ROLES OF VIRUSES IN AQUATIC ECOSYSTEMS

Telesphore Sime-Ngando and Jonathan Colombet
UMR CNRS 6023 LMGE, Laboratoire Microorganismes: Génome et Environnement, Clermont Université Blaise Pascal, Campus des Cézeaux, B.P. 80026, 63171 Aubière Cedex, France

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Content

Summary

Since the discovery around the 1980s that viruses of microbes are abundant in marine ecosystems, aquatic viral ecology has grown increasingly to reach the status of a full scientific discipline in environmental sciences. A dedicated ISVM society, the International Society for Viruses of Microorganisms (http://www.isvm.org/), was recently launched. Increasing studies in aquatic viral ecology are sources of novel knowledge related to the biodiversity of living things, the functioning of ecosystems, and the evolution of the cellular world. This is because viruses are perhaps the most diverse, abundant, and ubiquitous biological entities in the world's oceans and freshwaters. They exhibit various lifestyles that intimately depend on the deep-cellular mechanisms, and are ultimately replicated by members of all three domains of cellular life (Bacteria, Eukarya, Archaea), as well as by giant viruses of eukaryotic cells. This establishes viruses as microbial killers and manipulators in the world's aquatic ecosystems. The present chapter sought to review the literature on the diversity and functional roles of viruses in these ecosystems, targeting a wide group of audiences, in an effort to help weaken the gap between the growing number of on-going laboratory studies and ideas, and the benchmark of teaching and learning. This chapter is focused

primarily but not solely on prokaryotic viruses (i.e. phages) in marine ecosystems, which form the bulk of our knowledge in modern aquatic viral ecology.

1. Introduction

Since the discovery around the 1980s that viruses of microbes are abundant in marine ecosystems, aquatic viral ecology has increasingly grown to reach the status of a full scientific discipline in environmental sciences, with the recent launch of a dedicated ISVM society, i.e. the International Society for Viruses of Microorganisms (http:// www.isvm.org/). As potential infectious agents of all types of living cells, viruses are the most abundant biological entities in the biosphere. They are omnipresent components of the microbial food web dynamics in a great variety of environments, including the most extreme ecosystems such as hydrothermal vents, hypersaline waters, deserts, etc. Moreover, in spite of the difficulties of routinely observing and describing biological nanoparticles, combined with the absence of conserved evolution tracers such as RNA ribosomal genes, we now consider viruses to represent the greatest reservoir of non-characterized genetic diversity and resources on earth. They contain genes that code for essential biological functions such as photosynthesis, making their hosts powerful vehicles for genetic exchanges in the environment. Because lytic viruses kill their hosts, they play fundamental roles in cycling nutrients and organic matter, structuring microbial food webs, governing microbial diversity and, to a lesser extent, being a potential food source for protists. As symbionts, viruses can also form long-lived associations with their specific hosts, reducing their fitness, or allowing infected hosts to remain strong competitors through a mutualistic relationship or symbiosis. In addition, the discovery and characterization of the unique group of archaeal viruses is influencing the field of prokaryotic virology, increasing our knowledge on viral diversity and changing perspectives on early stages of evolution.

It is thus obvious that recent studies in aquatic viral ecology are a source of novel knowledge related to the biodiversity of living things, the functioning of ecosystems, and the evolution of the cellular world. This is because viruses exhibit various life strategies that intimately depend on the deep-cellular mechanisms, and are ultimately replicated by members of all three domains of cellular life: Bacteria, Eukarya, and Archaea. This establishes viruses as both microbial killers and manipulators in the aquatic ecosystem. The present chapter sought to review the literature on the diversity and functional roles of viruses in aquatic ecosystems, targeting a wide set of audiences. The author believes that this is timely, will help to narrow the gap between the growing number of laboratory studies and ideas, and the benchmark teaching and learning. This chapter is focused primarily but not only on prokaryotic viruses (i.e. phages) in marine ecosystems, which form the bulk of our knowledge in modern aquatic viral ecology.

