

Marine viruses — major players in the global ecosystem

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Abstract | Viruses are by far the most abundant ‘lifeforms’ in the oceans and are the reservoir of most of the genetic diversity in the sea. The estimated 10^{30} viruses in the ocean, if stretched end to end, would span farther than the nearest 60 galaxies. Every second, approximately 10^{23} viral infections occur in the ocean. These infections are a major source of mortality, and cause disease in a range of organisms, from shrimp to whales. As a result, viruses influence the composition of marine communities and are a major force behind biogeochemical cycles. Each infection has the potential to introduce new genetic information into an organism or progeny virus, thereby driving the evolution of both host and viral assemblages. Probing this vast reservoir of genetic and biological diversity continues to yield exciting discoveries.

Virosphere

The portion of the Earth in which viruses occur or which is affected by viruses; sometimes called the virosphere.

Heterotrophic

Describes an organism that uses organic compounds for both energy and growth.

Autotrophic

Describes an organism that uses inorganic compounds for both energy and growth. In the oceans phytoplankton are the most common autotrophs.

The oceans cover more than 70% of the Earth's surface. They control the climate, provide a significant amount of the protein that is consumed globally and produce approximately half of the Earth's oxygen. Microorganisms are a major force behind the nutrient and energy cycles in the world's oceans and constitute more than 90% of the living biomass in the sea. It is estimated that viruses kill approximately 20% of this biomass per day. As well as being agents of mortality, viruses are one of the largest reservoirs of unexplored genetic diversity on the Earth.

The virosphere is probably inclusive of every environment on the Earth, from the atmosphere to the deep biosphere. However, nowhere is the importance of viruses more evident than in the world's oceans. The observation that millions of virus-like particles are present in every millilitre of ocean water¹, coupled with evidence that viruses are substantial agents of mortality in heterotrophic and autotrophic plankton^{2,3}, has focused attention on the enormous underestimation of the effects of viral infection in the sea. It has become apparent that viruses are major players in the mortality of marine microorganisms and, consequently, affect nutrient and energy cycles as well as the structure of microbial communities.

Although the story of marine viruses is still emerging, it is becoming increasingly clear that we need to incorporate viruses and virus-mediated processes into our understanding of ocean biology and biogeochemistry. Progress in our understanding of marine viruses and their effects has been rapid and has been summarized in several comprehensive reviews^{4–8}. However, many challenges remain. This Review examines our current knowledge of marine viruses, and highlights areas in

which marine virology is advancing quickly or seems to be poised for paradigm-shifting discoveries.

The abundance of marine viruses

Although there was persuasive evidence in the late 1970s that viruses are abundant in the sea⁹, it was not until a decade later that quantitative estimates revealed that each millilitre of seawater contains millions of these particles¹. Most of the first estimates of abundance were based on electron microscopy of virus particles that had been removed and concentrated from seawater (BOX 1). Although such studies provided convincing evidence that the particles were virus-like and present in high abundance, the estimates obtained were variable and inaccurate. This, in combination with the high costs and time that are associated with electron microscopy studies, led to efforts to develop more accurate, high-throughput methods that are based on epifluorescence microscopy^{3,10–12}. These methods were quickly adopted by the scientific community and, in general, have resulted in reproducible estimates of abundance, although methodological errors have led to significant underestimates in many cases^{7,13}. For example, estimates taken from the deep ocean only a few years ago were one order of magnitude less than those more recently obtained^{14,15}. At present, there is good agreement and a high level of confidence in the estimates of viral abundance in the water column, when procedures are carefully followed. Nonetheless, although viruses are clearly present in high numbers^{16,17}, the accurate and reproducible estimation of viral abundance in marine sediments remains challenging.

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The abundance of viruses exceeds that of bacteria and archaea by approximately 15-fold. However, because of their extremely small size, viruses represent only approximately 5% of the prokaryotic biomass (FIG. 1). Within any environment, the total viral abundance generally varies along with the prokaryotic abundance and productivity

(as reflected by the concentration of chlorophyll *a*)^{18–21}. Consequently, in the oceans, viral abundance decreases further offshore and deeper in the water column. Similar patterns are observed when the titres of specific groups of infectious viruses are determined^{22,23}. These trends are reflected in the ratio of virus particles to prokaryotic or

Box 1 | Methods for estimating viral abundance in aquatic systems

Five methods are used to estimate the abundance of viruses in aquatic samples: plaque assays (PAs); most-probable-number assays (MPNs); transmission electron microscopy (TEM); epifluorescence microscopy (Efm); and flow cytometry (FC). Which procedure is used depends on the question being addressed and the accuracy and sensitivity that is required.

PAs and MPNs¹⁶² are used to quantify the abundance of infectious units that cause the lysis of a particular host. Hence, the host cells must be cultivable, which is not the case for most of the microbial taxa in the ocean. PAs are used to estimate the titres of viruses that cause the lysis of bacteria, cyanobacteria and algae that can be grown on solid media. Mixtures of host cells and viruses are combined with molten agar, which is poured over a bottom layer that is made with a higher percentage of agar. Infectious viruses will form a clearing (plaque) on a lawn of host cells. The number of plaque-forming units in a given volume of water can be estimated using this method. MPNs are used for cells that are cultivable, but which cannot be grown on solid substrates, and use a series of dilutions, with ten or more replicates at each dilution. The replicates in which no growth, or growth followed by cell lysis, occurs are assumed to contain at least one infectious virus. The number of replicates at each dilution in which lysis occurred can be used to calculate the number of infective units in the original sample. PAs and MPNs are the only methods that can be used to directly determine the abundance of infectious viruses, and they can also be used to obtain and purify specific viral isolates. However, these methods provide no information on the total abundance of viruses in a sample.

