Host-hijacking and Planktonic Piracy: How Phages Command the Microbial High Seas

Description: A critical literature review drawing together evidence of the different ways in which bacteriophages control host metabolism in the marine environment. This review considers: (1) Marine-specific examples of phage-mediated suppression and promotion of substrate uptake; (2) Augmentation and redirection of host resources towards energy production by viruses in marine bacteria; 3) How virally-encoded auxiliary metabolic genes and active lysogeny may confer an increase in host fitness in certain environments; 4) The strengths and limitations of new sequencing technologies to improve our understanding of the mechanisms used by viruses to hijack host metabolism.

Authors: Joanna Warwick-Dugdale[1,2], Holger H Buchholz[2], Michael J Allen[1,2], Ben Temperton*[2]

Addresses: [1]Plymouth Marine Laboratory, Prospect Place, The Hoe, Plymouth, PL1 3DH, UK. [2]University of Exeter, Geoffrey Pope Building, Stocker Road, Exeter, EX4 4QD, UK.

Email: Joanna Warwick-Dugdale - jwd@pml.ac.uk;

Holger Buchholz - hb513@exeter.ac.uk;

Michael J Allen - mija@pml.ac.uk;

Ben Temperton - B.Temperton@exeter.ac.uk

*Corresponding author

Abstract

Microbial communities living in the oceans are major drivers of global biogeochemical cycles. With nutrients limited across vast swathes of the ocean, marine microbes eke out a living under constant assault from predatory viruses. Viral concentrations exceed those of their bacterial prey by an order of magnitude in surface

water, making these obligate parasites the most abundant biological entities in the ocean. Like the pirates of the 17th and 18th centuries that hounded ships plying major trade and exploration routes, viruses have evolved mechanisms to hijack microbial cells and repurpose their cargo and indeed the vessels themselves to maximise viral propagation. Phenotypic reconfiguration of the host is often achieved through Auxiliary Metabolic Genes – genes originally derived from host genomes but maintained and adapted in viral genomes to redirect energy and substrates towards viral synthesis. In this review, we critically evaluate the literature describing the mechanisms used by bacteriophages to reconfigure host metabolism and to plunder intracellular resources to optimise viral production. We also highlight the mechanisms used when, in challenging environments, a 'batten down the hatches' strategy supersedes that of 'plunder and pillage'. Here, the infecting virus increases host fitness through phenotypic augmentation in order to ride out the metaphorical storm, with a concomitant impact on host substrate uptake and metabolism, and ultimately, their interactions with their wider microbial community. Thus, the traditional view of the virus-host relationship as predator and prey does not fully characterise the variety or significance of the interactions observed. Recent advances in viral metagenomics has provided a tantalising glimpse of novel mechanisms of viral metabolic reprogramming in global oceans. Incorporation of these new findings into global biogeochemical models requires experimental evidence from model systems and major improvements in our ability to accurately predict protein function from sequence data.

Keywords: AMGs, marine; cyanophage; nucleotide scavenging; biogeochemical cycling; host-virus interactions; lysogeny

Background:

Based on their extraordinary abundance and diversity, J.B.S. Haldane once quipped that '*The Creator would appear as endowed with a passion for stars, on the one hand, and for beetles on the other*' [1]. In comparison, the Creator's zeal for viruses would make stars and beetles appear to be a side-project performed with perfunctory indifference. It is estimated that there are a million viruses in the ocean for every star in the universe [2]. Assuming an average size of 100 nm in length, placed end-to-end marine viruses would stretch to our nearest neighbour star (Proxima Centauri, 4.22 light years away) and back. The vast majority of these viruses are obligate parasites of marine bacteria - the primary drivers of global carbon biogeochemistry [3].

With 10^{28} infections occurring per day in the oceans [4], these bacteriophages are responsible for up to 50% of bacterial mortality [5] and the daily release of 12.4 μg of organic carbon per litre of seawater, or an estimated 10 billion tons globally per day [5,6]. Release of cellular substrates through lysis have been shown to stimulate surviving members of the community and increase microbial productivity through nutrient recycling [7–9]. Thus, marine viruses are a major component of global carbon cycling.