2. Basic Knowledge on the World of Viruses

2.1. Definition and Lifestyles

Viruses are biological entities consisting of single- or double-stranded DNA or RNA surrounded by a protein and, for some of them, a lipid coat. In aquatic systems, most viruses are tailed or untailed phages, with a capsid diameter often smaller than 250 nm,

based on direct transmission electron microscopy observation. Viruses have no intrinsic metabolism and need the intracellular machinery of a living and sensitive host cell for all processes requiring energy. They have various life cycles, all starting with diffusive passive fixation on specific receptors (often transporter proteins) present at the surface of a host cell, followed by injection of the viral genome into the host cell. In the lytic cycle, the viral genome induces the synthesis of viral constituents, including the replication of the viral genetic material. A number of progeny viruses are then produced and released into the environment by the fatal rupture of the host cell.

In the chronic cycle, the progeny viruses are episodically or constantly released from the host cell by budding or extrusion, without immediate lethal events. This cycle is less well known in plankton, but is common in metazoan viruses such as Herpes and Hepatitis viruses or rhabdoviruses. Chronic viral infection is a dynamic and metastable equilibrium process which ends with the lysis of the host cell after serial budding of lipid membrane-coated viruses, as seen in hosts of the marine protist *Emiliana huxleyi*. Recently, chronic infection without host lysis has been reported for the first time in a marine primary producer *Ostreococcus tauri*, where the low rate of viral release through budding (1 to 3 viruses $cell^{-1}$ day^{-1}) allows cell recovery and the stable coexistence of viruses and their hosts.

In the lysogenic cycle, the viral genome integrates the genome of the host cell and reproduces as a provirus (or prophage) until an environmental stress to the immune host cell sets off a switch to a lytic cycle. Both the provirus and the host cell benefit from lysogeny. Lysogeny provides a means of persistence for viruses when the abundance of the host cells is very low. Prophages may affect the metabolic properties of host cells which can acquire immunity to superinfections and new phenotypic characteristics such as antibiotic resistance, antigenic changes, and virulence factors. A variant to the lysogenic cycle is the so-called carrier state or pseudolysogenic cycle, where the viral genome is not integrated with the host genome but rather remains in an 'inactive state' within the host cell. There is no replication of the viral DNA, which is segregated unequally into progeny cells, most likely for a few generations. Pseudolysogenic viruses probably occur in very poor nutrient conditions where host cells are undergoing starvation and cannot offer the energy necessary for viral gene expression.

2.2. Viruses and the Tree of Life

There are three domains of life – Archaea, Bacteria and Eukarya – that consist only of cellular organisms. Because viruses lack the ribosomal RNA nucleotide sequence upon which these cellular domains of life are based, they cannot be integrated into the cellular tree of life, although susceptibility to virus infection is a common feature of all members of the three domains of life. In the absence of universal evolution markers for the entire viral world, viruses have been grouped by many different methods, according to various criteria: the nature of the host, the characteristics of the free virions (phenotype, genotype, resistance to organic solvents for viruses with lipid coat…), or even the name of the related illness, the laboratory or the researcher working on the targeted viruses. Viral groupings recognized by the International Committee on Taxonomy of viruses (ICTV) include orders, families, subfamilies, genera, and species defined as a group of viruses that constitutes a replicative lineage and occupies a

particular ecological niche. Recently, Hurst (2000) introduced the idea that the taxonomy of viruses and their relatives could be extended to the domain level, and suggests the creation of an additional biological domain that would represent the acellular infectious agents that possess nucleic acid genomes. The proposed constituents of this domain are items such as satellite viruses, virusoids or viroids, and the proposed domain title is *Akamara*, whose derivation from the Greek (*a* + *kamara*) would translate as "without chamber" or "without void".

A new way to classify phages has also been proposed based on the complete sequences of 105 viral genomes. This so-called "phage proteomic tree" places phages relative to their neighbors and all other phages included in the analysis, which is a method that can be used to predict aspects of phage biology and evolutionary relationships, and to highlight genetic markers for diversity studies. The approach is useful for those phages whose complete genomes have been deciphered (i.e. a minority of environmental viruses); primarily those belonging to a common pool of genes (e.g. following genetic recombination), or which have evolved from a common ancestor. It is well known that the genomes of some phages, if not most, are mosaics of genes from various sources, including other phages and their hosts.

