

# Phage puppet masters of the marine microbial realm

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**Viruses numerically dominate our oceans; however, we have only just begun to document the diversity, host range and infection dynamics of marine viruses, as well as the subsequent effects of infection on both host cell metabolism and oceanic biogeochemistry. Bacteriophages (that is, phages: viruses that infect bacteria) are highly abundant and are known to play critical roles in bacterial mortality, biogeochemical cycling and horizontal gene transfer. This Review Article summarizes current knowledge of marine viral ecology and highlights the importance of phage particles to the dissolved organic matter pool, as well as the complex interactions between phages and their bacterial hosts. We emphasize the newly recognized roles of phages as puppet masters of their bacterial hosts, where phages are capable of altering the metabolism of infected bacteria through the expression of auxiliary metabolic genes and the redirection of host gene expression patterns. Finally, we propose the 'royal family model' as a hypothesis to describe successional patterns of bacteria and phages over time in marine systems, where despite high richness and significant seasonal differences, only a small number of phages appear to continually dominate a given marine ecosystem. Although further testing is required, this model provides a framework for assessing the specificity and ecological consequences of phage–host dynamics.**

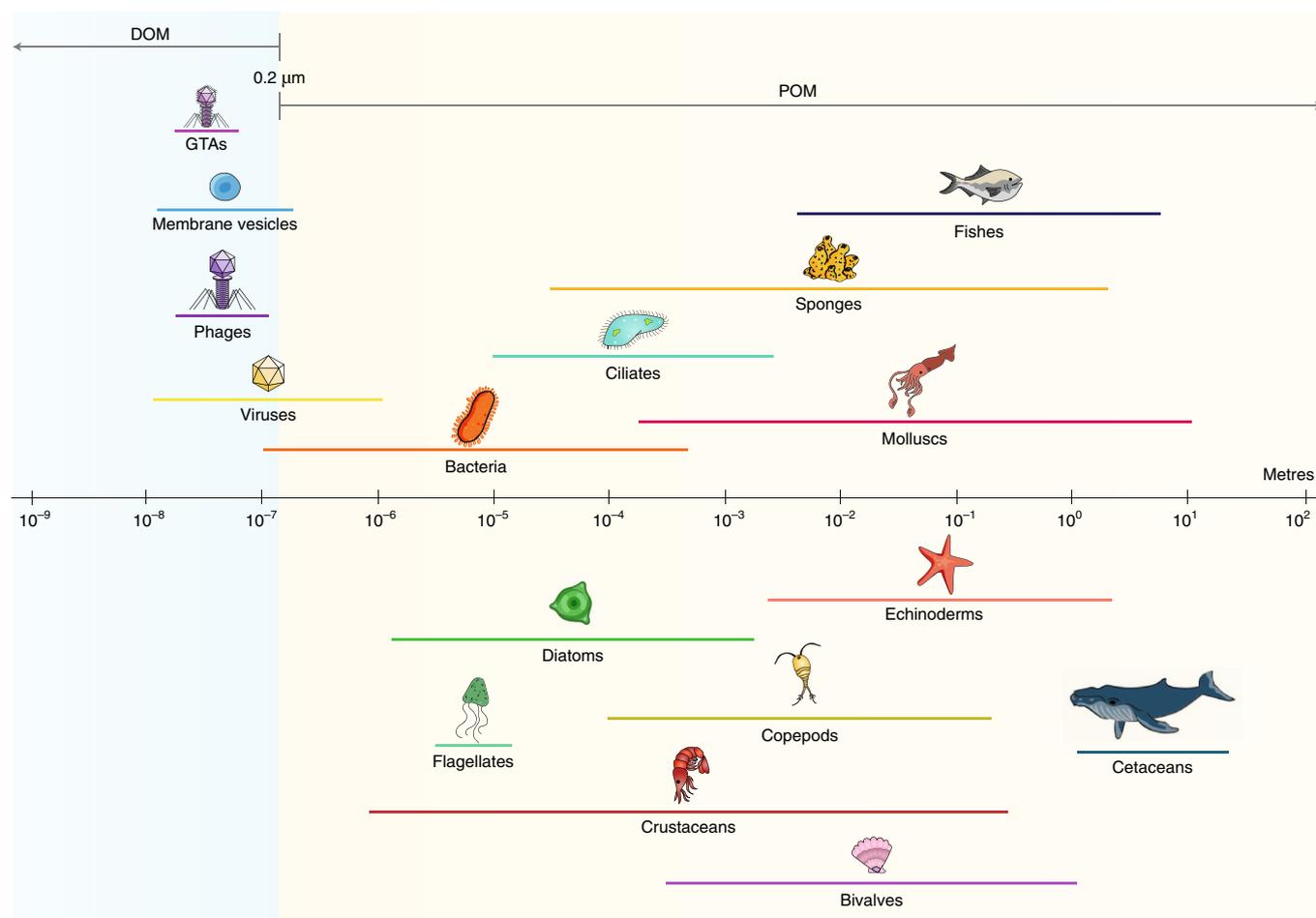
Viruses are the most abundant biological entities in the oceans, with average concentrations of 10 million per millilitre of surface seawater<sup>1</sup>. Viruses outnumber bacteria by approximately ten-fold, and are much more abundant than phytoplankton, zooplankton or organisms at higher trophic levels. To put this into perspective, the number of viruses in just one litre of surface seawater ( $\sim 10^{10}$ ) exceeds the current number of humans on the planet ( $7.6 \times 10^9$ ). Viruses represent the smallest known biological entities in the oceans, an ecosystem containing organisms with lengths spanning nine orders of magnitude (Fig. 1). While viruses represent 94% of the nucleic-acid-containing particles in the oceans, they only comprise a minuscule proportion of the total biomass due to their remarkably small sizes<sup>2</sup>. Although the size of marine viruses varies widely (Fig. 1), the average diameter of marine viruses is  $\sim 54$  nm (ref. <sup>3</sup>). To provide context, it would take  $\sim 55$  million viruses of this size to cover the period at the end of this sentence. Nonetheless, the oceans contain 100 billion times as many viruses as there are sand grains on Earth ( $\sim 10^{19}$ ), and if all  $\sim 10^{30}$  of these marine viruses were lined up side-by-side, they would extend for  $5.4 \times 10^{22}$  m, which is the equivalent of 12 trillion roundtrips from Earth to Mars<sup>4</sup>.

Viruses are presumed to infect all marine organisms, from small phytoplankton that play essential roles in global carbon cycling to more commercially valuable organisms such as invertebrates, fish and megafauna<sup>5</sup>. However, since bacteria are by far the most abundant cellular organisms, most of the viruses are likely to be bacteriophages (that is, phages: viruses that infect bacteria). The presence of phages in the oceans was noted over 60 years ago<sup>6</sup>, but their high abundance has been recognized for less than 30 years<sup>7,8</sup>. Marine viruses were originally quantified using transmission electron microscopy but this method has largely been replaced by techniques utilizing nucleic acid staining, such as epifluorescence microscopy and flow cytometry, to count 'virus-like particles' (VLPs)<sup>9</sup> (Fig. 2, Table 1). It is important to note that VLP counts visualize the concentrated nucleic acid stain, not the particles themselves, since the small size of viruses falls below the detection limit of light micro-

copy. As such, there are many inaccuracies possible with these abundance estimates, including inflated numbers resulting from counting small bacteria, gene transfer agents (GTAs) or membrane-bound extracellular vesicles that are in the same size range as viruses (Fig. 1). Potential underestimates can also result from the exclusion of large viruses and the inability to see small RNA or single-stranded DNA viruses<sup>10–12</sup>.