Lysis and release of cellular material as dissolved organic matter is only the final step of a complex host-virus interaction where the invading pathogen can manipulate host metabolism and alter its phenotype to favour viral replication at the expense of host function. Once a virus has overcome host defences, lytic viral infection typically involves a shut-down of host metabolism, followed by degradation of macromolecules and scavenging of intracellular resources. Virally encoded 'Auxiliary Metabolic Genes' (AMGs) are expressed during infection to augment and redirect energy and resources towards viral production [10–13]. These AMGs are often repurposed versions of host-genes picked up during ancestral infections, evolving separately to improve viral fitness [14,15]. Conservation of AMGs across phage lineages suggests that not only are such genes critical for viral success, but that viral modulation of marine nutrient cycling via metabolic hijacking is an important, but understudied component of biogeochemical cycles. Furthermore, viral replication through lysis is only beneficial to the virus if upon release, its progeny can infect other susceptible cells. This process is governed by host availability and density-dependent selection. In resource-limited or otherwise challenging environments viral fitness may be better served by maximising host fitness until such time as the conditions are met for a lytic approach to be favourable. Single amplified genomes of bacterial cells from marine environments have shown that ~1/3 contain viral sequences [16,17]. Between 28-71% of marine bacterial isolates contain inducible prophage, with greater occurrence of lysogeny associated with low-nutrient environments [18–21]. In extreme environments such as hot-springs, nearly all cells contained a viral signature [22]. In lysogenic cells, the viruses are not passive passengers, but actively promote the host fitness through expression of viral genes increasing metabolic flexibility, enabling antibiotic resistance, toxin production and immunity to similar viruses [23] (reviewed in [24]). The aim of this review is to illustrate the different ways in which bacteriophages alter the function of their hosts, emphasising how marine viruses modify the metabolism of environmentally relevant bacteria and in turn their impact on global carbon biogeochemistry.

We also discuss how novel molecular methods are providing greater insight into the breadth and scale of viral hijacking in the global ocean.

Plunder and Pillage - Redirecting cellular metabolism to maximise viral production

First, we will consider the classic view of viral predation, where the virus replicates by lytic infection and redirects host resources for the purpose of viral production that ends in cell lysis and release of viral progeny (the 'Plunder and Pillage' strategy, Figure 1a). Once a virus docks with a susceptible cell and successfully injects its genome and associated proteins into the host, hijacking of host metabolism is swift and efficient. Infection in marine T4-like cyanophages follows a similar pattern to that observed in T4 infections in *Escherichia coli*, with 3 distinct phases of transcription of viral genes: (1) defence neutralisation and host metabolic rewiring; (2) synthesis of viral components; and (3) viral assembly [25]. These stages are referred to as 'early', 'middle' and 'late' stages, respectively. Examples of expression of early stage genes include the infection of the marine Bacteroidetes *Cellulophaga baltica*. Infection with a specialist phage was initiated with expression of 15 genes putatively involved in overcoming host defences. Of these, one was an anti-restriction defence gene thought to provide viral defence against host degradation of viral DNA. The remaining 14 genes had unknown function but shared a promoter with the anti-restriction defence and were located in the same genomic neighbourhood, inferring a complementary functional role [13]. A study of the transcriptional response to infection by the cyanophage Syn9 in multiple *Synechococcus* hosts showed that within the first 30 minutes following infection, almost all transcription is associated with phage encoded genes and within the first hour of infection ~80% of cellular DNA has been scavenged for resources [25]. Reconfiguration of cellular transcription machinery is often achieved either by altering host RNA polymerases to favour viral promoters using phage factors packaged within the viral capsid and delivered during infection, or by direct delivery of a virally-encoded RNA polymerase [25–28].

Once the cellular machinery has been repurposed for viral production, the 'middle' stage genes are expressed, which include mechanisms to manipulate host metabolism. Termed "Auxiliary Metabolic Genes" (AMGs), many of these viral genes encode for central metabolic proteins and are conserved across different marine phage lineages, signifying their importance to viral success [29]. Two different classes of AMG have been described (reviewed in [30]): to be categorised as Class I, a gene must encode a protein with a central metabolic

function, for example in photosynthesis, carbohydrate metabolism or amino acid metabolism, and it will appear in a Kyoto Encyclopaedia of Genes and Genomes (KEGG) metabolic pathway [31]; Class II AMGs have been defined as those which encode proteins that have only general, undefined metabolic roles, or peripheral roles (examples include assembly or membrane transport functions) and therefore do not appear in KEGG metabolic pathways [30,32]. Table 1 provides numerous examples of metabolic pathways manipulated by virally-encoded genes in marine and non-marine systems, as identified from both experimental and metagenomic analyses.