3. Viruses Form a Huge Reservoir of Uncharacterized Biological Diversity

3.1. General Considerations

The most recent 9th report of the ICTV (http://www.ictvonline.org/index.asp?bhcp=1) includes 6 orders, 87 families, 19 subfamilies, 349 genera and 2284 virus and **viroid** species. This is mostly based on isolated hosts in laboratory cultures which, in the case of environmental samples, may not exceed 1% of the total prokaryotes. This implies that the diversity of environmental viruses is huge, although the bulk of the estimated 10^{31} viruses in the biosphere are unknown. Molecular approaches are thus critical and offer windows to the largest uncharacterized reservoir of diversity on the earth. PCR-based methods are restricted to chosen viral groups as no gene is universally conserved among viruses, while part of the existing diversity of these viral groups is missed because PCR primers are based on previously identified sequences described in public databases. Viral metagenomics gives access to an exhaustive view of uncultured viral diversity, and has so far revealed an important unknown diversity and an unexpected richness of viral communities.

3.2. Morphs and Phenotypes

The first descriptions of the global diversity of viruses are from the general forms of virus-like particles observed via transmission electron microscopy (Figure 1). In aquatic samples, viral phenotypes are limited, mainly including tailed or untailed particles with capsid heads, characteristics of bacteriophages. Tailed phages belong to the order *Caudovirales*, all of which are double-stranded DNA viruses that generally represent 8 to 43% of the total abundance of viruses in aquatic systems. Within *Caudovirales*, three families emerge as quantitatively dominant: *Siphoviridae* with long non-contractile tails (e.g. Phage lambda), *Podoviridae* with a short non-contractile tail (e.g. Phage T7), and *Myoviridae* with contractile tails of variable length (e.g. Phage T4) (Figure 1). In most

studies, untailed capsids dominate viral abundances. This may be an artifact due to the effects of mechanic shocks resulting from handling, primarily ultracentrifugation, because 96% of the 5500 specimens of described bacteriophages are tailed particles. In harsh environments, e.g. hot or anoxic, the diversity of viral forms increases with the increasing importance of archaeal hosts (see Section 3.4 below)

