Chapter 5
Marine Viruses

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Abstract With an estimated global abundance of 10^{30}, viruses represent the most abundant biological entities in the ocean. There is emergent awareness that viruses represent a driving force not only for the genetic evolution of the microbial world but also the functioning marine ecosystems. Culture studies advance our understanding how viruses regulate host population dynamics, but retrieving virus and host in pure culture can be difficult. Recent developments in high-throughput sequencing provide insights into the diversity and complexity of viral populations. This chapter describes current milestones in the burgeoning field of marine viral ecology, including the different aspects of marine viral action, viral diversity, ecological and biogeochemical implications of marine viruses, the cultivation of virus-host systems, and biotechnological applications of these astonishing microorganisms.

5.1 Introduction

Marine viruses are like any other viruses defined as small infectious agents, consisting of a core of nucleic acids (RNA or DNA) in a protein coat, that replicate only in the living cells of a host. A lipid envelope may be found outside or inside of

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the capsid. In the strict sense, complete viral particles found outside host cells and constituting the infective form are called virions, however, throughout this chapter the term viruses will be used for both the intracellular and extracellular forms. Viruses are classified by their genome type (DNA or RNA, single or double stranded, segmented or not, circular or linear) and size (from few Kbp to 2.5 Mbp), particle structure (e.g., icosahedral or helical) and size (from ~20 to 500 nm), capsid coat protein composition, whether it is enveloped or not, the replication strategy, the latent period (i.e., the time until progeny viruses are released from the host cell), sensitivity to physicochemical factors (e.g., temperature, pH, UV), and last but not least the host they infect. There are no universal oligonucleotide primers for marine viruses due to their huge genetic diversity, but the presence of specific conservative genes aids to the detection and classification of marine viruses, e.g., primers for fragments of the conserved cyanophage structural gene g20, or for the DNA polymerase gene (*pol*B) are used to detect large dsDNA algal viruses (Chen and Suttle 1995; Short and Suttle 2005).

Unable to replicate without a host cell, viruses are generally not considered living organisms or alive (Forterre 2010). The finding of large genome-sized viruses displaying a localized viral factory in the host's cytoplasm where the viral genome is replicated and virions are produced (La Scola et al. 2003; Santini et al. 2013), together with the discovery of viruses infecting the viral factory (c.q. virophages meaning virus eaters; La Scola et al. 2008; Fischer and Suttle 2011), renewed the discussion as to whether viruses are alive. Both ways, viruses have the ability to pass on genetic information to upcoming generations and as such they play an important role in biodiversity and evolution. Due to their mode of replication, viruses can take some genetic material from their host in their progeny but can also add their own genetic material to the host, thereby increasing genetic diversity and biodiversity as a whole. Actually, viruses can in a way be considered part of the genetic reservoir of their host, i.e., the genomes of viruses are part of the larger pan-genome (e.g., Bacteria or Archaea) like other extra-chromosomal elements. Given their rapid reproduction rates, and often high mutation rate, viruses represent an important source of genetic innovation. Besides the enormous reservoir of uncharacterized genetic diversity (Suttle 2007), viruses have the potential to be interesting material for biotechnological use (Sánchez-Paz et al. 2014).

The research on marine viruses, their population dynamics, diversity and ecological relevance has expanded exponentially over the last years. Marine viruses are the most abundant biological entities in the seas and oceans, ranging between 1 and 100,000 $\times$ 10^6 L^{-1}, whereby the higher abundances are generally found in the more coastal, eutrophic surface waters and the lowest numbers in the deep ocean. Per unit volume, viruses in marine sediments (benthic viruses) with the higher load of bacterial hosts exceed pelagic viruses by one or more orders of magnitude (Danovaro et al. 2008; Glud and Middelboe 2004; Hewson et al. 2001). The numerically dominant hosts are the marine microorganisms belonging to all three domains of life, i.e., Bacteria, Archaea, and Eukarya. With the development of sensitive nucleic acid-specific dyes in combination with epifluorescence microscopy (Noble and Fuhrman 1998), pelagic viruses could be more easily counted

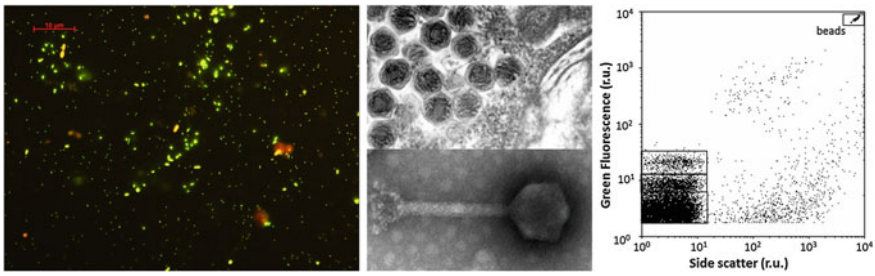

Fig. 5.1 Different techniques to detect marine viruses, i.e., epifluorescence microscopy upon staining the viral nucleic acid (*left panel*), transmission electron microscopy (TEM) thin section of host cell showing virions PgV infecting the phytoplankton *Phaeocystis globosa* (virus 150 nm diameter; *mid upper panel*), TEM of free bacteriophage (virus head diameter 100 nm, tail length 180 nm; *mid lower panel*), and flow cytometry of natural seawater sample showing several virus clusters (*right panel*)

than by transmission electron microscopy (Fig. 5.1). Moreover, the availability of fluorescent dyes allowed counting of the total viral community and not just the infectious viruses for specific hosts in culture as determined traditionally by plaque assay (using agar plates) and most probable number (end-point dilution of liquid cultures). More recently, staining of the viral nucleic acids combined with flow cytometry allowed a more objective and faster analysis (Brussaard et al. 2000; Brussaard 2004a) opening further the research field of marine viruses to a better spatial and temporal sample coverage, as well as higher degree of replication. Besides these methods for direct detection of virus particles, there are molecular approaches helping to identify and quantify (by qPCR) marine viruses (Sandaa and Larsen 2006; Tomaru et al. 2011a, b, c). Combining techniques such as, for example, flow cytometry sorting of virus and/or infected host with molecular sequencing will further advance prospective research aiming at answering questions on viral diversity and adaptation (Labonté et al. 2015; Martínez-Martínez et al. 2014; Zeigler et al. 2011). Metagenomic analysis has even revealed new virus families, e.g., ssDNA viruses infecting a wide range of marine hosts (Labonté and Suttle 2013a, b).

Although essential, quantification and identification by itself is not enough to answer the major research questions in marine viral ecology, e.g., who infects whom, what is the impact of viral infection on host population dynamics and subsequent species succession, and how do viruses affect ecosystem efficiency and consequently biogeochemical fluxes? One needs to be able to determine actual viral infection and lysis rates in the field. At the same time, there is need to isolate and bring into culture more (and new) virus-host model systems in order to allow optimal understanding of the mechanisms underlying successful infection and alterations in virus–host interactions due to change in environmental factors.

Table 5.1 Overview of some of the milestones obtained in marine virus ecology during the last 1–2 decades

Milestone	Impact
Counting viruses using sensitive dyes	Opened up the field as it allowed for more replicates and better spatial and temporal coverage
Viral lysis rates in the field	Demonstrate that virally induced mortality at least as important as traditional loss by grazing
New dsRNA virus family	Unique dsRNA virus infecting marine protist
Use of PCR primers	Specific virus groups can be detected
Full genome sequencing	Discovery of new metabolic pathways
Metagenomics	Deep coverage of viral diversity and discovery of new virus families (e.g., ssDNA viruses)
Large genome-sized virus discovery	With their huge size (1–2.5 Mb) and cellular trademark genes, these viruses are evolutionary challenging
Virophages	Viruses infecting viruses, thereby decreasing mortality of the host organism
Viral shunt	Realization that viral activity promotes flux of organic carbon and nutrients, making remineralized nutrients directly available for phytoplankton

The outcome of viral infection has implications for ocean function and it is an exciting time of discovery (some milestones are listed in Table 5.1). This chapter describes some of the milestones in marine viral ecology (Table 5.1) and outlines the different aspects of marine viral action, i.e., the different viral infection strategies, horizontal gene transfer, viral diversity, ecological influence on host mortality, microbial biodiversity, food web structure, organic carbon and nutrient cycling, the culturing and storage of virus-host systems, and concludes with several biotechnological applications.

5.2 Viral Infection Strategies

Microbial host cells and their viral predators have been evolving together for billions of years. At first glance, one might think that their interactions are simpler than those of other predator or parasite-host pairs but they are actually extremely complex. The main predators of microbes typically discriminate for prey size, along rough lines of taxonomy and morphology and can eat many individual prey of diverse genomic make up throughout its individual cell life span (Sintes and Del Giorgio 2014). In contrast, viruses are single opportunity killers and highly host-specific. One virus particle has only one chance to reproduce and requires a refined adjustment to the cell machinery on which it depends for ultimate replication and virion component. Therefore, viruses are specialized parasites that are fine-tuned to the host structure and metabolism (Leggett et al. 2013). Actually, the delicate discriminatory power of viruses has been used classically for typing, for

example, bacteria. Precise host cell recognition is an essential step in the life cycle of any virus. Accordingly, viruses have refined molecular recognition modules that allow discrimination of encountered cells before the infection process in triggered (Garcia-Doval and van Raaij 2013). These molecular recognition devices are among the most sensitive in nature and match in complexity, and probably in diversity as well, the vertebrate immune system (Fraser et al. 2007). Mirroring this diversity, host cells have an enormous diversity of virus potential receptors. They can be any outermost exposed structure of the cell, frequently proteins such as pillins, porins or transporters but, above all, polysaccharides or the glycosidic moiety of glycoproteins. Actually, the interaction with viruses might be behind the commonality of polysaccharides and glycoproteins in exposed structures of Bacteria, Archaea, and Eukarya.

Through the lytic cycle, viruses can reproduce inside the host cell directly upon penetration, causing destruction of the host cell to release the progeny viruses into the surrounding water. Alternatively, viruses may integrate into the host genome as a provirus and propagate together with the host. Thus, in contrast to lytic viruses, these temperate (or latent) phages establish a stable, but reversible, relationship with their host, which is termed lysogeny. For the prophage being inside the host provides protection against virion decay by environmental factors (Mojica and Brussaard 2014). Although at first glance it does not seem beneficial for the host to carry a prophage, it generally protects from infection by similar and related phages. Furthermore, prophage may provide useful genes and improve host fitness (Paul 2008). This transfer of genes via prophages is a form of horizontal gene transfer (HGT) that aids to the genetic diversity within the host species. The prophage can be released again by an inducing event triggering a lytic cycle. The environmental factors responsible for the transition from a lysogenic to a lytic cycle are not well identified. UV and pollution have been reported to induce the lytic stage, potentially to produce progeny viruses before potential cell death of the host (Paul et al. 1999). Alternatively, the triggering factor may represent more optimal growth conditions for the host (e.g., release of P-limitation; Wilson et al. 1996), whereby the shift from prophage to lytic phage is a favorable one as many more progeny viruses can be produced than under the lysogenic life style (one progeny every time the host divides). In agreement with this potential mechanism, the percentage lysogeny of total mortality of bacteria was found highest under oligotrophic conditions with low host abundance, both geographically as well as seasonally (McDaniel et al. 2002; Weinbauer et al. 2003).

Besides lytic and lysogenic cycles, the host cell can also release viruses by continuous or intermittent budding or extrusion without its immediate lysis. This replication strategy, known as chronic cycle or carrier state, is far less investigated in marine environments, yet it has been described for viruses that infect marine eukaryotic photoautotrophs (Mackinder et al. 2009; Thomas et al. 2011), but also for archaeal viruses (Geslin et al. 2003; Gorlas et al. 2012).