Virus-directed augmentation and redirection of host energy and resources to increase viral progeny production has been observed in both marine heterotrophs and phototrophs, with the latter the focus of much of the seminal research in this area. The lytic cyanophage Syn9 encodes photosystem II core protein D1 (which enables transfer of electrons from water to plastoquinone), as well as genes catalysing the synthesis of deoxyribonucleotides (*nrdA/nrdB*) using NADPH as a terminal electron donor [10]. Indeed, viral encoding of genes associated with photosystem II is common in cyanophages, with 88% of cyanophage genomes containing *psbA* and 50% containing both *psbA* and *psbD* [33]. Phages encoding genes associated with photosystem I were identified in metagenomic data and postulated to increase ATP concentrations to power viral production by promoting cyclic photophosphorylation, at the expense of reducing power for CO_2 fixation [34]. Interestingly, *in silico* modelling of the advantage conferred by a phage encoding a *psbA* gene suggested that the increase in energy available to the virus for replication is negated by the increased cost of encoding an extra gene. However, under high-light conditions, the virus gains an advantage by replacing photosystem II machinery damaged by light stress [35,36]. Thus, the presence of photosystem genes on viral genomes is a function of host ecotypic distribution and irradiance sensitivity. Recently a novel *Prochlorococcus*-infecting cyanophage (P-TIM68) was isolated that encodes viral genes for photosystem II as well as a cassette for photosystem I (*psaABCDEFJK*) - the first known example of a phage to manipulate both photosystems during infection. Predictions of enhanced cyclic electron flow around photosystem I were also confirmed [37]. Typically, these genes are co-expressed during early and middle infection and result in a decoupling of photosynthetic 'light reactions' from 'dark reactions', directing ATP and reducing power away from CO_2 fixation and towards nucleotide biosynthesis for phage replication [10,37].

142 Re-routing of photosynthesis-derived energy to increase viral production is just one of many strategies viruses
143 employ during infection to plunder the cellular resources of their hosts. The primary limiting nutrients in
144 marine environments are phosphate (P), nitrate (N) and iron (Fe) [38–40]. Therefore it is perhaps unsurprising
145 that marine viruses encode AMGs to augment host uptake of these resources during infection. Genomes of
146 viruses isolated from P-limited environments have been found to contain more AMGs associated with P-uptake
147 than those from P-replete environments [41]. T4-like cyanophages, S-SM1 and S-SM2, encode an alkaline
148 phosphatase, putatively enabling cleavage of phosphate from organic phosphate sources [42]. Under P-
149 limitation, hosts increase production of the phosphate transporter PstS to maximise uptake, and in P-limited
150 marine environments PstS is one of the most abundant proteins identified in metaproteomic datasets [43]. Both
151 virally-encoded *pstS* and *phoA* were over-represented in viral genomes from the North Atlantic Subtropical
152 Gyre, and occurred at similar frequencies to signature core genes. In the North Pacific Subtropical Gyre, *phoA*
153 was much less abundant in viral genomes, providing evidence of niche-separation of AMGs corresponding to
154 limiting nutrients [41]. Using model cyanophage/host systems, Zeng and Chisholm showed that both virally-
155 encoded *pstS* and *phoA* AMGs are upregulated during infection of P-limited hosts [15]. The phosphate-regulon
156 associated gene, *phoH*, is so common in viral genomes it has been proposed as a signature gene for measuring
157 phage diversity [44]. However, it is worth noting that the function of virally-encoded *phoH* is not yet clear,
158 and *phoH* expression in phosphate limited conditions appears to vary between hosts [45,46].
159
160 Viral manipulation of cellular nitrogen and sulfur levels during infection is perhaps less well documented in
161 cultured host-virus systems than phosphate regulation, but recent research is providing evidence that it is no
162 less important. Phage genomes have been found to encode numerous proteins to manipulate concentrations of
163 2-oxoglutarate, which in turn, regulates cellular N-limitation response via the promotor NtcA [42]. Beyond
164 cultured representatives, a recent global study of viral metagenomics identified 243 putative viral AMGs
165 including genes for photosystem II, ammonium transporters (*amt*) and ammonia monooxygenases (*amoC*), as
166 well as genes associated with sulfur reduction (*dsr*) and oxidation (*sox*) [47]. Screening of marine cellular
167 metagenomes from the Tara Oceans cruises revealed that not only do *amoC* genes encoded by viruses infecting
168 Thaumarchaeota form a distinct phylogenetic clade, they can also comprise up to half of the total abundance
169 of *amoC* in some metagenomic datasets [48]. Nitrate reductase genes (*nar*) have also been found in viromes
170 from deep-sea hydrothermal vents [49]. Evidence of virally-encoded components of dissimilatory sulfite