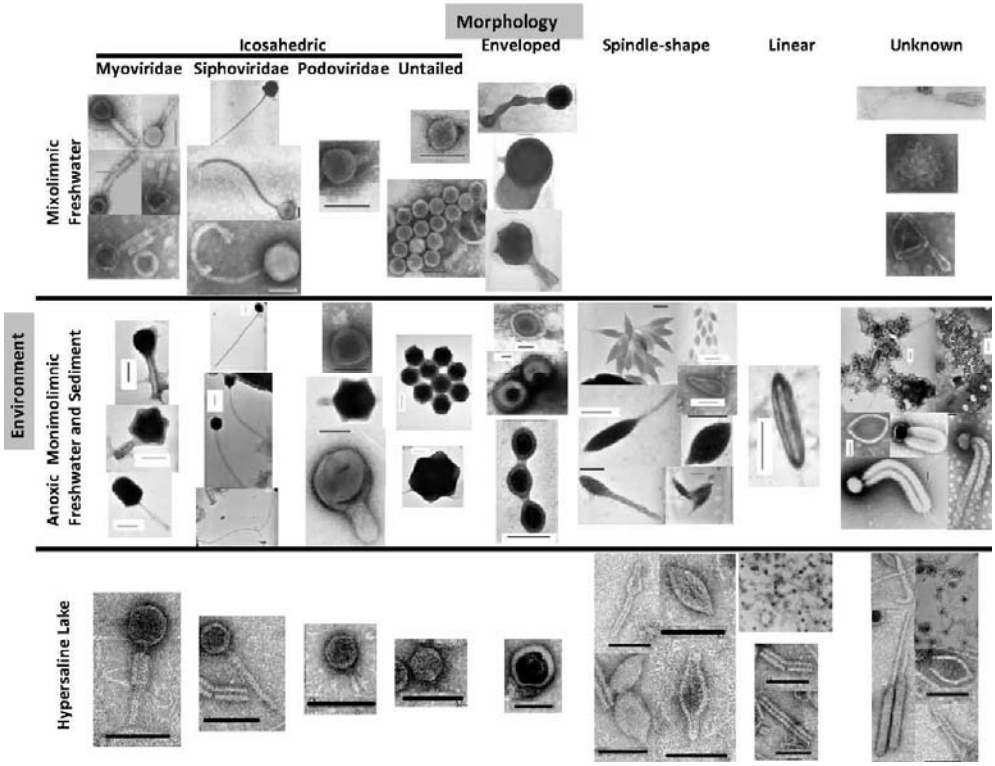

Figure 1. Aquatic ecosystems, here the deep freshwater Lake Pavin (France) and the hypersaline Lake Retba (Senegal), are characterized by highly diverse forms of virus-like particles, whose phenotypic complexity increases with environmental constraints such as oxygen and salt contents. In typical oxygenated pelagic waters, such as in the surface layers of freshwaters, typical phages (myo-, sipho- and podoviruses, and untailed and enveloped viruses) are encountered. In the permanently anoxic freshwaters and sediments, additional complex shapes of viruses (lemon, ellipsoid, spherical, cubic…) occur, similar to archaeal viruses found in hypersaline lakes. Scale bars = 100 nm.

Phenotypic traits and viral morphs in aquatic viruses are cryptic of the selective pressures faced by these communities, and provide insight into host range, viral replication and function. For instance, myoviruses are mostly lytic with a large spectrum of sensitive hosts, which is a competitive advantage that can be assimilated to *r*-strategist species thriving with high proliferation rates in fluctuating environments. In contrast, podoviruses are more highly-specific to their hosts, with siphoviruses being intermediate between myo- and podoviruses. In addition, several siphoviruses can encode their genome into their hosts for several generations (i.e. lysogeny), which can be rather assimilated to *K*-strategist species' characteristics of stable environments. Combined with the capacity of viruses to potentially face almost all types of

environments and the related interfaces, the ability of viruses to develop along the *r*-*K*-selection selection continuum, i.e. from typical *r* (e.g. prokaryotes) to *K* (e.g. vertebrates) strategist organisms, may help to explain their ubiquity, hence the notion of the virosphere (i.e. viral biosphere).

3.3. Genomic Diversity

Whole genome comparisons have shown that there are conserved genes shared amongst all members within certain viral taxonomic groups. These conserved genes can be targeted using PCR-amplification and sequencing for diversity studies of groups of cultured and environmental viruses. Examples of such genes are structural proteins such as *gp20,* which codes for the capsid formation in T4 phage-like viruses, DNA polymerases for T7-like podophages, or the RNA-dependent RNA polymerase fragment, which has been used to identify novel groups of marine picornaviruses. All of the conserved gene studies suggest that environmental viral diversity is high and essentially uncharacterized.

For the whole environmental communities, molecular fingerprinting approaches that separate polymerase chain reaction (PCR)-generated DNA products, such as denaturing gradient gel electrophoresis (DGGE) and pulse-field gel electrophoresis (PFGE), have been widely used but with limited results, restricted to double-stranded DNA viruses. With this approach, the genome size of aquatic viruses fluctuates from 10 to about 900 kb, with mean ranges of 10-630 and 10-660kb in marine and freshwater systems, respectively. The frequencies of the distribution of genome size classes are multimodal, with peaks in the interval <70kb and a mean at 50kb. It is likely that there is a relationship between the capsid and genome sizes, in response to adaptive pressure typical of planktonic realm where prokaryotic hosts are smaller in size compared to those in laboratory cultures.

A recently introduced fingerprinting approach adapted to viruses, randomly-amplified polymorphic DNA PCR (RAPD-PCR), allows sampling of viruses at the genetic level without requiring viral isolation or previous sequence knowledge. RAPD-PCR is accurate in assessing DNA viral richness in water samples by using single 10-mer oligonucleotide primers to produce amplicons (PCR-generated DNA fragments) and banding patterns, with each likely representing a single amplicon that originates from viral template DNA. Such an approach has been demonstrated to match observations from other community profiling techniques, revealing more temporal than spatial variability in viroplankton assemblages. Hybridization probes and sequence information can also be easily generated from single RAPD-PCR products or whole reactions, providing a tool for routine use in high-resolution viral diversity studies by providing assemblage comparisons through fingerprinting, probing, or sequence information.