There are typically approximately 10 VLPs per bacterial cell in surface seawater<sup>1,13</sup>; however, recent meta-analyses have demonstrated that the virus-to-bacteria ratio (VBR) often ranges between 1 and 100 (refs <sup>14–16</sup>). Although the mechanisms and ecological implications are not understood, it has been shown that the VBR is not linear across a wide range of host concentrations, and in particular that VLPs are relatively less abundant (VBR <10) at higher host cell densities<sup>14,16</sup>. Being a bulk measurement that does not consider diversity, host range or spatial heterogeneity, the VBR is limited in its ecological meaning. Despite being more challenging to measure, an understanding of lineage-specific interactions is essential for predicting the maximum contact rate between viruses and susceptible hosts<sup>17</sup>. The general assumption that there are approximately ten VLPs for each bacterial cell is also complicated by the fact that these counts are usually made on sample volumes of several millilitres, which is a tremendous amount of space for these tiny entities. It is important to remember that microbial diversity in the water column is heterogeneous, resulting in patches of activity<sup>18</sup>. Taking a parcel of seawater and filtering it down to a homogenous, two-dimensional sample probably misrepresents interactions occurring in local microenvironments (Fig. 2).

The few studies that have examined viral and bacterial abundance on extremely small spatial scales support this hypothesis. These studies have revealed a large degree of heterogeneity in viral and bacterial abundance, which results in a wide VBR range that starkly contrasts the relatively stable VBR observed in bulk measurements. For example, at millimetre-level scales, no correlation is seen between bacterial and viral abundance, and the different



**Fig. 1 | The size of biology in the oceans spans nine orders of magnitude from the smallest viruses (~17 nm) to the largest charismatic megafauna (blue whale, ~33 m). The operational definition between DOM (<0.2  $\mu\text{m}$ ) and POM (>0.2  $\mu\text{m}$ ) is also shown to provide context. The overlap in sizes between the smallest bacteria (*Pelagibacter ubique*, 120 nm diameter) and the largest phages (*Bacillus megaterium* phage G, 160 nm capsid diameter) and viruses (*Pithovirus*, 1.5  $\mu\text{m}$  capsid diameter) complicates filtration-based strategies to separate these microbial groups. In addition, the size range of viruses completely encompasses that of GTAs and membrane-bound extracellular vesicles, making it impossible to distinguish these entities by size alone.**

'hotspots' in abundance of these groups result in a highly variable VBR at small spatial scales<sup>19–22</sup>. Combined with documented small-scale heterogeneity in the diversity of bacterial hosts<sup>23</sup>, the spatial heterogeneity of viral and bacterial abundance suggests that ecologically relevant contact rates and interactions (that is, those between viruses and susceptible hosts) are patchy and occur in dynamic microniches.

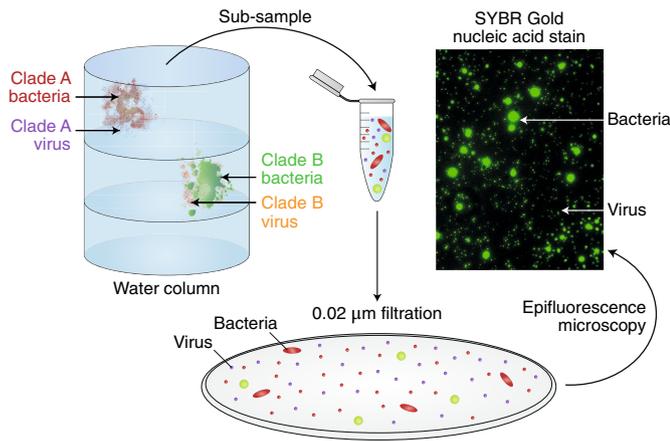
### Lytic versus lysogenic modes of phage infection

The role of phages in oceanic biogeochemistry is most frequently mediated through lytic infection, in which the injected DNA of lytic phages directs the host cell to replicate phage DNA, leading to cell lysis and the release of progeny phage particles. An average burst size, the number of phage particles produced through lysis of a bacterial host, is 24 for marine environments, but this value is highly variable and generally increases in eutrophic conditions and with higher percentages of infected cells<sup>15,24</sup>. However, a lytic infection is only one lifestyle that marine phages can exhibit. In contrast to lytic infection, temperate phages lysogenize their hosts, either integrating into the bacterial chromosome or in some cases being maintained as an extrachromosomal element, where they replicate with the bacterial cells as prophages until an environmental or cellular trigger causes them to enter the lytic cycle (Fig. 3). Finally, although

virtually nothing is known about the role of chronic phage infections in the oceans, some phages can replicate and produce progeny phage particles without death of the host bacterial cell<sup>25</sup>.

The factors influencing the lysis–lysogeny decision have been studied in detail for some model phage–host systems<sup>26</sup>; however, the mechanisms of this phenomenon are largely unexplored for most marine phages<sup>25</sup>. The growth rates and stress levels of bacterial cells are thought to be major drivers of the lytic–lysogeny decision in marine phages. Lysogeny is suggested to protect phages from decay during times of low host abundance and growth rates. Prophages potentially enhance the survival of their bacterial hosts in unfavourable conditions through the suppression of non-essential host metabolic activities<sup>27</sup>. Although not yet observed in marine systems, two recent papers have demonstrated novel mechanisms for the coordination of phage lifestyles. Trinh et al.<sup>28</sup> used fluorescence reporting to capture individual lambda phage 'voting' to lysogenize or lyse host cells, while Erez et al.<sup>29</sup> demonstrated a quorum-sensing-like mechanism where the concentration of a phage-specific short peptide communication signal determined the lytic–lysogeny decision for new phage progeny. Future studies should determine if marine phage–host systems utilize similar techniques.

One technique commonly used to determine the prevalence of lysogeny in the marine environment is the induction of prophages



**Fig. 2 | Spatial heterogeneity in the abundance of bacteria and VLPs may exist on extremely small scales in the oceans.** The procedure of removing a sample of seawater from the water column, homogenizing it by mixing and then filtering onto a 0.02 µm filter results in a more ubiquitous two-dimensional picture of marine bacteria and viruses once their nucleic acids are stained and visualized under an epifluorescence microscope. In this scenario, two different clades of bacteria and viruses that were patchy in situ would appear on a slide as a homogenous sample, with counts yielding an average VBR ratio of ~10 for surface seawater. Although more difficult to study, the highly variable VBR observed on small spatial scales is probably more representative of ecologically relevant contact rates and patchy virus–host interactions that occur in dynamic microniches.

using mutagens such as mitomycin C (ref. <sup>30</sup>). This technique introduces DNA damage through cross-linking, activating bacterial stress responses and triggering induction of integrated prophages to undergo the lytic cycle. Although mitomycin C is the most common inducing agent used in laboratory studies, this method is limited because mitomycin C does not naturally occur in marine systems and this chemical may underestimate induction rates compared to natural factors such as ultraviolet irradiation or anthropogenic pollutants such as pesticides and oil<sup>31</sup>. Individual mitomycin C induction experiments in marine ecosystems have suggested that lytic infection predominates during favourable conditions (that is, those of rapid cell growth), whereas lysogeny is more common during periods of lower growth and cell abundance<sup>27,32</sup>. However, a recent meta-analysis of the fraction of cells inducible by mitomycin C across numerous environments and studies did not reveal any linkages between host density and the prevalence of lysogeny<sup>33</sup>, indicating that this hypothesis should be reassessed. Other studies have examined the prevalence of lysogeny through sequence-based analyses, relying on the ability to identify genes characteristic of temperate phages, for example integrases and excisionases<sup>34</sup>. Results from this technique are difficult to interpret because there are two possible explanations for the high abundance of temperate phage hallmark genes recovered in viral metagenomes. Intuitively, a higher abundance of temperate genes indicates a higher prevalence of lysogeny, and the two are often linked in the literature<sup>16</sup>. In contrast, because only intact viral particles are sequenced in viral metagenomics, more genes characteristic of temperate phages might actually indicate the opposite: these genes are abundant in phage particles because the prophages have been induced and are undergoing lytic infection<sup>35</sup>. Under this hypothesis, prophages remain incorporated in the host DNA in environments where lysogeny dominates, resulting in a low abundance of temperate phage hallmark genes in the phage particles sequenced using viral metagenomics<sup>35</sup>. A more effective method may be the comparison of the sequence composition of free phage particles (that is, ambient viral metagenomes) to that of induced viral metagenomes. Induced viral metagenomes are pre-

pared by first reducing the concentration of ambient virus particles, then using mitomycin C to induce prophages, followed by purification of the induced phage particles. This technique has been used to determine the prevalence of lysogeny in various marine environments, with decreased overlap between ambient and induced viral communities indicative of more lysogeny<sup>36,37</sup>.