5.3 Virus Characteristics

The characterization of virus isolates typically includes a phenotypic analysis (particle morphology, symmetry and size; genome type and size; composition of structural proteins) in order to assign a taxonomical status to these isolates. Additionally, parameters related to virus life cycle, specificity pattern, environmental stability or resistance, are other important viral features. The information generated by one-step growth experiments, also referred to as "single burst experiments," have proven essential in this respect. This classical procedure developed by Ellis and Delbrück (1939) is designed to quantitate and monitor the growth of lytic viruses and it provides two fundamental viral properties: the latent period (that is the duration between virus adsorption and extracellular release from the infected host cell) and the burst size (that is the number of progeny viruses released per infected host cell). These viral parameters vary depending on the virus isolate and the host they infect. Collectively these variables constitute important components to model virus–host interactions in the ocean. For example, the infection strategy of a given virus largely determines the ecological impact of virus interactions. Gaining fundamental knowledge on the infection strategy of relevant virus-host model systems, the conditions that induce the transition between these different modes of infection, and the potential influence environmental factors have on the intensity and the kinetics of infection are thus a prerequisite for understanding the impact of viral infection on microbial assemblages.

The infection range (the range of action of a phage measured in terms of the varieties of bacteria in which it can grow) has been a major conundrum of virus studies. The host specificity of bacteriophages (i.e., viruses that infect bacteria; there are also referred to as phage or bacteriovirus) was recognized very soon after their discovery in the early twentieth century (Abedon 2000). The idea of phage therapy was based on this early discovery and was the main driver of the original interest on bacteriophages, later displaced by the enormous role played by phages in the development of genetics and molecular biology. The success or failure of adsorption and subsequent lysis of the host typically, but not always, determines the host infection range. There is a wide diversity of mechanisms within the cell to prevent phage replication, including receptor variation, restriction–modification, abortive infection, lysogenic immunity, or innate immunity conferred by CRISPRs (see for example Stern and Sorek 2011). In the laboratory, host specificities are determined by pairwise infection, in which a clonal virus lineage is added to a collection of host cultures, infection is scored positive if lysis occurs. These infection assays suggest that marine viruses exist along a continuum of specialists to generalists. The specialist viruses can only infect a restricted range of strains, sometimes even only the one used for their initial isolation. The generalist viruses can infect many different strains and occasionally can infect also isolates from different species and even different genera (e.g., Baudoux et al. 2012; Johannessen et al. 2015; Matsuzaki et al. 1992; Sullivan et al. 2003; Suttle et al. 1995). However, in general the genus is the taxonomic unit that encompasses the most sensitive

strains for the vast majority of phages. It is sensible to say that the boundary of natural host range of most phages is the genus.

Cross-infections between virus and hosts are challenging to interpret. In spite of 25 years of investigation, there is as yet no consensus on either the global pattern of viral infection or the environmental drivers that shape these patterns of interactions. For example, to what extent does virus–host infection vary in space and/or time, are there factors that favor generalists upon specialists, and are virus–host interactions structured at all (Avrani et al. 2012)? It is presently inconceivable to bring into culture and test the specificity pattern of all individual virus-host model systems dispersed in marine systems. As an alternative, theoretical approaches that rely on empirical data have been proposed to study of virus–host interactions as networks (Flores et al. 2011, 2013; Weitz et al. 2013). Network-based analysis tests whether virus–host interactions are structured (i.e., they possess a structured pattern) or random. The most common patterns in ecological networks are being nested or modular. Nested virus–host interaction networks are characterized by a hierarchy of resistance among hosts and infection ability among viruses (Flores et al. 2011; Weitz et al. 2013). This pattern is thought to result from coevolutionary arms race (Red Queen hypothesis) where viruses evolve to broaden host ranges and hosts evolve to increase the number of viruses to which they are resistant (Flores et al. 2011). In contrast, modularity is characterized by groups of viruses and hosts (referred to as modules) that preferentially interact with another (Flores et al. 2011; Weitz et al. 2013). This ecological pattern should emerge if virus–host interactions result from evolutionary processes that lead to specialization.

A network-based analysis of cross-infections of 215 phages against 286 bacteria collected across the Atlantic Ocean (Moebus and Nattkemper 1981) revealed that infection network possessed a multiscale structure (Flores et al. 2013). At a global network scale, virus–host interactions network displayed a modular structure. This modularity was explained, at least in part, by geography. In other words, viruses isolated from a given sampling site were more likely to infect cooccurring hosts, supporting the need to account for biogeography in the analysis of viral diversity. However, individual modules were either further modular or nested, which suggests that different coevolutionary processes drive virus–host interaction at different scale (Flores et al. 2013). Network-based analyses of virus–host interactions have emerged quite recently. Yet, these analyses represent a promising tool to quantify the functional complexity of viral infections in natural systems and to identify the drivers of microbial species interactions.

5.4 Virus and Host Diversity

The role of viruses in shaping the evolution of their hosts is acquiring new dimensions both as selective pressure that keeps populations diverse (Rodriguez-Valera et al. 2009) and as sources of genetic innovation (Suttle 2007). Viruses may carry metabolic genes of the host to enhance infection performance, but can also

introduce genetic diversity to the cognate pathways. Microbiology has failed to provide a sensible representation of the diversity of microbes using only the pure culture approach. Although great advances are being made, until recently the vast majority of microbes in nature has been inaccessible to microbiologists. If this is the situation of cellular microbes, the status of the viruses preying on them is even direr. Most of what we know about viruses derives from the studies of human, cattle and plant crop viral pathogens. Lately large efforts to sequence more virus genomes of different hosts have been made (Pope et al. 2015), and the approaches based on high-throughput sequencing metagenomics and single cell genomics are also applied to marine viruses (Angly et al. 2006; Rodriguez-Brito et al. 2010).

The advent of high-throughput sequencing technologies permitted to determine the complete genome sequence of more than one hundred cultured marine viruses over the past 15 years and many more are currently being analyzed. Virus genomics provides invaluable insights into the understanding of how viral infection and virus evolutionary relationships function. One notable feature is that marine viruses encode remarkable panoplies of biological functionalities. They display core genes, found in most viruses, involved into the replication of viral DNA and the structure and the assembly of virions. Genome analyses also indicate that viruses encode and transfer a variety of auxiliary metabolic genes (AMG) derived from their hosts (Rohwer et al. 2000; Sullivan et al. 2005; Wilson et al. 2005). Although the ability to shuffle genetic information through HGT is commonly reported in viruses, the repertoire of host-derived genes encode by marine viruses differ from those of non-marine origins. The most emblematic AMG is probably the *psb*A photosynthesis gene reported in cyanophage genomes (Mann et al. 2003; Sullivan et al. 2005). This gene encodes the protein D1, which forms part of the photosystem II reaction center in the host cell. During the course of viral infection, the D1 proteins derived from the host decline dramatically while those encoded by the cyanophages increase (Clockie et al. 2006). This led to the hypothesis that the expression of virus-derived *psb*A helps maintaining photosynthesis throughout the lytic cycle, which, in turn, 'boosts' the host metabolism to support virus replication. The analysis of AMG in virus genomes suggests that marine viruses have evolved a wide range of strategies to hijack host metabolism towards their own replication (Hurwitz et al. 2013). AMG are widespread and they appear to be involved into phosphate scavenging, carbon, or nitrogen metabolism (Brum and Sullivan 2015; Hurwitz et al. 2013; Rohwer et al. 2000; Sullivan et al. 2005). The genome analysis of viruses that infect the bloom-forming alga *Emiliania huxleyi* (EhV) also revealed a unique cluster of sphingolipid biosynthetic genes (Wilson et al. 2005) that facilitate virus replication and assembly (Rosenwasser et al. 2014). Genomics of marine virus isolates provides unprecedented knowledge about complex metabolic pathways that play a pivotal in role in host–virus interactions.

In parallel to genome analysis of cultured isolates, the development of culture-independent tools was a breakthrough in virus ecology in the ocean. Metagenomics largely contributed to unveil the extent of viral diversity and the dynamics of viral communities (Angly et al. 2006; Breitbart et al. 2004; Brum et al. 2015). The genome sequence of relevant virus models is thus essential to build a

reference database in order to identify the members of the viral communities and, importantly, whom they infect (Kang et al. 2013; Zhao et al. 2013). Despite its success, a general problem in the application of metagenomics techniques is that although the number of viral particles is high, the amount of viral DNA in natural environments is much smaller than cellular DNA. Therefore, since the beginning, DNA amplification approaches were applied to retrieve the required amount for Next Generation Sequencing (NGS) (Angly et al. 2006). In addition, the short NGS reads complicate enormously the already shaky grounds of viral genome annotation. Finally, one classical major limitation of viral metagenomics or metaviromics was assembly. By assembly of high coverage metagenomes large genome contigs can be reconstructed that are much more reliably annotated and classified. However, for unknown reasons, assembly works much more poorly with phage genomes in metaviromes. This problem can be partially by-passed by using cellular metagenomes. Cellular DNA is also rich in viral DNA derived from cells that are undergoing the lytic cycle. In metabolically active communities such as the deep chlorophyll maximum (DCM) 10–15 % of the bacterial and archaeal DNA derives from such phages (Ghai et al. 2010). For example, by using advanced binning approaches, a bacteroidetes phage that seems to be extremely widespread and conserved in the human microbiome could be assembled (Dutilh et al. 2014). By using metagenomic fosmid libraries Mizuno et al. (2013) described large genomic fragments from more than a 1000 viral genomes obtained from a single sample from the Mediterranean DCM. Fosmids have similar size to typical *Caudovirales* genomes and many of these could be retrieved. They could be gathered by genome comparison into 21 major sequence groups and a vast genomic diversity was present within each group. Host could be assigned to some of the 10 totally novel groups by comparison to putative host genomes what allowed assigning the first phages detected to marine Verrucomicrobia and the recently described marine actinobacteria Actinomarinales (Ghai et al. 2013). This work also revealed high microdiversity among the highly related sequence groups (Rodriguez-Valera et al. 2014), particularly in the host recognition modules. Specific single stranded (ss) DNA amplification has allowed the reconstruction of ssDNA viruses that are prevalent in marine habitats and have small genomes that simplify their reconstruction from metaviromes (Labonté and Suttle 2013a, b). The newly developed technology of single cell genomics is also providing data about viruses taking advantage of the same phenomenon, i.e., sorted cells are often undergoing lysis and eventually the active virus genome is abundantly represented in the resulted amplified genome (Labonté et al. 2015). This technique allowed the description of the first genome of a virus preying on the ubiquitous group I marine archaea or Thaumarchaeota.