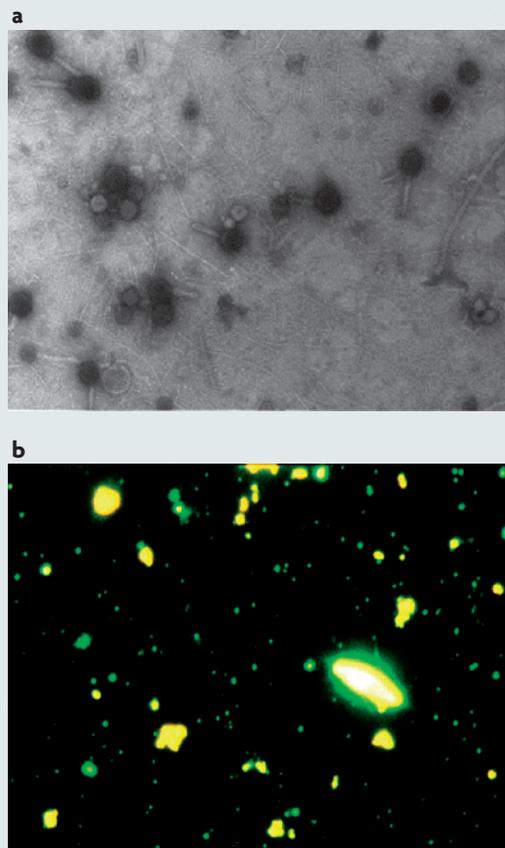
TEM is the only method that provides data on both the abundance and morphology of virus-like particles¹⁶³ (a). The viruses must be concentrated from seawater, deposited on a supporting grid and stained with an electron-dense material, such as uranyl acetate. This approach has the advantage that particles that resemble viruses can be identified and quantified. However, there are many technical aspects that are involved with concentrating, staining and visualizing the viruses, which can lead to variable and inaccurate estimates of the total abundance. The TEM approach has largely been superseded by Efm, except where data on the morphology of the virus particles are required.

Efm is currently the most widely used approach for estimating the total abundance of virus particles. In this method, the viruses are concentrated on a membrane filter, their nucleic acids are stained with a brightly fluorescent dye and the abundance of viruses is estimated by Efm (b). The first estimates of viral abundances that were made by Efm used DAPI (4', 6-diamidino-2-phenylindole)^{3,164,165}, although the fluorescence was near the limit of detection for many microscopes. Subsequently, a new generation of brightly fluorescent dyes, such as YO-PRO¹¹ and SYBR Green¹², have made accurate and high-precision counts routinely obtainable. However, many estimates have been derived from samples that were inappropriately preserved for Efm, and consequently much of the data in the literature are underestimates^{7,13}.

Most recently, FC has been used to estimate viral abundances^{27,28}. This accurate high-throughput method also allows the quantification of subpopulations of viruses that differ in their characteristics of fluorescence and light scattering. FC allows large numbers of samples to be analysed quickly, which should begin to supply us with a synoptic picture of the distribution and abundance of viruses in the sea.

There is now high confidence in the estimates of the abundance of free double-stranded DNA viruses that are provided by Efm and FC. However, even our current estimates are too low because of the presence of RNA¹³⁶ and single-stranded DNA¹³³ viruses that occur in the sea but that cannot be resolved using the currently available methods¹⁶⁶. In addition, viruses that are attached to particles can be abundant, but are difficult to quantify by Efm and will be missed by FC. Despite these caveats, our ability to accurately quantify viruses in aquatic samples has improved vastly over the past 15 years.

Of the images, a shows a transmission electron micrograph of a natural virus community and b shows an epifluorescence micrograph of a seawater sample that has been stained with YO-PRO-1.



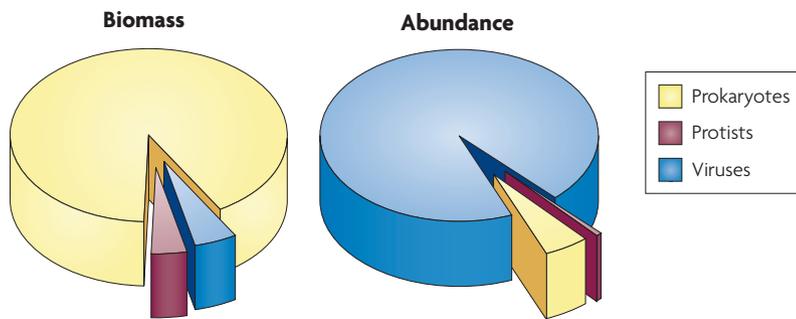


Figure 1 | Relative biomass and abundances of prokaryotes, protists and viruses. Viruses are by far the most abundant biological entities in the oceans, comprising approximately 94% of the nucleic-acid-containing particles. However, because of their small size they comprise only approximately 5% of the biomass. By contrast, even though prokaryotes represent less than 10% of the nucleic-acid-containing particles they represent more than 90% of the biomass. Protists can represent as much as half the biomass in surface waters¹⁶⁹, but in the meso- and bathypelagic depths of the ocean they only comprise a few percent or less of the biomass¹⁷⁰. Consequently, overall, their biomass probably represents even less than that of the viruses.

bacterial cells (VBR). However, marked differences have been reported in the relationship between the viral and prokaryotic abundance in different marine environments. For example, in the surface waters of the Pacific and Arctic Oceans the VBR is ~40 and ~10, respectively, and in lakes the average VBR is less than 5 (REF. 21). By contrast, in the deep waters of the Atlantic Ocean the ratio often exceeds 100 (REF. 15). The reasons for these differences are unknown, although in freshwater environments the loss rates of virus particles may be greater²¹, resulting in a higher abundance of viral particles, whereas the high VBR in the deep ocean might reflect a zone of viral accumulation^{15,24,25}. These are large-scale patterns that are controlled by environmental differences, but ultimately, viral production occurs at microbial hot spots and on spatial scales of individual cells. This is evident by the order-of-magnitude variations in viral abundance and VBRs on spatial scales of centimetres that occur in aquatic environments²⁶.

Our view of the distribution and abundance of viruses in the sea is enhanced by flow cytometry, which is a high-throughput method in which the fluorescent staining of nucleic acids allows virus particles to be counted, even though they are too small to scatter light in a predictable way^{27–30}. FC allows sub-populations of both viruses and potential host cells to be discriminated, based on the characteristics of their fluorescence and scatter. Although the data are limited, in the Arctic Ocean biome the most abundant sub-population of viruses had a lower fluorescence and was most highly correlated with the heterotrophic prokaryotes, which had a higher nucleic-acid content (J.P. Payet and C.A.S., unpublished observations). It has been argued that this sub-group represents the most active members of the prokaryotic community^{31–33}, although this interpretation has been disputed^{34,35}. By contrast, viruses that have more fluorescence and scatter are characteristic of the Phycodnaviridae family, which infect eukaryotic phytoplankton. Viruses with these characteristics were the most tightly coupled to the chlorophyll *a* concentration, which is an indicator of

the abundance of photosynthetic cells (J.P. Payet and C.A.S., unpublished observations).

