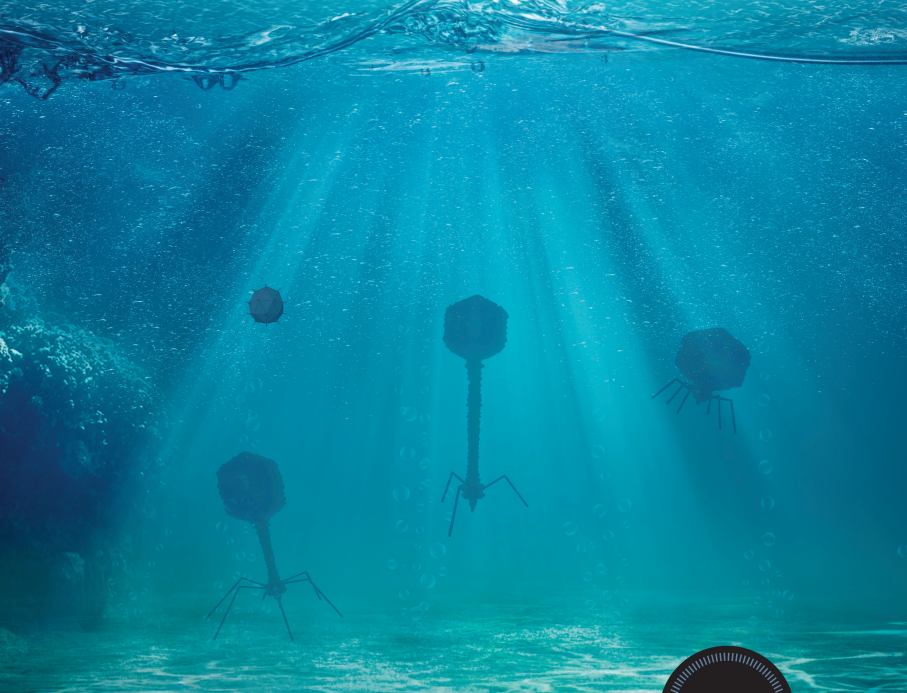




ENJEUX SCIENCES

MARINE VIRUSES

SIMPLE PARASITES
OR MAJOR PLAYERS
IN AQUATIC ECOSYSTEMS?

STÉPHAN JACQUET,
ANNE-CLAIRE BAUDOUX, YVES DESDEVISES, SOIZICK F. LE GUYADER

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Quæ

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In memory of Markus Weinbauer

This book, published in 2026 as a translation of its French edition released in 2023, is dedicated to the memory of our colleague Markus Weinbauer, whose work profoundly shaped aquatic microbial ecology and opened new perspectives on the role of viruses in the oceans. Through a scientific outlook that was both rigorous and deeply curious, Markus helped reveal the immense importance of marine viruses in the functioning of ecosystems. At a time when these invisible entities were still largely overlooked, he demonstrated that they play an intimate role in the cycling of matter, the dynamics of microbial communities, and, more broadly, the balance of the oceanic biosphere. Beyond his major scientific contributions, those who had the privilege of working alongside him remember a generous and enthusiastic researcher who trained a great number of young scientists. The pages of our book devoted to marine viruses owe much to the path he helped chart. His influence can be seen in the questions we ask today, in the methods we employ, and in the ever-renewed sense of wonder inspired by the study of the microbial world. May this work, in its own way, extend the scientific momentum he helped inspire and remind us how curiosity, rigor, and generosity can shape a discipline in lasting ways.



WHAT IS A VIRUS, PARTICULARLY A MARINE VIRUS?

The study of viruses is relatively recent. It is less than 100 years since viruses were detected and characterized using the first electron microscopes (1937). As the word virus comes from the same Latin word meaning “poison”, they were first, and for a long time, considered and studied as pathogens, in plants, animals and, particularly, humans. A virus is an obligate intracellular parasite that requires another living organism to replicate.

The study of viral diversity in ecosystems, including aquatic ecosystems, as components of microbial communities appeared much later. Indeed, it was not until the 1980s that this type of research began. To understand viral diversity, new methods had to be developed to identify and, when possible, quantify non-observable and mostly non-cultivable entities. Initially, microscopy highlighted the numerical predominance of viruses in aquatic environments, but these approaches remained limited in their capacity to describe viral diversity, as the shapes and sizes of different viruses are often very similar. Awareness of the enormous diversity of viruses is quite recent, especially due to developments in molecular biology and high-throughput sequencing from the 2000s onwards. Over the past two decades, metagenomic analyses (see box: “*Omics*” in a nutshell) of the non-cellular fraction of marine environments that contains viruses have significantly expanded our knowledge and revealed a vast genetic diversity of both DNA (deoxyribonucleic acid) and RNA (ribonucleic acid) viruses.

Marine viruses are not fundamentally different from terrestrial viruses, but the global ocean is incredibly vast and difficult to access, containing a potentially enormous diversity of viruses that remain largely unknown. In addition, in extreme marine environments, such as hydrothermal sources, a wide variety of unique viruses has been shown to exist, as synthesized in the publications of Lossouarn *et al.* (2015) or Gil *et al.* (2021).

Extremophilic microorganisms (e.g. those living in very hot or very acidic environments), such as archaea, are associated with viruses with totally unusual forms and unique genetic signatures. In the ocean, viral genes code for functions not typically found in terrestrial viruses. It is now estimated that 50–70% of the genetic information contained in the genomes of marine viruses encodes unknown functions, with some of these genes never having been recorded in the databases. Therefore, there may be differences between aquatic and terrestrial viruses. However, it is important to note that there is no strict dichotomy between marine (or more generally aquatic) and terrestrial viruses.

METAGENOMICS

It is now possible to determine the incredible diversity of microorganisms in a seawater sample on the basis of their DNA, whereas so-called conventional techniques (typically microscopy) only allowed a glimpse. Combining genetics, ecology, and computer science, metagenomics is a high-throughput sequencing approach coupled with bioinformatics analysis that, unlike genomics (which focuses on complete genomes from single or limited organisms), enables the study of genomes from multiple individuals across different species. It is thus possible to obtain the composition of a microbial assemblage, i.e. which species are present, their abundances and diversity, in a given sample.

Technical reasons and economic or public health interests have led terrestrial viruses of vertebrates to be given much more attention than aquatic viruses. However, our knowledge of the diversity and evolution of viruses of vertebrates is most often limited to those found in mammals or birds and associated with pathologies. The study of RNA viruses, known for their rapid evolution and very wide diversity of hosts, has quite logically shown a strong link between viruses and the evolution of their hosts. The history of these hosts most often began in the oceans, where they evolved over millions of years. The viruses associated with them have followed their evolution. Palaeovirology (the science that studies ancient viruses and the co-evolutionary

history of viruses and their hosts) shows that most RNA viruses infecting vertebrates have a marine origin. It therefore emphasizes the value of continuing studies to describe taxonomic diversity in the marine environment to help us understand the history of viral evolution. Such approaches challenge the conventional view that a single virus or viral type is associated with a single, specific host, especially since these hosts may share a common evolutionary ancestor. To advance understanding in this field, it is important to acquire more data on viral diversity within hosts, but also over time.

DEFINITION AND LIFE CYCLE

A virus is defined as a biological entity consisting of a single- or double-stranded DNA or RNA molecule surrounded by a protein shell (called a capsid) and, in some cases, a lipid envelope or layer. The definition also states that a virus does not breathe, divide or actively move. Viruses come in a wide variety of sizes and shapes, ranging from just a few hundredths of a micron to over one micron (μm), and take forms as diverse as filaments, bottle shapes, or the lunar-lander-like structures typical of bacteriophages. They also do not have their own metabolism, making them obligate parasites that rely entirely on the intracellular machinery of a living host to replicate and produce progeny. The first step of the infection of a host cell by a virus is quite universal: it consists of passive contact, recognition of the host targeted by the virus, then fixation of the virus to specific receptors, such as membrane transport proteins, and injection of genetic material into the cell. Subsequently, there are different replication methods: true lytic cycle, ineffective lytic cycle (where the virus does not lyse the cell), chronic cycle (production of virions¹ without lysis), lysogenic cycle or pseudo-lysogenic cycle (Figure 1).

1. Virion: The “free” form of a virus, therefore consisting of the capsid and the viral genome it contains.

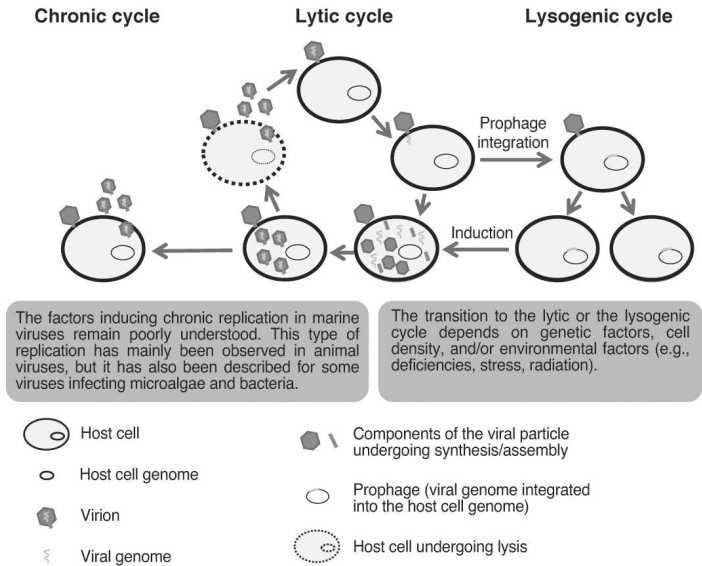


Figure 1. Major viral cycles or strategies.

Lytic and lysogenic cycles predominate in marine viruses. As shown in this diagram, there is no strict barrier between cycles, as the transition from one to the other can take place depending on the physiology of the host cell and environmental conditions.

The lytic cycle has been the most extensively studied in the marine environment, as it is quickly recognized for its significant and possibly measurable functional role: that of cell mortality and the redistribution of matter in the surrounding environment of infected organisms. Indeed, during the lytic cycle, the viral genome induces the synthesis of viral constituents, including the replication of viral genetic material. A certain number of viruses are then produced inside the host cell (this number, called the burst size, varies from a few to several thousand units) and their release takes place through the burst and hence the death of the cell that housed them. It is presently estimated that mortality induced by viruses affects at least 10% of microalgae (excluding mortality at the

termination² of blooms³, where this estimate can almost reach 100%) and 40% of bacteria (again, the percentages are variable, depending on a multitude of environmental factors, or the physiological state and the resistance capacity of the host). It is more difficult to estimate virus-induced mortality for metazoan organisms (typically zooplankton), for which there is still very little information. Note that the lytic cycle may be ineffective when blocked at one or more of its stages (e.g. adsorption to the receptor, entry of the viral genome, synthesis of the viral progeny or their release). This type of cycle, which does not lead to the production of infectious offspring, could correspond to a cul-de-sac or, more simply, to the abortion of a lytic cycle.

The second most well-known mode of replication is the lysogenic cycle, in which the viral genome integrates into the host genome. This is called a provirus or prophage that reproduces and persists in this state until an environmental event (stress, shock, thermal or nutrient limitation) induces the transition to a lytic cycle. Lysogeny can provide the virus with a means of maintenance, a refuge to survive in when conditions are unfavourable to the host's metabolism, but only up to a certain point. If the host cell is about to die, then the virus is forced to abandon ship! This mode of replication could benefit the virus if the host is weakly abundant. The host cell may also find it "advantageous" to be occupied by a lysogenic virus, since the latter may offer it some resistance or immunity to a new viral infection (which is known as immunity to superinfection), or new functions brought about by the viral genome, such as virulence factors (toxins) or factors enabling the adaptation to environmental fluctuation.

A variant of the lysogenic cycle that has been reported for the marine environment is pseudolysogeny, which refers to when a virus infects a host cell, but its genome does not integrate into the cellular genome and remains in what appears to be an inactive

2. Termination: when a bloom disappears, which can have various causes, including viral lysis.

3. Bloom: rapid increase in the concentration of generally phytoplanktonic microorganisms (microalgae or cyanobacteria) in an aquatic environment. This proliferation often causes water coloration (green, red, etc.) due to the pigment of the species concerned.

state inside the cell. This type of viral persistence would appear when the host cell is in a very slowed down physiological state, for example under very limiting nutrient conditions, so that no viral replication occurs. To our knowledge, the question of whether a true lysogenic cycle can be re-established if the host cell finds itself in a more favourable environment has not yet been answered.

Another mode of replication that has been shown in some viruses is the chronic cycle, especially in some phytoplankton genus or species (e.g. *Ostreococcus tauri*, *Emiliania huxleyi*, *Micromonas* spp.). In this case, the newly formed viruses inside the host cell are released in a constant or episodic manner without bursting the cell and thus without its death. This release takes place by extrusion or budding of part of the cell membrane. Two scenarios have been reported in the literature depending on the type of host, some of which lead after several cycles to the classical lytic cycle and the death of the host, while others seem to establish a stable coexistence between host and virus (but with very low viral production, i.e. less than three viruses released per cell per day).

In the marine environment, habitats are diverse and environmental conditions vary spatially and temporally. These variations profoundly alter organism physiology and it is now known that the physiological state of the host influences the kinetics and/or alternation of viral replication cycles, as synthesized in the review of Mojica and Brussaard (2014). Thus, it is easy to understand that viruses can shift from one cycle to another. This has been repeatedly shown for the bacterium-bacteriophage pair, in which host abundance and cell physiology are critical and involved in the establishment of the lysogenic cycle in relation to the lytic cycle. It appears that productive environments are dominated by lytic viruses, whereas low-production, oligotrophic (nutrient-poor) or anoxic (dissolved oxygen-deficient) environments are rather typical of lysogenic viruses. At least half of the so-called cultivable marine bacteria (i.e. those that can be isolated and cultured in the laboratory, probably less than 1% of the total actual bacterial diversity) have been shown to carry prophages that can be induced by chemical stresses; in other words, these bacteria are in a lysogenic state. In 2008, John Paul put forward the idea that these phages, if integrated into the cellular genome,

are true “time bombs” because they can become virulent and, when conditions are favourable, cause the death of a huge proportion of cells/populations.

It was long believed that reproductive modes or cycles are very independent, allowing viruses to be classified according to their mode of reproduction. Today, these cycles are seen more as a continuum: viruses can use different infection strategies depending on population density, host cell physiology and, more generally, their environment, which influences all biotic interactions. However, the precise mechanisms that trigger transitions between lysogenic, lytic or chronic cycles in marine viruses remain largely unknown.

Among the scenarios, it is often noted that lytic infections suppress high-growth bacterial hosts, leaving part of the ecological niche vacant for the development of prophage-carrying bacteria, or lysogens (bacteria harbouring dormant, integrated viruses known as prophages) to proliferate. These prophages provide their bacterial hosts with protection against lytic phages. As the population density of these lysogens increases, lysogenic phages may acquire mutations to overcome superinfection or homoimmunity (a mechanism by which a prophage protects its host from secondary infection by a closely related phage) and thereby regain control of the lysogenic population. The ongoing development of “omics” techniques, encompassing genomics, transcriptomics, proteomics and metabolomics, promises to reveal whether prophages are active or dormant and to provide detailed insights into the frequencies of lysis and lysogeny in the natural environment and on their dependency on environmental factors.

During pseudolysogeny, the phage enters neither the lytic nor the lysogenic phase but remains in the cell as an extra-chromosomal element that does not replicate and is not passed to both daughter cells after division. This mode of reproduction seems to be found in both DNA and RNA viruses. It can be observed when the host cell is in a resource-limiting environment. As soon as conditions become more favourable, the lytic cycle becomes the norm. It has been suggested that this viral strategy allows phages to protect themselves from environmental conditions such as the deleterious effect of ultraviolet (UV) radiation.

Among bacteria, 35–50% are susceptible to several phages. Thus, phages belonging to different taxonomic groups can infect a common host. Co-infections can also occur, complicating host-virus interactions, with different consequences for their ecology and evolution.

"OMICS" IN A NUTSHELL

Genomics is the science that studies the genome.

Transcriptomics is the science that studies how the overall expression of genes in an individual or set of organisms varies under varying environmental, experimental or pathological conditions.

Proteomics is the study of the proteome, the complete suite of proteins constituting a living organism.

Metabolomics is the comprehensive study of metabolites (small molecules, e.g. sugars, lipids, nucleic acids or vitamins, produced or modified during cellular metabolism within an organism or a tissue.

DIVERSITY AND STUDY METHODS

Initially, viruses were classified according to their morphological characteristics. While this classification is still used today to a limited extent, molecular approaches have greatly complemented it. Viruses with an elongated tail structure, used for host cell recognition (also known as tailed viruses), are still generally distinguished from those without a tail. In 2013, Brum *et al.* reported that tailless viruses dominated marine samples, comprising 50–90% of detected viruses. Among the tailed viruses, bacteriophages are the most prevalent, and these are still categorized depending on the size and flexibility of their tails. Specifically, siphoviruses have long, non-contractile tails; myoviruses have long, contractile tails; and podoviruses have short tails (Figure 2). However, this type of phenotypic classification captures only a small portion of the remarkable genetic diversity of marine viruses, which is now known to be exceptionally high and remains largely uncharacterized.

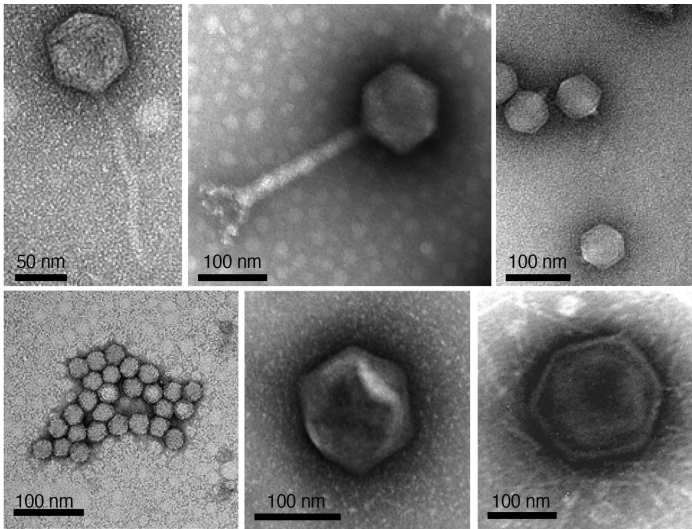


Figure 2. Major viral morphotypes.