### Viral shunt versus viral shuttle

With an estimated abundance of  $10^{29}$  cells in the world's oceans, bacteria play critical roles at the base of the marine food web<sup>38–40</sup>. While individual bacteria are not visible with the naked eye, the biomass of bacteria in the oceans is greater than the mass of zooplankton and fish combined. Furthermore, photosynthetic bacteria generate approximately half of the oxygen in the atmosphere<sup>38</sup>. There are two main mechanisms of bacterial mortality, grazing by protists or lysis by phages, which are each thought to be responsible for approximately half of bacterial death. Grazing moves carbon up through the trophic levels when bacteria are consumed by protozoa, which are then consumed by zooplankton, fish and larger organisms (Fig. 4). This food chain contributes particulate organic matter (POM), which sinks into the deep ocean and sequesters carbon through a process known as the biological pump<sup>41</sup>. By contrast, in the pathway known as the viral shunt<sup>42</sup>, bacteria are lysed by phages, releasing dissolved carbon and nutrients. This dissolved organic matter (DOM) is thought to be remineralized by bacteria within the microbial loop, essentially functioning as the ocean's recycling system and detracting from the biological pump<sup>43</sup>. Conversely, there is evidence that viruses can contribute to the biological pump through creating sticky lysates that aggregate and sink, effectively shuttling organic carbon from the surface to deep ocean and enhancing the efficiency of the biological pump<sup>17,44,45</sup>. A recent global survey found specific phages strongly associated with carbon export in the oceans<sup>46</sup>, suggesting that the role of viruses in marine food webs merits further evaluation to determine the circumstances under which viruses act as a shunt (keeping carbon in the surface waters) versus a shuttle (contributing to the biological pump through aggregation, mixing or enhanced grazing)<sup>45</sup>.

Although the exact contribution of phages to bacterial mortality is dependent on local environmental conditions and is likely to vary between geographic regions, all available studies suggest that phages exert a significant top-down control on bacterial numbers in the oceans. Approximately 25% of primary production in the surface ocean is estimated to flow through the viral shunt, leading to rapid recycling of DOM by increasing community respiration and decreasing the transfer of carbon to higher trophic levels<sup>42,43,47</sup>. Between 20–30% of marine bacterial cells are thought to be infected by phages at any given time, with the viral shunt releasing up to 3 gigatons of carbon into the oceans per year<sup>2</sup>. An estimated  $10^{28}$  viral infections occur per day in the oceans<sup>2</sup>. To put this into perspective, in every blink of an eye (0.3 seconds), the number of viral infections in the ocean ( $3 \times 10^{22}$ ) equals the number of stars in the universe. In the time it took you to read that sentence,  $\sim 10^{24}$  viral infections occurred in the oceans. These numbers emphasize the importance of phage lysis as a major force in the rapid recycling of carbon and nutrients within the oceans.

### Viral contribution to the DOM pool

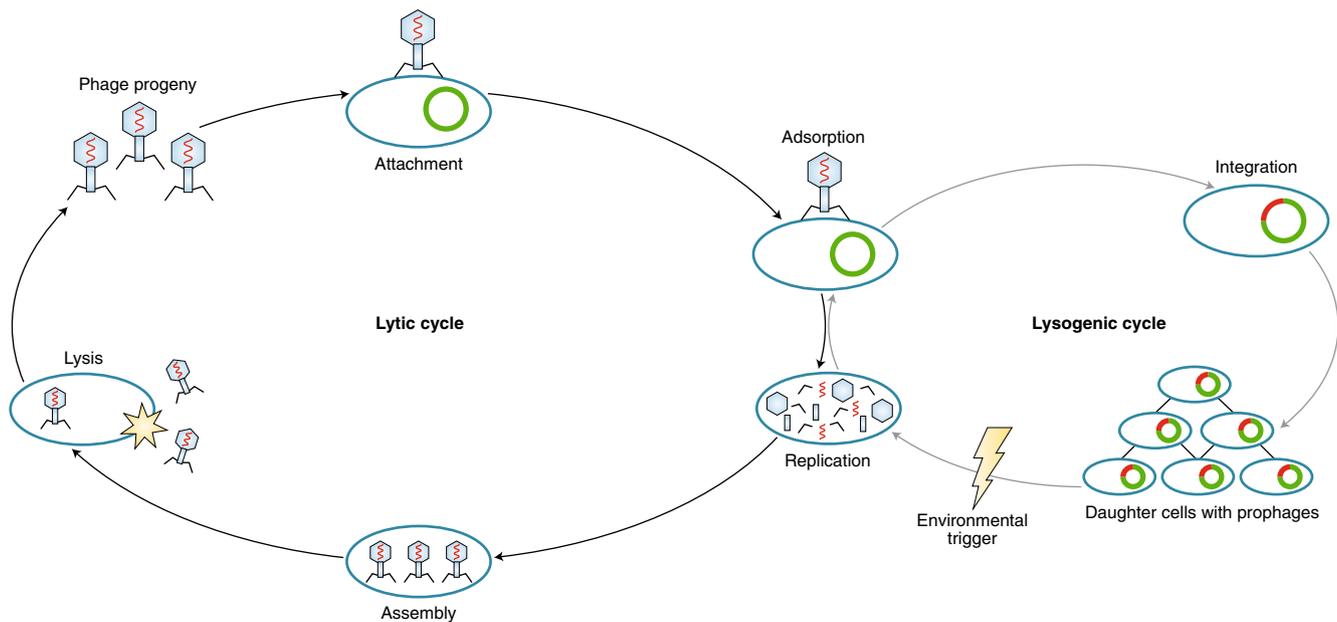
Due to their small size, the vast majority of viruses fall under the oceanographic definition of DOM (operationally defined as the ability to pass through a 0.2 µm filter; Fig. 1). Since viruses are clearly not 'dissolved', this creates an enigma and the contribution of viral particles to dissolved carbon as well as macro- and micro-nutrient pools has only recently become a topic of interest<sup>48,49</sup>. By extrapolating estimates of the carbon, nitrogen and phosphorus contained within individual phage particles to the marine ecosystem scale, Jover et al.<sup>48</sup> suggested that viruses could make a significant contri-

**Table 1 | Descriptions and applications of a few common and emerging viral techniques for virus quantification, diversity analyses and host identification (adapted from ref. <sup>134</sup>)**