5.5 Ecological Importance

Under natural conditions the richness of microbial species is high, while laboratory research indicates that competition for the same resources ultimately will lead to the dominance of the best competitor and the extinction of the other competitor(s). The most renowned example is the Plankton Paradox (Hutchinson 1961), whereby according to the competitive exclusion principle only a small number of plankton species should be able to coexist on the growth-limiting resources while in reality large numbers of plankton species are found to coexist within small regions of open sea. Because ecological factors stay hardly ever constant, spatio-temporal heterogeneity, but also selective grazing, symbiosis and commensalism have been suggested as factors responsible for resolving the Plankton Paradox. Another top-down controlling factor more recently acknowledged is viral infection. Marine viruses have typically narrow host ranges, which makes them highly relevant as underlying cause of coexistence of microbial species (Suttle 2007; Thingstad 2000). As viral infection is dependent on contact with the host, higher abundances of susceptible host species are expected to favor successful infection. This Killing-the-Winner (KtW) model describes how the abundance of the competition strategist is top-down controlled by viruses, thereby ensuring coexistence of competing species since the growth-limiting resources can be used by others (Thingstad 2000). Then again, a high abundance of host is not necessarily the result of rapid growth as host species might be resistant to top-down control (by zooplankton predators and/or viral lysis), or there might be substantial loss of viral infectivity or virus particles that prevent further infection of the developing host population. The traditional rank-abundance curves (illustrating the relative abundance of species) have to be read differently then, i.e., the least abundant taxa are not rare (or dormant) but are instead actively growing but at the same time also predated and/or lost by viral lysis (Suttle 2007). They illustrate r-selection, with high reproductive output, while the few most abundant taxa are K-selected with low maximum growth rates, better competitors for resources and less sensitive to cell loss (Suttle 2007; Våge et al. 2013). At the same time, the marine viruses rank-abundance curve shows a contrasting pattern with most viruses r-selected, being virulent with small genome sizes and high decay rates. The low abundance virus taxa are K-selected with larger genomes, longer-lived, and potentially forming stable associations with their host.

Blooms of phytoplankton exemplify situations where there is an unbalance between growth and loss processes, either because the algal species is able to outgrow infecting (at least temporarily) or because it is (largely) resistant to infection. Blooms of *E. huxleyi* illustrate the concept of KtW well, because during the development of the bloom the percentage of infected cells is increasing to a point that causes the collapse of the bloom (Bratbak et al. 1993; Brussaard et al. 1996a; Martínez-Martínez et al. 2007). Interestingly, the cell morphotype of *E. huxleyi* seems to affect the chance of infection. A laboratory study showed that the diploid calcifying coccolith-bearing cells were virally infected but the haploid cells were not (Frada et al. 2008). This finding led to the proposition of the Cheshire Cat

escape strategy, where viral infection indirectly promotes a resistant haploid phenotype and thus sexual cycling (Frada et al. 2008). However, the genetically distinct communities of the *E. huxleyi* host and the co-occurring viruses under natural conditions were stable over several years, which seems to be inconsistent with the Cheshire Cat hypothesis (sexual reproduction would actually reshuffle the genes in *E. huxleyi*; Morin 2008). Moreover, a study of a natural bloom of *E. huxleyi* showed that the haploid organic scale-bearing cells (haploid by nature, Laguna et al. 2001) were also visibly infected by viruses (using transmission electron microscopy; Brussaard et al. 1996a). It needs to be seen whether these differences between the studies (1) indicate that sex does not act as an antiviral strategy (Morin 2008), (2) are due to growth-controlling factors (i.e., the natural bloom was nitrogen depleted while the laboratory study used nutrient replete cultures), (3) different virus types (the natural study showed co-infection of two different viruses), (4) genetically distinct algal host strains (and thus potentially different sensitivity to infection), or (5) the haploid *E. huxleyi* cells in the laboratory study being different than the natural scale-bearing cells.

Another example of how blooms are formed by (largely) viral infection-resistant cell morphotypes is the algal blooms of the phytoplankton genus *Phaeocystis* (Brussaard et al. 2005). *Phaeocystis* can make colonies provided that there is enough light available to allow the excess organic carbon to be excreted in the form of carbohydrates forming a colonial matrix (Schoemann et al. 2005). The colonial cell morphotype has a much lower chance of becoming infected (or grazed) than a single cell (Jacobsen et al. 2007; Ruardij et al. 2005) and therefore a considerable increase in biomass is possible. At the moment, the cells become shed from the colonial matrix (due to low light or nutrient depletion; Brussaard et al. 2005) the liberated single cells are readily infected and eaten and the bloom crashes (Baudoux et al. 2006).

Viruses can be so specific that they only infect certain strains. This provides the potential for intraspecies succession of host and virus within a phytoplankton bloom (Baudoux et al. 2006; Tarutani et al. 2000). For example, the different sensitivities of *Heterosigma akashiwo* clones to viral infection resulted not only in changes in the total abundance of the virus infecting *H. akashiwo* but also the clonal diversity of the algal host. Another example of high differential sensitivity to viral infection within a species is the blooms of *Synechococcus* spp. that were composed of many (genetically) different populations of host and cyanophage (Mühling et al. 2005; Suttle and Chan 1994; Waterbury and Valois 1993). Differences in infectivity properties of the host strains may be directly due to changes in the composition of the attachment sites on the host's cell surface (Marston et al. 2012), or may be the indirect result of altered virus proliferation as a consequence of variation in host cell metabolic traits between the host strains (Brussaard, unpublished data). Resistance is thought to come with a cost, i.e., a resource competitive disadvantage compared to viral infection susceptible strains (Lenski 1988; Thingstad et al. 2015). Furthermore, host growth-controlling factors have been shown to affect virus latent period, viral burst size, the level of infectivity of the viral offspring, and even viral life strategy (Baudoux and Brussaard 2008; Bratbak et al. 1998; Maat et al. 2014; Wilson et al. 1996). Altogether, variations in host susceptibility to viral infection

and viral production rate and yield add not only to biodiversity but also to spatial and temporal variability of microbial hosts and viruses.

Although many studies have indicated that viral infection is a significant loss factor, accurate measurements of viral lysis rates of microorganisms in the field are still few (Suttle 2005). Furthermore, rates of virally mediated mortality are assessed using different approaches (results are therefore not necessarily comparable), and most methods rely on different assumptions and conversion factors. The first approach, using visibly infected cells obtained from transmission electron microscopy analysis, is dependent on good estimation of the proportion of the lytic cycle during which virus particles are visible in the infected host cells (Brussaard et al. 1996a; Proctor and Fuhrman 1990). This factor is difficult to assess as the lytic virus growth cycle is highly variable between viruses and it can also differ within a virus depending on host metabolism. Estimating viral lysis rates using (fluorescent) virus tracers requires estimates of burst sizes (Heldal and Bratbak 1991; Noble and Fuhrman 2000; Suttle and Chen 1992). Using viral decay rates to estimate production rates also requires the assumption that there is a steady-state situation. The use of synthesis rates of viral DNA requires many conversion factors (Steward et al. 1992). Measuring viral production rates is most widely used but does need an estimate of burst size and comes with substantial sample handling (Weinbauer et al. 2010). Many of these methods, except the visibly infected host cell assay, measure viral lysis of the total community, because the different virus (and often also host) populations cannot be discriminated. Because algal viruses typically represent a few percent of the total virus abundance, the same methods are not well suited for phytoplankton viral lysis rate estimates unless the algal virus can be separately counted. For phytoplankton, viral lysis rate can be assessed using a modified dilution assay (Baudoux et al. 2006; Evans et al. 2003; Kimmance and Brussaard 2010), but here the restriction is that the algal host cells are newly infected and lysed within the time of incubation (typically 24 h because of synchronized cell division of many phytoplankton species).

The available viral lysis rate measurements in the field demonstrate that viral lysis is similar to predation rates (Baudoux et al. 2006, 2007; Mojica et al. 2015). The pathways of cellular organic matter and energy through the food web will, therefore, follow different ways (Suttle 2005). Upon predation organic carbon and nutrients are transferred to higher trophic levels, while upon viral lysis the host's cellular content is released into the surrounding waters as dissolved and detrital particulate organic matter. Viruses are as mortality agents causing accelerated transformation of organic matter from the particulate (lysed host cells) state to the dissolved phase, upon which heterotrophic microbial communities mineralize the largely (semi-) labile organic matter directly (Lønborg et al. 2013). The diversion of organic matter towards the microbial loop (microbial recycling) results in enhanced respiration (Suttle 2005). Cell lysis of microorganisms has been reported to sustain the heterotrophic bacterial carbon demand (Brussaard et al. 1995, 1996b, 2005). Globally, viral lysis seems to negatively affect the efficiency of the biological pump (i.e., the transfer of photosynthetically fixed carbon to the deep ocean as dead organisms or feces). The rapid release and remineralization of nutrients in the photic

zone may increase the ratio of carbon relative to nutrients exported to the deep. Conversely, viral lysis may potentially short-circuit the biological pump by stimulating the formation of transparent exopolymer particles (TEP) and aggregates (Brussaard et al. 2008; Mari et al. 2005), as well as release elements that could as such stimulate primary production (e.g., ligand-bound iron and organic phosphorus; Gobler et al. 1997; Løvdal et al. 2008; Poorvin et al. 2004). With respect to the global anthropogenic climate change (greenhouse effect but also alterations in the physicochemical characteristics of the water masses, e.g., temperature, salinity, light, nutrients), it is urgent to improve our understanding of how viral activity affects the processes involved in the natural sequestration of carbon dioxide. A study in the northeast Atlantic Ocean showed for instance that the share of viral lysis (as compared to grazing) of phytoplankton increased under conditions of temperature-induced vertical stratification (Mojica et al. 2015).

5.6 Isolation, Culture, and Characterization of Marine Viruses

Almost from their first discovery in 1917, cultured virus–host systems (VHS) were viewed as relevant models for microbiologists and geneticists who investigated the nature of genes and heredity. This early era of virus research constituted the foundations of molecular biology. It was not until the mid-1950s that marine viruses were discovered with the isolation and the description of a phage lytic to the fish gut associated bacterium, *Photobacterium phosphoreum* (Spencer 1955). Although the potential of bacteriophages to control host populations emerged quite rapidly, virus isolation from oceanic settings was only reported sporadically and most studies focused on the biology rather than the ecological implications of these model systems (Hidaka and Fujimura 1971; Hidaka 1977; Spencer 1960). The breakthrough discovery that viruses are the most abundant biological entities in the ocean marked a turning point in the field of virus ecology and literally renewed interest in isolating, culturing, and characterizing marine viruses.

Several protocols describing procedures for the isolation of lytic viruses have been reported from various laboratories. These protocols include three main steps: (1) A natural sample is added to a selection of prospective host cultures, (2) the lysis of this host is visualized and, (3) in case of positive lysis, the lytic agent is cloned and stored appropriately until characterization. In the following section, we provide a thorough description of these steps and recommendations to optimize successful virus isolation. Although these features are applicable to most viruses, viral strains may differ in their development, and therefore isolation protocols may have to be adapted accordingly. In theory, at least one, and usually multiple, viruses, can infect any given organism. However, empirical data have shown that some strains are more permissive to viral infection than others. It is, therefore, preferable to use several clonal host lineages in order to increase the chance of

successful isolation. When possible, one should isolate the host strains just prior to virus isolation from the same location in order to increase success rate in case the virus has a narrow host range. Furthermore, owing to their parasitic nature, viruses largely rely on the metabolism of their hosts for production. In most cases, well-growing hosts tend to be more susceptible to viral infection.

Prior to the isolation, it is recommended to remove particles larger than viruses by low-speed centrifugation and/or pre-filtration through low protein binding membrane filters (e.g., polycarbonate, polyethylsulfone, polyvinylidene fluoride) with a preferable pore size of 0.45–0.8 μm (filtration through 0.2 μm could exclude larger virus particles). However, this may result in loss of viruses and therefore it is recommended to also use a non-treated sample. For sediment or any other type of porous samples, viral particles need to be transferred into a buffer solution prior to centrifugation and/or pre-filtration (Danovaro and Middelboe 2010). If the targeted virus population is expected to be low in abundance, tangential flow filtration/ultrafiltration or ultra-centrifugal units with a retention cutoff of at least 100 kDa can concentrate viral particles. As an alternative to these techniques that can become time consuming and costly, the host-enrichment technique also increases the probability of successful isolation considerably. This enrichment step consists of amending the viral community to be screened with 1–5 prospective host strains and host growth medium. Upon incubation this mixture, viruses lytic to these hosts should propagate and hence this procedure facilitates isolation. After incubation, the suspension can be clarified by centrifugation or pre-filtration and, if necessary, further concentrated by ultrafiltration as described above. Once prepared, the extracted viral community can be stored at 4 °C until the isolation procedure.