The viruses presented here have a typical shape (with an icosahedral protein capsid) and may or may not possess a tail, which can be short or long, contractile or non-contractile. The upper part of the figures shows the three typical representatives of the tailed bacteriophage family (from left to right; Siphoviridae, Myoviridae and Podoviridae). The shapes in the bottom row are more characteristic of viruses that infect protists⁴ (from left to right: Picornaviridae, Phycodnaviridae and an uncultivated giant virus). N.B.: there are other, often rarer, filamentous or pleomorphic morphologies (e.g. those of certain archaeal viruses, see Table 1). Source: © Baudoux A.-C./Photothèque, Station Biologique de Roscoff; © Schoehn G./Photothèque, Institut Biologie Structurale.

The analysis of nucleotide sequences (DNA or RNA) of marine viruses with metagenomic tools (see boxes: *Metagenomics* and *Studying marine viruses using environmental DNA*) provides new insights into ocean viral diversity. Data from the *Tara Oceans* mission (Figure 3) revealed an extraordinary diversity of viral

4. Protists are not a phylogenetically defined group. However, for reasons of clarity and legibility, we have decided to use this term without adding quotation marks. This editorial choice also applies to the terms prokaryotes, fish, algae, reptiles and invertebrates that are used in this book.

sequences in marine environments. When the *Tara* expedition began in 2009, viruses were known to be abundant (1-100 billion viruses per litre of seawater) and it was estimated that they killed about one-third of the microbial cells in seawater every day. However, these findings were largely derived from counting microscopic particles that resembled viruses and laboratory incubation experiments with environmental samples. Over the past decade, rapidly advancing sequencing technologies and molecular biology techniques have enabled systematic and quantitative studies of oceanic “viromes” (the total viral genomic content in a sample) on a global scale. These capabilities significantly expanded our understanding of oceanic viral genomes: from only 39 publicly available isolated genomes prior to the *Tara Oceans* mission to approximately 200,000 different populations of predominantly double-stranded DNA viruses (metagenome-derived sequences) in the global oceanic virome (GOV2).



Figure 3. The research schooner *Tara* photographed during one of its numerous research missions on viruses and other planktonic organisms.

Tara Oceans and the ship's subsequent expeditions have contributed a great deal to the discovery of marine plankton, its diversity and the interactions between microorganisms, including viruses. The schooner has travelled thousands of kilometres around the globe and collected several thousand samples of seawater and plankton from several hundred sites corresponding to a wide variety of ocean biogeographic provinces. For more information, visit the *Tara Oceans* Foundation website (<https://fondationtaraocéan.org/>). Source: © Latreille F./Fondation Tara Océan.

To achieve this, the *Tara* travelled for three years across the different oceans that represent about 70% of the surface of the planet. During this mission, more than 35,000 water samples were collected from around the globe (see box: Tara Oceans *discoveries*). The initial objective was to obtain basic data to understand the interaction between populations of phyto- and zooplankton, bacteria, archaea, heterotrophic unicellular eukaryotes and, of course, viruses. The samples were fractionated to enable population analyses using different targeted metagenomic approaches (most often on part of the 16S RNA or 18S RNA). It emerged that viral communities seem structured (probably indirectly through their hosts) by temperature and oxygen, and are passively transported by ocean currents, in accordance with the notion that everything is everywhere *a priori* and that it is the environment that “sorts”, or rather promotes one or another viral population through its abundance (thus taking up the concept of “Everything is everywhere, but the environment selects.”, an idea initially formulated more than 80 years ago by Baas Becking). Structure has been identified between populations (macrodiversity) and at finer scales within populations (microdiversity), the latter retracing the most recent ecological and evolutionary changes. The analysis of these data revealed that the oceans comprise five ecological zones overall. In addition, modelling helps explain their biodiversity in terms of latitude, providing a consistent framework for understanding the low diversity of the Southern Ocean and higher diversity in other ocean regions. These models explain the diversity of micro- and macro-organisms. Beyond these large-scale patterns, ocean viruses are now also linked *in silico* (by computer) to ecologically significant marine microbial hosts, providing fundamental hypotheses that can be tested by experimental virus-host binding methods. In other words, viruses and microbial species are associated as potential host-virus pairs through computer analysis of variations in their abundances in a very large number of ocean samples. These data provided world maps of virus-host infection, and for cyanobacteria, they have advanced our understanding of how viral infections are associated with the daily dynamics of host communities, such as cycles

occurring over the course of the day. These data also revealed key auxiliary metabolic genes encoded by viruses, indicating extensive host metabolic reprogramming and likely direct modulation of biogeochemical cycles, greatly complementing existing datasets.

TARA OCEANS DISCOVERIES

In 10 years of exploration, *Tara Oceans* has made it possible to discover the incredible diversity of the constituent bodies of plankton. Apart from viruses, another example is that 150,000 genetic types of eukaryotic plankton have been identified, revealing unsuspected diversity (compared to just over 12,000 species described at the time) and largely unknown (more than 1/3 of this diversity could not be associated with any of the large known eukaryotic lines). Interestingly, the genetic types that fit into the eukaryotic tree life turned out to be mostly single-celled organisms (or protists), including many kinds of parasites, symbionts or predators.

Analysis of ballast tank⁵ water by metagenomics methods is a complementary element to these approaches, showing the diversity of viruses and a possible impact of biogeographic models. A study prior to the *Tara Oceans* campaign showed that non-compliance with the regulation of mid-ocean ballast water exchanges can lead to the movement of viromes (including potential viral pathogens) specific to geographical and environmental niches. Given the intense growth in global trade and maritime traffic, the discharge of these waters into port areas could promote the spread of diverse and non-cosmopolitan DNA viruses, and potentially RNA viruses, from one part of the world to another. Ballast water could thus be a factor contributing to ocean virome transport and potentially to increased exposure of the aquatic biosphere to viral invasions.

5. Ballast tank: large-capacity water tank built into some vessels. It is intended to be filled or drained of seawater in order to optimize navigation. The operation of emptying, carried out in poor conditions may cause ecological problems. It is now highly regulated.

Apart from these human-induced movements, the absence of perceptible geographical barriers to genetic dispersal in the marine realm (such as mountains would be on land) has led to the suggestion that organisms are easily transported by currents and thus proliferate everywhere. That said, ocean currents are now commonly recognized as barriers to microbial dispersion. Virome analysis of samples taken from the Indian and Southern Oceans showed a statistical difference in their microbiomes, with a higher presence of large nucleocytoplasmic DNA viruses (named NCLDV for nucleocytoplasmic large DNA viruses, now called Nucleocytoviricota, which are mainly Phycodnaviridae and Mimiviridae) observed in the Southern Ocean, while members of the Caudovirales were more evenly distributed.

MICRODIVERSITY

While the contributions of viral metagenomics have allowed us to considerably expand our knowledge of the diversity of marine viruses, it is worth noting that most of these analyses have relied on methods to recover mainly the genomes of the dominant viruses. In other words, viral microdiversity (based on small nucleotide differences within the same viral species) is still poorly understood to date, which could have important consequences for the estimation of overall diversity, but also for the ecological understanding of microbial community structure.

STUDYING MARINE VIRUSES USING ENVIRONMENTAL DNA

The diversity of viruses in the marine environment is now most often estimated by directly sequencing their genomes. In this case, we are dealing with virus assemblages, which *a priori* have a high genetic diversity. There are several approaches to this:

Metagenomics (or non-targeted metagenomics)

This technique involves sequencing all the DNA (or RNA) of the genomes of all biological entities found in a volume of water. Here, it will be viruses that have previously been separated from the cell

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fraction by filtration (< 0.45 or $< 0.2 \mu\text{m}$) or (ultra)centrifugation of the sample. Sequencing all the genomes present gives an idea of the diversity of viruses present and even the functions encoded in their genomes, if the genes discovered have been previously characterized and referenced in genomic databases. However, it should be noted that it is only possible to sequence small DNA fragments, representing only a small part of the genome, which must therefore first be "broken into pieces". These pieces must then be reassembled to reconstruct the original genomes, which is no easy task! Indeed, it is a bit like having a single large box containing a mixture of many jigsaw puzzles of many more or less similar pictures but without having any idea what each of them represents. In addition, each puzzle is often incomplete because not all fragments are usually sequenced. Bioinformatics methods make it possible to reconstruct long fragments of genomes, and sometimes even complete ones (metagenome assembled genomes: MAGs). It is truly through this approach that we do environmental genomics. If, instead of analyzing genome assemblies in this way, we do it on the RNAs transcribed by these genomes, then the technique we are using is metatranscriptomics, which provides information on the instantaneous functional activity of the studied assembly and also reconstructs the genomes of RNA viruses.

Metabarcoding (also known as targeted metagenomics, amplicon sequencing or metagenetics)

Metabarcoding consists of targeting by PCR (i.e. specifically amplifying by polymerase chain reaction) a molecular marker (such as a gene) common to the group of taxa whose diversity one wishes to study. This marker is often called a barcode. For example, in bacteria and eukaryotes, one most often takes a region of DNA that codes for ribosomal RNA, which all cells possess to allow them to translate messenger RNAs (themselves transcribed from DNA) into proteins. We then sequence the diversity of barcodes obtained. The slight variations in the sequences of these markers make it possible to estimate the diversity of the entities that carry them, which can sometimes be identified if these sequences are referenced in databases. The problem with viruses is that there is no common molecular marker for all of them. It is therefore necessary to target certain groups for which there is prior knowledge about their genomes, which necessarily biases the vision of the viral diversity obtained.

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The data generated by metabarcoding give an idea of the diversity of the biological entities studied and an approximation of their relative abundances, since it is expected that a specific variant will be amplified more or less as a function of its initial presence in the sample (although the technique sometimes leads to a biased amplification of certain variants).

Genome sequencing of individual cells

It is possible to sequence the genome of isolated microorganisms (e.g. after sorting on a flow cytometer) into single amplified genomes (SAGs), which for viruses results in vSAGs derived from the genomics of isolated viruses, or single-virus genomics (SVG). Many methods now exist to obtain SAGs. Of course, the great diversity of viruses prevents such approaches from being used to estimate it, but the technique provides a detailed understanding of the viral strains that may dominate certain marine microbial ecosystems. It is also useful for assisting genome assembly methods, for example, from fragments obtained in metagenomics.

ddPCR

Quantitative PCR techniques can accurately measure the abundance of a specific molecular marker; for example, related to a particular viral strain. The most recent technique, which is both sensitive and affordable, is digital droplet PCR (ddPCR), which splits the sample into tens of thousands of nano-sized droplets, each containing a PCR amplification. All these amplifications are analyzed and the proportion of those containing the target marker is calculated, making it possible to quantify the targeted taxon. This allows precise monitoring of the abundance of particular viruses in the marine environment. Naturally, this process is only effective if the target sequence is perfectly characterized.

In marine cyanophages, a small number of genetic changes could operate to generate phenotypic diversification, affecting the infection of different strains of the picocyanobacterial host *Synechococcus*, one of the most ubiquitous and abundant marine organisms in the biosphere. Until recently, this microdiversity has been somewhat neglected, especially since it is quite difficult to understand.

The double-stranded DNA virus named vSAG 37-F6 has been described as probably being the most abundant marine virus in temperate and tropical waters of the open ocean, occupying a wide variety of ecological niches, from surface to deep waters. Nevertheless, despite its great abundance, the genome of this virus could not be reconstructed (assembled) from metagenomic data (to make what is called a metagenome-assembled genome: MAG). The host of vSAG 37-F6, *Pelagibacter* spp., was then discovered using unicellular genomic data mining, as related viral “contigs” (viral genome fragments, continuous and ordered genomic sequences, generated by assembling clones from an overlapping genomic library) were present in different single amplified genomes (SAGs) of *Pelagibacter* spp. Thus, this virus is thought to be responsible for channelling a huge amount of carbon through the viral short circuit (or viral shunt, see section *Marine viruses and biogeochemical cycles*), which has a major impact on a global scale. The microdiversity of this virus has recently been elucidated, revealing more than a thousand different genotypes in a single sample. This high diversity creates a myriad of potential infection cycles: characterized by variable efficiency in both space and time. Each vSAG 37-F6 virus species probably, therefore comprises hundreds or thousands of different strains, and each of these in turn produces several thousand viral particles per millilitre.

This microdiversity does not seem to be related to the variability of host strain defence mechanisms. It could be evolutionarily neutral due to a large population size and a high number of individuals/strains that each fluctuate with continuous and perpetual infection cycles. These observations therefore imply that pelagiphages exhibit truly gigantic effective population sizes, where permanent microdiversity is ancient and maintained over long periods of time. They also suggest that microdiversity is not substantially affected by selective forces, such as selective sweeps, which are often thought to have a significant impact on viral evolution. One reason that could explain the high microdiversity of viruses is therefore probably related to the high diversity of their hosts.

In viral ecology, unlike bacterial ecology, we are still far from answering some fundamental questions, such as the *in situ* abundance of co-occurring strains/species related to the structure of the viral community. The future lies in sequencing the entire genome of all co-existing viral variants in a sample to capture the full microdiversity of each virus.

THE CONCEPT OF VIRAL SPECIES

Viruses differ fundamentally from other organisms, explaining the specificity of the nomenclature adopted for the former, in particular parasitism and the absence of sexual reproduction. However, the very definition of viral identity remains a subject of ongoing debate.

A viral species is thus regarded as a biological entity that forms a polythetic class of viruses, constituted by their progeny and delimited by the occupation of a particular ecological niche. "Viral quasi-species" refers to different subpopulations of the same virus infecting the same host when these replicate at high speed and with a high mutation rate. This results in a vast genetic diversity.

THE OUTSTANDING WORK OF THE ICTV

The International Committee on Taxonomy of Viruses (ICTV) is an authoritative committee on virus classification in the virological community. It publishes an annual revision of the viral classification and has a database (ICTVdB), in which all known isolated viruses (i.e. several thousand belonging to more than 100 different families) are referenced and characterized.



ARE VIRUSES ABUNDANT IN THE OCEAN?

The answer to whether viruses are numerous in the ocean is a definitive yes. Indeed, the study of aquatic viruses really began when this exceptional abundance was demonstrated. In 1989, Bergh *et al.* published an article in the prestigious journal *Nature*, which remains to this day the reference and starting point for research on the importance and role of viruses in aquatic environments. Since then, viruses have been accepted and repeatedly confirmed to be the most abundant and dynamic biological entities found in aquatic ecosystems. These characteristics lead them to play a number of crucial roles as cell mortality agents, regulators of nutrient and carbon flows, regulators of the genetic diversity of marine microbes, gene transfer agents, etc. (see section *What roles do viruses play in marine ecosystems?*).

There are, on average, between 10^8 and 10^{11} viral particles per litre of seawater; we will go into further detail on this figure later. For illustration and comparison, it is estimated that there are 2×10^{11} stars in the Milky Way! If we reduce our volume to 1 millilitre (i.e. 1 cm^3 of water, the volume of a thimble), there are therefore between 100,000 and 10,000,000 viruses. So it turns out we are swallowing a lot more than just water when we get a mouthful of the sea! This figure sounds incredible, but it is indeed real and, over the last 30 years, has never been contradicted. Although... (see section *Viruses that fly under the radar*).

In contrast, bacteria are typically 5 to 25 times less abundant, despite being the most prevalent cellular entities and the primary hosts for viruses known as bacteriophages (literally, “bacteria eaters”). Each of these values is, of course, a snapshot at a given time and place, and there can be many variations and exceptions (depending upon the environment). Indeed, this ratio between viruses and cellular microorganisms can fluctuate from values below 1 to above 160!

As already mentioned, Bergh *et al.* were the first to report this high figure for natural aquatic environments. Before this, the estimates were much lower, which explains why there was much less interest in this biological community, whose role was thought to be negligible. As early as the mid-1950s, Spencer reported the existence of marine viruses by making the first isolation. It was not until the end of the 1970s that Torella and Morita gave a first quantified estimate, but this first count of marine viruses in an environmental sample already revealed 10^4 viruses per millilitre!

It is therefore understandable that with their seminal paper, aptly titled *High abundance of viruses found in aquatic environments*, Bergh *et al.* revolutionized the prevailing knowledge of the time. They reported concentrations of 4.8 to 9.9×10^6 viruses per millilitre in various Norwegian fjords, 10.1×10^6 in Chesapeake Bay, and 14.9×10^6 in the Atlantic Ocean. However, in some environments such as other fjords or the Barents Sea, the reported concentrations were lower (less than 10^5 viruses per millilitre), but the authors stated, almost apologetically, that these samples had been obtained during winter (compared with the others, obtained in the productive period) and that the season had surely caused the lower abundances! The highest concentration reported in this study was 2.5×10^8 viruses per millilitre; however, this sample did not come from a salt water environment but from a German eutrophic lake, thus revealing that fresh waters are obviously also home to high numbers of viruses. This book is only concerned with marine viruses, but there is much to be said about viruses in continental aquatic environments (including freshwater bodies: lakes, ponds, rivers, groundwater, lagoons, estuaries, etc.). Finally, the Bergh *et al.* study also indicated that the viruses appeared to be predominantly bacteriophages between 30 and 100 nanometres (nm) in size.

As a result of this work, the number of articles on marine viruses has steadily grown, and most environments have been studied: from the equator to the poles, from closed seas to the open ocean, from coastal waters to great depths, etc. An internet search on search engines such as Google Scholar, PubMed or Web of Science, using keywords such as *virus*, *viral*, *virio**plankton*,

marine, ocean, allowing all combinations, reveals between 60,000 and 80,000 references over the period 1955–2020. Keeping only the term *oceanography* (with *virus*, *viral*, *virio plankton*) in Web of Science, a clear evolution of this number can be seen over time, as illustrated below (Figure 4).