Finally, metagenomics has revolutionized microbiology by paving the way for a culture-independent assessment and exploitation of microbial and viral communities present in complex environments. Metagenomic viral analyses or virome studies suggest that environmental viral diversity is high and essentially uncharacterized. Metagenomic analyses of 184 viral assemblages collected over a decade and representing 68 sites in four major oceanic regions showed that most of the viral DNA

and protein sequences were not similar to those in the current databases. Global diversity was very high, presumably several hundred thousand species, and regional richness varied on a North-South latitudinal gradient. However, most viral species were found to be widespread, supporting the idea that viruses are widely dispersed and that local environmental conditions enrich for certain viral types through selective pressure.

Interrogation of microbial metagenomic sequence data collected as part of the Sorcerer II Global Ocean Expedition (GOS) also revealed a high abundance of viral sequences, representing approximately 3% of the total predicted proteins in the 0.1-0.8 μm size fraction of the plankton. Viral sequences revealed hundreds to thousands of viral genes, encoding various metabolic and cellular but mostly structural functions. Quantitative analyses of viral genes of host origin confirmed the viral nature of these sequences and suggested that significant portions of aquatic viral communities behave as reservoirs of such genetic material. Distributional and phylogenetic analyses of these host-derived viral sequences also suggested that viral acquisition of environmentally relevant genes of host origin is a more abundant and widespread phenomenon than previously appreciated. The predominant viral sequences identified within microbial fractions originated from tailed bacteriophages and exhibited varying global distributions according to viral family. The recruitment of GOS viral sequence fragments against 27 complete aquatic viral genomes revealed that only one reference bacteriophage genome was highly abundant and was closely related, but not identical, to the cyanobacterial myovirus P-SSM4 of *Prochlorococcus* hosts, suggesting that this virus may influence the abundance, distribution and diversity of one of the most dominant components of small phytoplankton in oligotrophic oceans.

Overall, metagenomic analysis of viruses increasingly suggests novel patterns of evolution, changes the existing ideas on the composition of the virus world, and reveals novel groups of viruses and virus-like agents. The gene composition of marine DNA viromes is dramatically different from that of known bacteriophages. The virome is dominated by rare genes, many of which might be contained within virus-like entities such as gene transfer agents (GTA). Analysis of marine metagenomes thought to consist mostly of bacterial genes revealed a variety of sequences homologous to conserved genes of eukaryotic nucleocytoplasmic large DNA viruses, resulting in the discovery of diverse members of previously undersampled groups and suggesting the existence of new classes of virus-like agents. Unexpectedly, metagenomics of marine RNA viruses showed that representatives of only one superfamily of eukaryotic viruses, the picorna-like viruses, dominate the RNA virome.

3.4. The Particular Case of Archaeal Viruses

About one century of research on viruses of *Bacteria* and *Eukarya* has resulted in a profound understanding of different aspects of their biology. However, exploration of viruses associated with the third domain of life, the *Archaea*, is still in its infancy. The domain *Archaea* comprises two established phyla at the highest taxonomical level, *Euryarchaeota*, and *Crenarchaeota*, and three proposed phyla the status of which is debated: *Korarchaeota, Nanoarchaota* and *Thaumarchaeota*. The *Euryarchaeota* include methanogens, extreme halophiles, thermoacidophiles and hyperthermophiles, and the *Crenarchaota* include exclusively hyperthermophiles. The *Nanoarchaeota* and

Koracheaota are also hyperthermophiles, whereas the cultured members of *Thaumarchaeota* are mesophilic ammonia-oxidizers (i.e. that grow in moderate conditions). The picture of viral diversity differs significantly when one examines habitats where *Archaea* dominate (e.g. extreme geothermal environments), where viruses exhibit diverse unusual morphologies, different from head-and-tail phage particles characteristic of mesopelagic waters.