Such observations might help us to understand some of the emergent properties of viral infection. For example, most models that try to estimate the impact of viral infection on marine microbial mortality assume that every member of the prokaryotic community is equally affected by viral infection^{2,36–38}. However, if viruses preferentially infect cells that are growing more rapidly this will, in turn, affect nutrient cycling and, potentially, the efficiency with which carbon is transported from where it is fixed in surface waters to the deep ocean.

Viruses, mortality and elemental cycling

As agents of mortality, viruses have a range of effects on the world's oceans, from altering geochemical cycles to structuring populations and communities. However, quantifying the effect of viruses on host populations remains difficult⁷. Poorly constrained estimates indicate that, on average, viral lysis in surface waters removes 20–40% of the standing stock of prokaryotes each day³⁶, and is approximately equal in importance to grazing as a source of microbial mortality³⁹. However, estimates of viral lysis vary widely among studies and the methods that are available produce variable and uncertain results^{40,41}. In addition, there are few estimates of viral-mediated mortality for microbial communities in sediments⁴² or the subsurface waters that constitute most of the world's oceans¹⁵. Although over long time periods viral-mediated mortality must approach a steady state, in which mortality and production are balanced, this is frequently not the case for the timescales over which experiments are conducted. Sometimes this is obvious, for example, during large-scale lytic events that can lead to the termination of phytoplankton blooms⁴³, but in most cases the effects of viral infection on phytoplankton blooms are more subtle^{44–46}. In addition, the observations of diel and seasonal shifts in viral production^{47,48}, and temporal shifts in the composition of viral communities^{49–51} and the organisms they infect⁵² imply that viral infection is not at a steady state in the marine environment. The fact that virus replication rates increase in conjunction with increases in host growth rates emphasizes that viral-mediated mortality is not in a steady state, and that some subsets of the host community will be disproportionately affected. An increase in the rate of viral reproduction in response to an increase in the growth rate of host cells is a strong feedback mechanism that would probably prevent dominance by the fastest growing taxa. Reports that bacteriophage abundance is most strongly correlated with the most active subset of the prokaryotic community (J.P. Payet and C.A.S., unpublished observations) is further evidence that it should not be assumed that the effects of viral infection are spread evenly across the microbial community. The lack of straightforward and reliable approaches for estimating the rates of mortality that are imposed by viruses on marine prokaryotic and eukaryotic heterotrophic and autotrophic communities remains one of the biggest obstacles for incorporating viral-mediated processes into global models of nutrient and energy cycling.

Biome

An ecological area that contains similar groupings or communities of organisms.

Diel

A 24-hour period that corresponds to a cycle of light and darkness.

Box 2 | Viruses and the biological pump

The biological pump (BP) is a combination of processes that leads to the sequestration of carbon in the deep ocean as the result of the sinking of particulate organic matter from surface waters. The amount of carbon that is exported by the BP has direct implications for the concentration of carbon dioxide in the atmosphere. The carbon that is exported from surface waters includes living and dead cells, faecal pellets from zooplankton, and detritus. Viruses alter the pathways of carbon cycling in the sea as the result of cell lysis⁷, which converts living particulate organic matter into dead particulate organic matter and dissolved organic matter. In particular, carbon from cell lysis will sink more slowly and be retained to a greater extent in surface waters, where much of it will be converted to dissolved inorganic carbon by respiration or solar radiation. However, the amount of living particulate organic carbon in surface waters is controlled by the availability of nutrients such as nitrogen, iron or phosphorous, which limit the growth of the primary producers. Consequently, the amount of carbon that is exported is a function of the amount of the growth-limiting resource that is supplied to the photic zone. The efficiency of the BP increases as the ratio of carbon relative to the amount of the limiting resource (or resources) increases. Viruses can increase the efficiency of the BP if they increase the export of carbon relative to the export of the limiting resource (or resources).

The biological pump becomes more efficient if the ratio of exported carbon relative to the nutrient (or nutrients) that limits primary productivity is increased. There are several ways by which viruses can enrich or reduce the relative amount of carbon in exported production. For example, virus-mediated cell lysis could liberate elements that were complexed with organic molecules in approximately the same ratio as they occur in the organisms they infect. However, the chemical composition of the excretion and faecal pellets from zooplankton can differ markedly from that of the phytoplankton that they ingest, depending on the elemental assimilation efficiency^{167,168}. Furthermore, the mineral elements that are liberated during viral lysis, such as iron, are rapidly re-assimilated⁶², and the viral particles that are released are rich in nitrogen and phosphorous. The selective retention of viruses and mineral elements in the photic zone, relative to carbon-rich components, such as cell-wall material, potentially increases the efficiency with which carbon is exported to below the pycnocline. With as much as one-quarter of the primary production in the ocean ultimately flowing through the viral shunt⁴, there is a crucial need to accurately quantify the nature and fate of the products of viral lysis, and incorporate these processes into models of global geochemical cycles.

Owing to the overwhelming dominance of microbial biomass in the oceans, the geochemical effects of viral lysis are translated, directly and indirectly, by how they influence the prokaryotic and protistan assemblages. By the simplest approximation, the viral shunt^{4,7} moves material from living organisms into the particulate and dissolved pools of organic matter^{53–56}, where much of it is converted to carbon dioxide by respiration and photo-degradation^{4,5,7,8,57}. However, the effects can also be more profound and potentially include the release of dimethyl sulphide^{58–61}, a gas that affects the Earth's climate, and the remobilization of the organically complexed iron that limits primary production in much of the world's oceans^{62,63}. For example, the viral lysis of prokaryotes liberates sufficient amounts of biologically available iron to support the needs of phytoplankton⁶². Ultimately, it is both the quantity and composition of the material that is released by viral lysis that affects microbial communities and global geochemical cycles.

As well as increasing the amount of respiration in the system, the shunting of organic material from organisms to the dissolved pool by viral lysis potentially influences the amount of carbon that is exported to the deep ocean by the biological pump⁷. This is a globally significant process that sequesters approximately 3 gigatonnes of

carbon per year (BOX 2). Viruses can transform microbial biomass into dissolved and particulate organic matter within the photic zone by lysis or can export more carbon and other organic molecules out of the photic zone by the accelerated sinking rates of virus-infected cells⁶⁴. Accelerated sinking, as the result of viral infection, might be a mechanism that enhances the export of the smallest primary producers from surface waters. The controls on the biological pump are complex⁶⁵, but ultimately, the export of nutrients other than carbon must be balanced by the influx of new nutrients. Hence, viral lysis can only affect the efficiency of the biological pump by altering the proportion of carbon that is exported relative to the nutrients that limit primary productivity. However, as lysis releases highly labile cellular components, such as amino acids and nucleic acids, that can be rapidly incorporated by living organisms (FIG. 2), this should have the stoichiometric effect of retaining more nitrogen and phosphorous in the photic zone than would occur if the cells were to sink, thereby increasing the efficiency of the biological pump.