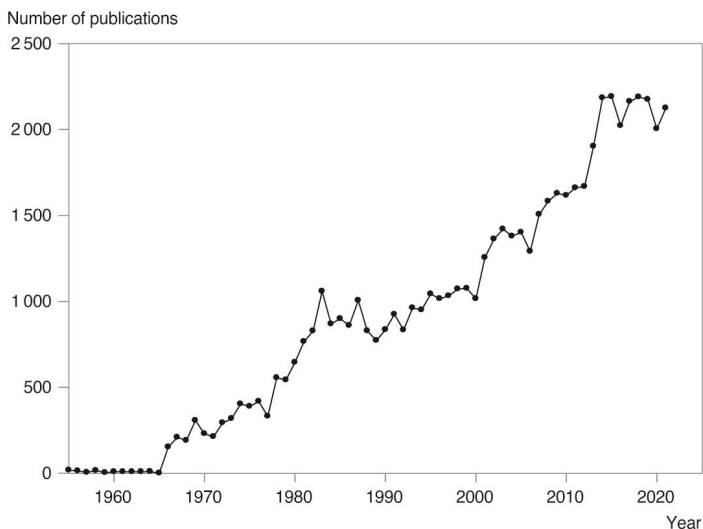


Figure 4. Evolution of the average annual number of publications on marine viruses in the Web of Science database, obtained by combining the term *oceanography* with the term *virus*, *viral* or *virio plankton*.

Naturally, numerous review articles have regularly assessed the current state of knowledge on the quantitative importance of viruses and their diverse roles. If we stick to the purely quantitative aspect, figures vary from a little less than 10^6 viruses per millilitre to a little more than 10^8 viruses per millilitre, depending on whether we are at the bottom of the oceans or in rich coastal waters. At the scale of the whole earth, this means that there would be up to 4×10^{30} viruses in the seas and oceans, this figure being simply calculated by multiplying the total volume of water in the seas and oceans (1.3×10^{21} litres) by the average abundance of these particles (3×10^9 viruses per litre). Total terrestrial viral abundance has recently been revised, and the

figure given above, commonly cited in the literature for several years, has been confirmed as a good approximation. This viral count, which arguably should be termed the “Hendrix estimate” in tribute to the author who provided one of the first, and remarkably accurate, estimates (Mushegian, 2020), has recently been revised to around 10^{31} particles, thus close to the estimate above. It is widely associated with the ocean, which contains 4 to 5×10^{30} virus-like particles (mostly bacteriophages). One hundredth of this huge quantity would be “renewed” every day. It is estimated that 10^{28} viruses are produced daily in the global ocean. If one writes this figure out with all its zeros (10,000,000,000,000,000,000,000,000,000), one understands better the extent of this process. Of course, there are large variations depending on ecosystems, habitats, etc., and the daily production values actually vary between 10^5 and 10^8 viruses per millilitre per day, i.e. the time required for total viral community renewal (turnover) being from 0.05 to 30 days.

Other astronomical figures can also be put forward. If we consider that each viral particle has an average size of 100 nm, and that all of these particles found in the ocean are put end to end like a pearl necklace, this would then be about 10 million light-years long, a hundred times the diameter of our galaxy! When expressed in units of biomass (the total organic matter within living organisms), the viral contribution remains negligible compared with the cellular world. This is due to their minute size, especially in contrast to bacteria and archaea, which account for over 90% of this biomass in terms of carbon units. Even so, viruses account for 200 million tonnes of carbon, the equivalent of 75 million blue whales!

In general, the viral abundance observed at any given time represents a dynamic balance between production and loss processes, and tends to increase with ecosystem productivity (i.e. it is higher in ecosystems where the highest biomass growth rates are recorded). Thus, in the marine environment, concentrations generally decrease from the coast to the open ocean and from the surface to deep water (from 10^8 to 5×10^6 viruses per millilitre on average). High concentrations (typically 10^8 viruses per millilitre) can also be found in surface microlayers or in and on particles

suspended in water. However, it should be noted that in some cases (e.g. deep oases with hydrothermal fluids), viral abundance may be significantly higher (in the above example, abundances at depth are higher than in overlying surface waters). These particles will also generally be the only microbial “predators”.

Virus abundance does not therefore remain constant over time but tends to fluctuate on all time scales studied (from a minute to a year). On a seasonal scale, peak concentrations are often recorded in spring and summer in surface waters, followed by a subsequent decline in autumn-winter periods. This phenomenon can easily be explained. In spring, when photoperiod and water temperature increase, there is higher photosynthetic activity and increased exudation of dissolved organic carbon from algae. This leads to an increase in bacterial production, which, in turn, promotes viral proliferation. Autumn, with its strong mixing of surface waters, is also often characterized by high viral concentrations, although “viral load dilution” will also take place through the water column. Seasonal changes in virus concentrations are naturally correlated with those of their hosts (bacteria, phytoplankton). This correlation is hardly surprising since viruses, which are obligatory parasites of cells, need a host in order to multiply. This host can only be encountered as a result of incidental events, in a manner similar to the diffusion of particles into the environment. Since bacteria and phytoplankton are the most abundant organisms in aquatic ecosystems (eubacteria and archaea account for up to 90% of the total biological carbon found there), it is easy to understand why there are close relationships between viruses and bacteria (Figure 5). Strong relationships have also been observed between viruses and the phytoplankton community, often quantified using indirect measurements of chlorophyll *a*. However, other factors can also be involved and contribute to changes in viral abundance in aquatic systems.

While surface waters are generally richer in viruses than deep waters, it is important to note here that the upper layer of the oceans is also a place where a steep viral decline is recorded. Viral particles are sensitive to solar radiation, in particular UV radiation, which can remove the infectivity of these particles and degrade them.

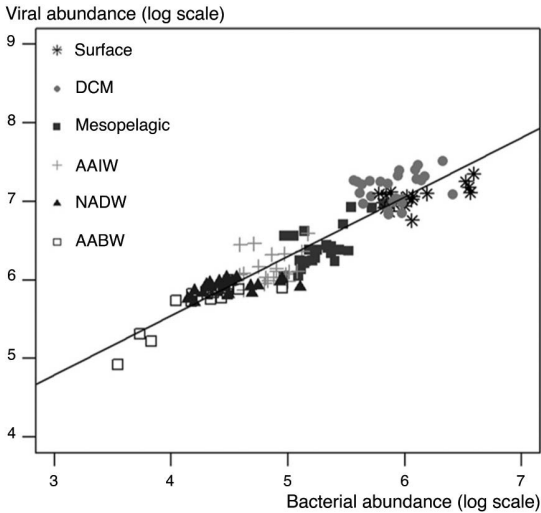


Figure 5. Correlation between viral and bacterial abundance, expressed on a logarithmic scale.

The symbols represent different parts or depths of the water column, such as the surface, the mesopelagic zone, the maximum chlorophyll layer, etc. The strong correlation observed in the graph is fairly typical and is found to a greater or lesser extent in most aquatic ecosystems. Here, the authors have distinguished between different zones (the surface, the depth of maximum chlorophyll concentration, the deep zone, etc.).

Source: Eggleston and Hewson, 2016 (CC BY 4.0).

Overall, viral abundance appears lower in winter than in other seasons and its increases are recorded at key periods (early spring, mid-summer, early autumn), while seasonal trends during the rest of the year are quite variable. Although viral abundance fluctuates between seasons, the frequency of observation, usually between once a week and once a month, reflects the very strong dynamics of these entities on shorter time scales. The turnover times of viral communities are very fast: from a few hours to a few days. Ten minutes may be enough for their concentration to be multiplied by four! Working on short time scales clearly reveals the extent to which these particles are highly dynamic. However, few studies have yet focused on this short-term variability. The patterns observed at the temporal scale are also mirrored at the spatial scale, and so a large variability in viral abundance

can be recorded over a distance of a few centimetres, or even on a smaller scale when examining microzones and aggregates.

The predominance of viral over bacterial abundance can easily be observed and is generally defined using the virus-to-bacteria ratio (VBR), often used as a descriptor of the relationship between viruses and their potential hosts. As already mentioned, this ratio generally ranges from 1 to 160 for marine ecosystems (with an average value of around 25). The highest values are often recorded in lakes and are indicative of viruses actively infecting microorganisms, which themselves must be metabolically active. Virus-to-bacteria ratio is therefore logically higher for more nutrient-rich and productive environments. This observation suggests that host bacterial populations produce a greater number of viruses under environmental conditions that promote rapid growth and high productivity. These increases in viral production in nutrient-rich environments are also partly due to higher infection rates and a higher number of viruses produced per infected host, also known as burst size or yield. These two biological parameters can strongly influence VBR. In sediments, the VBR often remains very low (although still ranging from 0.03 to 410). Values have rarely been reported and low viral abundances in such situations could be explained by high rates of loss (decomposition) due to high temperature, UV radiation, digestion by extra-cellular enzymes, absorption of viruses onto other types of particles, predation of viruses (called virivory) by small nanoflagellates, etc. Some of these processes remain very little studied.

VBR *VERSUS* VPR

Rather than virus-to-bacteria ratio (VBR), it is more accurate to use the term virus-to-prokaryote ratio (VPR). Indeed, abundance values have often been obtained from counts made by epifluorescence microscopy or *via* flow cytometry, techniques that do not separate bacteria from archaea. The designation VBR is often used in the literature, but it almost certainly refers to more than just bacteria *stricto sensu*. Thus, in the figure below, taken from the article by Parikka *et al.* (2017), the authors sought to analyze everything “hidden” behind the value of that ratio, clearly preferring to use the term VPR and explaining why.

While VBR increases as one goes down through the water column, this measure, although interesting and still in frequent use, has sometimes led to inconsistent and opposite conclusions. An analysis by Parikka *et al.*, aimed at elucidating the factors underlying this relationship, revealed that the ratio is highly dependent on a multitude of variables (Figure 6).

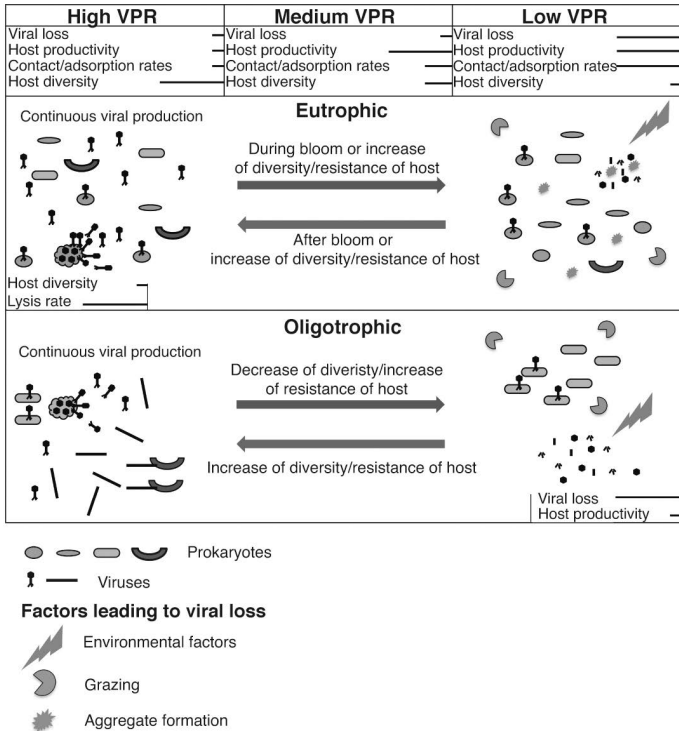


Figure 6. Schematic summary of trophic conditions and other factors that may influence the virus-to-prokaryote ratio (VPR) value.

The VPR may depend on several factors, among which host productivity and viral loss (e.g. decay, adsorption onto particles, virivory, etc.) are probably the most influential. Eutrophic and oligotrophic ecosystems are illustrated in the figure with shared characteristics (second row) and characteristics specific to each (third and fourth rows). These characteristics may vary within each ecosystem (e.g. host production rates may be high or low in oligotrophic systems).

Source: Parikka *et al.*, 2017 © 2016 Cambridge Philosophical Society.

ARE OCEAN SEDIMENTS A RESERVOIR OF VIRUSES?

Viruses are therefore very abundant in all the seas and oceans of the planet. Moreover, we have so far only considered the water column, i.e. the liquid medium in which the viruses live in suspension. An even larger viral reservoir exists in the sediments. Made up of particles coming from the surface or from the continents, which have been deposited on the seabed under the effect of gravity, often in variable successive layers or strata, sediments constitute an ecosystem in their own right. Various independent studies (summarized notably by Danovaro *et al.*, 2008, and Pinto *et al.*, 2013) concluded that viruses are abundant and active in these benthic ecosystems (as opposed to the planktonic component that occupies the water column from the surface to the bottom), with current measurements of viral abundance generally indicating wide ranges, from 10^8 to 10^{11} viruses per gram of dry sediment, and viral production rates of the order of 10^6 to 10^8 viruses per gram per hour. The same applies to benthic prokaryotes, which have concentrations in the range of 10^7 to 10^9 cells per gram of dry sediment. This also means that, in terms of volume, the viral abundances on and below the surface of the sediments exceed those of the water column by 10 to 1,000 times. It is therefore understandable that the ocean floor is an important reservoir of sedimentary particles, especially since viruses adsorbed on these particles have a multiplied lifespan. On a global scale, this viral reservoir contains in the order of 3×10^{29} viruses, including the superficial sedimentary part alone.

Virion concentrations in the marine sediment surface are generally higher than in the water immediately above. As in the water column, viruses become less abundant with depth in sediments but remain detectable at depths of 4,100 metres (m) below the seabed and in sediments estimated to be hundreds of thousands of years old, or more.

IN THE DEEP SEA

High viral abundances reported for the ocean are often those of surface waters. This leads to the question of abundances in deep water, in the hadal zone, beyond 6,000 m depth: cold, dark

and high-pressure environments accounting for 1% of the oceans. Whereas most organisms find these habitats inhospitable, many microorganisms thrive in such extreme niches, exhibiting remarkable resilience where others cannot survive. It is difficult to give viral abundance figures because studies are still rare. However, in samples of fluid from the Juan de Fuca rift, viral concentrations of 0.2 to 2×10^5 viruses per millilitre have been reported, the viruses being predominantly archaeal viruses known to be able to withstand extreme conditions, such as can be imagined here, in terms of pressure and temperature ($> 60^\circ\text{C}$). Recently, a study was conducted at depths of up to 10,000 m in the Kermadec Trough and Atacama Trough regions by Schaubberger *et al.* (2021). These authors estimated viral and prokaryotic abundances. While these are, admittedly, much smaller than those close to the surface and decrease as one goes deeper, there are still between 10^3 and 10^4 prokaryotes (bacteria and archaea) and between 10^4 and 10^5 viruses in each millilitre of water, with a clear correlation between these abundances.

ESTIMATED NUMBERS OF VIRUSES ON EARTH

1.2×10^{30} viral particles in the whole ocean
 10^{28} viruses produced every day across the whole ocean
 2.6×10^{30} viruses in soils
 3.5×10^{30} viruses below the ocean surface (including sediments)
 Between 0.25 and 2.5×10^{31} viruses in the terrestrial subsurface
 Total virosphere equal to 10^{31} particles

OXYGEN MINIMUM ZONES

Marine oxygen minimum zones (OMZs) can also be considered as “extreme environments”. Today, these account for around 7% of ocean volume and have increased significantly due to global climate change over the last 50 years, especially in the North and Equatorial Pacific where the volume of water considered anoxic (dissolved oxygen, O_2 , is below the detection limit) has quadrupled.

The expansion of OMZs is expected to have positive feedback effects on climatologically active trace gases, typically methane (CH_4),

nitrous oxide (N_2O) and dimethylsulfide (DMS), resulting from chemotrophy by unique microbial assemblages present in these regions. Microorganisms in these areas deplete bioavailable nitrogen by anaerobic oxidation of ammonium and denitrification, accounting for 30-50% of ocean nitrogen removal. Recent research in OMZs has largely focused on the cycle of these major nutrients, as well as on the taxonomically and functionally distinct microorganisms responsible for key metabolic processes. Conversely, information about viral communities in these regions has remained scarce.

Recently, it has been revealed that although viral abundance tends to be lower in these areas with low oxygen content, this is not the case for the frequency of infected cells (for which the burst size remains high). In addition, the diversity of viral communities differs between this type of habitat and elsewhere in the water column. Thus, the auxiliary metabolic genes (AMGs, see box: *AMGs in brief*) found in viruses are higher in number in the oxygenated part of the water column, linked with the increase in host metabolism. In OMZs, the requirement seems to be rather to rationalize genomes and retain only essential genes, in response to the limitation of physical space, energy and materials needed for DNA replication. Thus, when host metabolism is sufficient to support viral replication, viruses may not need to encode AMGs related to the predominant biogeochemical reactions performed by their hosts in this environment, but rather selectively encode any gene that increases viral replication.

AMGS IN BRIEF

Auxiliary metabolic genes (AMGs) are found in many bacteriophages but come from bacterial cells. They are known to become involved in host cell metabolism during infection so that the phage can replicate more efficiently. For example, bacteriophages that infect the most abundant marine cyanobacteria (namely the genera *Synechococcus* and *Prochlorococcus*), which are called cyanophages, carry AMGs acquired from their immediate host, as well as more distantly related bacteria. In cyanophages, AMGs support a variety of functions, including photosynthetic carbon metabolism, nucleic acid synthesis and metabolism (see box: *Photosynthetic genes in cyanobacterial viruses*).

VIRUSES THAT FLY UNDER THE RADAR

Viruses are therefore detected and abundant wherever there is life, but are the same viruses found everywhere? Globally, the majority of viruses are recognized as bacteriophages (most commonly referred to as phages), with the bacterial compartment being the most abundant host. However, in some environments or habitats, it seems possible that other types of viruses are predominant, with the predominant hosts being, for example, archaea or eukaryotes.

Everything we have said so far has mainly concerned double-stranded DNA viruses, i.e. those viruses that are “easily” detectable by the techniques discussed in the box *Different methods for counting free viruses*. With the “omics” tools that have been developed over the last two decades and the discovery of the incredible diversity of viruses, it has also been found that some types have been largely overlooked and underestimated, namely single-stranded DNA viruses (Holmfeldt *et al.*, 2012) and RNA viruses. In 2009, Lang *et al.* found that RNA viruses, in addition to their diversity of hosts in the marine environment, had a high chance of being as abundant, or even more abundant, than DNA viruses, which Steward *et al.* highlighted in a very original way a few years later (in 2013) in Hawaiian coastal waters. In summary, the authors compared the total mass of RNA and DNA in the viral fraction harvested from seawater and, using data on the mass of nucleic acid per virion containing RNA or DNA, were able to estimate the abundances of each. Their data suggested that the abundance of RNA viruses rivalled or exceeded that of DNA viruses. Also, the dominant RNA viruses were picorna marine viruses (after “pico”, meaning very small, and “rna”, for RNA), characterized by small genomes below the detection limit of common fluorescence-based counting methods (epifluorescence, cytometry) and infecting eukaryotes.