The hyperthermophilic archaeal viruses are so exceptional in their morphological and genomic properties that eight novel virus families were introduced for their classification, and some still remain unclassified. Moreover, the virion morphologies are astonishingly complex (Figures 1); not only morphotypes, but also genomes of hyperthermophilic archaeal viruses are unique. Often, none of the putative genes of a virus have any similarity to the sequences in extant databases; on average, more than 90% of their genes do not have homologues in databases. The lack of understanding of viral functions can be partially due to very limited knowledge on the biology of these viruses.

Only one virus of methanogens, the virus ΨM1 (and its deletion derivative ΨM2) of *Methanothermobacter* has been described in detail. Morphological and genomic properties of the virus are highly similar to those of head-tailed bacterial viruses from the family *Siphoviridae*. Similarly, a significant proportion of known viruses of extremely halophilic Archaea has virions typical of head-tailed phages of the families *Myoviridae* and *Siphoviridae*. Moreover, the contents of their genomes are similar, allowing evolutionary relationships to be assumed between head-tail viruses of *Archaea* and *Bacteria*. The studies of viral diversity in hypersaline waters of Lake Retba, Senegal, where extremely halophilic archaea are the major component of microbial community, revealed a remarkable morphological diversity of viruses, where the proportion of head-and-tail virus-like particles was only about 5% and viruses with the most unusual morphologies prevailed, supporting the notion of the exceptional nature of the archaeal virosphere. Unexpectedly, similar complex viral shapes were recently discovered in the deep aged freshwater sediments of the volcanic Lake Pavin, France. Indeed, some of these viruses (lemon, filament, ellipsoid-shaped, etc.) resembled double-stranded DNA viruses of hyperthermophilic and hyperhalophilic *Archaea*, while morphologies of others (spherical and cubic) were never recovered in environmental samples (Figures 1). Because archaeal viruses are largely undersampled compared to bacterial viruses, these observations suggest that the known morphological diversity of archaeal viruses could be only ‘the tip of the iceberg’ of a huge genetic resource in the biosphere.

4. Abundance, Distribution and Biogeography

Viruses were first suspected as abundant particles in the sea in the late 1970s, which was confirmed one decade later with the discovery that 1 milliliter of sea water contains millions of viruses. First estimates were variable and inaccurate because they were based on manipulated (i.e. ultracentrifuged) samples observed at high magnification using transmission electron microscopy. More accurate and reproducible estimates were provided later using direct epifluorescent microscopy or flow cytometry (Figure 2), yielding viral abundances that exceed those of *Bacteria* and *Archaea* by an overall

average of about 15-fold. However, because of their small size, viruses represent less than 5% of the carbon biomass of prokaryotes. Viral abundance generally increases with the increasing productivity of aquatic ecosystems and, as a consequence, decreases from freshwater to marine ecosystems, from costal to oceanic zones, and from the surface to the bottom of the euphotic layer. The abundance of viruses in individual aquatic systems appears to be independent of salinity but related to the biomass of primary and secondary producers, as well as to seasonal effects. In the dark ocean (i.e. meso- and bathypelagic zones), where about 75% of prokaryotic biomass and ca. 50% of prokaryotic carbon production in the world ocean occurs, high abundance of viruses was observed. Similarly, two deep marine sediment studies from Ocean Drilling Project samplings have reported abundant viruses and prokaryotes in >100 m sediment cores aged from 0 to 14,000 yrs and from 0.5 to 2 million yrs, respectively. On a volumetric basis, viral abundances in sediments exceed 10 to 1000 times that in the water column, representing active and mostly endemic components of benthic environments. Because the relative abundances of *Archaea* increase in the dark deep ocean and freshwater lakes, viruses of *Archaea* are also expected to be abundant there, as recently suggested by highly complex diverse morphologies observed in a deep-dark permanently anoxic freshwater lake (Figure 1). Thus, it is likely that the ecology of the deepest and benthic waters where eukaryotes are constrained by poor oxygen conditions is essentially driven by the dark viral loop (DOM-prokaryotes-viruses) processes.