Method	Description	Application	Advantages	Disadvantages
Single-cell genomics <sup>128</sup>	Mining single amplified genomes (all DNA molecules recovered from a single cell) for viral sequences	Diversity; host identification	Viral-host linkage without culturing	Database limitations
CRISPR loci <sup>140</sup>	Comparing viral metagenomes to CRISPR loci (a series of short repeats, interspersed by spacers) that function as a bacterial defence system against phages	Diversity; host identification	Viral-host linkage without culturing	Limited distribution of CRISPR systems in marine bacteria
PhageFISH <sup>141</sup>	Co-localization of host and virus through fluorescent in situ hybridization (FISH)	Quantitative; diversity; potential for host identification	Quantifying infected cells; potential for viral-host linkage without culturing	Requires previous sequence knowledge of virus and host to design primer/probes
Polonies <sup>142</sup>	Solid-phase PCR amplification that allows for simultaneous amplification of host and virus using degenerate primers to encompass the diversity within a viral group	Quantitative; diversity; potential for host identification	Quantifying diverse viral groups with degenerate primers; potential for viral-host linkage without culturing	Requires specialized equipment; requires previous sequence knowledge of virus and host to design primer/probes
Microfluidic digital PCR <sup>143</sup>	Co-localization of host and viral DNA in microfluidic chambers, which are simultaneously amplified during PCR	Diversity; host identification	Viral-host linkage without culturing	Requires specialized equipment; requires previous sequence knowledge of virus and host to design primer/probes
Quantitative PCR <sup>165</sup>	Quantifying DNA using fluorescence from either a SYBR green dye or a fluorescent probe during PCR	Quantitative	Fast and straightforward quantification	Requires previous sequence knowledge of virus to design primer/probes; only targets a narrow group of viruses
SYBR stain <sup>9</sup>	All nucleic acids in a sample are stained, abundance of VLPs can be quantified by counting under epifluorescence microscope or flow cytometry	Quantitative	Able to quantify total abundance of VLPs and bacteria; fast and straightforward quantification	Counts strictly based on size, thus large viruses or small bacteria may be miscounted, possible misidentification of GTAs and vesicles as VLPs; the stain is biased towards dsDNA viruses; provides no insight into diversity of bacteria or viruses; homogenized counts, thus VBR is misleading
Metagenomics <sup>89,92</sup>	Shotgun sequencing from communities of purified viral particles (that is, viral metagenomics) or mining information from cellular metagenomes, where viral signals are captured from sequencing of lysogens, cells infected with lytic phages (virocells), large virions or viral particles attached to larger particles	Quantitative; diversity	Does not require previous sequence knowledge; recovers large diversity of viruses; archived sequences available for re-analyses; provides ecological insight	Database limitation, high percentage of unknown sequences; computational/bioinformatic limitations; difficulties determining if genetic material is of viral origin
Signature gene amplification and sequencing <sup>109</sup>	Targeting a viral signature gene (such as the major capsid protein) through PCR amplification and sequencing	Diversity	Fast, straightforward and accessible; useful for targeted studies, can give insights into previously described groups	Requires previous sequence knowledge of virus and host to design primers
Phage isolation and culture <sup>166</sup>	Obtaining phage isolates for in-depth characterization	Diversity; host identification	Facilitates studies of genome, structure, lifestyle, burst size	Difficulty culturing

bution to the phosphorus reservoir in environments where phosphorus is limited. This study also demonstrated a stoichiometric mismatch between phage particles and their bacterial hosts, with

phages more enriched in nitrogen and phosphorus relative to carbon compared to their bacterial hosts. In addition, a recent study by Bonnain et al.<sup>49</sup> hypothesized that phages could serve as organic



**Fig. 3 | The two main lifestyles of marine phages: lytic versus lysogenic.** In lytic infection, the phage injects its DNA into the cell, which subsequently directs the host cell to replicate phage DNA, leading to cell lysis and the release of progeny phage particles. In contrast, temperate phages lysogenize their hosts, either integrating into the bacterial chromosome or in some cases being maintained as an extrachromosomal element, where they divide as prophages with the bacterial cells until an environmental or cellular trigger causes them to enter the lytic cycle. Although not shown here, some phages can undergo a chronic infection in which phage particles are produced without death of the host bacterial cell<sup>44</sup>.

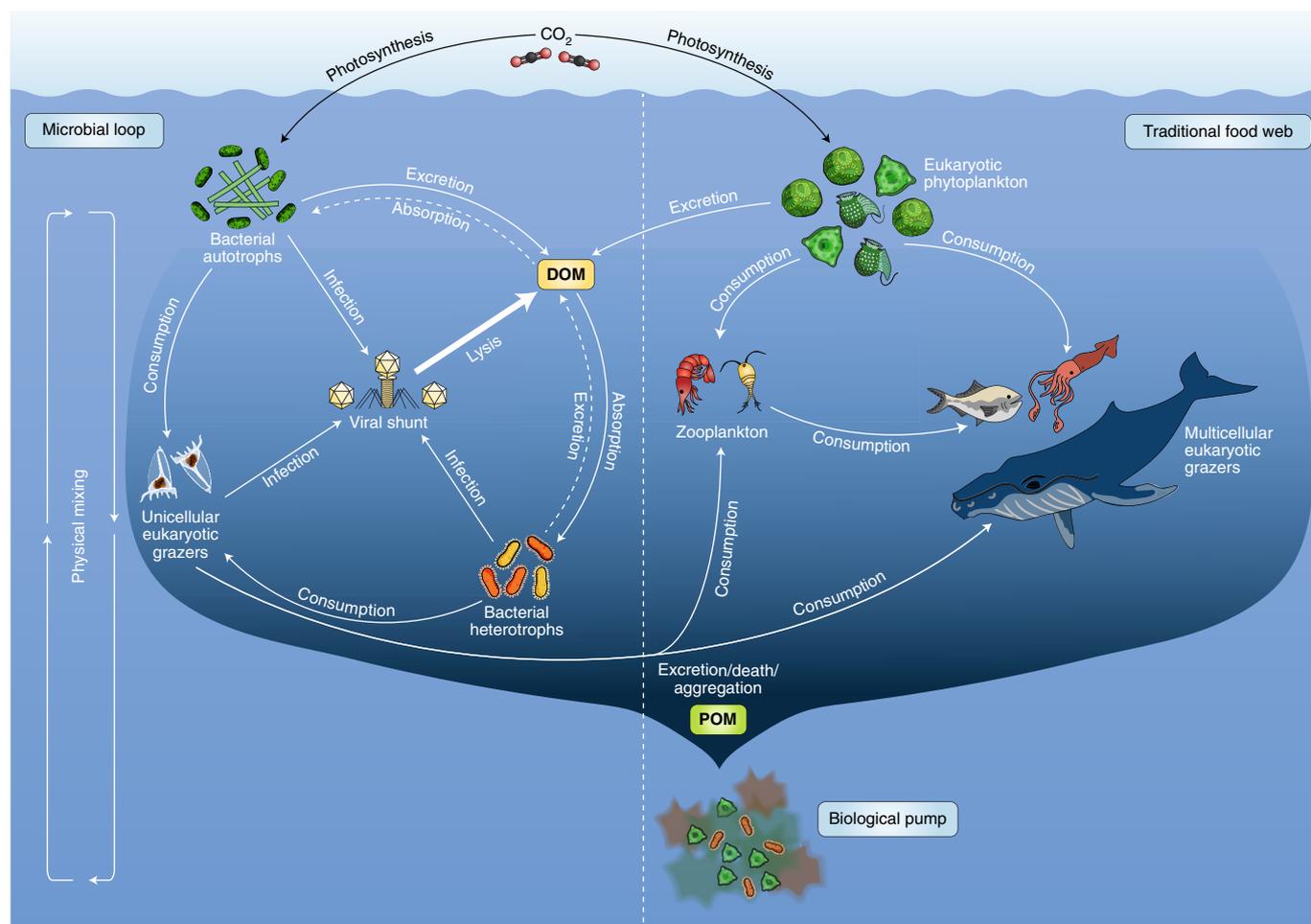
ligands for iron, acting as an important reservoir of the trace metals that frequently limit primary production. Modelling studies predict that most of the phosphorus contained in the host bacterial cell can be sequestered in phage particles, while these particles contain only a small proportion of the host-derived carbon and nitrogen<sup>48</sup> and up to 14% of the cellular iron reserves<sup>49</sup>. Together, these studies indicate the potential contributions of free phage particles to marine DOM pools and have implications for the composition and bioavailability of lysates (that is, the dissolved and particulate matter produced through phage lysis of a bacterial cell).