DIFFERENT METHODS FOR COUNTING FREE VIRUSES

Two techniques are used to quantify the abundance of infectious viruses, i.e. those causing lysis of one or more particular hosts. They require that these host cells are cultivable, which is not the case for most microbial taxa in the ocean.

The **plaque assay approach** is used to estimate the abundances of viruses that cause lysis of bacteria, cyanobacteria and microalgae that can be grown on solid media. To do this, host cells and viruses are combined with molten agar, which is poured over a bottom layer containing a higher percentage of the same agar, typically in a Petri dish. Infectious viruses will form a plaque on a lawn of host cells. The number of plaque-forming units formed in a given volume of water can be estimated using this method.

The **most probable number approach** is also used for host cells that are cultivable but not on solid substrates. Here, a dilution series is performed, typically in tenfold increments, with several replicates of each dilution. Dilutions in which no growth—or growth followed by cell lysis—is observed 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 infectious units in the original sample.

While both these methods can be used to directly determine the abundance of infectious viruses, they do not provide any information on the total abundance of free viruses in a sample.

Transmission electron microscopy is the only method that provides data on both the abundance and morphology of viral particles. Viruses need to be concentrated from seawater, placed on a support grid and stained with electron-dense material, such as uranyl acetate. This approach has the advantage that virus-like particles can be identified and quantified. However, there are many technical biases, such as virus losses during concentration, colour quality and visualization of viruses, which may lead to inaccurate estimates of total abundance. For virus enumeration, this approach has been largely replaced by epifluorescence microscopy and flow cytometry, which provide more reliable estimates.

Epifluorescence microscopy has long remained the most widely used approach for estimating the total abundance of viral particles. In this method, viruses are concentrated on a membrane filter, their nucleic acids are marked with a luminous fluorescent dye, and the abundance of viruses can then be calculated.

.../...

.../...

The first estimates of viral abundances made by this method used the dye DAPI (4',6-diamidino-2-phenylindole), although the fluorescence was close to the detection limit for many microscopes. Subsequently, a new generation of luminous fluorescent dyes, such as YO-PRO11, especially the SYBR family (Green I, Green II, Gold and Safe), made the counts accurate.

Since 1999, **flow cytometry** has made it possible to obtain rapid and reliable counts of aquatic viruses and to discriminate up to 4 or 5 subgroups within this community, called VLP1, VLP2, VLP3, VLP4 (for virus-like particle 1, etc.). This precise, high-throughput method also makes it possible to analyze a large number of samples and obtain extensive data on the abundance of free double-stranded DNA viruses. However, this method is not optimal for obtaining counts of RNA or single-stranded DNA viruses, the quantitative importance of which is now known in ecosystems.

An alternative means can be provided by **quantitative PCR (qPCR)**, which can be used to quantify the abundance of a given virus (whether DNA or RNA) present in a sample. In short, viral nucleic acids are purified from the sample and then—using a virus-specific primer pair—a PCR is performed in the presence of either a dsDNA dye marker or a sequence-specific reporter such as a TaqMan probe. A pre-retrotranscription step (i.e. RNA-to-DNA conversion) is necessary if the virus under investigation is an RNA virus. As DNA amplification increases, the fluorophore signal increases. The rate of this increase is proportional to the amount of initial nucleic source material present. The number of (thermo)cycles required to produce a minimum level of fluorescence (called the Ct or Cq value) is compared with that of a serially diluted standard of known concentration. This comparison gives the number of genomes compared to the viral standard. This approach has many advantages: it is relatively fast, very specific and uses equipment readily available in a molecular biology laboratory. However, it also has several disadvantages, the main one being the requirement to have virus-specific primers to carry out the PCR reaction and to obtain a reliable enumeration of a given population. If you are dealing with many types of viruses or strains that vary with the seasons, for example, the primers must be specifically designed for each virus or virus strain. Finally, it should be noted that all these methods remain problematic for viruses that are attached to particles, which can be particularly abundant. Regardless of the method chosen, only the free fraction of the viral community is considered. This represents only a portion of the total, as integrated viruses are effectively “overlooked”.

The significance of RNA viruses was recently confirmed. Following the analysis of water samples collected between 2009 and 2013 during the *Tara Oceans* scientific expedition, which had already identified at least 200,000 distinct DNA viruses, RNA viruses were shown to be both ubiquitous and abundant across the global ocean. Their diversity also proved to be far greater than previously estimated. In addition to the 4,300 previously classified RNA virus species, 5,500 new species were discovered. Remarkably, many of these novel viruses did not belong to any known phylum, potentially representing a missing link in the evolution of RNA viruses. In fact, the five ranks of the RNA virus classification that already existed were not sufficient, and scientists had to create five more phyla that could include thousands of other species. It is understandable that with this result, published in spring 2022, the evolutionary tree of RNA viruses was completely modified, doubling its number of branch lines (including two present throughout the global ocean). Furthermore, the role of RNA viruses in the evolution of early life forms on Earth has been clearly highlighted, as have the essential ecosystem services they provide, such as oxygen production and ocean carbon sequestration.

Since the 2010s, “viromic” studies have made it clear that we can only see the tip of the iceberg: most viral sequences are surely yet to be discovered and the majority of those revealed are still unidentified (i.e. they cannot be classified taxonomically). Today, the question remains of how this exceptional diversity is generated.

Marine viromes change more with space than time. Thus, in the ocean, they vary significantly with depth (both in number of species and gene content), whereas they appear more stable and persistent over time. This has been clearly observed for some cyanophages (cyanobacterial phages).

Another uncertainty about what is counted and what is not, is caused by those elements that resemble viruses but which are not (Forterre *et al.*, 2013; Colombet *et al.*, 2020)! In aquatic environments, within the very closed group of femtoplankton (i.e. biological organisms or particles smaller

than 0.2 μm), there are not only viruses, but also extracellular vesicles, organomineral biomimetic particles, star-shaped or archaic nanoparticles (Aenigmarchaeota, Nanoarchaeota, Nanohaloarchaeota, Parvarchaeota). The high abundances of these entities found in some situations raise the question of the actual quantitative contribution of viruses. Most estimates of viral abundance are based on the counting of “viral particles” by positive labelling of nucleic acids. These estimates probably lead to an overestimation of true viruses by counting all the potential nucleic acid carriers described above, bearing in mind that “free” DNA can be found in the environment, and that virus DNA is frequently integrated into genomes of cellular organisms, which could thus sometimes be detected as viruses. The extent of this overestimation could have a fundamental impact on the ecological role of viruses. Soler *et al.* (2015) suggested that extracellular vesicles may be more numerous than true viral particles in some aquatic environments (work revealing concentrations of 10^5 to 10^6 vesicles per millilitre) and Colombet *et al.* (2020) reported that star-shaped nanoparticles (aster) may account for up to 40% of the virioplankton counted by transmission electron microscopy. Aster-like nanoparticles (ALNs) are femtoplankton-sized entities (such as viruses), recently discovered in different aquatic environments, and whose intrinsic nature and ecological characteristics remain to be determined. The abundances of ALNs can be significant and show a marked seasonal dynamic (from undetectable to several million particles per millilitre). It appears that some ALNs are strongly associated with certain bacterial phyla, suggesting a broad and nonspecific relationship. Through their seasonal dynamics and their potential link with prokaryotes, ALNs could be an important ecological actor in the functioning of aquatic ecosystems and could have been confused with viruses themselves for many years. It is therefore possible that all the above figures have been largely overestimated, so new estimates would be welcome.

Finally, it should be noted that the scientific literature available in 2022 shows that, while we have a good idea of the importance of viruses in regulating marine microbial diversity and certain functions, there are still significant knowledge gaps about

the factors that regulate the abundance of viral populations. Typically, many questions remain concerning how viruses are renewed in the water column. Admittedly, it is known that their abundance and infectivity are sensitive to certain near-surface factors, such as UV rays, or that part of their decline can be attributed to non-selective grazing by filter feeders or to sedimentation by attachment to cells or particles that sink to the depths. Recently, the possible role of certain organisms has been highlighted, such as appendicularians during phytoplankton blooms terminated by significant lytic viral action. This was shown with *Oikopleura dioica*, which is capable of removing up to almost 90 viral particles per millilitre per day, although it has also been observed that some of the ingested viruses are released and retain their infectivity. This type of finding echoes the fact that picophytoplankton and microbial abundance are often reported as the main factors controlling viral populations in the epipelagic and mesopelagic layers of the ocean, supporting the idea that viral distribution in the surface ocean depends primarily on host availability. Conversely, deeper in the bathypelagic or hadal zone, viral abundance is more often found to be weakly related to host abundance, suggesting that other environmental and/or biological variables may control viral distribution and virus-host interactions in the ocean depths. Intuitively, one would think that the viruses that best exploit their hosts, thus lysing them most efficiently by rapidly producing the most virions, would perform better in terms of evolution than less virulent variants (in terms of abundance and distribution). However, a study from 2022 in the Red Sea shows, by comparing two related groups of cyanophages, that it is the least well “performing” group in terms of the characteristics mentioned above that is much more abundant (in the order of 10 times more). It therefore seems, at least in this case, that the ecological success of marine viruses is favoured by intermediate virulence. Here, it is suggested that these viruses have a larger reservoir of hosts to infect, hosts whose abundance would drop too if faced with viruses possessing higher virulence. Of course, this dynamic is most likely related to the complexity and characteristics of each microbial ecosystem, as well as to the degree of specificity of the viruses concerned and

their mode of transmission. We must therefore be careful not to generalize this result; furthermore, it illustrates the inherent risk of extrapolating observations derived from overly reductionist approaches.



ARE ALL MARINE ORGANISMS AFFECTED BY VIRUSES?

In principle, all living organisms, from bacteria to whales, are susceptible to infection by one or more viruses. The rise of genomics has revealed that the presence of viral genes acquired during evolutionary history in the genome of many marine organisms is evidence of past infections.

VIRUSES OF WHAT?

The major categories of marine (and more broadly, aquatic) viruses currently in the spotlight and driving scientific debate primarily include the viroplankton, giant viruses and their virophages, and finally, animal viruses. As described above, viroplankton includes the fraction of viruses suspended in the water column, which is found in very high abundance, up to a hundred billion per litre of seawater. One of the characteristics of this viroplankton, and viruses in general, is that it does not have the ability to move. It diffuses passively into the surrounding water until the ideal encounter with a suitable host. Because cellular/microbial life forms (bacteria, archaea, microalgae) dominate marine ecosystems, they are naturally prime targets for viroplankton. Since the discovery of the first marine virus in 1955, research in this field has brought to light viruses of a remarkable diversity of shape and size. For example, approximately 60 viruses have been identified that infect microalgae, belonging to 6 phyla and 10 classes. These viruses are characterized by high natural variability, encompassing diverse genetic compositions (DNA or RNA), nucleic acid architectures (double- or single-stranded, linear or segmented), and genome sizes ranging from approximately 10 kilobases (kb) in the smallest RNA viruses to nearly 600 kb (and potentially beyond) in giant viruses. The number of viruses isolated and diversity of hosts they infect are, of course, tiny compared with the estimated 64,500 microalgal

species (belonging to 14 phyla and 51 classes). It is likely that most, if not all, algal species are hosts of one or more types of viruses. In addition, the overwhelming majority (usually 90%) of sequences from viruses detected in marine viromes still remain taxonomically unassigned, suggesting that an enormous viral diversity remains to be discovered.

For multicellular organisms, there was initially only a real interest in viruses when high mortalities were observed, typically those of fish on farms and when this type of aquaculture became commercially important and solutions had to be found to such problems. Besides this, a few studies and the media regularly report mortalities of marine mammals (especially dolphins and pinnipeds). While such events remain anecdotal compared with induced mortality in the microbial world, the pathogenic role of these viruses should not be minimized, particularly considering recent examples of dolphin and seal mortality. One example is the discovery and possible emergence of a new eukaryotic virus belonging to the genus *Morbillivirus*, which contaminates Fraser's dolphins, a very social cetacean species likely to spread the disease to their congeners and other species (of dolphins or whales) around the world. Although *Morbillivirus* examples have been known for many years, this is a new type. The threat is taken so seriously that vaccination of as many animals as possible is being considered and already being done in some countries (e.g. Hawaiian monk seals, a small and relatively localized population).

Another recent example demonstrating the permeability of the species barrier is the infection of sea otters by pinniped distemper, also caused by a *Morbillivirus*. This disease, easily transmissible in marine mammals, results in breathing difficulties, nasal and ocular discharge, fever and erratic swimming. In this case, the reason was found to be related to the retreat of the pack ice and resulting change in the otters' range, revealing yet another negative consequence of climate change. With the melting of the sea ice and opening of new migration routes for animals, who are always searching for new territories and food, there is a significant concern that this type of disease will spread from one ocean to another.

Many viruses associated with marine vertebrates have been recently identified. These belong to various groups (e.g. Arenaviridae, Astroviridae, Filoviridae, Flaviviridae, Hepadnaviridae, Matonaviridae, Paramyxoviridae, Reoviridae, Rhabdoviridae), suggesting that marine animals have natural, diverse and still largely unknown viromes. Given that marine invertebrates are the most diverse known group of animals on the planet, it is also not surprising that many RNA viruses have been found to be associated with them (several hundred).

THE EXAMPLE OF THE OCEAN ABYSS

The ocean abyss is a specific environment, which is cold and dark, with the weight of the water column creating high pressure. The ocean's great depths have often been described as extreme environments, and rightly so, because not everything can live there. Some microorganisms, bacteria and archaea (such as *Thaumarchaeota*), are perfectly at ease, as are their viruses. The diversity of the latter is still largely unknown, and the vast majority of the sequences reported is absent from existing databases. What is known and striking is the extraordinary morphological diversity of archaeal viruses (which can have the shapes of bottles, ampoules, lemons, etc., Table 1).

An even more specific habitat, also generally characteristic of the deep seabed, is that of hot thermal vents, where hydrothermal fluids escape from the bowels of the Earth at temperatures sometimes exceeding 300°C (the ambient pressure preventing this liquid from boiling). These fluids are none other than seawater which has penetrated the ocean floor and then heated by contact with the rock and become enriched with various elements. Again, archaeal viruses were isolated and described as infecting *Pyrococcus abyssi*, a species itself isolated from a hydrothermal fluid at 2,000 m depth. This virus was the first from the abyss to be isolated and characterized.

Another remarkable deep-sea discovery is *Tupanvirus*, a member of the exclusive group of giant viruses that is fundamentally challenging our perspective on the place of viruses in the living

Table 1. Schematic morphological diversity of archaeoviruses.

Viral morphology	Family	Host species
Brooch	Fuselloviridae 	<i>Sulfolobus</i> , <i>Acidianus</i> , <i>Haloarcula</i> , <i>Pyrococcus</i> , <i>Aeropyrum</i> , <i>Stygiolobus</i> and <i>Thermococcus</i>
	Bicaudoviridae 	<i>Acidianus</i>
	Spiraviridae 	<i>Aeropyrum</i>
Spherical	« Halosphaerovirus » 	<i>Haloarcula</i> and <i>Halorubrum</i>
Pleomorphic	Pleolipoviruses 	<i>Haloarcula</i> , <i>Halorubrum</i> and <i>Halogeometricum</i>
Head and tail	Myoviridae 	<i>Halorubrum</i> , <i>Natrialba</i> , <i>Halobacterium</i> , <i>Haloarcula</i> and <i>Methanobacterium</i>
	Podoviridae 	<i>Haloarcula</i>
	Siphoviridae 	<i>Haloarcula</i>
Bottle	Ampullaviridae 	<i>Acidianus</i>
Shape of a bacillus	Clavaviridae 	<i>Aeropyrum</i>
Drop	Guttaviridae 	<i>Sulfolobus</i> and <i>Aeropyrum</i>
Linear	Lipothrixviridae 	<i>Acidianus</i> and <i>Sulfolobus</i>
	Rudoviridae 	<i>Sulfolobus</i> , <i>Stygiolobus</i> and <i>Acidianus</i>
Spherical	Globulaviridae 	<i>Pyrobaculum</i> and <i>Thermoproteus</i>
Icosahedral	« Turriviridae » 	<i>Sulfolobus</i>

Source: Snyder et al., 2015.

world (see box: *The incredible story of giant viruses*). *Tupanvirus*, about 1 μm in size, was not isolated from liquid media but from deep sediments. Its host is likely a eukaryote, such as a protist or an amoeba, yet almost nothing is currently known about its ecology.

VIRUS RESISTANCE

Viruses exert significant pressure on their hosts, particularly when high virulence reduces host fitness or leads to cell death, as seen with lytic viruses infecting bacteria and marine microalgae. In this situation, virus-resistant hosts have a very large advantage and resistant strains are expected to emerge regularly.

It is possible to experimentally generate host strains resistant to a marine virus, for example in microalgae. When the culture of a microalgae is infected with a specific viral strain, the density of microalgae decreases very rapidly. However, the hosts do not completely disappear; a few cells survive, which can be transplanted and grown. This is how a resistant strain is selected.

The mechanisms of virus resistance and cellular defence are varied. In prokaryotes, it should be noted that more than 60 defence or resistance systems have been genetically identified and are known to be conserved up to the “higher” vertebrates (mammals). Among these mechanisms is the now-famous CRISPR-Cas system (clustered regularly interspaced short palindromic repeats-associated protein), in which viral sequences are integrated into the host genome to serve as an immunological memory. This allows the host to recognize subsequent viral infections and eliminate them using Cas proteins. Other mechanisms involve the surface proteins of the hosts, which are the “locks” into which the specific viral “keys” are inserted, allowing infection. Any disappearance, modification or decrease in the number of these receptors therefore makes it possible to resist viruses. It is also possible to limit viral multiplication in the host cell through modification of the proteins involved in this process. The “Cheshire Cat” strategy is another resistance mechanism used by *Emiliana huxleyi* against its viruses (see box: *Emiliana huxleyi: a model organism in more ways than one*). Sometimes,

cells do not prevent the entry and replication of the virus, but the progeny are released without destroying the host, which then acts as a “resistant-producer”.