reductase complex (Rdsr) have been found in viruses infecting the uncultivated chemoautotrophic gammaproteobacterial clade SUP05 [16,50,51]. SUP05 are important drivers of sulfur cycling [52] and peaks in abundance of virally-encoded *dsrC* were observed during SUP05 blooms. Investigators concluded that phage-encoded DsrC is likely to function in the sulfur cycling of infected SUP05, and may modulate vital electron transfer reactions. Evidence indicating increased viral infection of SUP05 with increasing depth, and decreasing O_2 levels, underlines the potential significance of this finding [16].

Virally-encoded genes associated with carbon uptake have also been identified in both model experimental systems and viral metagenomes, including those involved in uptake and metabolism of amino acids (*speD*, *cysK/M*, *metK*, *dapC*) and carbohydrates (*manA*, *rpiB*, *glgA*) [30,53]. Cyanophage genomes include genes (*talC*, *zwf*, *gnd*) to divert carbon towards the pentose phosphate pathway by converting glyceraldehyde-3P to fructose-6P, which is subsequently converted to reducing power and the synthesis of dNTPs for phage replication [10]. Viral metagenomics also identified other virally encoded genes involved in glycolysis (*manA*) and a glycogen synthase (*glgA*), with the latter identified in all examined viral metagenomes [53]. In this work, Hurwitz and colleagues postulated *glgA* was used to trigger a starvation response in the host in order to push carbon through non-glycolytic pathways that promote dNTP biosynthesis. Manipulation of succinate through the glyoxylate shunt is thought to facilitate energy production at the expense of amino acid synthesis, particularly in nutrient limited, deep ocean samples. Intracellular carbon is also redirected during viral infection for energy via virally-encoded genes for the Entner-Doudoroff pathway, the TCA cycle and fatty acid metabolism [53].

So how does expression of viral AMGs during infection influence cells at the fundamental level of metabolite synthesis and utilisation? The influence of viral hijacking at the metabolite level lacks a representative study in marine systems, but De Smet and colleagues performed an exemplary study in *Pseudomonas aeruginosa* PAO1 and compared responses across host-virus infections between one host and six different phages [12]. Following infection, concentration of 92 out 375 detectable host metabolites were significantly altered, showing a major rewiring of host metabolism. Only 9 of these metabolites, all associated with nucleotide metabolism, were altered by all six tested phages. This indicates that beyond nucleotide synthesis for viral replication, the impact of viral hijacking on host metabolism is not conserved even within a single host species.

Larger phage genomes encoded a larger suite of metabolic machinery for viral production, whereas smaller genomes relied more on host machinery and scavenging of resources. Phages which encoded one or more RNA polymerase shut down host transcription, and initiated their own transcription machinery. Phages with smaller genomes rewired host RNA polymerases directly to increase promoter specificity towards phage-specific promoters. Interestingly, hijacking of host metabolism appeared to favour pyrimidine production, with purine synthesis enriched only in phage YuA. Infection with YuA (genome size of 58.6 kb) also resulted in major depletion of cellular resources, recycling them for phage synthesis. In comparison, phage phiKZ (which has a 280kbp genome encoding 306 open reading frames) had almost no impact on host metabolite concentrations [12].