Structuring microbial communities

The molecular diversity of prokaryotic communities in the oceans is enormous^{66–68}, although the underlying ecological basis is unknown^{69–71}. One proposal is that the host-specific, often strain-specific, nature of viral infection makes viruses powerful agents for controlling the community composition^{6,72–74}. This has been incorporated into a model^{75,76}, in which the diversity of the microbial community is maintained by viral infection and microbial abundance is controlled by the nonspecific nature of protozoan grazing⁷⁷. This 'killing the winner' model is attractive, because of its dependence on the rapid propagation of viruses on taxa that become abundant⁷⁸. However, how viruses regulate microbial diversity in nature remains ambiguous, and it is unclear whether the differences in viral cellular receptors⁷⁹, which, in part, regulate the strain specificity of viruses, translate into broader measures of host genotypic diversity.

Perhaps the best evidence for the killing-the-winner scenario has come from studies on protistan phytoplankton⁸⁰. For example, during blooms of a single species, such as *Emiliania huxleyi*^{43,81}, *Phaeocystis globosa*⁸² or *Heterosigma akashiwo*^{44,83}, the high proportion of visibly infected cells, along with other evidence, has been used to infer high levels of viral-mediated mortality. This can result in bloom collapse⁴³, which can produce greater species diversity.

However, a high host abundance does not necessarily lead to the collapse of a taxon, even when the concentrations of infectious virus are high. In the case of the cyanobacterium *Synechococcus* spp., even though virus titres increase dramatically when the host-cell abundance exceeds $\sim 10^3$ per ml, high numbers that are resistant to virus infection persist^{22,23}. This implies that blooms of *Synechococcus* spp. are composed of many populations that probably differ in their viral receptors. Observations that the genotypic composition of *Synechococcus* spp. and cyanophages co-vary⁸⁴ support the view that viruses regulate the genetic diversity of

Pycnocline

The depth of the ocean at which the maximum change in density occurs owing to changes in the temperature or salinity.

Viral shunt

The viral-mediated movement of nutrients from organisms to pools of dissolved and non-living particulate organic matter.

Photic zone

The area of the ocean to which light penetrates.

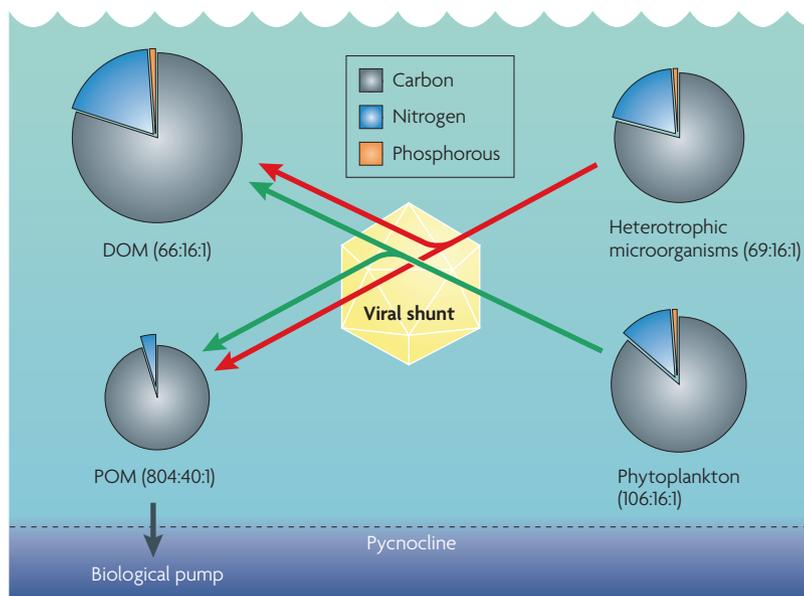


Figure 2 | Shunt and pump. The viral shunt moves material from heterotrophs and photoautotrophs (represented by red and green arrows, respectively) into particulate organic matter (POM) and dissolved organic matter (DOM). In this process there is a stoichiometric effect, such that the chemical composition of the POM and DOM pools are not necessarily the same as the composition of the organisms from which the material was derived. Highly labile materials, such as amino acids and nucleic acids, tend to be recycled in the photic zone, whereas more recalcitrant carbon-rich material, such as that found in cell walls, is probably exported to deeper waters. Thus, the material that is exported to deeper waters by the viral shunt is probably more carbon rich than the material from which it was derived. This would increase the efficiency of the biological pump. The numbers in parentheses are the estimated ratios of carbon:nitrogen:phosphorous (in atoms).

the cyanobacterial community. Other studies have also found that viruses can influence bacterial diversity^{84,85}. For example, in one study in which the concentration of viruses was reduced to lower contact rates⁸⁶, taxa that were usually rare increased in abundance, whereas the taxa that were most abundant declined. This indicates that the taxa that were initially numerically dominant were competitively inferior to the rare taxa, which were held in check because they were highly susceptible to viral infection. However, in other experiments the effects of manipulating viral abundance have been inconsistent and relatively minor^{87–89}. There can also be interactive effects of viral lysis and protozoan grazing on microbial diversity⁹⁰.

These inconsistent results might, in part, be due to methodological differences, but it is also probable that much of the variability is real. Viruses can influence microbial diversity either directly or indirectly⁷³. The most obvious direct influence is by selectively killing the competitively dominant taxa, which are probably the most active members of microbial communities. A less obvious direct effect is the introduction of new genetic traits by the horizontal gene transfer that can be acted upon by natural selection. Potentially important indirect effects include the release of predation pressure by the lysis of grazers and the stimulation of the growth of subsets of the microbial community by the recycling

of organic substrates. In nature, there are strong temporal and spatial gradients that have the potential to affect the influence of viruses on microbial diversity. All of these effects are dependent on transient matches between assemblages of hosts and viruses. As a result, it is not surprising that the influences of viruses on host populations are spatially and temporally variable.