### Phage puppeteers tinker with bacterial metabolism

In addition to affecting oceanic biogeochemistry through their physical contribution to DOM and the lysis of their bacterial hosts, phages can also affect the diversity and function of marine microbial populations through the incorporation and expression of auxiliary metabolic genes (AMGs)<sup>50</sup> (Fig. 5). When host-derived genes were first observed in temperate phages, they were originally termed 'lysogenic conversion factors'<sup>51</sup>; however, today, AMG is a broader term that also encompasses metabolic genes found in lytic phages. AMGs involved in photosynthesis, nucleotide metabolism and phosphorus cycling were initially identified in the completely sequenced genomes of cultured phages infecting marine heterotrophs<sup>52</sup> and cyanobacteria<sup>53,54</sup>. The number and functional diversity of AMGs has rapidly increased through inclusion of metabolic genes identified by careful analysis of viral metagenomes<sup>55–57</sup> and a classification system for AMGs based on their presence or absence in KEGG pathways has been proposed<sup>58</sup>. The AMGs found in marine phages include genes involved in antioxidation, carbon metabolism, cell protection, cycling of nutrients (such as nitrogen, phosphorus and sulfur), fatty acid metabolism, iron–sulfur clusters, photosynthesis, purine and pyrimidine metabolism, and protein synthesis<sup>59–61</sup>. The most enriched metabolic pathways in marine viral metagenomes are those involved in nucleotide biosynthesis, showing how phage infection shifts host metabolism toward phage DNA synthesis through multiple mechanisms, including the degra-

dation and recycling of host nucleotides<sup>62</sup>. Most AMGs are thought to be acquired from bacterial hosts; however, the phage-encoded versions of the genes often form a distinct phylogenetic cluster from their bacterial homologues, suggesting that they have evolved separately and in some cases may share a globally connected phage AMG pool<sup>63</sup>. Some AMGs have been shown to correlate with temperature, environment (nearshore versus open ocean) and the isolation host for the phage, demonstrating their importance in environmental adaptation<sup>60,64</sup>. For example, cyanophages isolated from regions low in phosphate are more likely to contain AMGs involved in phosphorus acquisition, including the phosphate-binding *pstS* gene and the alkaline phosphatase *phoA* gene, than those isolated from phosphate-replete regions<sup>65</sup>.

Since phages require active host cells to complete lysis, it is thought that AMGs sustain or supplement critical host metabolism during infection, contributing to the success of certain phages in marine environments. The cost of maintaining additional genetic material is presumably high for small phage genomes; therefore, only the most essential AMGs are likely to be maintained. A recent analysis of 60 cyanophage genomes hypothesized that common AMGs may have been acquired early during evolution and then maintained due to positive selection, while rare AMGs may be frequently gained and lost due to fluctuating selection pressures, such as those that vary by habitat or season<sup>60</sup>. For example, during *Prochlorococcus* infection in phosphate-limited conditions, increased transcription of cyanophage-encoded AMGs involved in phosphorus acquisition was observed<sup>66,67</sup>. While the function of some AMGs may be as simple as supplementing a rate-limiting step or replacing a rapidly degraded host protein<sup>68</sup>, more complicated mechanisms have also been proposed to explain the unique suite of known AMGs. This includes involvement in sequential reactions within a given pathway or participating in reactions that are essential to multiple pathways<sup>62</sup>. It has also been proposed that phage AMGs activate pathways within host bacteria to mimic carbon or nutrient-limited environmental conditions, resulting in rapid changes in host metabolism that promote phage replication<sup>69</sup>.



**Fig. 4 | The marine food web, emphasizing the central role of the microbial loop and viral shunt in recycling DOM.** Photosynthesis in the oceans is carried out by both bacterial autotrophs and eukaryotic phytoplankton, both of which produce fixed carbon. DOM, the largest reservoir of carbon in the oceans, is only available for uptake by bacteria. There are two main forms of bacterial mortality: predation by unicellular eukaryotic grazers or lysis by phages. Grazing moves carbon up through the trophic levels from bacteria to protozoa to zooplankton to fish and larger organisms, with all trophic levels of this food chain contributing to the biological pump through sinking of POM. In contrast, when bacteria are lysed by phages, carbon and nutrients flow through the viral shunt and are remineralized by bacteria within the microbial loop, making the viral shunt function as the ocean's recycling system. As recent research has implicated a role for viruses as a shuttle for carbon to the deep ocean, we have also indicated the potential stimulation of particle aggregation by phage lysis and indicated water column mixing as a physical mechanism to sequester DOM.

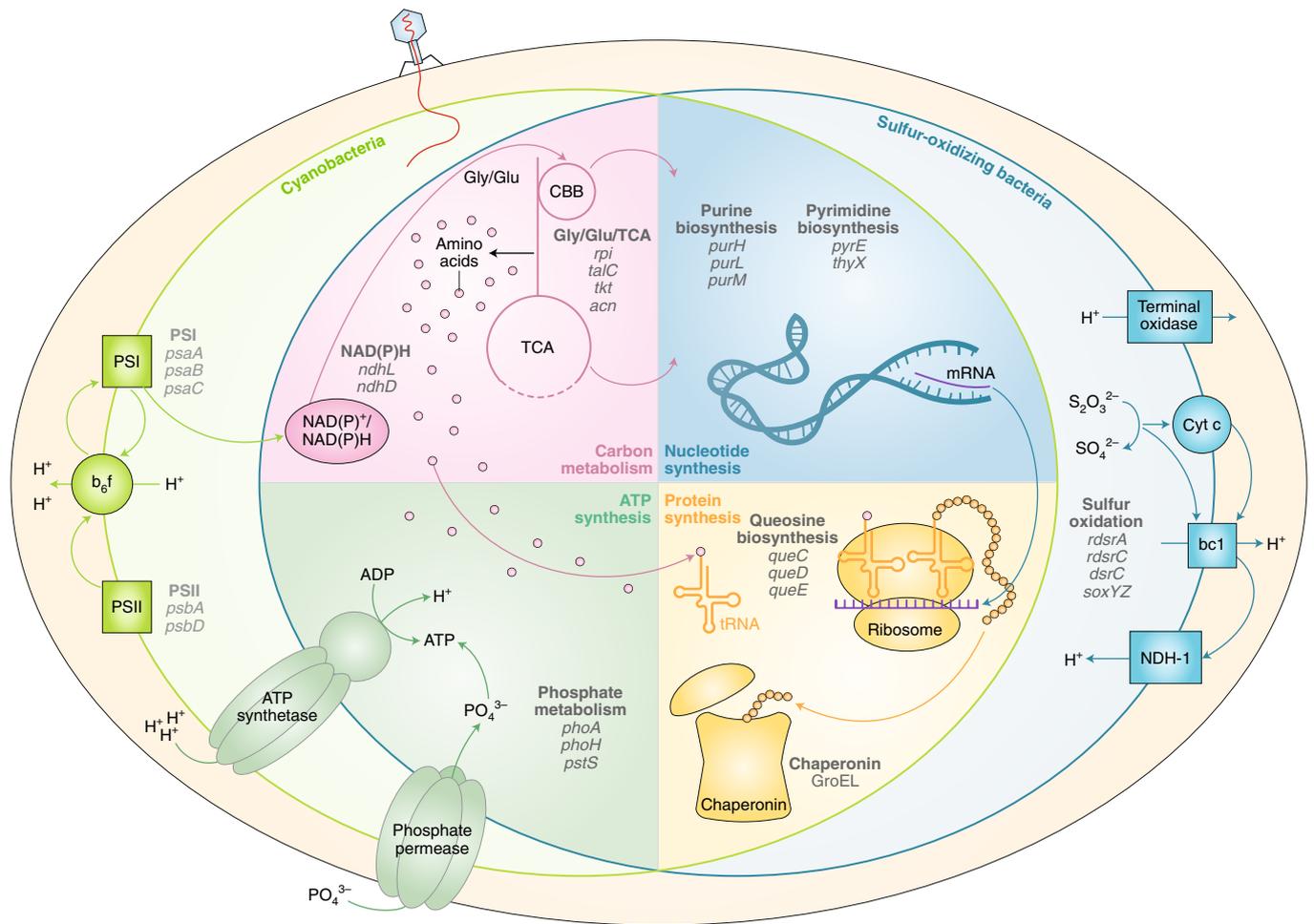
Puxty et al.<sup>59</sup> suggested that cyanophages may alter the flow of electrons from the photosynthetic electron transport chain during infection. Additionally, the identification of genes from the queuosine biosynthesis pathway in phages<sup>70,71</sup> creates the possibility that phages could be modifying their DNA to escape host restriction endonucleases or could be increasing translation efficiency by redirecting global host protein synthesis through queuosine-containing transfer RNAs (tRNAs)<sup>72</sup>.