5.6.1 Detection of Lytic Viral Lysis and Virus Purification

There are several approaches to visualize cell lysis of the targeted host due to lytic viral infection. One common technique is the plaque assay, which has been successfully applied to the isolation of viruses of bacteria and microalgae (Fig. 5.2). This technique relies on combining an inoculum of the viral sample and the targeted host culture in soft agar overlay on agar plates. On incubation, the host develops a homogenous lawn, except where virus lysis occurs, resulting in localized translucent plaques, referred to as a plaque-forming unit (PFU, Fig. 5.2). PFU typically originates from one infectious virus and displays a well-defined morphology. Different viruses infecting the same host can coexist in the same sample. It is therefore recommended to pick as many individual plaques as possible and to repeat the plaque assay for another two times to produce as many clonal lineages of these isolates. Alternatively, the lysis of infected host can be detected in liquid medium. In this case, the propagation of viruses is not limited by the diffusion in semi-solid agar media. Upon attachment on a susceptible host, the viral progeny is released in liquid medium and the newly produced virions can initiate another lysis cycle and propagate until complete lysis of the cultured host. Lysis is detected by complete

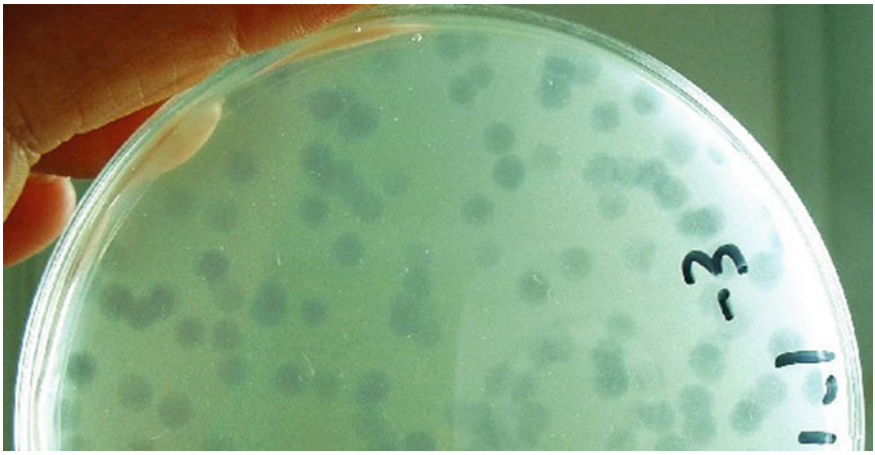

Fig. 5.2 Petri dish showing a plaque assay for viruses infecting the picoeukaryotic phytoplankton *Micromonas* sp. (*photo courtesy* Nigel Grimsley)

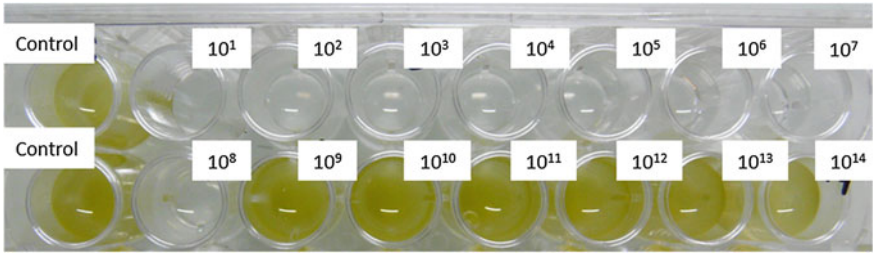

Fig. 5.3 Aquatic serial dilution set-up using microwell plates whereby a suspension of *Micromonas* virus RCC4265 was diluted from $\times 10^{1}$ down to $\times 10^{-14}$ in *Micromonas* host culture RCC829. Lysis is scored positive until dilution $\times 10^{8}$. Non-infected controls (*first column*) were taken along for reference

clearing of the host suspension as shown in Fig. 5.3. The resulting lysate can comprise a mixture of viruses that need to be separated as soon as possible to obtain clonal viral lineages. This can be achieved by extinction dilution methods in multiwell-plates (Fig. 5.3). The principle of this procedure is to serially dilute (usually using tenfold dilution increment) the lytic agents in vigorously growing host culture until complete extinction (i.e., no lysis detected anymore). The lysed culture from the most diluted dilution is selected and the complete procedure is repeated twice to ensure a clonal virus. While the plaque assay can lead to the isolation of several viral strains infecting the same host, isolation in liquid medium generally selects for the most abundant viral strain lytic for the targeted hosts. However, this latter procedure requires only non-specialized equipment, consumables and minimal sample handling.

5.6.2 Isolation of Temperate Viruses

The most common procedure to induce lysogens and isolate temperate viruses consists of inducing lysogens with the antibiotic mitomycin-C (Paul and Weinbauer 2010). The recommended mitomycin-C concentration varies depending on the lysogen strains but usually ranges between 0.1 and 2 μg mL^{-1}. This treatment usually induces the collapse of lysogenized culture and the release of temperate virus within 12 h. Cellular debris and larger particles can be clarified from the suspension by low-speed centrifugation and/or filtration through 0.45 μm low protein binding membrane filters. The filtrate containing temperate viral isolates can then be stored at 4 °C in the dark until further characterization. Alternatively, lysogens can also be induced upon short (30 s) exposure to UV-C radiation (Jiang and Paul 1998; Lohr et al. 2007; Weinbauer and Suttle 1996). These procedures demonstrated that lysogeny was frequent among cultured marine bacteria (Jiang and Paul 1998; Lossouarn et al. 2015; Stopar et al. 2004) yet it has been seldom reported in microalgae (Lohr et al. 2007) and archaea. However, one should bear in mind that lysogeny represents a complex and stable relationship between the virus genome and that of its hosts. The induction of lysogens upon chemical or physical induction does not take into account the biological principle of this symbiosis (Paul 2008). It is thus likely that some lysogens are not induced following these treatments.

5.6.3 Isolation of Chronic Viruses

Chronic viruses that are released by the host cell by continuous or intermittent budding or extrusion are less investigated than their lytic and temperate counterparts, yet they have been reported in marine eukaryotic photoautotrophs *Emiliania huxelyi* (Mackinder et al. 2009) and *Ostreococcus* sp. (Thomas et al. 2011), but also for viruses of the hyperthermophilic euryarchaeote *Pyrococcus abyssi* (Geslin et al. 2003) and *Thermococcus prieurii* (Gorlas et al. 2012). Chronic viruses are typically detected by the examination of host culture by transmission electron microscopy. The isolation of these viruses mostly relies on extracting the virus particles from the host culture. This can be done either by low-speed centrifugation and/or filtration through low protein binding membrane filters with pore size ranging from 0.2 to 0.45 μm.

5.6.4 Maintenance and Storage

The most common procedure to store virus stocks is to keep them refrigerated (4 °C) in the dark. However, viral strains differ considerably with respect to their stability in the cold. While some isolates can be kept for years, others decay when stored

refrigerated. Therefore, appropriate storage conditions should be designed for each individual type of virus. For the most challenging isolates, repeated transfers into fresh host culture can be considered for maintaining viruses. Finally, cryopreservation can be an alternative for, at least, some isolates. Bacteriophages affiliated to the order *Caudovirales* are often stored at −80 °C in 10–20 % sucrose, 10-20 % dimethyl sulfoxide (DMSO). These concentrations of cryoprotectant were also appropriate to store viruses of phytoplankton; however, storage temperature has to be modified according to the isolate stability (Nagasaki and Bratbak 2010). Conversely, to viruses pathogenic to human, animals or plants, marine virus isolates are rarely deposited in culture collections. Except for the Roscoff Culture Collection (RCC), which curates and supplies viruses of marine microbes, most VHS are maintained in personal culture collections of individual laboratories.

5.7 Marine Virus-Host Model Systems in Culture

During the past three decades few hundreds of viruses have been isolated from marine systems and brought into culture. Cultured viral isolates have been reported for many species emblematic of heterotrophic and phototrophic marine bacteria such as Proteobacteria, Bacteroidetes, Cyanobacteria (e.g., Holmfeldt et al. 2013, 2014; Moebus and Nattkemper 1983; Sullivan et al. 2003, 2005, 2009) and algae (Brussaard and Martínez-Martínez 2008; Short 2012; Tomaru et al. 2015 and references therein), whereas thus far only few viruses infecting archaea (Geslin et al. 2003; Gorlas et al. 2012), or heterotrophic protozoa (Arslan et al. 2011; Fischer et al. 2010; Garza and Suttle 1995) were brought into culture. Among the latter, the virus of the protozoa *Cafeteria roenbergensis* (CroV) was even parasitized by a small virophage (Mavirus) during CroV host infection (Fischer and Suttle 2011).

The isolation and characterization of these VHS unveiled an extraordinarily high level of morphological, taxonomical, and functional novelty. The characterization of 31 cellulophages demonstrated considerable divergence with known counterparts (Holmfeldt et al. 2013). The morphological and genomic analysis of these isolates showed that they represent 12 novel genera globally distributed in the ocean and together they comprise the largest diversity of phages associated to a single marine host (Holmfeldt et al. 2013). Likewise, marine protists host a wide diversity of viral pathogens. The majority of these viruses is complex, possesses a large dsDNA genome, and belongs to the Nucleo-Cytoplasmic Large DNA Viruses (NCLDV), for which the order Megavirales has been proposed (Colson et al. 2013). NCLDV from marine environments belong to 2 main families. The Phycodnaviridae family includes the majority of viruses that infects photosynthetic protists among which the haptophytes *E. huxleyi* (Schroeder et al. 2002; Wilson et al. 2005), *P. globosa* (Baudoux and Brussaard 2005; Brussaard et al. 2004b), the prasinophytes *Micromonas*, *Bathycoccus,* and *Ostreococcus* (Baudoux et al. 2015; Derelle et al. 2008, 2015; Martínez-Martínez et al. 2015; Zingone et al. 2006), or the raphidophyte *H. akashiwo* (Nagasaki et al. 1999).

Marine NCLDV also classify within the Mimiviridae family. This clade, named after the discovery of Mimivirus (Raoult et al. 2004), originally included viruses with exceptionally large dsDNA genomes. With a sophisticated shell 0.7 μm and a huge genome of 1.2 Mb, *Megavirus chilensis,* isolated from coastal waters of Chile, represents the largest Mimiviridae and it surprisingly propagates on a freshwater acanthamoeba (Arslan et al. 2011). Viruses with smaller genome sizes but sharing several genetic features with these giant viruses were added to this virus family; Mimiviridae includes now the virus CroV that infects the marine protozoa *Cafeteria roenbergensis* (750 kb, Fischer et al. 2010), but also viruses that infect photosynthetic protists such as the haptophytes *Phaeocystis pouchetii* (485 kb, Jacobsen et al. 1996), *Phaeocystis globosa* (460 kb PgV-16T, Santini et al. 2013), *Haptolina ericina* (formely named *Chrysochromulina ericina*, 530 kb, Sandaa et al. 2001; Johannessen et al. 2015), *Prymnesium kappa* (507 kb, Johannessen et al. 2015), the prasinophyte *Pyramimonas orientalis* (560 kb, Sandaa et al. 2001) and the pelagophyte *Aureococcus anophagefferens* (371 kb, Moniruzzaman et al. 2014) are awaiting assignment to this virus family. The discovery of Mimiviridae initiated intensive debates on the concept virus. Their huge and astonishingly complex genome was found to encode trademark cellular functions, which literally blurs the boundaries between viruses and cellular organisms. The upper limits of the viral world both in terms of particle size and genome complexity have been pushed out with the isolation of Pandoraviruses from coastal marine waters and a freshwater pond (Philippe et al. 2013). These amoeba viruses exhibit dsDNA genome of 2.5 Mb lacking similarities with known NCLDV.

