *et al.*, 2008), we still do not have a general idea of the rates and regulation of viral production, despite numerous studies employing a variety of experimental approaches to estimate this fundamental variable in viral ecology. Using a modified dilution-culture approach, Tijdens *et al.* (2008) found that viruses caused high mortality of both bacterio- and phytoplankton, suggesting that viral lysis can exert a strong control on microbial communities in eutrophic lakes. Lymer *et al.* (2008a) provide the first comparative analysis of viral-induced bacterial mortality for 21 lakes differing in trophic status and humic content. According to this study, the importance of viruses for bacterial mortality is typically highest in humic lakes of moderate trophic status and it tends to be greater in the hypolimnion than in the epilimnion, as has been previously pointed out (Weinbauer & Höfle, 1998; Colombet *et al.*, 2006). Consequently, viruses may indeed have a significant impact on microbial mortality in lakes. However, the data are still much too scant to draw firm conclusions on the role of viruses in fresh waters compared with other habitats, again emphasizing the need for significantly more basic information on viral activity in fresh waters.

What happens to freshwater viruses and bacteria when they are discharged from rivers into the ocean? Cissoko *et al.* (2008) addressed this question in an estuary in tropical Africa, where viruses appear not to be a prominent agent for bacterial mortality. The consequences for both bacteria and viruses were tested in short-term experiments in which fresh water and sea water were mixed. Dilution of freshwater microorganisms with particle-free seawater resulted in a rapid and dramatic decrease in bacterial production and cell numbers, followed by the recovery of part of the community and subsequent production of viruses. In contrast, freshwater addition to seawater samples favoured marine bacterial growth and probably activation of lysogens.

Host-virus interactions

In addition to the whole-community approach, detailed studies of specific virus-host interactions can provide important insight into the diversity, dynamics and regulation of viruses and host populations. This approach was applied by several studies focusing on cyanophages (Deng & Hayes, 2008; Dillon & Parry, 2008), but also on the fish-pathogenic koi herpesvirus (Matsui *et al.*, 2008), and it has contributed details, sometimes unexpected, on the properties, dynamics and impact of specific viral populations. Development of tools for analysing viruses in aquatic ecosystems is essential for obtaining accurate measurements of their activity and for predicting the consequences of these activities. For example, the paper by Miki *et al.* (2008) presents a new model of viral impact on bacterial diversity and the potential consequences for organic matter cycling. The model takes into account the functional diversity of bacterial host populations with respect to substrate specificity and resistance against viral infection, thus extending previous models on virus-host interactions (Thingstad & Lignell, 1997; Middelboe *et al.*, 2001). The model outcome indicates that host-specific mortality induced by viral infection changes the bacterial utilization of different types of organic matter.

Viruses and genetic exchange

Microbial genomes are constantly modified by horizontal exchange of genetic material, and transduction is potentially an important mechanism promoting this exchange. In his review, Ogunseitan (2008) provides an account of the importance of transduction in fresh waters and highlights that this virus-mediated genetic exchange between bacterial cells is likely to be a major driving force behind the structural and functional diversity of prokaryotic populations and communities. He also reviews the historical demonstrations of transduction in freshwater systems, the theoretical approaches for understanding the impact of transduction on bacterial diversity. Finally, the paper addresses several unanswered questions and suggests future directions for research on transduction, concluding that 'only the top of the iceberg has been explored so far'.

Outlook

Considerable strides have been made during the last decade in understanding the role of viruses in freshwater ecosystems. As pointed out by Raven (2006), 'aquatic virology is clearly an expanded field of research with advances on many fronts'. However, despite relatively intensive research and an attempt in this special issue to compare commonalties and differences in viral ecology between freshwater and marine systems (Clasen *et al.*, 2008; Danovaro *et al.*, 2008; Wilhelm & Matteson, 2008), it is painfully clear that a number of rather major aspects remain unresolved. Open questions include integration of complex interactions among various types of organic matter and diverse groups of bacteria, viruses and higher trophic levels in aquatic food webs. Other important gaps are the unknown extent and ecological consequences of viral genetic diversity. Strikingly, no large metagenomic studies similar to those initiated in marine environments (Breitbart *et al.*, 2004) have been conducted for freshwater viruses. Further, the influence of viral activity on host diversity (e.g. by selective mortality, lysogenization and transduction) and on biogeochemical cycled are issues that have only marginally been raised. Although central pieces are continuously added to the puzzle, new studies at the same time tend to reveal even greater complexity, diversity and variability in the interactions between viruses and their hosts. These are signs that we have only just begun to explore and comprehend the enormous genetic and functional diversity harboured in viral communities. Clearly, we are still far from a deep understanding of the mechanisms underlying the influence of viruses on microbial diversification and biogeochemical processes.

Collaboration and interactions among individuals and laboratories can play an important role in fostering scientific progress. However, current research in viral ecology is mostly carried out in small research groups rather than as larger collaborative efforts. This is witnessed, not least, by the diverse list of topics and authors in this special issue. The result is a somewhat fragmentary input to the emerging conceptual view of viral ecology. Nevertheless, Working Group 126 of the Scientific Committee for Oceanographic Research (SCOR), which deals with the 'role of viruses in marine systems', represents a first attempt at providing a platform for interaction and coordination of research in viral ecology at the international level. At the national level, attempts to coordinate research in viral ecology have also been made in the U.K. (Virus Ecology Group: VEG) and, more recently, in France (Réseau français de Virologie Aquatique, de la Génomique à l'Ecologie: RAVAGE). Extending such initiatives and seeking coordination through networks and larger projects that include both marine and freshwater viral systems could be an effective way forward in the attempt to bundle resources and expertise of the various research groups active in viral ecology and, thus, to continue making swift progress in the field.

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