Future research needs to examine the functional roles of specific AMG in cultured phage–host systems to elucidate their expression patterns, functional roles and potential fitness advantages. For example, a phage-encoded peptide deformylase that acts on photosynthetic D1 proteins was found to be more active and efficient than its bacterial counterpart<sup>73</sup>. Likewise, a study with two *Prochlorococcus* phage isolates demonstrated that the phage gene (*pebS*) was able to catalyse a phycoerythrobilin-synthase reaction that performs a four-electron reduction involved in biosynthesis of photosynthetic pigments requiring two genes (*pebA* and *pebB*) in the *Prochlorococcus* host<sup>74</sup>. Other environmental phages have been found to contain *pebS*, but this version has not been identified in bacterial cells<sup>74</sup>. The combination of two distinct steps into one

phage AMG is consistent with the selection for genome reduction in phages, and that the lengths of phage AMGs are typically one third shorter than their bacterial homologues<sup>75,76</sup>. Finally, it is also important to note that the phage AMGs may serve different functions than their bacterial homologues. For example, Ledermann et al.<sup>77</sup> identified phycobilin synthesis proteins in the genomes of phage that most likely infect alpha-proteobacteria, suggesting that phycobilins may be performing different roles in heterotrophic bacteria. Profiling the expression patterns and documenting the functional implications of phage AMGs during infection has not kept pace with the rapid discovery of novel AMGs, but it is clear that expression of these genes is one major way in which phages can alter their hosts' behaviour, making this an exciting area for future investigation.

#### Differences between infected and uninfected bacteria

Recent studies demonstrating the distinct physiology and metabolism of infected cells (that is, virocells) from those of their uninfected counterparts suggest that biogeochemical models might need to consider these two infection states separately<sup>78,79</sup>. Due both to the expression of phage-encoded AMGs and the ability of phages



**Fig. 5 | Venn diagram showing cyanobacterial (left) and sulfur-oxidizing (right) cells.** Pictured are colour-coded metabolic pathways, including: carbon metabolism, nucleotide metabolism, protein synthesis, ATP synthesis, photosynthesis and sulfur oxidation. The pathways in the central circle represent functions shared by both cells. The genes (in italics) serve as a few examples of AMGs associated with various cellular processes<sup>55,61,69,71,67</sup>. Gly, glycolysis; Glu, gluconeogenesis; CBB, Calvin-Benson-Bassham cycle; TCA, tricarboxylic acid cycle; PSI/II, photosystem I/II; b<sub>6</sub>f, cytochrome b<sub>6</sub>f complex; Cyt c, cytochrome c; bc1, cytochrome bc1 complex; NDH-1, type I NAD(P)H dehydrogenase.

to drive differential gene expression in the host, the metabolism of infected bacteria may be significantly different than that of uninfected cells. One way that phages can influence host metabolism is by shutting down select host pathways to redirect host efforts toward phage production. For example, some *Prochlorococcus* phages inhibit carbon dioxide fixation while photosynthetic electron transport remains functioning to support phage replication within the cell<sup>80</sup>. Alternatively, many phages degrade and repurpose host nucleic acids for the synthesis of phage DNA<sup>81</sup>. Intricate solutions may also exist to differentially regulate the fate of host versus phage products. For example, a recent study demonstrated that while *Prochlorococcus* phage P-SSP7 increased the expression of host RNase E to recycle host RNA during infection, it also produced phage antisense RNAs to create double-stranded RNA and protect its own transcriptome from degradation<sup>82</sup>.

A metabolomics study of a phage-infected marine heterotroph throughout the infection cycle in culture revealed increases in the intracellular concentrations of many different metabolites (for example, sugars, amino acids, lipids, nucleotides), consistent with the generally elevated metabolic activity measured in the cells by stable isotope flux measurements<sup>83</sup>. This enhanced metabolic activity helps support phage infection, with a large proportion of the host cell contents incorporated into phage progeny<sup>83</sup>. Despite the increased metabolite concentrations in infected cells, the majority

of compounds measured in lysed cultures were present at lower concentrations compared to the extracellular concentrations measured in uninfected control cultures. Presumably this is due to the fact that surviving cells in the infected culture rapidly utilized components of the lysate<sup>83</sup>. These findings support previous studies that showed rapid uptake of highly bioavailable lysis products by the remaining phage-resistant cells in a pure culture<sup>84,85</sup>, simple co-culture<sup>86</sup>, or by other members of marine bacterial communities<sup>87,88</sup>.

### Phage diversity is largely uncharacterized

The advent of viral metagenomics (that is, virus particle purification followed by metagenomic sequencing)<sup>89</sup>, combined with the increased throughput and decreased cost of next-generation sequencing technologies, has greatly expanded our knowledge of marine viral diversity<sup>3,90-93</sup>. Major advances have been made both in sequencing the complete genomes of cultured marine phages<sup>60,70,94</sup>, as well as in describing the diversity and biogeography of uncultured viral communities<sup>91,93,95</sup>. In addition, significant insight into marine viruses has been gained from mining cellular metagenomes, where viral signals are captured from sequencing of lysogens, cells infected with lytic phages (virocells), large virions, or viral particles attached to larger particles<sup>64,96</sup>. While the diversity of marine phages is still highly debated<sup>97</sup>, a few common themes have emerged. First, despite the high diversity of phages on a local scale, for example

in an individual water sample, the presence of a shared oceanic phage gene pool tempers this diversity on a global scale<sup>95,98</sup>. Second, while niche differentiation exists in different oceanic regions, for example the photic zone versus the aphotic zone, there is significant downward flux of marine viruses, presumably carried by sinking particles<sup>58</sup>. Third, viral community composition and distribution appears to follow major oceanographic currents<sup>95</sup> and can be driven by water column stratification<sup>99</sup>. Finally, our knowledge of marine phage diversity is constantly expanding and is highly dependent on methodological biases; for example, while this manuscript was in review, a major group of previously overlooked non-tailed double-stranded DNA (dsDNA) phages with relatively broad host ranges was discovered<sup>94</sup>.

Given the large degree of sequence overlap between geographic regions and the well-described propensity for phages to undergo modular evolution and gene swapping, there has been debate as to whether designations such as strains, species or populations can be applied to marine phages. This determination has important implications for our ability to assess overall phage diversity in the oceans, link phages to their hosts, and perform traditional ecological and evolutionary assessments. On one hand, the presence of 'hybrid' genomes with features of multiple viral families and GTAs highlights the mosaicism and genetic exchange between phages<sup>100</sup>. On the other hand, the analysis of hundreds of cultured cyanophages from coastal marine waters produced well-supported whole-genome clusters and sub-clusters that remained constant for over a decade<sup>101</sup>. These clusters function as stable ecotypes<sup>101</sup> and viral-tagging experiments could definitively discern population structure of natural uncultured *Synechococcus* phages<sup>102</sup>. These results suggest that, at least for cyanophages, discrete populations exist within phage sequence space, presumably because constraints on gene transfer or other selective pressures prevent a continuum of phage genetic information<sup>103</sup>. Recent data have also confirmed that these discrete phage populations are temporally stable, with nearly identical phage genomes observed several years apart and most of the sequence differences occurring in AMGs, restriction-modification enzymes, and virus structural proteins that are probably involved in receptor recognition<sup>101,104,105</sup>.

Time-series studies of phage diversity at several marine sites based on signature gene sequencing has provided insight into the stability of phage communities over time. Community profiling of the major capsid protein of T4-like phages in a North Atlantic fjord revealed clear, annually recurring, seasonal patterns in diversity<sup>106</sup>. Analysis of this same phage group at the San Pedro Ocean Time-Series (SPOT) site revealed diverse phage communities with recurring seasonal patterns in community structure<sup>107</sup>. Likewise, analysis of several viral groups at the Bermuda Atlantic Time-Series Study (BATS) site found that water density was a significant driver of viral community structure, with repeatable seasonal patterns in viral communities linked to seasonally recurring patterns of water column stratification and mixing at this site. The strong seasonal patterns in phage diversity may be indicative of phages controlling host abundance; however, network analysis of data from SPOT suggested that phage diversity was following that of their hosts (as opposed to driving it) for a majority of monthly and seasonal interactions<sup>108</sup>.