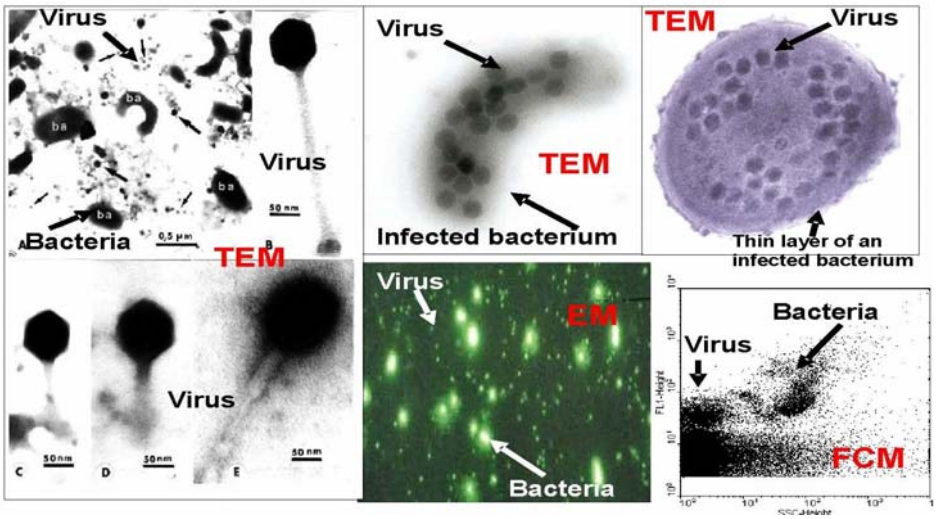

Figure 2. Quantitative methods for accessing the numerical importance and the infectivity of viruses in environmental samples, including transmission electron microscopy (TEM) for virus and infected bacteria observations, epifluorescent microscopy (EM), and flow cytometry (FCM).

According to time, viral abundances fluctuate on diverse scales, from minutes to years, often in association with prokaryotes, which offer the major reservoir for hosts. Surprisingly, there is only one to two orders of magnitude variation in virus abundance among systems (c. 100-fold; 10^9–10^{11} virus particles L^{-1}) in spite of more than three orders of magnitude variation in the planktonic biomass, as one ranges from either coastal to offshore or from surface to deep-water environments. This may help to explain why the virus-to-prokaryote ratios (VBRs) fluctuate substantially, with an

overall increase from 3-10 in oligotrophic marine systems to 6-30 in productive freshwaters where the burst size (i.e. the number of viruses release per lysed host cell estimated from those cells completely filled with viruses, Figure 3) and the contact and infection rates are generally higher, although this is not always the case. The higher VBRs in productive lakes may also reflect the increasing relative abundance of nonbacteriophage viruses along the trophic gradient of aquatic systems. Virus abundances in freshwaters appear to vary more strongly on seasonal scales than in marine environments, especially in lakes that undergo pronounced seasonal cycles, although the linkages between seasonal cycles and virus abundance remain unresolved in the absence of long-term studies. Evidence that viral abundance across oceans and lakes is driven by different factors was provided based on case studies, including bacterial and cyanobacterial abundances, and chlorophyll-*a* concentration as significant variables in lakes, bacterial and cyanobacterial abundances for coastal Pacific Ocean, and bacterial abundance and chlorophyll-*a* concentration for coastal Arctic Ocean.

On a global scale, the forces that shape the biogeography of viruses have received very little attention. It is of interest to search for general patterns of microbial and viral biogeography because general ecological theories, actually known solely from 'macroscopic' or visible species (e.g. the positive relationship between diversity and area sampled, or the negative one between local abundance and body size), will offer predictive tools in the context of global change. Microorganisms and their viruses have long been considered as ubiquitous, without a biogeography of any sort. This is because their dispersal is thought to be unlimited due to small size, large absolute abundances and the formation of resistant or dormant stages. The ubiquity tenet for microorganisms is the so-called Baas Becking statement ‘everything is everywhere, but the environment selects’. The assumption that viruses are ubiquitous across habitats is currently being evaluated and some phages could be globally distributed, while others could be unique and perhaps endemic to specific habitats, primarily to extreme environments such as deserts or deep-dark permanently anoxic volcanic lake sediments (Figure 1). It was also extrapolated from metagenomic data that viral diversity could be high on a local scale but relatively limited globally, and that viruses promote horizontal gene transfers by moving between environments. Further work is required to fully resolve and confirm the drivers of viral large scale distribution, in conjunction with the improvement of taxonomy, methods, and sampling effort for both viruses and their hosts.