Not all protist viruses are of the dsDNA type. The prasinophyte *Micromonas*, which hosts many Phycodnaviridae, can also be infected by dsRNA virus (Brussaard et al. 2004b). This reovirus displays unusual morphology and genetic features compared to known Reoviridae, which led to the proposition of a new genus, *Mimoreovirus*, within the family Reoviridae (Attoui et al. 2006). Moreover, diatom viruses exhibit tiny particle diameter (< 40 nm) compared to Phycodnaviridae and belong to two main groups: the Bacilladnavirus (ssDNA viruses, Kimura and Tomaru 2013, 2015; Nagasaki et al. 2005; Tomaru et al. 2008, 2011a, b, 2013b) and the Bacillarnavirus (ssRNA, Kimura and Tomaru 2015; Nagasaki et al. 2004; Shirai et al. 2008; Tomaru et al. 2009, 2012, 2013a).

Together, these novel VHS demonstrate that we have barely scratched the surface of viral diversity. Characterizing these model systems is essential to gaining knowledge about the functional role of this tremendous taxonomic diversity. Importantly, marine environments have been inadequately sampled. Most VHS cultured thus far originate from temperate coastal marine environments, while open-ocean, tropical and extreme marine systems have been largely under-explored. Likewise, VHS mostly include dsDNA virus that infect emblematic marine bacteria or protists. By comparison archaeal viruses, RNA viruses, or ssDNA are under-represented while some of these groups seem to comprise an important fraction of the viral community (Culley et al. 2006; Labonté and Suttle 2013a, b; Labonté et al. 2015). Hence, there is no question that more efforts for isolating novel viruses need to be pursued.

5.8 Marine Viruses and Biotechnological Applications

Metagenomic studies of marine viruses reveal the presence of many new genes and novel proteins and as such the vast amount of genetically diverse marine viruses is a largely untapped genetic resource for biotechnological applications. Interesting findings from studying marine viruses thus far involve the algal virus EhV infecting *E. huxleyi*, which has a unique cluster of seven genes that are involved in the sphingolipid biosynthesis, leading to ceramide formation (Wilson et al. 2005). Ceramides are the key structural lipids of skin, nails and hair as an effective epidermal barrier against water evaporation and entry of microorganisms. Use of ceramides by the cosmetic industry depends still largely on biomolecules obtained from natural sources, and thus the expression and production of viral ceramides may be a promising alternative source of natural ceramides (Wilson et al. 2005).

Other exciting recent discoveries are the viruses infecting viruses which showed that the infection with such virophage ('eaters of viruses') not only helps the survival of the cellular organism by decreasing the yield of the larger virus, but it also illustrated the potential of the virophages as source of biotechnologically interesting features (Fischer and Suttle 2011; La Scola et al. 2008). For example, the name of the virophage Mavirus is derived from Maverick virus as its genome encoding 20 predicting coding sequences of which seven have homology to the Maverick/Polinto family of DNA transposons. Furthermore, the DNA genome of the virophage Sputnik contains genes related to those in viruses infecting the three domains of life, and may as such serve the transfer of genetic material among viruses.

In the marine aquaculture industry, viruses are typically directly linked to (lethal) diseases and therefore are considered a major economic burden (Suttle 2007). However, the beneficial use of lytic viruses as mortality agents and source of lytic enzymes has been explored for multiple purposes. In aquaculture, *Chlorella* viruses were exploited to improve the extraction efficiency of algal lipids for biodiesel production (Sanmukh et al. 2014). The stringent host specificity of virus is another feature that has been exploited in aquaculture. Cultured fish, shellfish, or crustaceans, like any animal, are the target of bacterial infections that can have a dramatic economic impact. The use of chemotherapy is a rapid and efficient way to limit these infections. Yet these treatments are also lethal for beneficial bacteria and, importantly, they have led to the evolvement of multidrug-resistant bacterial strains that are a serious threat for farming industries. By comparison, lytic viruses are efficient antimicrobial agents that specifically infect and propagate on a limited number of host strains, leaving the co-occurring microbial community untouched. They have thus emerged as potential therapeutic agents to prevent and treat bacterial infections. Phage therapy has already been implemented to treat several fish pathogens and its effectiveness is currently tested for the treatment of other cultured animals in experimental conditions (Karunasagar et al. 2007; Martínez-Díaz and Hipólito-Morales 2013; Nakai and Park 2002; Oliveira et al. 2012 and references therein).

The application of viruses as therapeutic agents goes beyond aquaculture. In natural ecosystems, the combined effect of anthropogenic pressure and global change severely threatens the health of coral reefs. The increased surface temperature and pollution has been associated with the development of infectious diseases that lead to the demise of infected reefs. Phage therapy reached a new milestone with promising tests accomplished on the scleractinian corals *Favia favus* infected by *Thalassomonas loyana*, the causative agent of the white plague disease (Atad et al. 2012). Inoculation of the marine phage BA3 (Efrony et al. 2007, 2009) with diseased corals prevented the progression of the white plague disease and its transmission to healthy corals in the Gulf of Aqba, Red Sea. This promising study constitutes the first application of phage therapy to cure diseased corals in situ.

Another emerging field of interest is the detection and identification of viruses in ballast water. Ballast water discharges from ships are responsible for introducing invasive species and their viruses to other marine regions. The knowledge of marine microorganisms spread by ballast water discharge is still largely understudied but has potential of spreading disease agents. There are a few examples (already) of specific viruses that were introduced via ships' ballast water, i.e., the Infectious Salmon Anemia Virus (ISAV) in salmon farms of Scotland were linked to vessel visits, and marine cyanophages were introduced to the Great Lakes, USA (Murray et al. 2002; Wilhelm et al. 2006). Given the increasing volume of shipping and rapidly growing aquaculture industry, introduction of pathogenic viruses to near-coast ecosystems and aquaculture farms is a realistic threat. At the same time, increasingly strict regulations on ballast water discharge force the shipping industry to make use of on-board ballast water treatment systems in order to decrease the abundance of organisms and viruses. Effective methods of virus concentration and virus detection are needed to comply with the new quality standards.

The high yield genes found as AMG can be used by synthetic biology to create more efficient artificial cells in the future. It is clear that the vast genetic diversity contained in the viral biosphere compartment will eventually be used for biotech applications or nanotechnology (Fischlechner and Donath 2007).

5.9 Future Perspectives

Viruses play a major role in the natural history and population dynamics of most living organisms and this is even truer for Bacteria, Archaea, and protists. Marine viruses might contribute decisively to the marine ecosystem functioning and to the stability and performance of the microbial communities. Therefore, it is important to strive to get a meaningful representation of all the groups involved in the community behavior and that includes viruses. If we are to understand the biology of these microbes we have to advance in the knowledge of their viruses. Culture-independent approaches are allowing fast advances, but do not exclude the need for in situ viral lysis rate measurements, as well as experimental studies of viral–host interactions.

Viruses are probably the largest reservoir of novel genes in the biosphere and this novelty is important for their host biology. The role that viruses play in the evolution of microbes is considered to be fundamental. Cells can outsource their most innovative evolutionary experiments to their viruses that are not under the pressure of optimizing growth parameters to compete. The number of genes shared by (microbial) hosts and their viruses has been continuously increasing. As more viral genomes become available, the list of AMG genes increases and is now including multiples aspects of the host physiology (Breitbart 2012).

It has been proposed that viruses are a part of the bacterial and archaeal pan-genomes (Rodriguez-Valera and Ussery 2012) and that as such are impossible to extricate from the cellular lineages from an evolutionary perspective, i.e., to form a single selection unit. In the formidable task that microbiologists face when struggling to describe microbial diversity and its ecological function, viral diversity and activity can be considered the last but important frontiers that need to be overcome.

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References

Abedon ST (2000) The murky origin of Snow White and her T-even dwarfs. Genetics 155:481–486

Angly FE, Felts B, Breitbart M, Salamon P, Edwards RA, Carlson C, Chan AM, Haynes M, Kelley S, Liu H, Mahaffy JM, Mueller JE, Nulton J, Olson R, Parsons R, Rayhawk S, Suttle CA, Rohwer F (2006) The marine viromes of four oceanic regions. PLoS Biol 4:e368

Arslan D, Legendre M, Seltzer V, Abergel C, Claverie JM (2011) Distant Mimivirus relative with a larger genome highlights the fundamental features of Megaviridae. Proc Natl Acad Sci 108:17486–17491

Atad I, Zvuloni A, Loya Y, Rosenberg E (2012) Phage therapy of the white plague-like disease of *Favia favus* in the Red Sea. Coral Reefs 31:665–670

Attoui H, Mohd Jaafar F, Belhouchet M, de Micco P, de Lamballerie X, Brussaard CPD (2006) *Micromonas pusila* reovirus: a new member of the family Reoviridae assigned to a novel proposed genus (Mimoreovirus). J Gen Virol 87:1375–1383

Avrani S, Schwartz DA, Lindell D (2012) Virus-host swinging party in the oceans: Incorporating biological complexity into paradigms of antagonistic coexistence. Mobile Genetic Elements 2:88–95

Baudoux A-C, Brussaard CPD (2005) Characterization of different viruses infecting *Phaeocystis globosa*. Virology 341:80–90

Baudoux A-C, Brussaard CPD (2008) Influence of irradiance on viral-algal host interactions. J Phycol 44:902–908

Baudoux AC, Noordeloos AAM, Veldhuis MJW, Brussaard CPD (2006) Virally induced mortality of *Phaeocystis globosa* during two spring blooms in temperate coastal waters. Aquat Microb Ecol 44:207–217

Baudoux A-C, Veldhuis MJW, Witte HJ, Brussaard CPD (2007) Viruses as mortality agents of picophytoplankton in the deep chlorophyll maximum layer during IRONAGES III. Limnol Oceanogr 52:2519–2529

Baudoux A-C, Hendrix RW, Lander GC, Bailly X, Podell S, Paillard C, Johnson JE, Potter CS, Carragher B, Azam F (2012) Genomic and functional analysis of vibrio phage SIO-2 reveals novel insights into ecology and evolution of marine siphoviruses. Environ Microbiol 14:2071–2086

Baudoux A-C, Lebredonchel H, Dehmer H, Latimier M, Edern R, Rigaut-Jalabert F, Latimier M, Moreau H, Simon N (2015) Interplay between the genetic clades of micromonas and their viruses in the Western English Channel. Environmental Microbiology Reports 7:765–773

Bratbak G, Egge JK, Heldal M (1993) Viral mortality of the marine alga *Emiliania huxleyi* (Haptophyceae) and termination of algal blooms. Mar Ecol Prog Ser 93:39–48

Bratbak G, Jacobsen A, Heldal M, Nagasaki K, Thingstad F (1998) Virus production in *Phaeocystis pouchetii* and its relation to host cell growth and nutrition. Aquat Microb Ecol 16:1–9