In microalgae of the Mamiellophyceae group, including the genus *Ostreococcus* (known as the smallest free eukaryote), a virus-resistance chromosome has even been shown to be present in the genome! This is a small chromosome with a very different composition from the rest of the genome (hence its name: small outlier chromosome or SOC) and whose genes, coding mainly for membrane surface proteins, are expressed significantly during a viral infection, generating a modification of surface proteins that inhibits viral infection. The size of the SOC varies between resistant and susceptible host strains, suggesting mechanisms for rapid evolution of virus resistance.

Resistance is often associated with lower growth, and this cost prevents resistant cells from dominating populations. In addition, an arms race takes place between hosts and viruses, with great variation coupled with natural selection allowing the latter to bypass the defences of the host, which will then produce new ones, and so on. This type of process is an illustration of what is called the “Red Queen” hypothesis, which accounts for the continuous evolution of a given species to maintain its ability to cope with the evolutions of other species with which it co-evolves. It takes its name from a famous episode in Lewis Carroll’s *Through the Looking Glass*, where Alice and the Red Queen embark on a frantic race. Alice then asks: “*you’d generally get to somewhere else —if you run very fast for a long time, as we’ve been doing*”. The queen replies: “*now, here, you see, it takes all the running you can do, to keep in the same place. If you want to get somewhere else, you must run at least twice as fast as that!*” This metaphor, which therefore symbolizes the evolutionary race as a kind of arms race between species, can also be likened to what children love to do when they find themselves on an escalator. To stay in the same place on an escalator moving in the opposite direction, you have to keep moving forward, otherwise it carries you backwards!

Finally, viruses are often very specific to their hosts, and when resistance appears, it can only be in relation to one or a few

viral strains, whereas several viral strains can usually infect the same host, which cannot resist all of them! If we add to this the evolutionary processes mentioned above, we understand that the dynamics of host-virus associations is complex and difficult to predict.



WHAT ROLES DO VIRUSES PLAY IN MARINE ECOSYSTEMS?

VIRUSES AS DRIVERS OF GENETIC DIVERSITY

Viruses, as we have already seen, are currently the most abundant biological entities in marine ecosystems and perhaps also the most diverse. Over the last 20 years, hundreds of genomes of marine viruses have been studied as a result of the development of sequencing technologies and the falling cost of these analyses. This field of viral genomics consists of identifying genes contained in the genome, describing and comparing their organization, assigning them a function, understanding their relationship to other viruses or cellular organisms, or determining their expression during infection. Such studies shed light on the extraordinary diversity of marine viruses and have revolutionized our view of the ecological role of viruses in the ocean.

Virus genomes have a much higher information density than those of cellular organisms such as bacteria or eukaryotes. The genes carried by viruses, however, are not immutable. In fact, whether marine or terrestrial, all viruses have the ability to capture and integrate genetic material from their host, or other viruses (e.g. through co-infections), into their own genome. While some viruses have incorporated certain host genes into their own genomes, these transfers of genetic material generally occur when host DNA is, more or less accidentally, encapsidated along with, or instead of, the viral genome that typically goes in the capsid at the end of the viral replication cycle. The capsid retains its ability to inject its contents and also does this with the DNA “recovered” from its host, which will be transferred to its offspring during the next infection. However, the size of viral particles, which cannot be infinitely extended, imposes a limit on the integration of new genes.

Many genes formed in the viral world can be transferred to the cellular world. This process is called transduction. All cellular genomes have incorporated viral genes throughout their evolutionary history. In bacteria and archaea, 10-15% of the genomes consist of viral genes. This was also illustrated by genomic analysis of the alga *Bigelowiella natans*, which revealed integrated elements originating from both virophages (see box: *Can a virus itself get infected?*) and giant viruses. The researchers were able to deduce that this alga was targeted by one or more giant viruses (Blanc *et al.*, 2015), which were likely thwarted by these “viruses of viruses” capable of being transmitted from generation to generation. It should be noted that in *Bigelowiella natans*, the DNA polymerase responsible for replicating the nucleomorph (the vestigial nucleus of the endosymbiont) is also of viral origin, specifically from the NCLDV (nucleo-cytoplasmic large DNA viruses). The viruses themselves exchange genes. Advances in comparative genomics have revealed what is known as a mosaic architecture in viral genomes (particularly bacterial viruses), where similar gene blocks (surrounded by different sequences) can be found in many bacterial viruses that are not *a priori* related. This is due to a high level of horizontal gene transfer between viruses, resulting in complex evolutionary histories.

Although rare, a few studies have also revealed that the marine environment is a “hospitable” ecosystem for transduction. Chiura (1997) was apparently the first to demonstrate that virus-like particles produced by five marine isolates could mediate the transduction of various auxotrophic markers to *Escherichia coli* cells. This took place at a frequency of between 10^{-3} and 10^{-5} events per virus after infection of the isolates. One year later, Jiang and Paul found that transduction of plasmid DNA within concentrated marine bacterial communities from the Tampa Bay estuary in Florida could be high: of the order of one transduction obtained for 10^7 to 10^9 potentially recipient bacteria exposed to the viruses. This is equivalent to 1.3×10^{14} transduction events likely to occur every year, just in this small part of the ocean! When extrapolated to the entire ocean, these data suggest that 10^{24} genes would be displaced by viral transduction between host

cells each year. Although impressive, this figure probably does not reflect reality, as the actual amount of transduction by marine viruses and viral entities may be much greater than previously thought, in particular due to the action of gene transfer agents (named GTA, not to be confused with *Grand Theft Auto!*). Gene transfer agents are particles with a morphology similar to that of phages, but smaller (with a diameter of 30 to 50 nm and a DNA molecule of approximately 4 kb). What makes them unique is that they are encoded by host genes and not by a virus, and only package pieces of DNA from that host, providing a very efficient form of transduction. Genes coding for GTAs have been found throughout the sea and in various marine bacteria. As can be expected, transduction *via* GTAs is also likely to have important consequences for marine microbial ecology, but that is another story.

While the ability to acquire and transfer genes is a universal feature for viruses, analysis of marine genomes and viromes has revealed that viruses endemic to this environment have captured a range of genes quite different from their terrestrial counterparts. These genes, also known as “auxiliary metabolic genes” (AMG), generally code for key functions of host metabolism. The AMG acquired by the virus “replace”, reinforce or supplement those of the host that stop working. By expressing these genes during infection, viruses maintain the energy and resources needed to produce their progeny. This maximizes their infection and spread success. Depending on the hosts they infect and the environment in which they live, viruses select different types of AMGs: genes encoding photosynthetic proteins for viruses that infect certain photosynthetic organisms (see box: *Photosynthetic genes in cyanobacterial viruses*), genes involved in the metabolism of sulfur for viruses infecting bacteria growing near hydrothermal vents, or genes involved in the acquisition or transport of nutrients (nitrogen, phosphorus, carbon) that form the elementary bases of the proteins and nucleic acids composing viruses.

PHOTOSYNTHETIC GENES IN CYANOBACTERIAL VIRUSES

Until recently, photosynthesis was considered a process specific to autotrophic cellular organisms: those that make organic matter (sugars), using light energy, water and carbon dioxide (CO_2). In plants, algae and cyanobacteria, photosynthesis leads to the release of oxygen (O_2). Photosynthetic organisms thus affect the balance of our planet's atmosphere and therefore its climate. In 2004, the discovery of photosynthetic genes in the genome of cyanobacterial viruses (also called cyanophages) was a big surprise. Cyanophages have acquired a wide variety of genes from their hosts, involved in different stages of cyanobacterial photosynthesis, such as light capture, pigment synthesis, photosynthetic electron transport or carbon metabolism. The presence of these genes varies depending on the families to which the viruses belong and the hosts they infect. Two of these genes, *psbA* and *psbD*, are particularly common in viral genomes. These code for the proteins D1 and D2, which make up the core of one of the photosynthetic reaction centres: the photosystem II. This protein supercomplex makes it possible to capture light energy and initiate its conversion, through a chain of electron acceptors, into chemical energy in the form of ATP and NADPH. This process is accompanied by the lysis of water molecules (H_2O) into oxygen (O_2), protons (H^+) and electrons (e^-). During the infection process, the expression of the host D1 protein decreases drastically, while the viral D1 protein is overexpressed. It has been suggested that the expression of viral D1 protein compensates for the degradation of this protein in the host, while maintaining the functioning of photosystem II, and enhancing its stability. While the genes *psbA* and *psbD* are by far the best studied, approximately 15 other photosynthetic genes have been identified in viral genomes or metagenomes and their functions are being actively investigated. Current knowledge suggests that viral photosynthetic genes expressed during infection act in such a way as to improve chemical energy synthesis rates and divert this energy, originally used to convert CO_2 into sugars, towards the synthesis of nucleic acids required for the assembly of viral progeny genomes. One of the potential consequences of this photosynthetic piracy is an inhibition of CO_2 fixation by infected cells. Considering that cyanobacteria contribute 10% of global CO_2 fixation and that

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1-60% of cells are infected at some point in time, 0.02-5.39 gigatonnes of carbon per year may not be fixed due to infection. A more precise characterization of viral "photosynthesis" and its regulation is crucial for understanding the role of the oceans in climate regulation.

MARINE VIRUSES: FEARSOME PREDATORS

Infection by a "virulent" virus spreading by the lytic cycle leads to the release of viral progeny by lysis of the infected cell. Virulent viruses therefore cause death when the organism is, as in the case of most plankton, unicellular. Nearly a dozen approaches have been developed to quantify the number of cells infected and lysed by marine viruses. These approaches are based on different methods and hypotheses. Accurately measuring virus-induced mortality remains a major challenge to this day. However, all of these methods indicate that marine viruses are important life-threatening agents for microbial communities, including bacteria and phytoplankton. It is estimated that almost a third of microbes are infected at any given time in the global ocean.

For bacteria, direct observation of infected cells in water and marine sediments indicates that viruses typically destroy 10-30% of daily bacterial production, but estimates can reach 100%! The impact of viruses on bacterial communities is never constant; it fluctuates according to the time of day, the distance from the shore, and the water depth. It also varies based on the specific mix of bacterial populations found in a given area of the ocean. In surface waters, for example, viruses are estimated to eliminate 20-40% of bacterial production daily, with peaks of infection during the day likely synchronized with the cell cycle of the infected populations. However, it is in the ocean depths that the impact of viruses could be the greatest, as they are estimated to eliminate nearly 80% of bacterial production there. This may be due to the fact that viruses represent the primary agents of mortality for bacteria endemic to these environments.

Viruses also play an important role in regulating phytoplankton blooms that develop along the coast or offshore. From spring to

autumn, when environmental conditions are favourable, some microalgae proliferate dramatically, sometimes causing unusual changes to the appearance of the water, such as foam arriving on the shore or colouration of the surface. These events are ephemeral and sometimes suddenly disappear. For a long time, the disappearance of phytoplankton blooms was attributed to grazing by the upper trophic levels (microzooplankton) and/or to the depletion of nutritional resources. Since the early 2000s, viral infection has been considered a major controlling factor of these seasonal blooms. During blooms of phytoplankton species such as *Emiliania huxleyi*, *Phaeocystis globosa* or *Heterosigma akashiwo*, considerable rates of viral lysis mortality are recorded, as microalgae accumulate in the environment. At the end of the bloom, the observation of infected cells suggests that viruses alone may be responsible for its disappearance. Not all phytoplankton species develop blooms, however. Off our coasts, in the open ocean, a complex assemblage of phytoplankton, often dominated by small cells (referred to as picoplankton), colonizes the surface water. A vast diversity of viruses specific to these populations has been described and is thought to contribute to nearly half of the mortality within these assemblages.

Like bacterial populations, the viral lysis mortality of phytoplankton populations varies over time and space. Although not all waters of the globe have been sampled homogeneously (e.g. tropical waters remain largely undersampled), infection and viral lysis appear overall to be higher at low (subtropical) latitudes than at high (polar) latitudes. The reasons for these variations are not yet clear. There are many factors that regulate the speed and efficiency of viral infection. These can be abiotic factors (such as nutrient concentration, temperature, light intensity or UV radiation) that affect the metabolic status of hosts and/or the integrity of viral particles. Biotic factors, such as the ability to develop resistance, the morphology and abundance of hosts, or presence of grazers that can preferentially consume infected cells, are also involved in the regulation of infection. Environmental variability also influences viral replication strategies. Some studies have shown a higher proportion of temperate/latent viruses (replicating by lysogeny) in environments with low bacterial

abundance and activity. Conversely, a majority of virulent viruses (replicating *via* the lytic cycle) are found in nutrient-enriched and warmer waters where productivity is higher.

The specificity of viral infections (viruses infect a narrow host range and rarely cross the species barrier) makes them an important factor in structuring planktonic communities. The theoretical model known as “kill-the-winner” postulates that viruses preferentially regulate the most abundant species, which are considered to be the most competitive at acquiring nutritional resources. Indeed, viruses are not mobile, their encounter with a suitable host is random. Their probability of such an encounter is therefore all the greater if the host population is abundant, such as during phytoplankton blooms for example. Controlling or even eliminating dominant species allows less competitive ones to use resources and develop. Subsequently, a virus capable of infecting the newly dominant species emerges and the cycle continues, resulting in a diversification of the community. Various studies conducted from *in situ* infections or controlled laboratory experiments support the “kill-the-winner” hypothesis, which is a fundamental concept of microbial ecology research. However, there are a number of counterexamples that refute this hypothesis. For example, it has been repeatedly shown that populations of marine cyanobacteria, which are dominant in surface waters, are only weakly infected (e.g. Baudoux *et al.*, 2007; Mruwat *et al.*, 2021). Conversely, some smaller planktonic populations appear to be subject to high levels of viral lysis. These observations highlight the complexity of viral interactions and have led to a revision of current working hypotheses. This has driven the development of new theoretical models to better describe plankton-virus dynamics and predict their evolution as well as the consequences for the diversity of our current and future ocean.

ROLE OF VIRUSES IN MICROBIOTA

Many multicellular organisms host associated microorganisms in and on their bodies: this is the microbiota. An organism and its microbiota form what is called a holobiont. The microbiota is

essential to the physiological health of its host; a prime example is its role in digestion.

In the marine environment, this is the case for fish, coral, jellyfish, molluscs or macroalgae, to mention only those groups where the role of associated microorganisms has been studied. The microbiota is made up mainly, but not only, of bacteria. It also contains unicellular eukaryotes, archaea, and of course, viruses. The latter infect the cellular organisms of the microbiota and thus play an important role in its regulation and functioning. Corals host photosynthetic eukaryotic microorganisms in their cells. These are zooxanthellae, dinoflagellates of the genus *Symbiodinium*, which provide corals with oxygen and, where appropriate, play an important role in the construction of their limestone skeleton by providing them with the metabolic substances necessary for their development. Some stresses, such as a rise in temperature, cause zooxanthellae to be expelled from corals, causing coral bleaching and death. *Symbiodinium* can become infected with viruses that influence their host's tolerance to heat, lowering the threshold for bleaching. Similarly, viruses are abundant in the mucus found on the surface of corals. They are particularly active there (viral production is up to 9 times higher in coral mucus than in ambient water) and appear to be extremely diverse. The strong correlation with bacterial abundance suggests that the majority of the viruses present in this mucus infect bacteria. Various studies (including Barr *et al.*, 2013; Almeida *et al.*, 2019) show that mucus-associated viruses could play a key role in defence against pathogenic bacteria. These observations led to the creation of the “bacteriophage adhering to mucus” (BAM) model, which explains that the abundance of viruses in the mucus is linked to structural adaptations allowing adherence to this matrix and constitutes an immune barrier against pathogen invasion. In other words, this model suggests that viruses and the organism hosting them have a symbiotic relationship that, on the one hand, protects the host from infectious attacks and, on the other hand, allows the viruses to spread. Although many unknowns remain as to the exact nature of the relationships between viruses and the coral holobiont (of which they may be a part!), their multi-occurrence and persistence suggest an

important role in the maintenance of coral reefs, one of the richest ecosystems in the global ocean.

MARINE VIRUSES AND BIOGEOCHEMICAL CYCLES

Microorganisms play a key role in transporting and transforming the building blocks of life (carbon, nitrogen, oxygen, hydrogen) between the major reservoirs of our planet: land, water and atmosphere. Through their metabolism, microorganisms act as the engines of the major biogeochemical cycles. Take a breath, now a second... The oxygen you have just inhaled was produced largely in the ocean by phytoplankton. These organisms perform photosynthesis and use light, nutrients and CO_2 dissolved in seawater to transform them into living biomass and oxygen. As you will have understood, this process is essential for the functioning of our planet. On the one hand, phytoplankton “consume” atmospheric CO_2 to transform it into biomass and, on the other hand, restore O_2 to the atmosphere. The carbon fixed by phytoplankton is then consumed by zooplankton and the particles generated along this food chain (faecal pellets of zooplankton, dead cells, detritus) undergo a series of transformations. They can be recycled by heterotrophic organisms (bacteria) or sink to the ocean floor. A small portion reaches the deepest depths and is sequestered into the sediments for millennia. This is known as the biological carbon pump (Figure 7). It limits the amount of CO_2 in the atmosphere and helps to regulate the climate.

While the involvement of viruses in the functioning of large biogeochemical cycles and the biological carbon pump is undeniable, their role is intensely debated and the subject of particularly active research. The hypothesis that has prevailed until recently is that infection and viral lysis slow down the biological carbon pump. As mentioned earlier, viruses are major life-threatening agents for phytoplankton and bacteria. Infection leads to the release of their cellular content, rich in organic matter (composed of proteins, sugars, lipids, DNA, etc.), into the environment. By destroying these cells, viruses steal biomass destined for the higher trophic levels and transform

it into dissolved organic matter, then usable by the bacterial compartment. It has been estimated that 145 gigatonnes of carbon, 28 gigatonnes of nitrogen and nearly 5 gigatonnes of phosphorus released each year is concerned by this viral “short circuit” in the tropical and subtropical ocean alone. This diversion or short-circuiting of organic matter (called the “viral shunt”) results in a decrease in the production of the higher trophic levels, and therefore of the particles generated along the food chain, in favour of the microbial network.