The documented seasonal patterns at each of these sites clearly demonstrate that a subset of the phage community undergoes boom–bust cycles following bacterial host responses to changing environmental conditions, yet interestingly, each of these studies also found several operational taxonomic units (OTUs; clusters of highly related sequences) that were consistently present at relatively high abundances throughout the time-series, independent of seasonality. For example, deep sequencing of the AMG *phoH* (a gene of unknown function that belongs to the Pho regulon, which is activated in response to phosphorus limitation) at the BATS site showed that a small number of abundant OTUs dominated the viral com-

munity throughout ~15 depths, two seasons and three years<sup>109</sup>. The recurrence of identical phage sequences over long temporal scales is at first glance surprising given the current predictions of rapid phage–host coevolution<sup>110,111</sup>. However, it is important to consider that the conserved phage and host genes being analysed over time may not be relevant for determining phage–host interactions. For example, bacterial species are often delineated by 97% identity of their 16S rRNA genes, yet a bacterial species measured with this method is probably comprised of many different strains, each with differences in its phage susceptibility spectrum<sup>112,113</sup>. Likewise, in adapting to infect a different host, changes are unlikely to occur in conserved phage signature genes, but will most likely occur in phage genes whose products are implicated in host specificity or attachment, such as genes encoding tail fibre proteins, which are highly diverse in sequenced phage genomes<sup>114,115</sup>. Accordingly, data mining of environmental metagenomes demonstrated that many of the genes differing between bacterial strains encode products that are exposed extracellularly, which could serve as potential phage recognition targets<sup>116</sup>. Thus, the bacterial 16S rRNA genes and phage signature genes generally monitored in marine communities are unlikely to change in the process of developing or overcoming resistance, with the phage–host arms race primarily generating microdiversity in both phages and bacteria that is highly dynamic over time<sup>117–122</sup> (Box 1).

### Who infects whom?

The question of 'who infects whom' within marine microbial communities has continued to be recalcitrant, and the true degree of host specificity and polyvalency among marine phages remains unknown. Recent work has shown that commonly used methods for virus purification can bias against entire lineages of marine phages, including the newly discovered non-tailed dsDNA *Autolykiviridae*, which has a much broader host range than tailed phages typically recovered with plaque assays<sup>94</sup>. In addition, the biases of culture-based phage isolation via plaque assays originally led to the idea that some bacteria, such as the highly abundant yet slow growing SAR11 clade, were extreme defence specialists that could coexist with more rapidly growing competitor bacteria due to their resistance to phage infection<sup>2</sup>. For example, prior to 2013, no phages infecting the dominant marine heterotrophic clades SAR11 or SAR116 were known. However, once the genomes of phages infecting these hosts were described, mapping of marine viral metagenome sequences revealed their high abundances throughout the oceans<sup>123,124</sup>. In contrast to the dominance of SAR11 and SAR116 phage sequences in marine viral metagenomes, newly described phages infecting *Cellulophaga* spp. appear to be ubiquitous but present at low numbers in marine viral metagenomes<sup>70</sup>. All marine bacteria are probably susceptible to phage infection, but little is known about the degree of lytic phage pressure on various bacterial groups<sup>125</sup>.

A combination of single-cell genomics, enhanced sampling techniques, improved sequencing technology and new data-mining tools have led to the discovery of the first viruses infecting some important groups of marine microorganisms<sup>126–134</sup>. One extremely fruitful advance has been the assembly of complete phage genomes from marine viral metagenomes<sup>90,91,93,135,136</sup>, which provides insight into the genetic repertoire of marine phages, the organization and genomic context of AMGs, the evolutionary patterns of marine phages, and the overall level of marine viral diversity. Currently, the hosts for the majority of environmental viral genomes remain unknown, despite many newly emerging techniques for linking phages with their hosts, including single-cell genomics<sup>128,137–139</sup>, matching CRISPR spacers<sup>140</sup>, phageFISH<sup>141</sup>, viral tagging<sup>102</sup>, polonies<sup>142</sup>, microfluidic digital polymerase chain reaction (PCR)<sup>143</sup> and advanced computational approaches<sup>91,132,144–148</sup> (Table 1).

Recent meta-analyses of cultured phage–host interactions revealed nested patterns that suggest both generalist and specialist

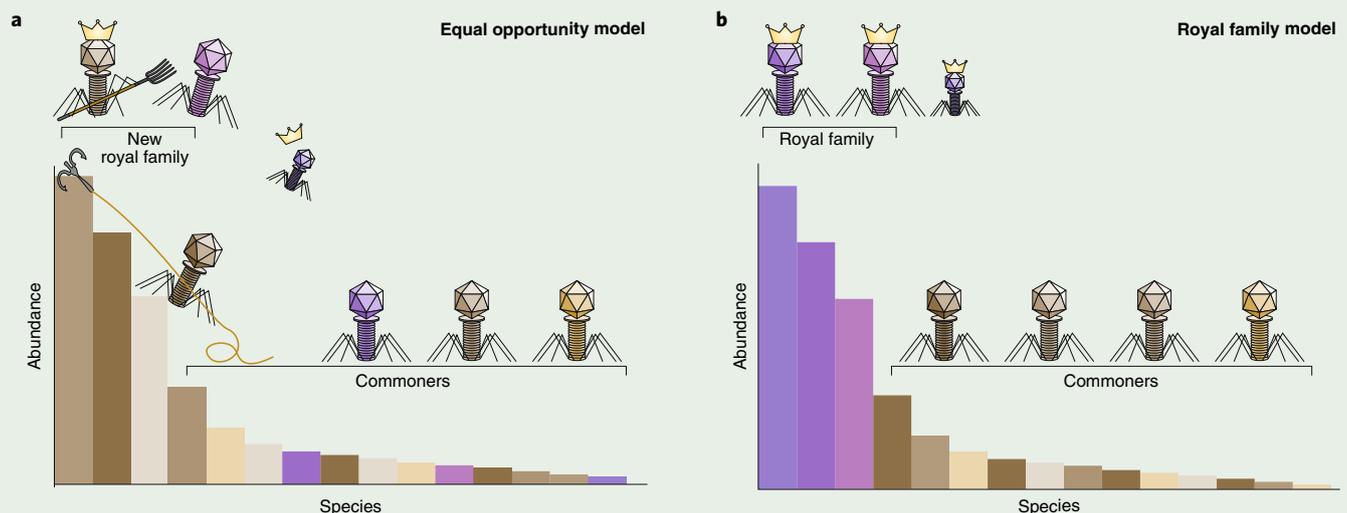
**Box 1 | Monarchy or anarchy: which phages rule the seas?**

For over two decades, research in marine microbial ecology has been guided by the conceptual framework of the kill-the-winner hypothesis, which mathematically describes the steady-state co-existence of many different phages and bacterial hosts in a given niche<sup>113,168–170</sup>. According to kill-the-winner dynamics, when a particular host becomes active, a phage that can infect that bacterial host will increase in abundance. Phage infection then causes the susceptible host population to decline, followed by a decline in the phage population as no more hosts are available. As the originally dominant host population declines, a new host, which is presumably resistant to the phage that infected the first host, will emerge to fill the now-vacant niche. Subsequently, a new phage emerges that is able to infect the newly dominant host, and the cycle continues. This gives rise to an interesting question: what do the ‘new’ hosts and phages in this kill-the-winner progression look like?