Breitbart M (2012) Marine viruses: truth or dare. Ann Rev Mar Sci 4:425–448

Breitbart M, Felts B, Kelley S, Mahaffy JM, Nulton J, Salamon P, Rohwer F (2004) Diversity and population structure of a near–shore marine–sediment viral community. Proc R Soc Lond B Biol Sci 271:565–574

Brum JR, Sullivan MB (2015) Rising to the challenge: accelerated pace of discovery transforms marine virology. Nat Rev Microbiol 13:147–159

Brum JR, Ignacio-Espinoza JC, Roux S, Doulcier G, Acinas SG, Alberti A, Sullivan MB (2015) Patterns and ecological drivers of ocean viral communities. Science 348:1261498

Brussaard CPD (2004a) Optimization of procedures for counting viruses by flow cytometry. Appl Environ Microbiol 70:1506–1513

Brussaard CPD, Martínez-Martínez J (2008) Algal bloom viruses. Plant Viruses 2(1):1–13

Brussaard CPD, Riegman R, Noordeloos AAM, Cadee GC, Witte H, Kop AJ, Nieuwland G, van Duyl FC, Bak RPM (1995) Effects of grazing, sedimentation and phytoplankton cell lysis on the structure of a coastal pelagic food web. Mar Ecol Prog Ser 123:259–271

Brussaard CPD, Gast GJ, Van Duyl FC, Riegman R (1996a) Impact of phytoplankton bloom magnitude on pelagic microbial food web. Mar Ecol Prog Ser 144:211–221

Brussaard CPD, Kempers RS, Kop AJ, Riegman R, Heldal M (1996b) Virus-like particles in a summer bloom of *Emiliania huxleyi* in the North Sea. Aquat Microb Ecol 10:105–113

Brussaard CPD, Marie D, Bratbak G (2000) Flow cytometric detection of viruses. J Virol Methods 85:175–182

Brussaard CPD, Noordeloos AAM, Sandaa R-A, Heldal M, Bratbak G (2004a) Discovery of a dsRNA virus infecting the marine photosynthetic protist *Micromonas pusilla*. Virology 319:280–291

Brussaard CPD, Short SM, Frederickson CM, Suttle CA (2004b) Isolation and phylogenetic analysis of novel viruses infecting the phytoplankter *Phaeocystis globosa* (Prymnesiophyceae). Appl Environ Microbiol 70:3700–3705

Brussaard CPD, Mari X, Van Bleijswijk JDL, Veldhuis MJW (2005) A mesocosm study of *Phaeocystis globosa* population dynamics. II. Significance for the microbial community. Harmful Algae 4:875–893

Brussaard CPD, Wilhelm SW, Thingstad F, Weinbauer MG, Bratbak G, Heldal M, Kimmance SA, Middelboe M, Nagasaki K, Paul JH, Schroeder DC, Suttle CA, Vaque D, Wommack KE (2008) Global-scale processes with a nanoscale drive: the role of marine viruses. The ISME Journal 2:575–578

Chen F, Suttle CA (1995) Amplification of DNA polymerase gene fragments from viruses infecting microalgae. Appl Environ Microbiol 61:1274–1278

Clokie MR, Shan J, Bailey S, Jia Y, Krisch HM, West S, Mann NH (2006) Transcription of a 'photosynthetic' T4-type phage during infection of a marine cyanobacterium. Environ Microbiol 8:827–835

Colson P, De Lamballerie X, Yutin N, Asgari S, Bigot Y, Bideshi DK, Cheng X-W, Federici BA, Van Etten JL, Koonin EV, La Scola B, Raoult D (2013) Megavirales, a proposed new order for eukaryotic nucleocytoplasmic large DNA viruses. Arch Virol 158:2517–2521

Culley AI, Lang AS, Suttle CA (2006) Metagenomic analysis of coastal RNA virus communities. Science 312:1795–1798

Danovaro R, Middelboe M (2010) Separation of free virus particles from sediments in aquatic systems. In: Wilhelm SW, Weinbauer MG, Suttle CA, Waco TX (eds) Manual of aquatic viral ecology. ASLO, pp 74–81

Danovaro R, Corinaldesi C, Filippini M, Fischer UR, Gessner MO, Jacquet S, Magagnini M, Velimirov B (2008) Viriobenthos in freshwater and marine sediments: a review. Freshw Biol 53:1186–1213

Derelle E, Ferraz C, Escande ML, Eychenié S, Cooke R, Piganeau G, Desdevises Y, Bellec L, Moreau H, Grimsley N (2008) Life-cycle and genome of OtV5, a large DNA virus of the pelagic marine unicellular green alga *Ostreococcus tauri*. PlosOne 3(5):e2250. doi:10.1371/journal.pone.0002250

Derelle E, Monier A, Cooke R, Worden AZ, Grimsley NH, Moreau H (2015) Diversity of Viruses Infecting the Green Microalga *Ostreococcus lucimarinus*. J Virol 89:5812–5821

Dutilh BE, Cassman N, McNair K, Sanchez SE, Silva GG, Boling L, Barr JJ, Speth DR, Seguritan V, Aziz RK, Felts B, Dinsdale EA, Mokili JL, Edwards RA (2014) A highly abundant bacteriophage discovered in the unknown sequences of human faecal metagenomes. Nature Communications 5:4498

Efrony R, Loya Y, Bacharach E, Rosenberg E (2007) Phage therapy of coral disease. Coral Reefs 26:7–13

Efrony R, Atad I, Rosenberg E (2009) Phage therapy of coral white plague disease: properties of phage BA3. Curr Microbiol 58:139–145

Ellis EL, Delbrück M (1939) The growth of bacteriophage. J Gen Physiol 22:365–384

Evans C, Archer SD, Jacquet S, Wilson WH (2003) Direct estimates of the contribution of viral lysis and microzooplankton grazing to the decline of a *Micromonas* spp. population. Aquat Microb Ecol 30:207–219

Fischer MG, Suttle CA (2011) A virophage at the origin of large DNA transposons. Science 332 (6026):231–234

Fischer MG, Allen MJ, Wilson WH, Suttle CA (2010) Giant virus with a remarkable complement of genes infects marine zooplankton. Proc Natl Acad Sci 107:19508–19513

Fischlechner M, Donath E (2007) Viruses as building blocks for materials and devices. Angew Chem Int Ed 46:3184–3193

Flores CO, Meyer JR, Valverde S, Farr L, Weitz JS (2011) Statistical structure of host-phage interactions. Proc Nat Acad Sci USA 108:E288–E297

Flores CO, Valverde S, Weitz JS (2013) Multi-scale structure and geographic drivers of cross-infection within marine bacteria and phages. ISME J 7:520–532

Forterre P (2010) Defining life: the virus viewpoint. Orig Life Evol Biosph 40:151–160

Frada M, Probert I, Allen MJ, Wilson WH, de Vargas C (2008) The "Cheshire Cat" escape strategy of the coccolithophore *Emiliania huxleyi* in response to viral infection. Proc Natl Acad Sci 105:15944–15949

Fraser JS, Maxwell KL, Davidson AR (2007) Immunoglobulin-like domains on bacteriophage: weapons of modest damage? Curr Opin Microbiol 10:382–387

Garcia-Doval C, van Raaij MJ (2013) Bacteriophage receptor recognition and nucleic acid transfer. Structure and physics of viruses. Springer, Berlin, pp 489–518

Garza DR, Suttle CA (1995) Large double-stranded DNA viruses which cause the lysis of a marine heterotrophic nanoflagellate (*Bodo* sp.) occur in natural marine viral communities. Aquat Microb Ecol 9:203–210

Geslin C, Le Romancer M, Erauso G, Gaillard M, Perrot G, Prieur D (2003) PAV1, the first virus-like particle isolated from a hyperthermophilic euryarchaeote,"*Pyrococcus abyssi*". J Bacteriol 185:3888–3894

Ghai R, Martin-Cuadrado AB, Molto AG, Heredia IG, Cabrera R, Martin J, Verdu M, Deschamps P, Moreira D, Lopez-Garcia P, Mira A, Rodriguez-Valera F (2010) Metagenome of the Mediterranean deep chlorophyll maximum studied by direct and fosmid library 454 pyrosequencing. ISME J 4:1154–1166

Ghai R, Mizuno CM, Picazo A, Camacho A, Rodriguez-Valera F (2013) Metagenomics uncovers a new group of low GC and ultra-small marine Actinobacteria. Sci Rep 3:2471

Glud RN, Middelboe M (2004) Virus and bacteria dynamics of a coastal sediment: implication for benthic carbon cycling. Limnol Oceanogr 49:2073–2081

Gobler CJ, Hutchins DA, Fisher NS, Cosper EM, Sanudo Wilhelmy SA (1997) Release and bioavailability of C, N, P, Se, and Fe following viral lysis of a marine chrysophyte. Limnol Oceanogr 42:1492–1504

Gorlas A, Koonin EV, Bienvenu N, Prieur D, Geslin C (2012) TPV1, the first virus isolated from the hyperthermophilic genus *Thermococcus*. Environ Microbiol 14:503–516

Heldal M, Bratbak G (1991) Production and decay of viruses in aquatic environments. Mar Ecol Prog Ser 72:205–2012

Hewson I, O'Niel JM, Fuhrman JA, Dennison WC (2001) Virus-like particle distribution and abundance in sediments and overlying waters along eutrophication gradients in two subtropical estuaries. Limnol Oceanogr 46:1734–1746

Hidaka T (1977) Detection and isolation of marine bacteriophage systems in the southwestern part of the Pacific Ocean. Mem Fac Fish Kagoshima Univ 26:55–62

Hidaka T, Fujimura T (1971) A morphological study of marine bacteriophages. Mem Fac Fish Kagoshima Univ 20:141–154

Holmfeldt K, Solonenko N, Shah M, Corrier K, Riemann L, Verberkmoes NC (2013) Twelve previously unknown phage genera are ubiquitous in global oceans. Proc Nat Acad Sci USA 110:12798–12803

Hurwitz BL, Hallam SJ, Sullivan MB (2013) Metabolic reprogramming by viruses in the sunlit and dark ocean. Genome Biol 14:R123

Hutchinson GE (1961) Theparadoxoftheplankton. Am Nat 95:137–145

Jacobsen A, Bratbak G, Heldal M (1996) Isolation and characterization of a virus infecting *Phaeocystis pouchetii* (Prymnesiophyceae). J Phycol 32:923–927

Jacobsen A, Larsen A, Martínez-Martínez J, Verity PG, Frischer ME (2007) Susceptibility of colonies and colonial cells of *Phaeocystis pouchetii* (Haptophyta) to viral infection. Aquat Microb Ecol 48:105–112

Jiang SC, Paul JH (1998) Significance of lysogeny in the marine environment: studies with isolates and a model of lysogenic phage production. Microb Ecol 35:235–243

Johannessen TV, Bratbak G, Larsen A, Ogata H, Egge ES, Edvardsen B, Eikrem W, Sandaa RA (2015) Characterisation of three novel giant viruses reveals huge diversity among viruses infecting Prymnesiales (Haptophyta). Virology 476:180–188

Kang I, Oh HM, Kang D, Cho JC (2013) Genome of a SAR116 bacteriophage shows the prevalence of this phage type in the oceans. Proc Natl Acad Sci 110:12343–12348

Karunasagar I, Shivu MM, Girisha SK, Krohne G, Karunasagar I (2007) Biocontrol of pathogens in shrimp hatcheries using bacteriophages. Aquaculture 268:288–292

Kimmance SA, Brussaard CPD (2010) Estimation of viral-induced phytoplankton mortality using the modified dilution method. Limnol Oceanogr Methods 7:65–73