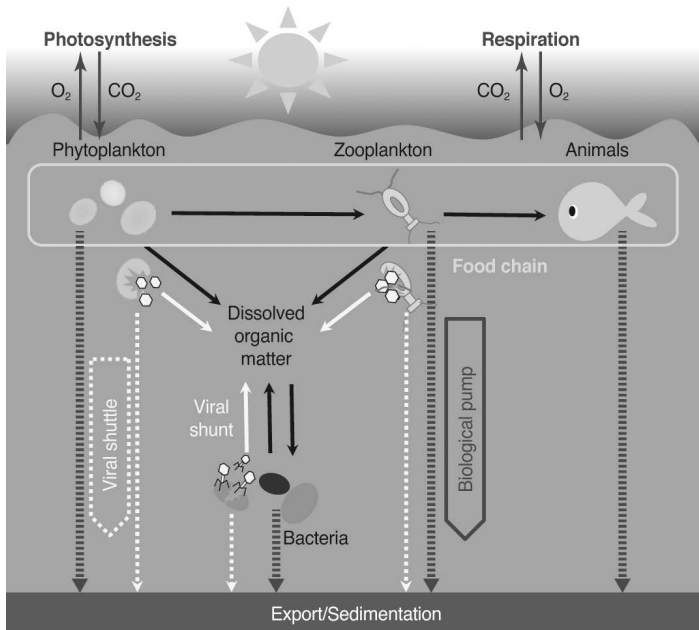


Figure 7. Simplified diagram of the role of viruses in the functioning of the biological carbon pump through the viral shunt and viral shuttle.

The white arrows show how viruses disrupt the “classic” food chain (in the light grey frame); white solid arrows show the viral shunt (production of excess dissolved organic matter in the water column); dotted white arrows show the viral shuttle (increased export of organic matter to deeper waters); dotted grey arrows represent the biological pump.

Viruses also influence the carbon cycle during infection (before lysis takes place), by hijacking the host metabolism for synthesis of viral components. This “reprogramming” profoundly changes the metabolic flows and energy level of the host cell. In cyanobacteria, one of the cellular processes deeply affected by viral infection is CO_2 fixation. As marine cyanobacteria are responsible for 10% of CO_2 fixation in the global ocean, viral infection could lead to a shortfall of 5 gigatonnes of carbon fixed each year.

Conversely, other studies indicate that viruses could accelerate the biological pump and carbon sequestration (“viral shuttle” hypothesis) by inducing, for example, the release of micronutrients (such as iron) during lysis, which limits primary production in nearly half of the world’s oceans. Infection and lysis of infected microbes could also induce the formation of different types of particles capable of sedimentation, such as aggregates consisting of viral lysis products, dormant spores or infected cells that lose their motility (Figure 7). Similarly, while viral lysis mainly leads to the release of labile organic matter, which is quickly usable by bacteria (this matter usually only resides in the environment for a few hours or days), it also generates a fraction (albeit a smaller one) of refractory organic matter (i.e. highly resistant to degradation), which will remain in the ocean for thousands of years because it cannot be assimilated by bacteria. Considering that 10^{29} viral infections occur every day in the ocean, a gradual accumulation of refractory matter could increase the sequestration of atmospheric carbon in the oceans.

As organic matter sinks from the surface to the seafloor, viruses continue to drive the biological pump. Recent research shows that viral production actually increases in deeper waters as viruses switch from a “hidden” (lysogenic) cycle to an active (lytic) one, essentially activating inside already infected cells. Interestingly, this does not lead to higher bacterial death rates at depth; the intense cold of the deep ocean slows down both bacterial growth and the speed at which viruses can burst their hosts. This process would explain, among other things, why VBR (the ratio between viral and bacterial abundances) increases with depth. In addition, viral lysis operating during particulate sedimentation regulates bacterial diversity and community structure, which consequently

influences the use and biological transformation of particulate and dissolved organic matter. It is increasingly clear that viruses are a means of transporting matter and therefore exporting carbon, and that the virus-organic matter-host organism triad should be given more consideration.

Do viruses slow down (shunt) or accelerate (shuttle) the efficiency of the biological carbon pump? It's still too early to decide. In fact, the relative contribution of shunt vs shuttle probably varies between infected populations and environmental conditions, which emphasizes the need to quantify viral infection *in situ* to better understand the fate of infected cells and to assess how natural variability modulates these processes.

Beyond their involvement in the carbon cycle, viruses are involved in other major biogeochemical cycles that can influence our planet's climate. Viral lysis may, for example, be accompanied by the production of a sulfur compound, dimethylsulfoniopropionate (DMSP), in seawater. DMSP is produced by phytoplankton and is a precursor to dimethyl sulfide (DMS). Once in the atmosphere, DMS oxidizes and leads to the formation of small particles that serve as condensation nuclei for clouds, therefore helping to increase cloud cover. The study of different phytoplankton populations (see box: *Emiliana huxleyi*: a model organism in more ways than one) has shown that viral lysis leads to the release of DMSP, although there has been no measurement of this process *in situ* to date. An animated film summarizing this link between the virus and the climate associated with *Emiliana huxleyi* is available at: <https://youtu.be/1AlxUII8f3s>.

EMILIANIA HUXLEYI: A MODEL ORGANISM IN MORE WAYS THAN ONE

A spectacular and well-studied example of the regulation of a phytoplankton bloom by viral action involves a microalga (more precisely, a haptophyte) with calcareous plates (calcium carbonate scales also known as coccoliths) on its surface and which has the pretty name *Emiliana huxleyi* (reclassified as *Gephyrocapsa huxleyi*).

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It is the most abundant and best-known species of coccolithophorid. This alga can proliferate and form blooms visible from space, particularly in the English Channel, North Sea and Norwegian Sea. What is remarkable is that this bloom never lasts very long and can disappear in a few hours or days. It is now known that the disappearance of these blooms is often due to a massive attack by large viruses (capsids up to 0.2 μm) logically called EhV (*Emiliania huxleyi* virus). These viruses, of great genetic diversity, are double-stranded DNA viruses of macroalgae, also known as phycodnaviruses ("DNA algae viruses"). Their action and diversity have been studied in the laboratory, in mesocosms⁶ and directly at sea.

Each infected *E. huxleyi* cell can release up to 500 viruses in a few hours (if environmental conditions are favourable), which can in turn infect more healthy cells. On the scale of a bloom spread over several hundred square kilometres and characterized by several million cells per litre, it is understandable that the bloom's disappearance, although generally quite rapid (a few days), is not instantaneous. In addition to contact in the water, the end of the bloom is also favoured by virus-laden aerosols that can travel greater distances in the atmosphere than in water and attack the bloom from another direction.

When there is a viral attack, regulation or termination of a bloom, there is inevitably a counterattack and resistance on the part of the host. Otherwise, this kind of proliferation would only happen once, and most viruses would no longer find hosts (since they would have killed every last one!). *Emiliania huxleyi* employs an original strategy to avoid population collapse: it develops a resistant form that is "invisible" to viruses, allowing the population to persist. This defence strategy involves the "species" ability to exist in a form distinct from the infected one—in this case, a haploid phase. These cells, which correspond to the microalga's gametes, are naked, flagellated and thus motile, whereas the diploid form is non-motile and armoured with coccoliths (which do not protect it in any way! see Figure 8).

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6. Mesocosm: a medium to large (semi-)closed experimental device for ecological and biological studies. Often deployed *in situ* (i.e. outdoors in the field), this is a scale model of an ecosystem to be studied. In the aquatic environment, it may consist, for example, of large bags (which may or may not be made of reinforced polypropylene) or cylinders (in Plexiglas or other material) filled with water from the surrounding environment.

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The radical difference in viral susceptibility between the stages of its life cycle provides *E. huxleyi* with an escape mechanism that allows genes from dominant diploid clones to be passed on to the next generation. This scheme has been dubbed the "Cheshire Cat", after the character in Lewis Carroll's *Alice's Adventures in Wonderland* who has the ability to appear and disappear at will. This evolutionary "strategy" makes it possible to remove the host from the pressure of the parasite, in connection with the Red Queen hypothesis of co-evolution.

In the ocean, the viral attack on *E. huxleyi* does more than just kill a large number of individuals of the target species; it also has wide-ranging repercussions on nutrient and matter cycling. Significant sedimentation of infected cells is observed, feeding the biological pump and carbon sequestration. This could go so far as to influence the local climate by the impact it would have on CO_2 . In addition, lysed *E. huxleyi* cells release dimethylsulfoniopropionate (DMSP), the precursor of a climate-active gas, dimethylsulfide (DMS), into the atmosphere. Upon contact with air, this gas transforms into sulfuric acid molecules that clump together and form aerosols that act as cloud condensation nuclei, facilitating the aggregation of water droplets and thereby seeding cloud formation. The more aerosols there are, the more condensation nuclei and small droplets form, thus increasing the reflectivity of clouds. These then send more solar radiation back into space, resulting in cooling. Intracellular DMSP has been shown to increase in *E. huxleyi* with temperature and CO_2 ; global environmental changes brought about by acidification and ocean warming could therefore lead to an increase in DMS. *Emiliania huxleyi* is thus a major species in the ocean carbon cycle, but also that of sulfur (DMSP and DMS being sulfur compounds). Like many organisms, *E. huxleyi* produces extracellular vesicles. These have multiple roles, some of which are still not well understood, and act on communication between the cells that produce them and the target cells. The production of these vesicles increases during viral infection, and their RNA content appears to specifically target genes related to the cell cycle and metabolism. Initial studies suggest that vesicles from infected cells (approximately 500 per cell) decrease the lifespan of the virus in a dose-dependent manner. This action likely takes place towards the end of the bloom, as the target population declines.

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Through a cascade effect, this would allow other organisms to gain an advantage in the competition for resources. In the wild, it therefore appears that *E. huxleyi* can protect itself by secreting vesicles capable of reducing the lifespan and infectivity of the viruses of which it is the host. However, once infection is initiated, a cascade of events inexorably leads to viral infection, and this happens concomitantly with the increase in the lifespan of the virus. This will also have an (positive or negative) effect on other phytoplankton species and thus on the succession of populations over time. This type of communication through extracellular vesicles during microbial infections of algal blooms could therefore have a significant impact on the growth and composition of associated marine populations.

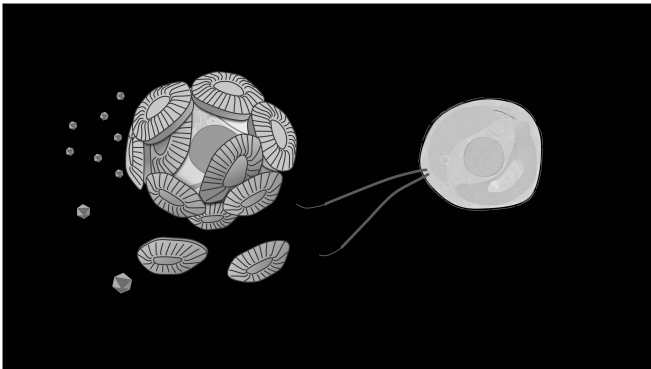


Figure 8. *Emiliana huxleyi* in its calcified (left) and “naked” flagellate (right) form, and viruses that infect only the calcified cell form.

Source: © Frada M., in Frada et al., 2008.

VIRAL RESPONSE TO THE IMPACTS OF CLIMATE CHANGE

Marine viruses, by acting on marine biogeochemical cycles, are biological entities essential to the balance of our planet. However, we need to ask how they respond to the consequences of climate change, such as ocean acidification, rising temperatures, deoxygenation, loss of biodiversity, or extreme weather events.

Since temperature is a key factor in metabolic processes and cell growth, it is logically expected to influence virus-host relationships. At first glance, since growth is stimulated, burst size is expected to increase and the duration of the lytic cycle to decrease, resulting in increased viral production. Higher temperatures are also expected to increase contact rates between viruses and hosts, through host multiplication and, consequently, increased infection rates. Laboratory and field studies on this issue are still rare, but generally show that lytic viral action and induced microbial mortality would increase with increasing temperature. However, there are nuances depending on geography, study models and the metabolic status of the infected microbial organisms and assemblages. This is well illustrated in the cosmopolitan microalga *Micromonas* and the viruses associated with it. This microalga, like all living organisms, has an optimal temperature range where it grows and thrives most effectively. Below the optimum growth temperature of *Micromonas*, viruses replicate by the lytic cycle, and their virulence gradually increases with temperature. At the host's thermal optimum, viruses establish a kind of "symbiosis", a "peaceful" relationship with their host, by replicating without detectable lysis. Beyond optimal growth, *Micromonas* is resistant to viral attacks. Such phenomena of resistance to viral attacks at high temperatures have also been observed for other microalgae. The initial metabolic state is, therefore, a key factor in predicting the response to temperature rise. The extrapolation of these results suggests that, in the future ocean, tropical regions could become "safe havens" for *Micromonas*, which would then escape infections by its viruses. There is no doubt that such a scenario would have significant consequences for the functioning of these ecosystems. Of course, this theoretical exercise needs to be validated *in situ*. In addition to direct effects of temperature on viral behaviour, reduced infectivity may occur due to conformational changes or increased exoenzymatic activity, which can essentially break down and destroy the virus particles. Furthermore, the intensification of the lytic cycle, which releases more nutrients into the water, acts alongside rising CO₂ levels and warming temperatures. Together, these shifting environmental conditions could alter

phytoplankton composition and amplify overall production. All of this would have consequences for sedimentation and deep carbon sequestration (Figures 7 and 9).

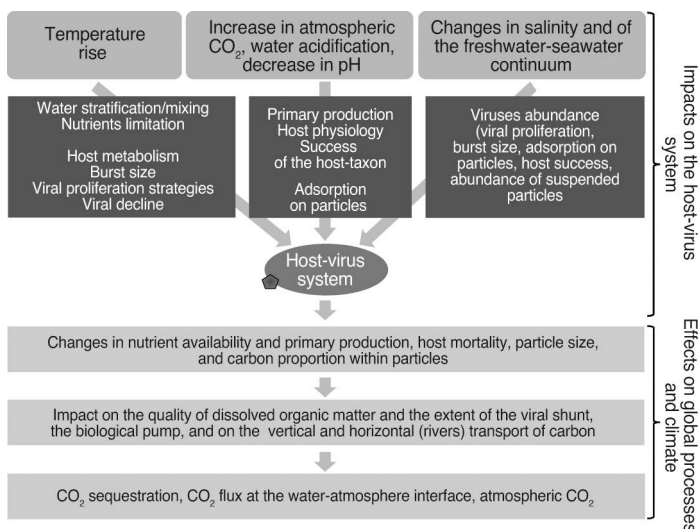


Figure 9. Schematic representation of the factors and processes controlling the host-virus system and how the interactions between viruses and hosts influence and modify the main processes taking place within marine ecosystems, ultimately impacting the climate.

Source: Zhang *et al.*, 2021 (CC BY 4.0).

Another factor likely to vary with global change is salinity, typically in polar waters plagued by melting glaciers and thus some desalination. It is known that salt concentration can affect the burst size of infected cells. Experiments have shown the possibility for some viruses to “cross” between very different biomes (freshwater to seawater and vice versa) and that systems with a lower salinity are characterized by a higher viral abundance. Ocean acidification, i.e. the decrease in pH due to the increase in atmospheric CO₂ generated by humans and partly absorbed by the ocean, adds to the processes likely to modify virus-host relations.

In light of all that has been discussed, there is little doubt about the importance of including the viral compartment in major

ocean-related climate models in the future. This is especially critical as it remains unclear whether viruses will tend to destabilize or, conversely, stabilize the system, as the concept of virus-host balance appears to be fragile, flexible and critical all at once.

A BRIEF SUMMARY

Microorganisms are now recognized as being at the heart of the energy and nutrient transformations that fuel the Earth's ecosystems—within soils, oceans and humans alike. Viruses modulate these microbial impacts, ranging from mortality and nutrient recycling to significant metabolic reprogramming during infection. While viruses are unanimously recognized as a major driving force in the evolution and functioning of ocean ecosystems, our knowledge is still very often only conceptual, based on a limited number of biological models (frequently lytic DNA viruses), and performed under controlled conditions. Too few studies have focused on the ecosystem-wide impact of viruses, which in turn limits the integration of this compartment into mathematical models to predict the consequences of climate change. It is now necessary to bridge the gap between laboratory findings and *in situ* observations in order to understand environmental feedbacks on viral processes and to fully grasp the complexity of the virosphere (the total diversity and presence of viruses in nature). It is true that while environmental virology strives to better understand the global virosphere, we face challenges in organizing this “sequence space” (creating a viral taxonomy based on nucleotide sequences), linking these viruses to their natural hosts (who infects who?) and establishing how virus populations are structured (ecological drivers) and have an impact on natural ecosystems. Overall, “viral ecogenomics” approaches can help map and understand viruses in complex systems, so that a new generation of ecosystem biology can be developed.

A major challenge in the coming years will also be to improve our understanding of the ecological and evolutionary role of temperate marine viruses, integrated into the genomes of their hosts.

Temperate phages are present in almost half of the bacterial genomes sequenced to date and in all environments sampled, from the poles to the tropics, as well as in all marine organisms. These viruses are often thought to have a beneficial effect on their host. For example, infection with a temperate virus may confer immunity to the superinfection or provide benefits to its hosts by allowing them to express particular characteristics carried by the integrated virus. There is currently a serious lack of knowledge about the mechanisms and conditions that lead a virus to enter a lytic or lysogenic cycle, as well as the influence of this life strategy on the functions and ecological niche of marine microbes.



WHY STUDY MARINE VIRUSES? REAL-WORLD APPLICATIONS

PHAGE THERAPY, A FUTURE-READY SOLUTION ALREADY IN USE

Over the last 50 years, the demand for aquaculture products (both plants and animals) has increased significantly. It will continue to grow to meet the food challenge we face. In parallel with this increase in production, limiting factors such as infectious diseases (due, *inter alia*, to pathogenic bacteria) lead to significant economic losses and a potential risk to the consumer, which must be taken into account. The limitation of the use of antibiotics in aquaculture in Europe since 2006 has prompted scientists to explore alternative solutions, leading to a rise or resurgence of interest in the use of more natural agents (e.g. organic) that can control, limit or destroy pathogens. Phage therapy or phage therapy is one of them.