Viruses of invertebrates and vertebrates

In some aspects, our knowledge of the marine viruses that infect invertebrates and vertebrates greatly exceeds our knowledge of those that infect other microorganisms. This is because the biology, pathology and diversity of many viruses that infect commercially important species (especially cultivated species) are well studied. However, in most cases, we know little about the reservoirs, sources and sinks of these viruses or the impact of viral infection on organisms that are not commercially significant. It is clear, however, that viral pathogens infect a broad range of evolutionarily divergent groups of marine organisms⁹¹. Most of our knowledge has been driven by the economic consequences of viral disease and the protection of stocks of commercial fisheries or at-risk species. In the marine aquaculture industry, viral diseases can cause enormous losses in production and revenue^{92,93}. It is remarkable that so many different pathogens can infect some already well-studied organisms, such as the panaeid shrimp⁹², and that previously unknown viruses are still routinely discovered. Some of these discoveries have been extraordinary; in the case of the white spot syndrome virus (WSSV), which infects panaeid shrimp, a new virus family has been recognized⁹⁴. Similarly, viruses that infect commercially significant finfish have been intensively studied, and have been found to encompass a wide range of viral families, including rhabdoviruses, birnaviruses, nodaviruses, reoviruses and herpesviruses.

In general, although there is a good understanding of the pathology of viral diseases, little is known about where these viruses occur outside of the host or their modes of transmission. Nucleic-acid technologies have demonstrated that there is considerable molecular diversity within many of these families. Some viruses have broad host ranges and appear to circulate between marine waters and freshwaters, making the transmission of viruses to new areas a serious threat. For example, phylogenetic analyses of isolates of infectious haematopoietic necrosis virus (IHNV) — a rhabdovirus that infects salmonids and is widespread in the northeast Pacific Ocean — provide strong evidence that the virus has not only been transmitted among fish stocks in North America, but has also been transmitted to marine and freshwater fish stocks in Europe and Asia⁹⁵. Viral haemorrhagic septicaemia virus (VHSV) is another rhabdovirus that is primarily associated with disease in trout farms in Europe, but has also been isolated from more than 40 species of marine fish⁹⁶, and has been implicated in mass mortalities of herring, hake and pollock in farms in Alaska⁹⁷. Phylogenetic analysis indicates that the European freshwater viruses had a common marine ancestor approximately 50 years ago,

and diverged from their North American marine and freshwater counterparts ~500 years ago⁹⁸. Most recently, VHSV has been detected in fish from lakes in Atlantic Canada⁹⁹, Michigan, United States¹⁰⁰, and the Great Lakes, where it has been associated with several mass mortality events that have affected different fish species¹⁰¹. Another example comes from the nodaviruses, which are pathogens of a wide range of fish species¹⁰². Based on nucleic-acid sequences from virus isolates there is evidence that disease is emerging in finfish aquaculture in Spain and Portugal¹⁰³.

Marine mammals are also susceptible to viral infections. The most widely reported example is the thousands of harbour seals that were killed in Europe in 1988 and 2002 by phocine distemper virus, a morbillivirus that is believed to circulate in Arctic phocid seals¹⁰⁴. Morbillivirus outbreaks have also been responsible for mass mortality events in dolphins and other cetaceans¹⁰⁵. As indicated by disease outbreaks and serological evidence, many other viruses, including caliciviruses, herpesviruses, adenoviruses and parvoviruses, circulate in marine mammal populations^{106–108}, and some of these can cause disease in humans¹⁰⁹.

Although much is known about the specific viruses that cause widespread mortality in commercial fisheries and at-risk mammal populations, little is known about their natural reservoirs. However, environmental genomic approaches are providing insights into the enormous genetic diversity of viruses in the sea, and hold promise for revealing the sources and sinks of these pathogens.

The diversity of marine viruses

Our appreciation of the genetic richness of viruses in the sea has greatly increased over the past decade. The first studies used restriction fragment length polymorphisms (RFLPs) and hybridization analyses to show that viral isolates that infect the phytoplankton *Micromonas pusilla*^{110,111} are not only widespread, but that the genetic similarity of isolates is not a function of geographical separation. A parallel study that also used RFLPs revealed diversity in the viruses that infect *Synechococcus* spp.¹¹² These early efforts were quickly followed by a range of methods that used PCR, such as denaturing-gradient-gel electrophoresis, pulse-field-gel electrophoresis and hybridization. These studies identified genetically rich viral communities without the need for culturing^{74,113–118}. Many of these approaches continue to shed light on the distribution, as well as the spatial and temporal dynamics, of environmental viral diversity. PCR-based gene-specific studies that target subsets of viral communities generally reveal that most of the diversity in virus communities is derived from sequences that are distantly related to those from cultured representatives^{119,120}. Many of these studies have been recently reviewed⁷⁸.

Cyanophage isolates that infect *Synechococcus* and *Prochlorococcus* spp. are exceptional, as they seem to be representative of the genetic diversity of cyanophages in nature¹²¹, although the function of a large proportion of their putative genes remains unknown^{122–124}. One of the most surprising discoveries to arise from the analysis of representative cyanophage genomes is

that many contain genes that encode core photosynthetic proteins^{125,126} that are expressed^{127,128} and have an evolutionary history that is distinct from that of their hosts¹²⁹.

Metagenomic approaches to viral diversity. Our knowledge of the diversity of viruses in the environment has been greatly increased by the use of metagenomic approaches to catalogue marine virus communities¹³⁰. Environmental virus samples are ideal candidates for metagenomic analyses. Although the genetic richness of natural viral communities is great, the small genomes of most viruses and the uneven distribution of genotypes in environmental samples indicates that the reconstruction of complete viral genomes will be considerably easier than the reconstruction of bacterial or archaeal genomes.

The first metagenomic studies of viral communities predicted that there would be thousands to more than a million different genotypes in samples of coastal waters and sediments^{131,132}. In a recent study, high-throughput pyrosequencing was used for a metagenomic analysis of viral communities that included between 41 and 85 individual samples from the Arctic Ocean, the coastal waters of British Columbia and the Gulf of Mexico, as well as a single sample from the Sargasso Sea¹³³. Of the approximately 1.8 million sequences that were obtained, more than 90%, on average, had no recognizable homology to previously reported sequences in GenBank. In part, the lack of recognizable homology can be attributed to the difficulty in identifying homologues using the approximately 100-base-pair sequence lengths that are produced by pyrosequencing. However, even among collections of longer-read viral-community sequence data, the BLAST (Basic Local Alignment Search Tool) homologue frequency to protein sequences within the GenBank non-redundant database is only approximately 30%, which is similar to that typically seen in phage whole-genome-sequence data^{130,131}. Nonetheless, the data show that viral diversity is poorly represented in the existing databases. There was also little sequence overlap among samples, and because three of the four metagenomes were composites of many samples the observed differences between environments were not the result of unrepresentative sampling within an environment. Surprisingly, at three of the four locations there were sequences with significant similarity to the single-stranded (ss) DNA viruses that belong to chp1-like microphages. This is the first evidence that ssDNA viruses are numerically significant members of the viroplankton.