Efficiency in host hijacking is thus not universal, and tends to be higher in infections between specialist phages and their preferred host (which are the focus of many host-phage model systems). Generalist phages that infect multiple hosts tend to have less efficient infections, fail to completely suppress host translation and transcription, have longer latent periods and decoupled translation and transcription [12,13]. The 'plunder and pillage' strategy was not observed in a generalist phage infection of *Cellulophaga baltica* [13], and it is possible that utilisation of AMGs for hijacking of host metabolism is not a feature of generalist infections. Yet, members of the abundant marine Myoviridae infecting *Synechococcus* and *Prochlorococcus* include many generalists, some capable of infecting both genera [54,55]. The fitness advantage to adopting a broad host range, at the expense of efficient infection makes ecological sense when one considers that temporal patterns in marine environments often result in transition from *Synechococcus*-dominated to *Prochlorococcus*-dominated communities as nutrient availability waxes and wanes over seasonal, depth and diel gradients [56–58]. Furthermore, recent evidence from an experimental evolution experiment suggests that infection of sub-optimal hosts increases mutation rates and diversification of phage populations compared to those infecting optimal hosts [59].

Batten down the hatches: Increased viral fitness through Increased host fitness

From the perspective of viral fitness, the benefit of driving host energy and metabolites towards viral production during lytic infection only confers an advantage if viral progeny released following a lytic event can successfully infect new, actively growing hosts. Imagine a band of naïve pirates in the doldrums of a seldom-travelled ocean

gyre. They seize a ship, kill the crew and strip its cargo with alarming efficiency, before scuppering the vessel and sailing off in rowboats. With no land in sight, nor new vessel to capture, their career as pirates would be short and they would soon succumb to the tropical sun, deprived of life-giving resources. In contrast, a savvy band of pirates would instead remain aboard the seized vessel, secure the mainmast during storms that may arise, ration the rum and steer her towards more profitable waters. Many marine viruses display a similar 'batten down the hatches' strategy by foregoing a lytic life cycle and instead increasing the fitness of the infected cell phenotype through metabolic manipulation (Figure 1b). Here, instead of producing viral progeny, the infecting virus is maintained either by integration into the host genome (prophage) or as an extrachromosomal element to convert the host into a lysogen. Lysogeny includes a broad range of mechanisms including chronic infection (slow release of viral particles without killing the host), pseudolysogeny (simultaneous high viral production without host lysis) and polylysogeny (infection of multiple viruses within the same host) (reviewed in [21]). Numerous studies of the prevalence of lysogeny in marine systems all suggest that lysogeny is favoured in low productivity environments [21,60,61]. Chemical induction of lytic viral production in natural marine communities using mitomycin C showed the switch only occurred at cellular concentrations > 10^6 cells per mL [62]. A significant decrease in viral abundance has been observed at depth, corresponding to host abundance an order of magnitude lower than those at the surface [63]. These lower virus-to-microbe ratios are often interpreted as evidence of increased lysogeny. A recent meta-analysis of virus-to-microbe ratios across global oceans showed that this ratio varies from 1.4 to 160 and suggested that host-virus ratios are shaped by complex feedback mechanisms between nutrient availability, evolutionary history and selection pressures [64].

Regardless of the mechanism used to maintain the virus within the host during lysogeny, the effect of phage on host phenotype has been extensively studied in important pathogens such as *E. coli* [65,66], *Vibrio cholerae* [67,68], *Vibrio harveyi* [69,70]*, Staphylococcus aureus [71]* and *Listeria monocytogenes [72]* (reviewed in [73,74]) (Table 1). However, relatively few studies into lysogeny in marine host-virus model systems have been performed. When Yu and colleagues isolated a *Pseudoalteromonas* strain from Arctic sea ice containing a filamentous phage, they noticed it had lower growth rates and cell density, and lower tolerance of NaCl and reactive oxygen species than when it was cured of the infection. Transcriptional analysis showed downregulation of succinyl-CoA synthetase and succinate dehydrogenase indicating virally-mediated suppression of central carbon metabolism. However, presence of the phage increased host motility. They postulated that the presence of the phage increased host fitness during the nutrient-limited polar winters by slowing down host metabolism and increasing its capacity to find new