The idea of using bacterial viruses, i.e. phages, against disease-causing bacteria is not new. It can be traced back to 1896, when antimicrobial activity against the *Vibrio cholerae* strain in the waters of the Ganges was demonstrated. The connection was finally made between 1915 and 1917 by researchers Twort and d'Hérelle. They demonstrated that these phenomena were caused by filterable and transmissible agents, which they identified and named “viruses”. Félix d'Hérelle then conducted numerous studies in the 1920s and 1930s to capitalize on these observations. He started fairly early by treating patients with dysentery, but not without having tested the safety of his phage treatment on himself and his family. Phages were then marketed by the pharmaceutical industry to treat pathologies linked to different bacterial infections. There are several advantages to using viruses as therapeutic tools: they are natural control agents for bacteria; they have a targeted spectrum of action, usually killing only one

bacterial species and thus preserving the microbiota associated with the diseased organism; finally, given their status as obligate parasites, once their host disappears, so do the viruses. However, the antibiotic era halted this type of therapy, and interest in phages waned—except in Eastern European countries.

Nevertheless, it is clear that the interest of phages in controlling infectious (bacterial) diseases in aquaculture has persisted. Indeed, fish reared in overpopulated conditions are prone to multiple infections, including bacterial ones, that can cause severe economic losses. The need to find alternatives to antibiotic treatments will promote the use of phages, in particular to combat infections associated with the *Vibrio* genus. While this approach has shown certain benefits in fish farms, interest is also growing in its use to combat *Vibrio* strains responsible for significant mortality in oyster hatcheries. Indeed, the mortality linked to infections by *Vibrio* bacteria has a strong economic impact on this commercial coastal activity. Mixing several previously characterized phage strains (referred to as a “cocktail”) represents a promising approach to limit mortality and thus ensure the continued commercial viability of oyster production. A similar approach has been proposed to limit the use of antibiotics in shrimp farms. Another application of phages is to combat strains of *Vibrio parahaemolyticus*, a marine bacterium that can cause severe human diseases associated to seafood consumption. The use of a phage cocktail as a biocontrol seems promising, considering both their rapidity of action and their bacterial specificity demonstrated *in vitro*.

The interest of phages in aquaculture is obviously not limited to *Vibrio* but also includes bacteria such as *Aeromonas*, *Pseudomonas*, *Acetobacter* and *Flavobacterium*. Despite its potential, phage therapy also presents limitations. The specificity of action, as phages are associated with specific bacteria, can be problematic. This necessitates the selection of new phages in response to the evolution of the bacteria. However, phages also evolve over time, and could, through an arms race, remain infectious for the evolving bacterium (or even for other bacteria). This also renders the composition of phage cocktails unstable, with an unpredictable character. It therefore seems appropriate to select

phages with the widest possible spectrum of activity, even though this decreases their bacterial lysis capacities. It is important to note that the resistance of certain bacteria to phages may occasionally result in a decrease in virulence due, for example, to a lower colonization power or an increased sensitivity to other phages. These observations confirm the important role of phages in the natural environment with regard to the microflora that surrounds them.

Another interest of bacteriophages is to combat biofilms. The use of specific phages or cocktails can significantly reduce bacterial proliferation, thus reducing the need for disinfectants in aquaculture.

Over the past several years, in response to the issue of coral bleaching, various solutions have been investigated, and here again, phages can be used to great advantage. While a combination of factors such as the effects of human activities, global climate change, and rising seawater temperatures are implicated in the decline of coral reefs, the influence of *Vibrio* bacteria on the bleaching phenomenon has been demonstrated at multiple sites. Thus, some phage strains, isolated from the environment, are interesting candidates to combat these particular *Vibrio* strains.

BIOTECHNOLOGICAL POTENTIAL OF MICROALGAE VIRUSES

Because they are fairly simple entities, viruses can be used and manipulated for useful and effective biotechnological applications. Algal viruses, in particular, are very promising in multiple biotechnological contexts (Figure 10). Algal biotechnology is booming, as sustainable sources of biomass are increasingly sought for the production of basic chemical precursors, biofuels, plastics and food supplements. Cultivated algae can also capture and sequester carbon. The use of viruses in algal cultivation may be a next step in biocontrol. The potential use of viruses as an energy-efficient method to induce cell lysis, facilitating downstream processing of bulk biomass, is a promising technology. After all, viruses have been breaking down cells for billions of years, so

why not exploit this property? Expanding upon this idea, viruses could be used to improve the biochemical or metabolic properties (and thus the value) of this biomass beyond simply facilitating the accessibility of its components. The concept of viruses as minimalist “bags of genes” has long been refuted: viruses possess a metabolic complexity that remains largely unexplored. In this context, microalgae technologies are a growing research area. This is the case, for example, with the use of photosynthetic microorganisms to enable sustainable production in the food, energy, chemical or pharmaceutical industries.

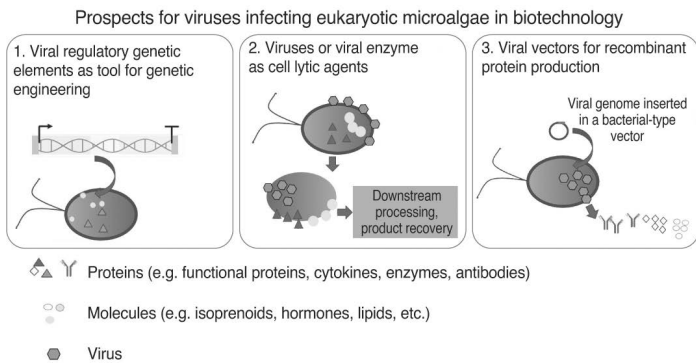


Figure 10. Schematic representation of the main prospects for the use of microalgae viruses in biotechnology.

(1) Viral genetic regulatory elements (e.g. promoters, terminators, ribosome binding sites, transcription enhancers, etc.) can be predicted and isolated from viral genomes and used as gene expression tools in genetic and metabolic engineering.

(2) Viruses or viral enzymes capable of degrading cell walls can be used as lytic agents, helping in the recovery of various cell products in biorefineries.

(3) Viral vectors can be generated and engineered for the production of recombinant proteins and biopharmaceuticals.

Source: D'Adamo *et al.*, 2021 (CC BY 4.0).

Since the 1980s, algal viruses have emerged as important subjects for ecological studies in the fields of marine science and aquatic environments, spurred by the identified link between virus-like particles and the dynamics of algal blooms. These observations raised new questions on the evolution and identification of these viruses. To date, fewer than 100 viruses infecting eukaryotic

microalgae have been isolated and cultured. While research in this field holds clear interest, the lack of pathogenicity for humans remains to be confirmed, as does their ability to fulfill industrial requirements. Microalgae are a future field of application for the establishment of production platforms. Their extraordinary diversity is an asset for the production of bioproducts (such as antioxidants, proteins, polysaccharides, vitamins, etc.), either naturally or after genetic modification. Although their production remains limited due to the prevailing high costs of cultivation and purification, viruses infecting these algae will play an important role in the emergence of these biotechnological applications. Indeed, technologies based on the use of viruses pave the way for rapid and programmable genetic modifications of eukaryotic cells in many research fields, ranging from applied science to biotechnology, or in medicine. Viruses are promising resources for the expression of recombinant proteins in gene therapy, for the production of viral vaccines, as nano-vectors for drug delivery or in nanomaterial technology.

Metagenomics data reveal the existence of a large number of unknown viruses, suggesting immense potential development. The staggering number of genes with unknown function that dominate viral datasets leads us to wonder the functions of all these viral proteins. It remains difficult to estimate the true extent of the discoveries yet to be made.

It is not only viral genes for which uses can be developed: attenuated viruses can be used as cell transformation vectors (such as in the AstraZeneca COVID-19 vaccine or the insect cell-baculovirus system) and new high-activity viral promoters can be used in expression cassettes (similar to the widely used T7 phage promoters) with termination sequences. Despite lingering concerns over production monitoring and biosecurity, facilitating access to viral strains, notably through the expansion of environmental virus collections, is crucial. This type of biobank is rare (only one has been built to date, which is located in Brittany [France]) and requires significant resources for the production of viruses in large quantities, host characterization and conservation methods to ensure genetic integrity. Nevertheless, it is a major step toward the use of viruses in biotechnology.

Indeed, in the coming years, it will be crucial for academic research to succeed in capturing the attention of industry by exploring the capabilities and applications of the newly identified viral strains and their genes.

FISHING FOR GENES

Coccolithoviruses (in particular, those infecting the cosmopolitan and calcifying microalga *Emiliania huxleyi*) have been shown to encode an almost complete biosynthesis pathway for a novel ceramide, a type of sphingolipid used in various skin and hair care cosmetics. This compound has reached a global market value of several hundred million dollars and has great potential in medicine for applications related to cell differentiation and tumour treatment.

Chloroviruses (which infect *Chlorella* spp., one of the most cultivated microalgae in the world, with an annual market expected to reach around \$400 million by 2028) have the genes needed to make hyaluronic acid, a sugar polymer with a global market of \$8.5 billion in 2020.

Both these types of viruses could be used to produce sustainable and vegan-friendly products, allowing metabolites to be handled naturally in a GMO-free setting.



WHAT MAJOR QUESTIONS REMAIN UNANSWERED?

The discovery and characterization of viruses are relatively recent (Lwoff, 1957) and their study has long remained confined to pathogenic strains of medical interest. In this context, it is not surprising that many questions remain unanswered, as viruses are invisible entities, small in size and diverse in structure and function. Nevertheless, the unknowns concern quite fundamental aspects of viruses, with questions remaining unanswered or responses being very controversial in the scientific community. Here we will address some of these unknowns.

ARE VIRUSES ALIVE? CAN THEY BE PLACED IN THE TREE OF LIFE?

These questions, which at first glance seem very simple, are not remotely so when applied to viruses. The first question also sets the daunting task of defining life itself. Definitions vary from author to author with no overall consensus. Here, we are talking only about scientific concepts of life, not philosophical or religious ones. It is generally accepted that life implies metabolic autonomy and self-replicating abilities. All known viruses, defined as strict cellular parasites, do not possess either of these two attributes and therefore do not logically meet this definition of living entities. No autonomous viruses are known (with no host allowing them to multiply). However, another way to answer this question is to investigate whether they can be placed in the tree of life (assuming such a tree even makes sense, though this is another question altogether!). Present viruses could descend from biological entities that were once living *stricto sensu*, but that lost their cellular attributes over a long period of time (they would then be “degenerate” cellular organisms), much like snakes became limbless tetrapods, or fleas became wingless pterygote insects. While there is little doubt that

viruses and cellular living things have a common origin, reflected in the structure of their nucleic acids and the universality of the genetic code, this does not imply that the different groups of viruses form distinct, well-defined branches of the tree of life. Indeed, a common origin of viruses is very unlikely due to the diversity of their structures and genetic material. They could be by-products of cells that evolved through transfers of genes “stolen” from their various hosts or from other viruses during co-infection of the same host cell, in addition to undergoing mutations (that have a high rate in viruses) and recombinations. All this would make the viruses an “evolutionary mosaic” with no definite origin. However, even if viruses had a given origin, this would not prevent the constitution of such evolutionary mosaics, as for the recent H1N1 virus! The analysis of the H1N1 genome clearly suggests that it is an assemblage of bird, pig and human viruses. In short, this issue is complicated, and this phylogenetic approach does not (yet) provide definitive evidence whether viruses are living entities.

Some large viruses belonging to the group Nucleocytoviricota (which are a branch of viruses also known as nucleocytoplasmic large DNA viruses: NCLDV) have been proposed as a fourth branch of the tree of life by Raoult *et al.* (2004) in addition to bacteria, eukaryotes and archaea (with the latter two domains perhaps forming only one), but this hypothesis is highly controversial and the presence of many horizontally transferred genes in these viruses makes it difficult to study the evolution of their genome. The evolutionary origin of viruses remains mysterious and is the subject of active debate among specialists, particularly in France, where competing teams have yet to reach a consensus. It is the discovery and study of novel viruses that may lead to a resolution.

In summary, viruses can be considered as entities produced by cellular living beings through evolution, much like segments of mobile genetic material that would have escaped from their hosts and then have an existence and evolution of their own, albeit remaining very dependent on those of their hosts. This view is supported by the fact that genomes are naturally abundant in mobile segments, such as transposons, which constantly

move from one location to another. A more recent hypothesis is that viruses are very ancient entities, predating even cellular organisms, and that they form their own branch of the tree of life, just like bacteria, archaea and eukaryotes (even if recent genomic studies make eukaryotes a branch derived from archaea, but this is another debate). This hypothesis comes from the discovery in the 2000s of giant viruses (see box: *The incredible story of giant viruses*), with genomes much larger and more complex than those of previously known viruses (currently up to 2.5 million base pairs [Mb] for *Pandoraviruses*). Above all, these genomes contain genes without counterparts in known cellular organisms, which are thus purely viral genes, incompatible with the fact that they were derived from their cellular hosts. The complexity of the giant virus genomes is also difficult to explain for simple cellular parasites.

THE INCREDIBLE STORY OF GIANT VIRUSES

Seas and oceans are therefore filled with viruses, in large numbers and of all sizes, probably capable of infecting any form of cellular life. Among them, viruses of very large size have long been ignored. Before discussing these extraordinary marine viruses, it is worth remembering that the discovery of the very first giant virus is relatively recent, dating from 2003. Initially confused with a bacterium observable by optical microscopy, what was eventually described as a giant virus and named *Mimivirus* (for "microbe mimicking virus") revolutionized the world of virology and beyond. Indeed, this virus and all those that would be discovered later led scientists to correct the very definition of "virus" and to imagine incredible evolutionary scenarios to explain their *raison d'être*, to the point of calling into question the definition of life. Today, it is known that these giant viruses are not uncommon, probably ubiquitous and characterized by a multitude of sizes and morphologies, genome sizes and gene contents, as well as replication strategies. *Mimivirus*, *Megavirus*, *Moumouvirus*, *Marseillevirus*, *Pandoravirus*, *Pithovirus*, *Mollivirus*, *Tupanvirus* soda lake, *Tupanvirus* deep ocean, *Megaklothovirus* and *Klothovirus* are some of the giant viruses isolated across the planet from very different sites: soils, water cooling towers, sediments and, of course, the ocean.

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What unites them in the first place, in addition to being double-stranded DNA viruses, is their extraordinary size compared with average viruses. They are greater than 300 nm and can reach or even exceed a micron. Apart from size, what makes them so unique is that they possess complex genomes with a very large number of genes about which little is yet known. For example, *Pandoraviruses* have genomes of comparable complexity to the smallest eukaryotic cells and more than 90% of the proteins encoded by their genomes do not resemble anything known. In general, for all these viruses, more than two thirds of the proteins they encode are said to be orphan (not related to any known proteins). It is now believed that at least some of these viruses have the ability to create *de novo* genes from parts of the genome called intergenic regions.

Today, there are four large families of giant viruses (Mimi- or Megaviridae, Pandoraviridae, Pithoviridae, Molliviridae), sometimes called "girus" (for giant virus). In each virus, more than a third of the genes code for completely original proteins, that have no known counterparts in any other form of life. It is this observation that raises the question of the origin of giant viruses and their evolution. Many surprises may be revealed by studying these giants, potentially elucidating the role they played in the appearance of life on Earth.

In the ocean, one of these large viruses goes by the acronym CroV, for *Cafeteria roenbergensis* virus. It is a parasite of the single-celled flagellate organism *Cafeteria roenbergensis*, which is a bacterivorous, flagellated heterokont alga of the Cafeteriaceae family. The CroV genome size is 730,000 base pairs (or 730 kb), encoding 544 proteins. Regulatory sequences identified within their DNA suggest a highly sophisticated protein expression system, temporally regulated and adapted to the stage of host infection. A wide variety of proteins have been identified, including DNA repair enzymes, enzymes involved in protein breakdown, and factors important for gene translation.

Megavirus chilensis has been discovered along the Chilean coast, off the marine biological station of Las Cruces. It has a shape comparable to *Mimivirus* and its envelope is also covered with fibres, giving it a hairy appearance. However, it is larger and more complex than its cousin, with a genome encoding 1,120 proteins. The two viruses have 594 genes in common and both have enzymes used to translate genes to make proteins (which viruses theoretically lack!).

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The amphora-shaped *Pandoravirus salinus* has also been isolated off the coast of Chile. With its 2.8 Mb and ability to code for 2,500 proteins, it is already an extraordinary particle! In addition, 90% of these protein sequences have no equivalent in current databases.

There are other large viruses, which often infect microalgae. For example, such viruses infect the phytoplankton species *Phaeocystis globosa*, which can cause visible blooms in coastal areas (taking the form of large amounts of whitish foam on beaches), particularly on the Atlantic, English Channel and North Sea coasts. With a genome size of 460 kb, coding for 434 proteins, this virus is known to be able to interrupt these blooms, making it a major player in the ecological balance.

Other very large viruses are known to infect other marine phytoplankton species such as *Haptolina ericina* and *Aureococcus anophagefferens*, playing a key role in regulating their populations. The world's smallest known free-living eukaryote, the microalga *Ostreococcus tauri*, is itself prey to viruses from the Phycodnaviridae family, which are close relatives of the "giant viruses" *stricto sensu*. It is a strange association: a dwarf cell infected by a (small) giant, whose diameter is a tenth of its host's. *Tupanvirus* deep ocean, a giant virus measuring 500 nm and related to *Mimivirus*, was discovered at a depth of 3,000 m in ocean sediments off Brazil and seems to have a more complete genetic arsenal than any other virus, with genes similar to those of known viruses and organisms in all three domains of life.

Finally, perhaps the strangest and largest of these giant viruses are the *Klothoviruses* and *Megaklothoviruses*, which can measure up to 4 µm! They are still poorly characterized, as their genomes have not been sequenced, and were first confused with bristles of their chaetognath hosts (planktonic fusiform predators that often measure several centimetres). Another unique feature of these viruses is that they contain ribosomes.