Metagenomic approaches can also be used to assess the diversity and richness of RNA viruses in environmental samples. As a follow-up to the primer-based approaches that revealed the great diversity of marine picorna-like viruses^{134,135}, Culley and colleagues used a metagenomic approach to determine the richness of RNA viruses in two coastal environments¹³⁶. There was no discernable overlap between the two viral communities. Although most of the sequences did not have any recognizable similarity to those recorded in the databases, many fell into one of three contiguous segments. This ultimately allowed the complete reconstruction of the genomes of three previously unknown viruses.

Pyrosequencing

A high-throughput method for sequencing DNA, in which light is emitted each time a nucleotide is incorporated into a complementary strand of DNA.

Virioplankton

Composed of un-attached (free) viruses in marine waters or freshwaters. Nominally defined as nucleic-acid-containing particles that can pass through a 200-nm pore-size filter.

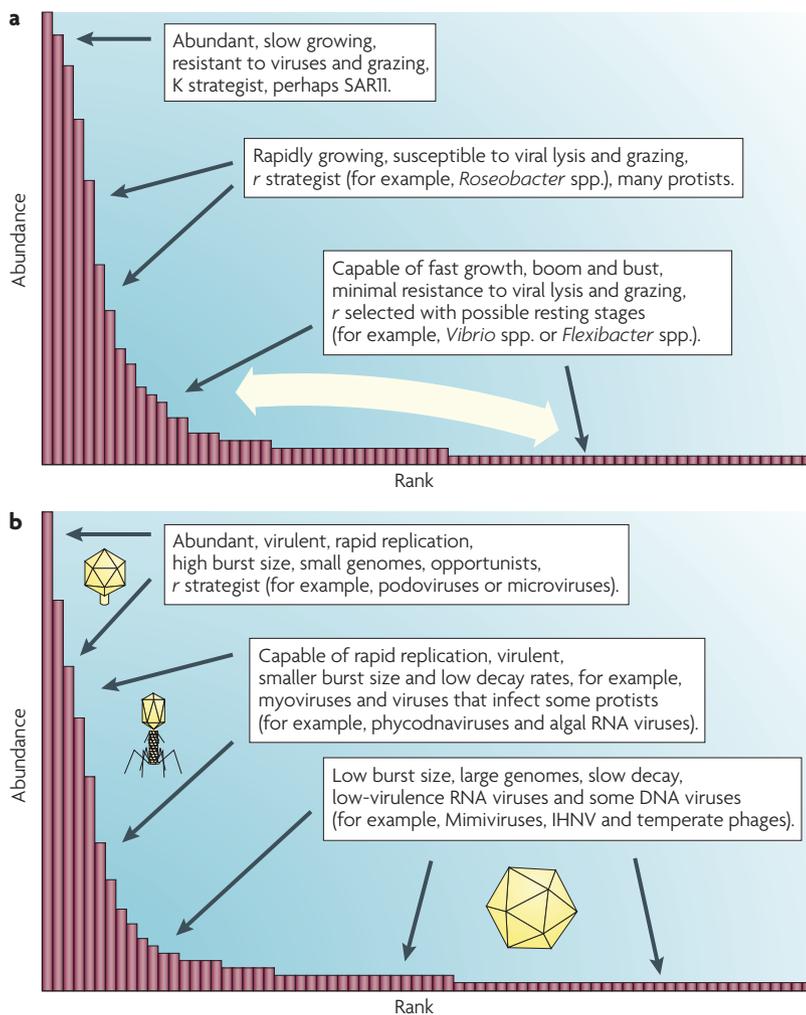


Figure 3 | The distribution of, and selective influences operating on, marine prokaryotes, eukaryotes and viruses. a | A rank–abundance curve showing marine prokaryotes and eukaryotes. The most abundant organisms in the ocean, such as SAR11, are probably K-selected organisms that have slow maximum growth rates but are resistant to viral lysis and grazing. By contrast, less abundant organisms, such as *Roseobacter* spp. and *Vibrio* spp., are capable of rapid growth but are highly susceptible to viral infection and grazing. Consequently, the rarer microorganisms are more r selected, whereas the microorganisms that dominate the biomass are the most K selected. The yellow arrow represents taxa that are typically present in low abundance and periodically encounter conditions that are conducive to rapid growth, but as their abundance increases the rates of viral infection also increase, resulting in lysis of the host cells and a return to low abundance. **b** | A rank–abundance curve showing marine viruses. In contrast to the most abundant prokaryotes, the most abundant viruses are r selected. They are virulent, have small genome sizes and are short-lived. The population structure is probably uneven, with many of the viruses at any given time being progeny from a limited number of lytic events. The rarer, more K-selected viruses have larger genomes, decay slowly and can form stable associations with their hosts. Also included in this group are some RNA and DNA viruses that are long-lived and have low virulence. IHNV, infectious haematopoietic necrosis virus.

Metagenomic approaches enable us to capture the genetic richness of marine viral communities, and assemble and characterize previously unknown viral genomes. However, the success of comparative analyses of metagenomic data will depend on the development of the infrastructure and analytical tools to handle the enormous datasets that are generated by these studies¹³⁷.

Diversity, viruses and r- and K-selection

The interactions between viruses and the organisms they infect control the genetic diversity of viruses and influence the composition of microbial communities. Examining the population structure of viruses and their hosts provides insight into the evolutionary strategies that shape these communities.

Rank–abundance curves and active populations. The diversity of microbial life in the oceans is enormous, but so far unquantified. The composition of prokaryotic and viral communities at a specific location follows a steeply declining rank–abundance curve (FIG. 3). This distribution is often interpreted to mean that there are a small number of active taxa that comprise most individuals and a large number of dormant taxa that are comprised of relatively few individuals^{70,120,130}.