Kimura K, Tomaru Y (2013) Isolation and characterization of a single-stranded DNA virus infecting the marine diatom *Chaetoceros* sp. strain SS628–11 isolated from western Japan. PLoS ONE 8:e82

Kimura K, Tomaru Y (2015) Discovery of two novel viruses expands the diversity of ssDNA and ssRNA viruses infecting a cosmopolitan marine diatom. Appl Environ Microbiol 81:1120–1131

La Scola B, Audic S, Robert C (2003) A giant virus in amoebae. Science 299:2033

La Scola B, Desnues C, Pagnier I, Robert C, Barrassi L, Fournous G, Merchat M, Suzan-Monti M, Forterre P, Koonin E, Raoult D (2008) The virophage as a unique parasite of the giant mimivirus. Nature 455:100–104

Labonté JM, Suttle CA (2013a) Metagenomic and whole-genome analysis reveals new lineages of gokushoviruses and biogeographic separation in the sea. Front Microbiol 4:404
Labonté JM, Suttle CA (2013b) Previously unknown and highly divergent ssDNA viruses populate the oceans. ISME J 7:2169–2177
Labonté JM, Swan BK, Poulos B, Luo H, Koren S, Hallam SJ, Sullivan MB, Woyke T, Wommack KE, Stepanauskas R (2015) Single-cell genomics-based analysis of virus-host interactions in marine surface bacterioplankton. ISME J 9:2386–2399
Laguna R, Romo J, Read BA, Wahlund TM (2001) Induction of phase variation events in the life cycle of the marine coccolithophorid *Emiliania huxleyi*. Appl Environ Microbiol 67:3824–3831
Leggett HC, Buckling A, Long GH, Boots M (2013) Generalism and the evolution of parasite virulence. Trends Ecol Evol 28(10):592–596
Lenski RE (1988) Dynamics of interactions between bacteria and virulent bacteriophage. In: Advances in microbial ecology. Springer, New York, pp 1–44
Lohr J, Munn CB, Wilson WH (2007) Characterization of a latent virus-like infection of symbiotic zooxanthellae. Appl Environ Microbiol 73:2976–2981
Lønborg C, Middelboe M, Brussaard CPD (2013) Viral lysis of *Micromonas pusilla*: impacts on dissolved organic matter production and composition. Biogeochemistry 116:231–240
Lossouarn J, Nesbø CL, Mercier C, Zhaxybayeva O, Johnson MS, Charchuck R, Farasin J, Bienvenu N, Baudoux A-C, Michoud G, Jebbar M, Geslin C (2015) 'Ménage à trois': a selfish genetic element uses a virus to propagate within Thermotogales. Environ Microbiol 17:3278–3288
Løvdal T, Eichner C, Grossart H-P, Carbonnel V, Chou L, Martin-Jezequel V, Thingstad TF (2008) Competition for inorganic and organic forms of nitrogen and phosphorous between phytoplankton and bacteria during an *Emiliania huxleyi* spring bloom. Biogeosciences 5:371–383
Maat DS, Crawfurd KJ, Timmermans KR, Brussaard CPD (2014) Elevated CO_2 and phosphate limitation favor *Micromonas pusilla* through stimulated growth and reduced viral impact. Appl Environ Microbiol 80:3119–3127
Mackinder LC, Worthy CA, Biggi G, Hall M, Ryan KP, Varsani A, Harper GM, Wilson WH, Brownlee C, Schroeder DC (2009) A unicellular algal virus, *Emiliania huxleyi* virus 86, exploits an animal-like infection strategy. J Gen Virol 90:2306–2316
Mann NH, Cook A, Millard A, Bailey S, Clokie M (2003) Marine ecosystems: bacterial photosynthesis genes in a virus. Nature 424:741
Mari X, Rassoulzadegan F, Brussaard CPD, Wassmann P (2005) Dynamics of transparent exopolymeric particles (TEP) production by *Phaeocystis globosa* under N- or P-limitation: a controlling factor of the retention/export balance? Harmful Algae 4:895–914
Marston MF, Pierciey FJ Jr, Shepard A, Gearin G, Qi J, Yandava C (2012) Rapid diversification of coevolving marine Synechococcus and a virus. Proc Nat Acad Sci USA 109:4544–4549
Martínez-Díaz SF, Hipólito-Morales A (2013) Efficacy of phage therapy to prevent mortality during the vibriosis of brine shrimp. Aquaculture 400–401:120–124
Martínez-Martínez J, Schroeder DC, Larsen A, Bratbak G, Wilson WH (2007) Molecular dynamics of *Emiliania huxleyi* and cooccurring viruses during two separate mesocosm studies. Appl Environ Microbiol 73:554–562
Martínez-Martínez J, Swan BK, Wilson WH (2014) Marine viruses, a genetic reservoir revealed by targeted viromics. ISME J 8:1079–1088
Martínez-Martínez J, Boere A, Gilg I, van Lent JW, Witte HJ, van Bleijswijk JDL, Brussaard CPD (2015) New lipid envelope-containing dsDNA virus isolates infecting *Micromonas pusilla* reveal a separate phylogenetic group. Aquat Microb Ecol 74:17–28
Matsuzaki S, Tanaka S, Koga T, Kawata T (1992) A broad-host-range vibriophage, KVP40, isolated from sea water. Microbiol Immunol 36:96–97
McDaniel L, Houchin LA, Williamson SJ, Paul JH (2002) Lysogeny in marine *Synechococcus*. Nature 451:496

Mizuno CM, Rodriguez-Valera F, Kimes NE, Ghai R (2013) Expanding the marine virosphere using metagenomics. PLoS Genet 9:e1003987

Moebus K, Nattkemper H (1981) Bacteriophage sensitivity patterns among bacteria isolated from marine waters. Helgoländer Meeresuntersuchungen 34:375–385

Moebus K, Nattkemper H (1983) Taxonomic investigations of bacteriophage sensitive bacteriaisolated from marine waters. Helgolhnder Meeresunters 36:357–373

Mojica KDA, Brussaard CPD (2014) Factors affecting virus dynamics and microbial host–virus interactions in marine environments. FEMS Microbiol Ecol 89:495–515

Mojica KDA, Huisman J, Wilhelm SW, Brussaard CPD (2015) Latitudinal variation in virus-induced mortality of phytoplankton across the North Atlantic Ocean. ISME J. doi:10.1038/ismej.2015.130

Moniruzzaman M, LeCleir GR, Brown CM, Gobler CJ, Bidle KD, Wilson WH, Wilhelm SW (2014) Genome of brown tide virus (AaV), the little giant of the Megaviridae, elucidates NCLDV genome expansion and host–virus coevolution. Virology 466:60–70

Morin PJ (2008) Sex as an algal antiviral strategy. Proc Natl Acad Sci 105:15639–15640

Mühling M, Fuller NJ, Millard A, Somerfield PJ, Marie D, Wilson WH, Scanlan DJ, Post AF, Joint I, Mann NH (2005) Genetic diversity of marine Synechococcus and co-occurring cyanophage communities: evidence for viral control of phytoplankton. Environ Microbiol 7:499–508

Murray AG, Smith RJ, Stagg RM (2002) Shipping and the spread of infectious Salmon Anemia in Scottish aquaculture. Emerg Infect Dis 8:1–5

Nagasaki K, Bratbak G (2010) Isolation of viruses infecting photosynthetic and nonphotosynthetic protists. In: Wilhelm SW, Weinbauer MG, Suttle CA, Waco TX (eds) Manual of aquatic viral ecology. ASLO, pp 92–101

Nagasaki K, Tarutani K, Yamaguchi M (1999) Cluster analysis on algicidal activity of HaV clones and virus sensitivity of *Heterosigma akashiwo* (Raphidophyceae). J Plankton Res 21:2219–2226

Nagasaki K, Tomaru Y, Katanozaka N, Shirai Y, Nishida K, Itakura S, Yamaguchi M (2004) Isolation and characterization of a novel single-stranded RNA virus infecting the bloom-forming diatom *Rhizosolenia setigera*. Appl Environ Microbiol 70:704–711

Nagasaki K, Tomaru Y, Takao Y, Nishida K, Shirai Y, Suzuki H, Nagumo T (2005) Previously unknown virus infects marine diatom. Appl Environ Microbiol 71:3528–3535

Nakai T, Park SC (2002) Bacteriophage therapy of infectious diseases in aquaculture. Res Microbiol 153:13–18

Noble RT, Fuhrman JA (1998) Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria. Aquat Microb Ecol 14:113–118

Noble RT, Fuhrman JA (2000) Rapid virus production and removal as measured with fluorescently labeled viruses as tracers. Appl Environ Microbiol 66:3790–3797

Oliveira J, Castilho F, Cunha A, Pereira MJ (2012) Bacteriophage therapy as a bacterial control strategy in aquaculture. Aquacult Int 20:879–910

Paul JH (2008) Prophages in marine bacteria: dangerous molecular time bombs or the key to survival in the seas? ISME J 2:579–589

Paul JH, Weinbauer MG (2010) Detection of lysogeny in marine environments. In: Wilhelm SW, Weinbauer MG, Suttle CA, Waco TX (eds) Manual of aquatic viral ecology. ASLO, pp 30–33

Paul JH, Cochran PK, Jiang SC (1999) Lysogeny and transduction in the marine environment. In: Bell CR, Brylinsky M, Johnson-Green P (eds) Microbial biosystems: new frontiers. Proceedings of the 8th international symposium on microbial ecology. Atlantic Canada Society for Microbial Ecology, Halifax, Canada

Philippe N, Legendre M, Doutre G, Couté Y, Poirot O, Lescot M, Arslan D, Seltzer V, Bertaux L, Bruley C, Garin J, Claverie J-M, Abergel C (2013) Pandoraviruses: amoeba viruses with genomes up to 2.5 Mb reaching that of parasitic eukaryotes. Science 341:281–286

Poorvin L, Rinta-Kanto JM, Hutchins DA, Wilhelm SW (2004) Viral release of Fe and its bioavailability to marine plankton. Limnol Oceanogr 49:1734–1741

Pope WH, Bowman CA, Russell DA, Jacobs-Sera D, Asai DJ, Cresawn SG, Jacobs WR, Hendrix RW, Lawrence JG, Hatfull GF (2015) Science education alliance phage hunters advancing, S. Evolutionary, R. Phage Hunters Integrating, Education and C. Mycobacterial Genetics. Whole genome comparison of a large collection of mycobacteriophages reveals a continuum of phage genetic diversity. Elife 4:e06416

Proctor LM, Fuhrman JA (1990) Viral mortality of marine bacteria and cyanobacteria. Nature 343:60–62

Raoult D, Audic S, Robert C, Abergel C, Renesto P, Ogata H, La Scola B, Suzan M, Claverie JM (2004) The 1.2-megabase genome sequence of Mimivirus. Science 306:1344–1350

Rodriguez-Brito B, Li L, Wegley L, Furlan M, Angly F, Breitbart M, Buchanan J, Desnues C, Dinsdale E, Edwards R, Felts B, Haynes M, Liu H, Lipson D, Mahaffy J, Martin-Cuadrado AB, Mira A, Nulton J, Pasic L, Rayhawk S, Rodriguez-Mueller J, Rodriguez-Valera F, Salamon P, Srinagesh S, Thingstad TF, Tran T, Thurber RV, Willner D, Youle M, Rohwer F (2010) Viral and microbial community dynamics in four aquatic environments. ISME J 4:739–751

Rodriguez-Valera F, Ussery DW (2012) Is the pan-genome also a pan-selectome? F1000Res 1:16

Rodriguez-Valera F, Martin-Cuadrado AB, Rodriguez-Brito B, Pasic L, Thingstad TF, Rohwer F, Mira R (2009) Explaining microbial population genomics through phage predation. Nat Rev Microbiol 7:828–836