As early as 2007, during the first major marine expeditions gathering environmental genomic data, such as the *Global Ocean Sampling*, giant virus sequences were already detected. Further study revealed a year later that many sequences were very close to those of *Mimivirus* and showed that this type of virus is an abundant, diverse and ubiquitous component among DNA viruses infecting oceanic eukaryotes.

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As a result, it is now known that giant viruses are numerous in the ocean and have the potential to infect a wide variety of organisms. Data from 2022 indicated that the diversity of giant viruses is still largely underestimated but could be much higher than that of marine bacteria (Schulz *et al.*, 2022). What delayed their discovery is that most environmental microbiology studies have filtration steps. Historically, the viral fraction was—and often still is—defined only by what passes through 0.2 or 0.45 μm filters, effectively leaving these giant viruses behind.

Why are these viruses so revolutionary? First and foremost, because of their size! A virus can no longer be systematically considered “tiny”. Some of them are clearly larger than single-celled organisms (bacteria or archaea). The study of giant viruses also shows that viruses can be complex. Viruses cannot then no longer be defined on the criteria of simplicity and parasitism as before. Certain viruses, possessing genomes exceeding 1,000 genes, serve as compelling evidence of this. Moreover, several hundred bacteria and archaea are far from having as many genes! Finally, another argument relied on the fact that protein production from DNA is based on two steps: first the transcription of the gene through a copy carried by messenger RNA, then the translation of this information to assemble the protein. Viruses do not usually have a translation mechanism: they use the enzymes of the host cells they infect for this purpose. The presence of these enzymes in giant viruses challenges a long-standing dogma and suggests that these giant viruses are in fact the descendants of an ancestral cell that had its own translation machinery. However, it cannot be ruled out that they “stole” it from a host during their evolutionary history.

The “virus” is traditionally regarded as the encapsulated element that contains the genetic material, and which comes to life only when it is in a host cell on which it is dependent by diverting the host’s metabolism for its own benefit. Some authors have reversed the problem by defining virions merely as dispersal stages (like gametes) and considering that the “living” phase of the virus is the infected host itself, opposing the concepts of *ribocell* and *virocell*.

Viruses have long been considered as by-products of biological evolution. This opinion is changing following a series of recent

discoveries, the most striking being that of giant viruses. Viruses now appear much older, more complex and diverse than previously thought. They can be defined as organisms that multiply by producing virions. This definition excludes infectious RNAs (sometimes officially classified alongside viruses) and implies that at least one viral protein must be present to qualify as a virion. Viruses therefore probably appeared after RNA cells and proteins, but before the last universal cellular ancestor (Luca), the last common ancestor of all modern cells. Moreover, the classical assimilation of the virus to the virion has been called into question. Infection leads to the formation of a virocell which has all the attributes of a living organism. The virocell concept focuses on the intracellular phase of the viral cycle, the moment when the virus's true "creativity" is expressed, leading to the emergence of entirely novel viral proteins. Viruses are the main source of variation and selection, the two pillars of Darwinian evolution. These data place viruses at the centre of the evolutionary process (as a driver of evolution) and could be considered as fully-fledged organisms.

CAN A VIRUS ITSELF GET INFECTED?

This question may seem absurd! We are used to consider that if an organism, a tissue, a cell can be infected and made "sick", then it must be alive. Given everything discussed above regarding the living or non-living nature of viruses, one might expect that a virus could not be infected if it were indeed a non-living entity. And yet, it can! There are viruses of viruses. They were discovered with the giant viruses, when it was revealed that the latter can be infected and regulated by counterparts much smaller in size and much simpler genetically. These are called virophages (literally meaning "virus eaters"), which can therefore be defined as viruses that infect other viruses in order to replicate.

Virophages are double-stranded DNA viruses of eukaryotic hosts, dependent on the giant viruses they infect and belonging to the family Lavidaviridae (*lavidaviridae* for *large virus dependent or associated*). By parasitizing giant viruses and inhibiting their replication, they serve as a defence mechanism of the eukaryotic host (the target of the giant virus).

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This interaction creates a complex *ménage à trois* between the host, the virus, and the virophage. These viral viruses have been found in various habitats including seas and oceans (many are also found in lakes), either directly (after isolation) or through the analysis of genetic sequences from metagenomic databases. This relatively recent discovery (in 2008) had the effect of a bombshell in the world of virology, as it was previously thought that viruses infecting cells could only be parasites and not themselves hosts of other parasites. Since then, many virophages associated with giant marine viruses have been found, such as in the bloom-causing microalga, *Phaeocystis globosa*. This alga is known to create coastal blooms that appear as whitish mucilage resembling seafoam. Among the biological actors likely to control these blooms are the viruses that infect *P. globosa*, but it is also understandable that virophages can challenge this viral control by protecting the host population through an infection of the virus or viruses that are supposedly regulating it! Another known virophage, *Mavirus*, is associated with CroV giant viruses infecting the marine heterotrophic protist *Cafeteria roenbergensis*, a relatively ubiquitous genus playing an important role in the ocean. *Mavirus* was the first marine virophage characterized and, since then, the discovery and characterization of virophages have become a regular occurrence. Moreover, it appears that, each time, there are several virophage genomes for each new virophage, suggesting that these viruses play an important role in the ecology and evolution of a category of planktonic organisms. The adage “The enemy of my enemy is my friend” finds a perfect application in virophages, which serve as defenders of eukaryotic cellular hosts afflicted by giant viruses. Beyond this regulatory role, which effectively protects the cellular hosts targeted by giant viruses, virophages also appear as ideal vectors for gene exchange between different viruses and their eukaryotic hosts—much like plasmids, which are the primary vectors for gene transfer in the bacterial world.

WHY DO WE FIND VIRUSES THAT HAVE NO HOSTS?

Environmental DNA sequencing in the marine environment has revealed many virus sequences whose hosts are unknown! Indeed,

the question of who is the host or hosts of viruses detected in the sea, in diverse and complex communities, is a non-trivial task that often remains unresolved. When cells are cultivable and a virus is isolated, it is easy to determine the ability of the virus to infect a particular organism and this has been frequently studied in the laboratory. Nature, however, is not easily tamed when it comes to microorganisms, since it is estimated that less than 1% of microbial diversity is currently cultivable, i.e. that it can be isolated and grown in liquid or solid media in the laboratory.

Giant viruses have so far been isolated and grown only from amoebae and microalgae, but the sequences of these viruses are so widespread in the environmental databases of the global ocean that they are very likely to also use other hosts. More generally, virus sequences are identified in large datasets of environmental sequences, without knowing the hosts of these viruses. It is now common practice, particularly in marine environments, to sequence the DNA of microorganisms present in water samples. Such operations have been carried out across the world's oceans, for example during the expeditions *Tara Oceans* and *Global Ocean Sampling*. By sequencing all the present DNA using high-throughput techniques, many sequences can be assigned to known organisms and the identity of unknown sequences can be estimated by their evolutionary proximity to previously identified entities through the reconstruction of phylogenetic trees. The continuous exploration of the marine environment will allow us to gradually unravel the mystery for many of these hosts, but there is no doubt that a large number of unknown viruses remain to be discovered.

Nevertheless, methods are being developed to link certain viruses to various potential hosts in environmental genomics data, techniques that are being refined with the growth of databases of genetic sequences identified in the environment. These methods may be statistically based in order to match certain viruses to certain hosts based on their genetic composition and/or population parameters. They may also involve experimental approaches.

It is possible to study viral sequences within microbial hosts (whether they are integrated or not) using existing sequence

datasets. This approach enables the identification of numerous potential hosts, and the study of this “dark matter” significantly increases the number of known sequences (12,000 in Roux *et al.*, 2015) in different environments, in particular in the ocean.

CAN MARINE (AQUATIC) VIRUSES BE DANGEROUS FOR HUMANS?

The COVID-19 health crisis has highlighted how reducing natural habitats and, as a result, reducing the boundaries between wildlife and human activities, promotes the spread of viruses that are potentially deadly to humans. The question of whether SARS-CoV-2-like viruses (Severe Acute Respiratory Syndrome Coronavirus 2) could have originated in the marine environment remains poorly documented. To date, no zoonotic marine viruses have been discovered, as all viruses involved in human diseases are of terrestrial origin. The risk therefore seems rather low, but it is certainly not zero, since certain marine viruses can pass from one environment to another and perhaps even from marine to terrestrial settings. We are therefore justified to wonder whether, among these viruses, there might be one that presents a danger to humans and plays a role in the emergence of new diseases.

This book describes an incredible viral diversity that remains very little analyzed. Is it this diversity that makes viruses so complicated to study and classify? Where should we start? Host specificity is one of the criteria used to classify “human viruses”, “bacterial viruses”, “marine viruses”, etc. Considering human viruses, some induce clinical signs and are even capable of creating pandemics such as that caused by SARS-CoV-2, while others are essential to our lives (some retroviruses have thus contributed to approximately 10% of mammalian genomic sequences and to the evolution of the placenta [Chuong, 2018]). Just as the oceans are home to an impressive number of viruses, our bodies also contain a great many. Let us recall the example already cited above: the number of viral particles in faecal and oral viromes is approximately 10^{11} , which is comparable to the number of stars in the Milky Way galaxy.

Estimating the impact of marine viruses on humans is difficult, as our understanding of the diversity and evolution of vertebrate RNA viruses is largely confined to those found in mammalian and avian hosts and associated with observable diseases. However, a large-scale meta-transcriptomic approach has uncovered more than 100 vertebrate-associated viruses in reptiles, amphibians, lungfish, ray-finned fish, cartilaginous fish and jawless fish. Newly discovered viruses appear in every family or genus of RNA viruses associated with vertebrate infection, including those containing human pathogens, such as the influenza virus, the Arenaviridae and Filoviridae families. The evolution of these viruses largely reflects the phylogenetic history of their hosts. RNA viruses in vertebrates tend to broadly follow the evolutionary history of their hosts, which began in the ocean and spans hundreds or even millions of years. These results, which apply to most families or genera of vertebrate RNA viruses, are consistent with recent analyses of viral evolution using palaeovirological data. They demonstrate the importance of conducting widespread taxonomic surveys on virus diversity, including marine viruses, when trying to trace their evolution. These findings also have broader implications for our understanding of the evolution of viruses. In particular, it is clearly simplistic and perhaps wrong to identify one specific host group at the origin of another, given that our sampling of the diversity of RNA viruses is still very limited. Sequence analysis shows that a very large number of virus genomes identified display phylogenetic proximity to viral families infecting humans. In the future, it is likely that possible links between marine viruses and human viruses will be identified.

Picorna viruses are a group of single-stranded RNA viruses that are major pathogens of animals, plants and insects. These viruses pose a significant threat to the economy and public health. In the past, Picorna viruses have been responsible for devastating epidemics in humans such as poliomyelitis, as well as in plants and insects. These viruses share a conserved RNA-dependent RNA polymerase (RdRp), a versatile enzyme essential for RNA synthesis and an excellent molecular marker for surveying the diversity of picorna-like viruses in nature. Intriguingly, metagenomic analyses of marine viral communities

have revealed a vast array of these sequences. These marine lineages differ significantly from known canonical picornaviruses and likely represent entirely new families. While this suggests that members of the *Picornavirales* are significant pathogens of marine phytoplankton, it would be an overstatement to suggest they pose a danger to humans. Similarly, very few coronaviruses have been isolated from aquatic organisms. These are not zoonotic and are relatively distant from human coronaviruses, so this type of virus would supposedly not contaminate humans through consumer products, for example.

ARE VIRUSES GOOD FOR THEIR HOSTS?

As paradoxical as it may seem, viruses can have beneficial effects on their hosts! This is the case when viruses allow their hosts to eliminate competitors or when they improve the fitness of their host, giving it advantageous characteristics and thus allowing the organism to colonise new niches.

Superinfection immunity is a mechanism whereby a cell already carrying a virus *via* a lysogenic cycle becomes resistant to subsequent infections by the same virus. This is often achieved through the expression of a specific repressor protein induced by the integrated prophage. The host therefore has a selective advantage over other non-immune cells. In this case, the first viral infection confers a benefit to the host cell.

Viruses can also provide benefit their hosts by allowing them to express other specific characteristics, including through the synthesis of certain compounds. To return to an example mentioned above, the bacterium *Vibrio cholerae* lives in the marine environment. Two toxins emitted by the bacterium make it an agent of cholera, but these can only be emitted if the bacterium has integrated a virus into its genome whose genes then express these toxins, thus conferring an advantage on its bacterial host. While no marine viruses are known to be directly pathogenic to humans, some may be pathogenic in indirect ways.



Conclusion

With the complex roles viruses play in the ocean, they can ultimately be considered as both angels and demons. While they are often likened to ruthless assassins (killing up to 40% of the bacteria in the ocean every day in the case of DNA viruses), they are also incredible helpers, transferring genes between a variety of hosts (bacterial or eukaryotic), which can support key processes such as photosynthesis. By killing their planktonic hosts, viruses also partially control certain key nutrient cycles and actively participate in maintaining biodiversity. The death of some clearly benefits many others. Their action also results in the deep transfer of carbon-rich organic matter away from the atmosphere, thereby helping to mitigate the effects of climate change.

Viruses are an integral part of human history, not only through the diseases they have caused and continue to cause, but also through their industrial exploitation: vaccine production, cell transformation, expression systems or therapies using phages and new enzymes. Biotechnologies using marine viruses are already in use, while many more offer promising avenues for future development. Viruses of algae, in particular, hold great promise in multiple biotechnological contexts.

While we have mostly focused on the largest reservoir of marine viruses, i.e. those that infect the most abundant hosts, bacteria and phytoplankton, much remains to be discovered about all the other viruses (those of fish, jellyfish, octopuses, turtles, dolphins, etc.). There is no shortage of questions. How many viruses are there? What explains this incredible diversity? What roles do viruses play in different ecosystems? How were they involved in the evolution of life on Earth?

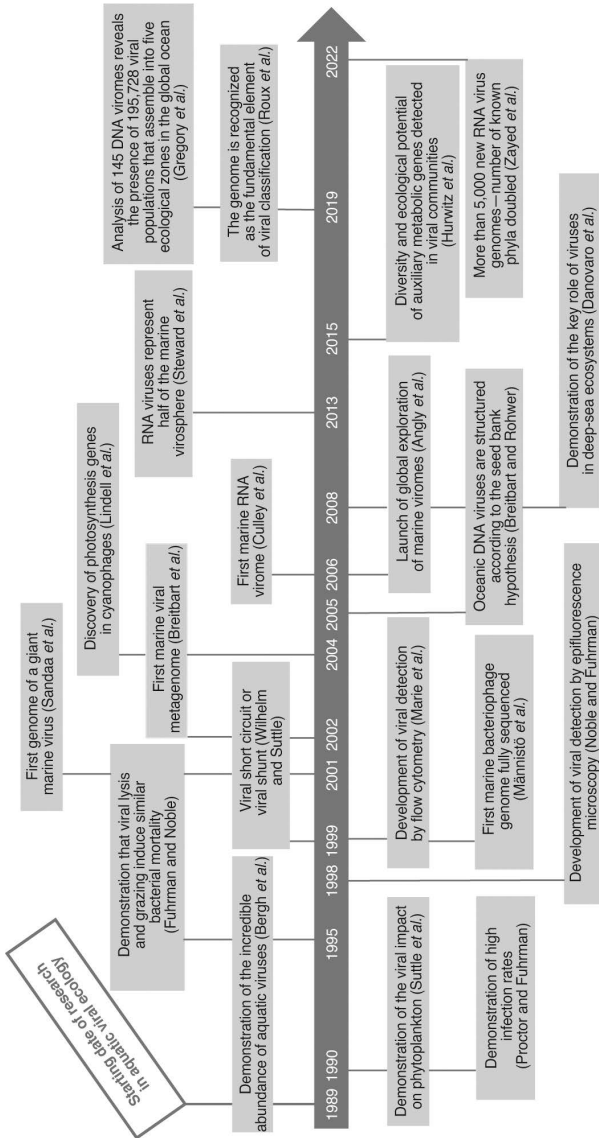
Although they also infect the macrofauna, viruses that regulate microbial communities are the true planetary machinery driving the major biogeochemical cycles. Therefore, it is almost a pity that these silent killers and facilitators are so little known, appreciated and studied, with most of our knowledge coming from

recent metagenomic studies and a handful of model systems. Another irony of science is that the evolutionary tree of life still has no real place for viruses, while without them, most likely, there would be no life at all. The simple truth is that, without their ceaseless activity, we would most certainly not be here, the global biogeochemical cycles would cease, the world would stagnate, and life would either stop altogether or be at least very limited. Let us remind ourselves once again—and truly grasp the scale—that there are more than 10^{30} viruses in the ocean (compared to only 10^{23} stars in the entire Universe), and that 1,000,000,000,000,000,000,000,000,000 infections occur every second, with all the possible or proven consequences that we have described above. These are of course only estimates and there are probably still several thousand species waiting to be discovered.

So how should we answer the question in the subtitle of this book?

The findings presented throughout this book regarding seas and oceans are closely mirrored in freshwater environments, especially lakes and reservoirs, where viral abundances and various functional roles attributed to viruses are often remarkably similar to those observed in the marine environment.

This timeline summarizes some important dates in the history of marine viral ecology.





Learn more (short bibliography)

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Viruses never cease to fascinate and frighten us because of their pathogenic nature for plants and animals. In seas and oceans, they are major players in the functioning of ecosystems. Viruses are the smallest, most abundant, and most diverse biological entities known to date in marine environments, where they perform multiple functions. In addition to their role in cellular mortality and the redistribution of matter, viruses are also powerful drivers of the evolution of their hosts and represent a major source of genetic diversity, offering rich opportunities for discoveries in both fundamental and applied research. The aim of this book is to highlight an invisible biological community that is essential for understanding and explaining how the oceans function. The authors, specialists in the ecology and evolution of aquatic viruses, show how viruses regulate microbial ecosystems—the foundation of oceanic food webs—and how their activity could, under certain circumstances, influence the climate.

This book is intended for students in life and earth sciences, teachers, researchers, and academics, as well as for the general public interested in the subject of viruses and the oceans.

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