nutrient sources in the heterogeneous structure of the sea ice in which it lived [75]. Using metagenomic analyses, Brum and colleagues postulated that a similar mechanism increased host fitness in Antarctic bacterial communities during times of low nutrients and explained an observed seasonal prevalence of lysogeny prior to the summer blooms [76]. The underpinning mechanisms are possibly similar to those observed in the *E. coli* phage λ. This lysogen maintains integration in the host genome via a phage-encoded repressor known as *cI*. *cI* also represses the host gene *pckA,* which encodes a protein for the conversion of oxaloacetate to phosphoenolpyruvate. Thus, lysogeny results in a decoupling of central carbon metabolism from cellular synthesis, reducing host growth rate and potentially conferring a selective advantage in hosts within nutrient-poor environments [66]. Integration of the viral genome into the host genome can interrupt metabolic genes and effectively act as regulatory mechanisms, either at the level of the individual cell by repeated integration and excision, or at the population level by non-reversible lysogeny suppressing genes in a subpopulation [74]. Although there are no known examples of the former in marine bacteria, viruses infecting the cyanobacteria *Anabena* spp. and *Nostoc* spp. appear to regulate nitrogen-fixation through active lysogeny. Here, prophages interrupting N-fixation genes (*nifD*, *fdxN* and *hupL*) are excised from the genome during N-limitation, re-activating the genes (reviewed in [74]). Many metabolic genes identified in viral metagenomes are predicted to confer a selective advantage to hosts including sulfur oxidation genes [16,50] and genes associated with adaptation to high-pressures associated with depth [30]. One study of viral metagenomes from hydrothermal vents identified virally-encoded genes associated with pyrimidine, alanine, aspartate, glutamine, nitrogen, amino and nucleotide sugar metabolism. Pathway analysis suggested that viral AMGs allowed for branched metabolic pathways to alternative products that would provide additional metabolic flexibility to the host, thus increasing host fitness [49].

Metabolic false flags

In March 1723, the pirate Captain Low approached a Spanish merchant ship in the Bay of Honduras under the Spanish colors. Once they drew near the vessel, they:

'*hauled them down, hoisted up their black flag, fired a broadside and boarded her*' [77]

Indeed, it was commonplace for pirates to sail under false flags of different countries in order to prevent their targets from identifying them as a threat until it was too late. Similarly, viruses encode genetic tools to prevent a hijacked host from recognising an infection and taking appropriate action. As internal concentrations of cellular substrates

289 decrease, cells can undergo a 'stringent response' where they down-regulate growth functions and, in some cases,
290 initiate programmed cell death, regulated by the toxin-antitoxin pair MazEF. Nutrient limitation results in uncharged
291 tRNAs, which triggers increasing concentrations of guanosine tetraphosphate (ppGpp). This in turn inhibits RNA
292 transcription and promotes transcription of the global stress response regulator RpoS, shifting the cell into stationary
293 phase [78]. Increasing concentrations of ppGpp also increases production of MazF, a toxin that inhibits protein
294 synthesis (resulting in cell dormancy). In some circumstances, MazF also initiates programmable cell death, with
295 different mechanisms used under different stressors such as antibiotic treatment or DNA damage [79,80].
296 Programmable cell death is thought to sacrifice a large proportion of a population, so that a small sub-population
297 can survive by recycling released nutrients [80]. Programmable cell death regulated by MazEF has been shown to
298 play a role in the survival of a bacterial population against phage infection. Hazan and Engelberg-Kulka showed
299 that 400 times more viral progeny were produced in Δ*mazEF E. coli* cells infected with phage P1 compared to wild-
300 type cells. In addition, when lysogens were mixed with non-lysogens, the Δ*mazEF* cells were susceptible to infection
301 and lysed, whereas the non-lysogen wild-type cells were not infected by the induced phage. They suggested that it
302 is likely the wild-type lysogens were killed by MazEF, preventing viral replication to the benefit of the population
303 [81]. Similar observations of the role of toxin-antitoxin mechanisms for phage resistance were observed in *Erwinia*
304 *carotovora*, where expression of the toxin-antitoxin system conferred resistance to a broad range of viruses [82].
305
306 Given its dual role as both a regulator of the stringent response and of MazEF-regulated resistance and cell death,
307 increasing cellular levels of ppGpp pose a clear threat to viral replication. Many viruses are dependent on host RNA
308 transcription for replication, and viral replication has been shown to be drastically reduced or suspended in infected
309 *E. coli* cells that enter stationary phase [83]. Cell death limits the spread of phages through a population by reducing
310 host density and purges lysogens from the population. Thus, is of little surprise that in the arms-race between viruses
311 and their hosts, the viruses have commandeered a ppGpp regulator to counter host defence mechanisms. MazG has
312 been shown to decrease cellular concentrations of ppGpp in *E. coli* and thus repress production of MazF [79]. Thus
313 in the host MazG serves as a switch to recover from a starvation response upon return to nutrient replete conditions
314 and to abort programmable cell death. *mazG* is common in viral genomes from marine environments and has been
315 reported in viruses infecting both heterotrophs [84,85] and phototrophs [14,86]. The oligotrophic nature of vast
316 areas of the ocean make it likely that a starvation state for host cells is the norm, resulting in high cellular
317 concentrations of ppGpp and minimal transcription. It is likely that phages overcome this limitation by using virally-
318 encoded MazG to deplete cellular ppGpp concentrations and thus force the infected cell to respond as if it were