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PCR primer : Is a strand of nucleic acid that serves as a starting point for **PCR**.

r/K selection : Theory relates to the selection of combinations of traits in an organism that trade-off between quantity and quality of offspring. r-selection makes a species prone to numerous reproductions at low cost per individual offspring, while K-selected species expend high costs in reproduction for a low number of more difficult to produce offspring.

Redfield stoichiometry : Is the atomic ratio of carbon, nitrogen and phosphorus found in plankton and throughout the deep oceans. This empirically developed stoichiometric ratio is found to be C:N:p=106:16:1. This term is named after the American oceanographer Alfred C. Redfield, who first described this ratio in an article written in 1934.

Retroviruses : Are enveloped viruses that belong to the viral family *Retroviridae*. Some are integrated and duplicated in a host cell while other, called endogenous retroviruses are integrated into the genome of the host and inherited across generations.

Satellite virus : A small virus that occurs in association with another virus (helper virus), upon which it is dependent.

Symbiosis (syn. Mutualism) : Defines the close relationship between two or more biological entities (symbionts) that live together to the advantage of one, both or all symbionts.

Viroids : Plant pathogens that consist of a short stretch (a few hundred nucleotides of highly complementary, circular, single-stranded RNA without the protein coat that is typical for viruses.

Virusoids : Circular single-stranded RNAs dependent on plant viruses for replication and encapsidation, considered as a satellite viruses because they depend on helper viruses.

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Biographical Sketch

Télesphore Sime-Ngando defended his PhD at the Université Blaise Pascal (UBP, Clermont-Ferrand II, France) in 1991, under the supervision of Hans Julian Hartmann and Claude Alain Grolière. His research topic was on the community ecology of ciliates and the microbial loop in lakes. He pursued his career as a postdoctoral fellow at the Oceanographic Center, Rimouski (1991-1992) and as Research Assistant at the GEOTOP laboratory, Montréal (1993-1994), Canada, working on the same topic but in oceanic ecosystems, including annual sea-ice of the Arctic Ocean, with Drs Kim Juniper and Michel Gosselin. In 1994 he was recruited as an Associate Scientist (CR) in the French National Center for Scientific Research (CNRS) and started working on aquatic viruses. In 2005 he was promoted to Senior Scientist (Director of Research) and initiated research on the molecular diversity and functional roles of heterotrophic flagellates in lakes, unveiling the incidence of zoosporic fungi (Chytrids) with neglected trophic modes (parasitism, saprotrophy, symbiosis) within the community of heterotrophic picoeukaryotes. Dr Sime-Ngando heads the Laboratory Microorganisms: Genome & Environment', UMR CNRS-UBP 6023, and the research team 'Viruses and Microbial Metabolisms'. He has more than 120 publications to his credit in peer reviewed scientific journals and acts as key reviewer for major journals in the field of aquatic microbial ecology. http:// www.lmge.univ-bpclermont.fr /spip.php?rubrique100.

Jonathan Colombet defended his PhD at the Université Blaise Pascal (UBP, Clermont-Ferrand II, France) in 2008, under the supervision of Télesphore Sime-Ngando. His research topic was on the community ecology of viruses (diversity and activity). He pursued his career as a postdoctoral fellow at the LIENSs laboratory, La Rochelle (2008-2009), working on the same topic but in oceanic ecosystems. In 2009 he was recruited as an Engineer in the Laboratory Microorganisms: Genome & Environment', UMR CNRS-UBP 6023. Since then he assisted scientists in their researches on viral ecology notably by developing methodologies for characterization of viral diversity. He has more than 15 publications to his credit in peer reviewed scientific journals.