However, this interpretation is not necessarily correct. For example, rare taxa can dominate if the pressure from grazers or viruses is reduced⁸⁶. This indicates that some rare taxa are metabolically active, but suffer high loss rates. Evidence for this came from a study in which the most abundant cells (belonging to the SAR11 group) were less active than rarer cells (such as *Roseobacter* spp.)¹³⁸. However, SAR11 can still have an important role in nutrient uptake and recycling because of their abundance^{138–140}. At other times their productivity appears to be equal to or even exceed that of other prokaryotes¹⁴¹. Cases in which SAR11 is abundant but has low activity might be indicative of resistance to viral lysis; this is consistent with the possibility that members of this group contain inducible prophage¹⁴². By contrast, viruses that infect *Roseobacter* spp. are readily isolated from seawater and roseophage-like sequences are common in the metagenomic data¹³³. Although *Roseobacter* spp. can be an abundant component of productive and polar marine microbial communities (up to 50% of the prokaryotes)^{143–146}, they appear to represent a much smaller fraction of the prokaryotes in oligotrophic waters¹⁴⁷. Such observations imply that active *Roseobacter* spp. populations are probably kept in check by viral lysis. By contrast, although SAR11 might be less active, it could be more abundant in oligotrophic waters because it is resistant to losses, including those from viral lysis (FIG. 3a).

In stable marine environments, the composition of microbial communities is often predictable and stable. This indicates that communities are close to a steady state, at least by our measurement of taxonomic composition. The idea that viruses kill the winner⁷⁶ is probably true during the onset of blooms, but selection for resistance means that, at the level of taxonomic resolution that we use, the result is not always evident. A good example is the temporal dynamics of *Synechococcus* spp. and infectious cyanophages, both of which can remain at high abundance for extended periods of time^{22,23,84}. Some of the phenotypic diversity in *Synechococcus* spp. is associated with phage resistance, but this is not necessarily reflected by the genetic distance between genotypes or at a perceivable taxonomic level. Consequently, there appears to be stable coexistence between host

Box 3 | Marine viruses and the *r*- and *K*-selection continuum

The basis of *r* and *K* selection is the idea that organisms vary in the degree to which they are selected to have a high reproductive output (*r* strategist) or be a better competitor for resources and have a lower reproductive output (*K* strategist). In general, *r* strategists are considered to be opportunists that are small, replicate quickly, have short life cycles and produce many progeny. They have evolved to quickly exploit abundant resources and are poorer competitors for resources that are in short supply. Many viruses are strongly *r* selected, in that they are virulent, reproduce quickly and produce many progeny. However, other viruses are *K* strategists as they can integrate into the host genome (as a temperate phage) or form low-level chronic infections that cause minimal disease in the host (for example, some herpesviruses and rhabdoviruses).

cells and infectious viruses, which persists against a background of high contact rates between viruses and host cells but a low infection efficiency. Protists — most of which are unicellular, such as phytoplankton and microzooplankton — might be better candidates to fit a model in which the most abundant taxa are rapidly growing, whereas the rarer taxa persist owing to low loss rates. Protist communities can be temporally dynamic in their composition⁵⁰ and can respond rapidly to environmental changes, as has been shown by the ephemeral blooms of photosynthetic protists. In addition, there is persuasive evidence that viruses can kill the winner in the case of some bloom-forming species^{43,80,148}. However, there are instances in which blooms persist even in the presence of the viruses that infect them¹⁴⁹. These arguments are not meant to imply that all rare taxa are rapidly growing and are kept in check by viral infection and predation, and that all abundant taxa are slow growers that are resistant to loss. However, the evidence does indicate that some, and perhaps most, taxa that are persistently dominant are resistant to viral predation, whereas some rarer taxa are rapidly growing and are probably highly susceptible to viral infection and/or grazing.

Rank–abundance data for virus communities are limited, but the metagenomic data for DNA and RNA¹³⁶ virus communities indicate that they fit a steeply declining rank–abundance curve (FIG. 3b). Although there are not enough data to determine the genotypic stability of viral community composition, the lack of overlap among communities^{133,136}, temporal variation in the composition of viral communities⁷⁴ and highly uneven population structures^{131,132,136} indicate they are dynamic. However, there are components of the viral community that appear to be stable. For example, studies on the temporal dynamics of the genetic richness of viruses that infect eukaryotic phytoplankton have indicated that they vary much less than the community of protists⁵⁰. In addition, the genomes of the most abundant viruses fall into discrete size classes that seem to be consistent across a wide range of environments^{117,118}. Most marine viruses seem to have genome sizes of 25–50 kilobases (kb), whereas less-abundant virus types have genome sizes that lie between approximately 60 kb and 150 kb.

The picture that emerges is of a microbial community in which the most abundant taxa are slow growing and resistant to viral infection and grazing, whereas taxa

that are capable of rapid growth are highly susceptible to viral lysis and grazing, and are subject to boom-and-bust cycles (FIG. 3a). However, the most abundant viruses have small genome sizes and are likely to be virulent, with high rates of viral production (FIG. 3b). The hosts for these viruses probably come from taxa that are rare, but which grow rapidly during transiently favourable conditions. These are the progeny viruses that have killed the winners.

***r* and *K* selection in the marine milieu.** Viruses and the organisms that they infect exist along a continuum of *r* and *K* selection^{8,150,151} (BOX 3). Many viruses can be considered to be *r* selected (those with large burst sizes and short generation times). However, other viruses have lifestyles that are more characteristic of *K* selection, for example, a temperate phage that can integrate its DNA into the genomes of host cells (lysogeny) or can establish other carrier relationships with its host (pseudolysogeny or latency). The most *r*-selected viruses will have rapid rates of replication and high burst sizes, and will kill their hosts. By contrast, the most *K*-selected viruses will coexist with their hosts for extended periods. Overall, viruses and microorganisms with high growth and loss rates and rapid responses to environmental changes are generally *r* selected. By contrast, fish and mammals that can integrate over large scales of time and space are the most *K* selected (FIG. 4).

For viruses infecting prokaryotes, it is predicted that *K*-selected phages with small genomes and burst sizes will infect the most abundant, slow-growing members of the prokaryotic community. By contrast, the most *r*-selected phages will be highly virulent, have large burst sizes and will rapidly take advantage of members of the microbial community that are growing rapidly in response to transient, favourable conditions.