nutrient-replete, enabling transcription so that it may be hijacked for viral replication [87]. In addition, suppression of programmable cell death may prevent the host population from sacrificing itself in order to limit viral infection. Recent work in has shown that phages can utilise host quorum sensing to coordinate a lytic-lysogenic switch using a phage-derived oligopeptide signal [88] . Given that programmable cell death has been shown to be orchestrated through quorum sensing [89], and that suppression of *mazEF* expression increases host resistance to phages, it is conceivable that infecting viruses can use MazG to manipulate ppGpp concentrations and thus maintain the susceptibility of hosts within a population to subsequent infection by viral progeny. In pirate terms, viruses have evolved the capacity to sail under a false flag, disabling host alarm systems until it is too late. Further experimental evidence of the use of MazG and its effects on marine populations is required to explore whether this phenomenon is observed in nature.

Future understanding of host-virus interactions

The current understanding of how viruses hijack host metabolism during infection is the result of both culturing experiments in model systems; and culture-independent techniques such as viral metagenomics. Culture-dependant techniques have significant advantages: Model systems enable us to study the relationship between host and viruses in controlled conditions; viral replication cycles and critical parameters (e.g. host range; burst size) can be defined, and the functionality of viral genes may be determined *in vitro*. In culture, predator-prey interactions can be isolated from those occurring in complex microbial communities. This reduction in complexity allows for investigation into how transcriptomes, proteomes and metabolites are altered during infection and thus enable a systems-biology approach to understanding complex metabolic cascades and regulation (e.g. [12,13,25,45,90]. However, many important marine taxa have, to date, resisted efforts to culture them [91]. Consequently, our model systems of host-virus interactions in marine systems are limited to a handful of taxa, with a major focus on the cyanobacteria and a limited number of heterotrophic hosts.

For systems outside of cultured representatives, viral metagenomic studies to date have provided major insights into to viral taxonomic and functional distribution and diversity [30,32,47,53]. Understanding the extent and mechanisms of metabolic hijacking by marine viruses using metagenomic data comes with its own challenges: Firstly, in any viral metagenome there is the possibility of contaminating cellular DNA or randomly packaged host DNA encapsulated in gene transfer agent particles [32,92]. As viral genomes are assembled from short read data, there is the possibility of cellular functions being misassembled into viral contigs. In such

circumstances, the function may be interpreted as a novel AMG acquired by the virus to improve fitness, rather than as an artefact of bioinformatic processing [93,94], with a concomitant over-estimation in the degree of viral piracy that occurs in marine systems. Secondly, some of the most cosmopolitan and dominant viruses on Earth are challenging to assemble using short-read technology and are under-represented in marine viral metagenomes [95,96]. Complementary approaches to construct viral genomes from environmental samples such as single cell genomics and the development of long-read viral sequencing can alleviate these problems to some degree. Assembly of short-read data from a genome amplified from a single cell or single virus cell vastly reduces the complexity of the De Bruijn Graph and captures taxa missing from shotgun metagenomic approaches. This approach has successfully to identify new viruses and novel AMGs [16,17,96]. Long-read viral metagenomics [97] offers the potential to accurately identify putative AMGs as viral, rather than cellular contaminants, by capturing the gene neighbourhood of the AMG to reliably assess its viral origins. Capturing full length viral genomes on single reads is now technically feasible and will provide a powerful tool to explore how AMGs are distributed within viral populations and how their evolution is impacted by recombination, shown to be the dominant form of mutation in some phages [98]. Long-read metagenomics from cellular fractions will better quantify the extent of lysogeny within a population by capturing integrated viral genomes on single reads.