Rodriguez-Valera F, Mizuno CM, Ghai R (2014) Tales from a thousand and one phages. Bacteriophage 4:e28265

Rohwer F, Segall A, Steward G, Seguritan V, Breitbart M, Wolven F, Farooq Azam F (2000) The complete genomic sequence of the marine phage Roseophage SIO1 shares homology with nonmarine phages. Limnol Oceanogr 45:408–418

Rosenwasser S, Mausz MA, Schatz D, Sheyn U, Malitsky S, Aharoni A, Weinstock E, Tzfadia O, Ben-Dor S, Feldmesser E, Pohnert G, Vardi A (2014) Rewiring host lipid metabolism by large viruses determines the fate of *Emiliania huxleyi*, a bloom-forming alga in the Ocean. Plant Cell 26:2689–2707

Ruardij P, Veldhuis MJW, Brussaard CPD (2005) Modelling the development and termination of a *Phaeocystis* bloom. Harmful Algae 4:941–963

Sánchez-Paz A, Muhlia-Almazan A, Saborowski R, García-Carreño F, Sablok G, Mendoza-Cano F (2014) Marine viruses: the beneficial side of a threat. Appl Biochem Biotechnol 174:2368–2379

Sandaa R-A, Larsen A (2006) Seasonal variations in virus-host populations in Norwegian coastal waters: focusing on the cyanophage community infecting marine *Synechococcus* spp. Appl Environ Microbiol 72:4610–4618

Sandaa R-A, Heldal M, Castberg T, Thyrhaug R, Bratbak G (2001) Isolation and characterization of two viruses with large genome size infecting *Chrysochromulina ericina* (Prymneriophyceae) and *Pyramimonas orientalis* (Prasinophyceae). Virology 290:272–280

Sanmukh SG, Khairnar K, Chandekar RH, Paunikar WN (2014) Increasing the extraction efficiency of algal lipid for biodiesel production: novel application of algal viruses. Afr J Biotechnol 13:1666–1670

Santini S, Jeudy S, Bartoli J, Poirot O, Lescot M, Abergel C, Berbe V, Wommack KE, Noordeloos AAM, Brussaard CPD, Claverie J-M (2013) Genome of *Phaeocystis globosa* virus PgV-16T highlights the common ancestry of the largest known DNA viruses infecting eukaryotes. Proc Natl Acad Sci 110:10800–10805

Schoemann V, Becquevort S, Stefels J, Rousseau V, Lancelot C (2005) Phaeocystis blooms in the global ocean and their controlling mechanisms: a review. J Sea Res 57:43–66

Schroeder DC, Oke J, Malin G, Wilson WH (2002) Coccolithovirus (Phycodnaviridae): characterisation of a new large dsDNA algal virus that infects *Emiliana huxleyi*. Arch Virol 147:1685–1698

Shirai Y, Tomaru Y, Takao Y, Suzuki H, Nagumo T, Nagasaki K (2008) Isolation and characterization of a singlestranded RNA virus infecting the marine planktonic diatom *Chaetoceros tenuissimus* Meunier. Appl Environ Microbiol 74:4022–4027

Short SM (2012) The ecology of viruses that infect eukaryotic algae. Environ Microbiol 14:2253–2271
Short CM, Suttle CA (2005) Nearly identical bacteriophage structural gene sequences are widely distributed in both marine and freshwater environments. Appl Environ Microbiol 71:480–486
Sintes E, Del Giorgio PA (2014) Feedbacks between protistan single-cell activity and bacterial physiological structure reinforce the predator/prey link in microbial foodwebs. Front Microbiol 5:453
Spencer R (1955) A marine bacteriophage. Nature 175:690–691
Spencer R (1960) Indigenous marine bacteriophages. J Bacteriol 79:614
Stern A, Sorek R (2011) The phage-host arms race: shaping the evolution of microbes. BioEssays 33:43–51
Steward GF, Wikner J, Cochlan WP, Smith DC, Azam F (1992) Estimation of virus production in the sea. II: field results. Mar Microb Food Webs 6:79–90
Stopar D, Černe A, Žigman M, Poljšak-Prijatelj M, Turk V (2004) Viral abundance and a high proportion of lysogens suggest that viruses are important members of the microbial community in the Gulf of Trieste. Microb Ecol 47:1–8
Sullivan MB, Waterbury JB, Chisholm SW (2003) Cyanophages infecting the oceanic cyanobacterium *Prochlorococcus*. Nature 424:1047–1051
Sullivan MB, Coleman ML, Weigele P, Rohwer F, Chisholm SW (2005) Three *Prochlorococcus* cyanophage genomes: signature features and ecological interpretations. PLoS Biol 3:e144
Sullivan MB, Krastins B, Hughes JL, Kelly L, Chase M, Sarracino D, Chisholm SW (2009) The genome and structural proteome of an ocean siphovirus: a new window into the cyanobacterial 'mobilome'. Environ Microbiol 11:2935–2951
Suttle CA (2005) Viruses in the sea. Nature 437:356–361
Suttle CA (2007) Marine viruses–major players in the global ecosystem. Nat Rev Microbiol 5:801–812
Suttle CA, Chan AM (1994) Dynamics and distribution of cyanophages and their effect on marine *Synechococcus* spp. Appl Environ Microbiol 60:3167–3174
Suttle CA, Chen F (1992) Mechanisms and rates of decay of marine viruses in seawater. Appl Environ Microbiol 58:3721–3729
Suttle CA, Chan AM, Cottrell MT (1995) Viruses infecting the marine Prymnesiophyte *Chrysochromulina* spp.: isolation, preliminary characterization and natural abundance. Mar Ecol Prog Ser 118:275–282
Taruntani K, Nagasaki K, Yamaguchi M (2000) Viral impacts on total abundance and clonal composition of the harmful bloom-forming phytoplankton *Heterosigma akashiwo*. Appl Environ Microbiol 66:4916–4920
Thingstad TF (2000) Elements of a theory for the mechanisms controlling abundance, diversity, and biogeochemical role of lytic bacterial viruses in aquatic systems. Limnol Oceanogr 45:1320–1328
Thingstad TF, Pree B, Giske J, Våge S (2015) What difference does it make if viruses are strain-, rather than species-specific? Front Microbiol 6:320
Thomas R, Grimsley N, Escande ML, Subirana L, Derelle E, Moreau H (2011) Acquisition and maintenance of resistance to viruses in eukaryotic phytoplankton populations. Environ Microbiol 13:1412–1420
Tomaru Y, Shirai Y, Suzuki H, Nagumo T, Nagasaki K (2008) Isolation and characterization of a new single-stranded DNA virus infecting the cosmopolitan marine diatom *Chaetoceros debilis*. Aquat Microb Ecol 50:103–112
Tomaru Y, Takao Y, Suzuki H, Nagumo T, Nagasaki K (2009) Isolation and characterization of a single-stranded RNA virus infecting the bloom-forming diatom *Chaetoceros socialis*. Appl Environ Microbiol 75:2375–2381
Tomaru Y, Fujii N, Oda S, Toyoda K, Nagasaki K (2011a) Dynamics of diatom viruses on the western coast of Japan. Aquat Microb Ecol 63:223–230

Tomaru Y, Shirai Y, Toyoda K, Nagasaki K (2011b) Isolation and characterisation of a single-stranded DNA Virus infecting the marine planktonic diatom *Chaetoceros tenuissimus* Meunier. Aquat Microb Ecol 64:175–184

Tomaru Y, Takao Y, Suzuki H, Nagumo T, Koike K, Nagasaki K (2011c) Isolation and characterization of a single-stranded DNA virus infecting *Chaetoceros lorenzianus* Grunow. Appl Environ Microbiol 77:5285–5293

Tomaru Y, Toyoda K, Kimura K, Hata N, Yoshida M, Nagasaki K (2012) First evidence for the existence of pennate diatom viruses. ISME J 6:1445–1448

Tomaru Y, Toyoda K, Kimura K, Takao Y, Sakurada K, Nakayama N, Nagasaki K (2013a) Isolation and characterization of a single-stranded RNA virus that infects the marine planktonic diatom *Chaetoceros* sp. (SS08-C03). Phycol Res 61:27–36

Tomaru Y, Toyoda K, Suzuki H, Nagumo T, Kimura K, Takao Y (2013b) New single-stranded DNA virus with a unique genomic structure that infects marine diatom *Chaetoceros setoensis*. Scientific Reports 3:3337

Tomaru Y, Toyoda K, Kimura K (2015) Marine diatom viruses and their hosts: Resistance mechanisms and population dynamics. Perspect Phycol 2:69–81

Våge S, Storesund JE, Thingstad TF (2013) SAR11 viruses and defensive host strains. Nature 499: E3–E4

Waterbury JB, Valois FW (1993) Resistance to co-occurring phages enables marine Synechococcus communities to coexist with cyanophages abundant in seawater. Appl Environ Microbiol 59:3393–3399

Weinbauer MG, Suttle CA (1996) Potential significance of lysogeny to bacteriophage production and bacterial mortality in coastal waters of the Gulf of Mexico. Appl Environ Microbiol 62:4374–4380

Weinbauer MG, Brettar I, Hofle MG (2003) Lysogeny and virus-induced mortality of bacterioplankton in surface, deep, and anoxic marine waters. Limnol Oceanogr 48:1457–1465

Weinbauer MG, Rowe JM, Wilhelm S (2010) Determining rates of virus production in aquatic systems by the virus reduction approach. In: Wilhelm SW, Weinbauer MG, Suttle CA, Waco TX (eds) Manual of aquatic viral ecology. ASLO, pp 1–8

Weitz JS, Poisot T, Meyer JR, Flores CO, Valverde S, Sullivan MB, Hochberg ME (2013) Phage–bacteria infection networks. Trends Microbiol 21:82–91

Wilhelm SW, Carberry MJ, Eldridge ML, Poorvin L, Saxton MA, Doblin MA (2006) Marine and freshwater cyanophages in a Laurentian Great Lake: evidence from infectivity assays and molecular analyses of g20 genes. Appl Environ Microbiol 72:4957–4963

Wilson WH (2005) The versatility of giant algal viruses: from shunting carbon to antiwrinkle cream. Ocean Challenge 14:8–9

Wilson WH, Carr NG, Mann NH (1996) The effect of phosphate status on the kinetics of cyanophage infection in the oceanic cyanobacterium *Synechococcus* sp. WH7803. J Phycol 32:506–516

Wilson WH, Schroeder DC, Allen MJ, Holden MT, Parkhill J, Barrell BG, Churcher C, Hamlin N, Mungall K, Norbertczak H, Quail MA, Price C, Rabbinowitsch E, Walker D, Craigon M, Roy D, Ghazal P (2005) Complete genome sequence and lytic phase transcription profile of a coccolithovirus. Science 309:1090–1092

Zeigler Allen L, Ishoey T, Novotny MA, McLean JS, Lasken RS, Williamson SJ (2011) Single virus genomics: a new tool for virus discovery. PLoS ONE 6:e17722

Zhao Y, Temperton B, Thrash JC, Schwalbach MS, Vergin KL, Landry ZC, Ellisman M, Deerinck T, Sullivan MB, Giovannoni SJ (2013) Abundant SAR11 viruses in the ocean. Nature 494:357–360

Zingone A, Natale F, Biffali E, Borra M, Forlani G, Sarno D (2006) Diversity in morphology, infectivity, molecular characteristics and induced host resistance between two viruses infecting *Micromonas pusilla*. Aquat Microb Ecol 45:1–14