To help conceptualize the bacterial–phage hierarchy, we propose two classes: the ‘royal family’ and the ‘commoners’. The royal family, which is distinct in different locations (that is, ‘kingdoms’), is analogous to the dominant bacterial and phage populations (that is, the highest ranks in a rank-abundance curve). Meanwhile, all the other bacterial and phage populations present within the ecosystem make up the commoners. Following the death of a bacterial monarch and subsequent decline in abundance of its phages following typical kill-the-winner dynamics, we hypothesize two possible scenarios that can occur. In the first scenario, here termed the ‘equal opportunity model’ (see figure, panel a), all hosts (and their respective phages), including the commoners, have an equal opportunity to step in and replace the empty niche, becoming royalty. Despite the fact that each of the commoners is relatively rare, when combined, there are many more commoners than members of the royal family. Therefore, in the equal opportunity model, it is highly unlikely that the same host and phage sequences would be abundant at two different times, at least on a broadly measured scale (that is, 16S rRNA and phage signature genes). One alternate possibility is the ‘royal family model’ (see figure, panel b), where members of the royal family are dominant because they are optimized to that specific niche. Therefore, the next host following the death of a bacterial

monarch is much more likely to be a descendant of the royal family (that has acquired resistance to its dominant phage predators), as opposed to a commoner that has scaled the steep rank-abundance plot to rise to dominance. Accordingly, in this model, the next phages to become dominant are likely to be variants of previous royal family members that have overcome the host’s newly developed resistance. This evolutionary arms race<sup>158,171,172</sup> has been demonstrated in culture-based studies where marine bacteria quickly acquire resistance to co-occurring phages, leading to microdiversity that often has functional consequences for bacterial physiology<sup>117</sup>. The results of these phage–host interactions can also be observed through examination of microdiversity in time-series datasets from natural marine systems<sup>118–121</sup>.

In the royal family model, kill-the-winner fluctuations on the level of interacting phage–host pairs drive changes in community composition over time, largely through arms-race selection for resistance and counter-resistance that results in fine-scale diversity changes while leaving coarse-level diversity (that is, the level typically monitored by conserved genetic targets in marine time-series studies) unchanged. This model is consistent with the constant-diversity dynamics model<sup>116</sup> and recent theoretical analyses<sup>112,113</sup>, which suggest that bacterial diversity emerges from the balance of competitive and defensive abilities against strain-specific phage predators in the environment. Moreover, this model is supported by the findings of Rodríguez-Brito et al.<sup>173</sup>, who demonstrated the persistence of broad viral and host taxa concurrent with kill-the-winner-type fluctuations at the level of host strains and viral genotypes in four aquatic ecosystems. While further time-series studies of bacterial and phage microdiversity in natural marine systems are needed to test this hypothesis, initial evidence supports the royal family model, where the hosts who are well-suited for the niche will stay in power, and changes in the throne will favour bacteria belonging to royal lineages that have managed to escape their abundant phage attackers. Nevertheless, these scenarios provide an important framework for thinking about phage–host interactions and the genomic scales (for example, signature genes versus whole-genome sequencing) on which these dynamics are measured.



**Proposed ‘equal opportunity model’ and ‘royal family model’ for describing the turnover of marine phages. a,b**, Following the death of a bacterial monarch and subsequent decline in abundance of its phages (following kill-the-winner dynamics), in the equal opportunity model (a), all hosts (and their respective phages), including the commoners, have an equal opportunity to step in and replace the empty niche, becoming royalty. Alternatively, in the royal family model (b), the next host following the death of a bacterial monarch is more likely to be a descendant of the royal family that has acquired resistance to its dominant phage predator, as opposed to a commoner that has scaled the steep rank-abundance plot to rise to dominance.

viruses exist and co-occur<sup>149–151</sup>. Interestingly, the marine phage–bacteria interaction networks indicated that within one location, phages could infect several different hosts, while phages from different geographic locations were mostly incompatible<sup>149,150</sup>. In addition, synthesis of multiple datasets indicated that co-infection (the simultaneous infection of a host by more than one phage) may be common in natural communities<sup>152</sup>. Global marine network analyses predicted that most phages only interact with a single or very limited set of closely related OTUs<sup>153</sup>. However, this phenomenon is particularly difficult to study in natural systems because the sequence-based techniques that are available for measuring bacterial and phage diversity cannot distinguish phage susceptibility nor host range patterns, respectively<sup>154</sup>.

While abundance measurements of specific phage–host interacting pairs in the environment are lacking, models suggest that strain-specific phages can drive the coexistence and balance between ‘competitor’ and ‘defender’ bacterial strains in natural communities<sup>112,113,155,156</sup>. Competitor bacterial strains grow rapidly but are highly susceptible to phage infection, while defender bacterial strains grow slower but are less susceptible to phage infection. Recent theoretical studies suggest that high-abundance, low-activity bacterial species are not necessarily competitors or defence specialists, but rather those species comprised of a large number of phage-controlled strains that use defence strategies without large competitive costs. In thinking about the co-evolutionary battle waged between individual phage–host pairs in the ocean, it is important to consider potentially unintended consequences for bacteria developing resistance to a given phage in terms of susceptibility to infection by other co-occurring phages. For example, development of resistance to one phage can alter the susceptibility and speed of infection dynamics by other phages in both directions (either providing additional phage resistance or increasing susceptibility to other phages)<sup>154,157,158</sup>. Intriguingly, recent evidence suggests that phage-resistant bacterial cells can become infected through transient acquisition of phage receptors via membrane vesicles produced by co-existing susceptible cells<sup>159</sup>, which has potential implications for temporarily expanding host range in complex environmental communities.

Importantly, culture-based host range studies have demonstrated that host range should not be thought of as a binary system of a failed or successful infection. In the case of a successful infection, a wide range of infection efficiencies may be observed on different hosts<sup>160–162</sup>. For example, quantitative infection studies of *Cellulophaga* phages showed that a single phage could infect as few as one or as many as 17 of the 19 host strains tested, with up to 10 orders of magnitude variation in infection efficiency (that is, number of plaque-forming units per millilitre of culture<sup>162</sup>). There may also be tradeoffs between host range and infection efficiency; culture-based studies of specialist and generalist phages showed that phages with broader host ranges tended to have reduced burst sizes<sup>160</sup>. In addition, the recent observation that infection parameters (burst size, plating efficiency) can be dependent on the previous host infected by that phage also suggests that there is a great deal of plasticity in infection efficiency that can be affected by factors that are not yet fully understood<sup>162</sup>.

Few studies to date have examined the genetic processes and consequences of infection of multiple hosts by the same phage. A recent study of the infection of three different *Synechococcus* strains with the cyanophage Syn9 showed that phage transcription was nearly identical during infection, while the expression patterns of strain-specific host genes located in hypervariable genomic islands varied significantly<sup>163</sup>. Likewise, experimental infection of two cultured strains of the marine heterotroph *Cellulophaga baltica* by a single phage revealed similar phage gene expression profiles, but one host strain upregulated the expression of genes involved in DNA degradation and downregulated genes involved in translation.

This resulted in inefficient phage infection from the reduction and delayed production of phage particles<sup>164</sup>. A key difference between the results in these two studies is that the variation in *Synechococcus* transcriptomes was driven by strain-specific genes<sup>163</sup>, while in the *Cellulophaga* system the genes with differential expression were present in both strains. The transcriptome differences in *Cellulophaga* were attributed to failure by the phage to repress host stress responses early in infection, as well as differing cellular environments during late infection<sup>164</sup>. If these phenomena are broadly applicable to marine phage–host systems, it would suggest that the infection strategy of a given phage is conserved across multiple susceptible hosts, but that the defence mechanisms of different hosts play a dominant role in dictating the progression of infection<sup>163,164</sup>.

## Concluding remarks

The numerical dominance of marine phages and their significant impacts on oceanic biogeochemistry are well-accepted; however, we are only beginning to illuminate the ‘black box’ of marine phage–host interactions. Emerging techniques for examining the contribution of phages to oceanic DOM, studying the impacts of phage infection of bacterial metabolism during infection, and linking phages to their hosts will yield powerful insights into the factors that govern phage infection dynamics and their subsequent ecological consequences.

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### Author contributions

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### Competing interests

The authors declare no competing interests.

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