In general, viruses that infect photosynthetic and heterotrophic protists are expected to be virulent and have high reproductive rates, so as to be able to take advantage of the high growth rates and rapid responses to environmental changes that are characteristic of many protists. As all cultivated viruses that infect marine protists are lytic, the virulent lifestyle is probably dominant among natural communities of viruses infecting protists. However, a limited number of viruses have been isolated to date, and viruses that are capable of latent infection might yet be discovered. Protists are also infected by RNA viruses with small genomes and large burst sizes^{152–154}, as well as double-stranded DNA viruses with large genomes and relatively small burst sizes^{155–158}, indicating that the degree of *r* and *K* selection differs among protist-infecting viruses.

Finally, many viruses that infect large organisms, such as crustacean zooplankton, fish and mammals, will be highly *K* selected and are expected to have a lifecycle that depends on a non-virulent and close association with that of its host. Outbreaks that result in significant mortality would be expected to occur only sporadically, and would probably be associated with a transmission that is outside the normal host range of the virus.

Protist

A eukaryotic photosynthetic and heterotrophic organism that belongs to the kingdom Protista.

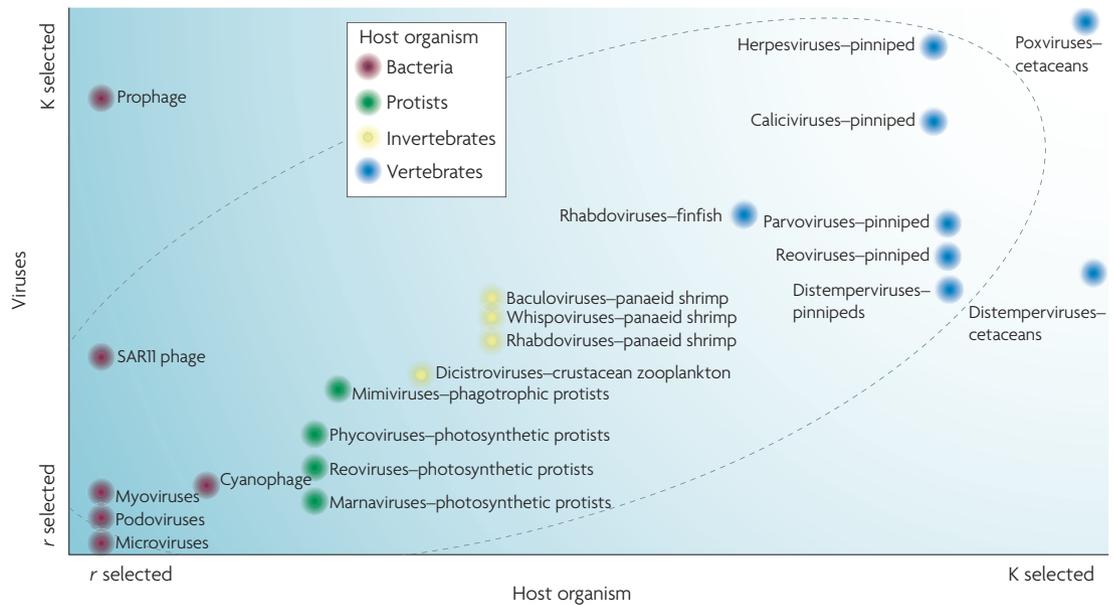


Figure 4 | **The distribution of marine viruses and their hosts along an r- and K-selection continuum.** It is proposed that viruses and the organisms they infect exist along a continuum of r and K selection. The axes have no units but represent a continuum that ranges from primarily r selected to K selected. In general, prokaryotes and the viruses that infect them are more r selected, even within groups, although there is considerable variation. For example, temperate phages that form stable associations with the hosts they infect are more K selected than lytic phages. In general, viruses that infect larger, longer-lived organisms are more K selected, tend to have lower virulence and, in some cases, form stable associations with the organisms they infect. The individual host–virus combinations should be considered as a ‘cloud’ rather than discrete points, and the position of each host–virus combination is strictly qualitative. The oval illustrates the general relationship between r and K selection in viruses and the organisms they infect.

Ultimately, a given organism is likely to be affected by a range of different viruses that vary markedly along the r–K-selection continuum. For example, mammals are infected by herpesviruses that form stable latent infections with their hosts and highly virulent morbilliviruses that cause distemper. Some viruses have bridged both ends of the r–K-selection spectrum. The best examples are temperate phages, in which the viral DNA is either stably integrated into the host-cell genome and replication occurs in conjunction with the host cell or viral replication occurs by a virulent lytic infection. Both strategies appear to be common in marine environments^{142,159–161}. Consequently, there appears to be two winning strategies that are exploited by marine viruses. At one end of the spectrum are highly virulent r-selected viruses, for example, lytic phage and protist-infecting viruses, which replicate and kill their hosts within minutes to hours. At the other extreme are viruses that are K specialists, which can form a stable association with their hosts for an indefinite period of time, such as prophage and latent herpesviruses.

Whether or not the scenarios outlined above are responsible for the highly uneven population structures that are characteristic of marine microbial communities, in which few taxa are numerically dominant, requires further exploration.

Conclusions

Despite the significance of viruses and viral-mediated processes in the ocean, quantitative estimates of the rates of infection and viral-mediated mortality remain poorly constrained. As a result, our understanding of the effects of viruses on emergent properties such as community structure or rates of nutrient cycling is far from complete. Similarly, we are far from being able to translate the genetic complexity of marine viruses into an understanding of biological potential. The future looks bright, however, as high-throughput methods of nucleic-acid fingerprinting and sequencing, as well as viral enumeration, are rapidly beginning to yield a broad view of the distribution and composition of viral communities in the sea.

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Acknowledgements

Thanks are owed to T. F. Thingstad for discussions on the biological pump. The comments of C. Pedros-Alio, K. E. Wommack, C. Winter and S. W. Wilhelm are much appreciated, as well as discussions with A. M. Chan, C. Chenard, J. L. Clasen, M. Fischer, J. Labonte, J. Payet and members of the Scientific Committee on Oceanic Research Marine Virus working group.

Competing interests statement

The authors declare no competing financial interests.

DATABASES

Entrez Genome: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genome>
 |HNV|VHSV|WSSV
Entrez Genome Project: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genomeprj>
 |Emiliana huxleyi|Micromonas pusilla

FURTHER INFORMATION

Curtis A. Suttle's homepage: <http://www.ocgy.ubc.ca/~suttle/>
 ALL LINKS ARE ACTIVE IN THE ONLINE PDF