It is worth noting however, that no matter how sophisticated sequencing methods become, perhaps the greatest barrier to understanding how marine viruses influence cellular metabolism during infection lies in our extremely limited capacity to identify the function of viral genes, in both cultured isolates and genomes constructed from environmental DNA. Whilst machine learning approaches are rising to meet this challenge [32,99], one must consider that the scale of the 'known unknowns' is vast, with 63-93% of protein sequence space lacking functional or taxonomic annotations [100]. <1% of viral populations in the Pacific Ocean Virome had a closely related taxonomic representative in culture [101]. Methods to identify viral host range through computational methods such as correlative abundance [102] or nucleotide composition [103,104], are undergoing rapid development, but must be used cautiously for inferring ecological patterns [105]. Despite these challenges, the last decade has seen a dramatic improvement in our capacity to generate and interpret viral metagenomic data, largely driven by efforts to understand marine systems (e.g. [47]). This improvement has revealed a growing body of evidence identifying viruses as important agents in global carbon biogeochemical cycles, through: 1) lysis-

dependent nutrient cycling and increased community productivity [7-9]; 2) influences on host-substrate interactions through auxiliary metabolic genes, shaped through viral evolution (reviewed in [11]). Viral metagenomics allows microbial ecologists to directly ascribe such functions to viruses and provides relative quantitation of viral populations and genetic diversity in a way that is challenging from cellular metagenomic data. More recently, these methods have been applied to medical microbiology and have similarly established viruses as an equally important component of the human microbiome alongside their cellular counterparts (reviewed in [106]). Indeed, there is a growing consensus that our view of microbial ecology must put viruses centre stage as key players in shaping community structure and function. Increasing interest in the role of viruses in microbiomes will undoubtedly catalyse a feedback loop that energises the development of novel bioinformatic and culturing methods. Such tools will ultimately overcome the technical limitations previously outlined, revealing more of the metabolic capacity for cellular piracy encoded within viral sequence space.

Conclusion

The contemporary image of pirates is typically one of swashbuckling, romantic characters of Robert Louis Stevenson's *Treasure Island* and J.M. Barrie's *Peter Pan*. Piecemeal accounts and a lack of historical records has enabled myth and legend to supersede the violent and unpleasant reality written in Charles Johnson's *A General History of the Robberies and Murders of the Most Notorious Pyrates* [107]. Similarly, the relationships between marine viruses and their hosts have been considered through the paradigm of predator and prey, with much research focused on viruses as agents of top-down control. Our understanding of viral impact on host metabolism in marine systems is derived from a few model systems, or inferred from model systems of medically relevant pathogens that have evolved in nutrient-rich environments supporting high cellular densities. It is now clear however that lysogeny is common in marine systems and has the capacity for reconfiguration of host metabolism and increasing host fitness during frequent periods of nutrient limitation. Viral metagenomics continues to offer tantalising evidence of putative mechanisms for viral piracy, but even the most advanced machine learning approaches are limited by comparison to existing model systems. Thus, if we are to better understand the impact of viral metabolic hijacking on global biogeochemical cycles, advances in computational methods must be matched with recent efforts to improve the culturing important marine taxa [108,109] and their associated viruses, followed by *in vitro* determination of mechanism and impacts on host and viral fitness. Our efforts will be repaid in full as data is fed back into computational approaches to facilitate the

accurate translation of experimentally observed phenotypic changes into impacts on global biogeochemical cycling in our current models.

Competing interests

The authors declare that they have no competing interests

Funding

This work was funded by the BIOS-SCOPE award from Simons Foundation International, and by Natural Environment Research Council (NERC) awards NE/R010935/1 and NE/P008534/1. JWD and HB were funded by NERC GW4+ Doctoral Training Partnerships.

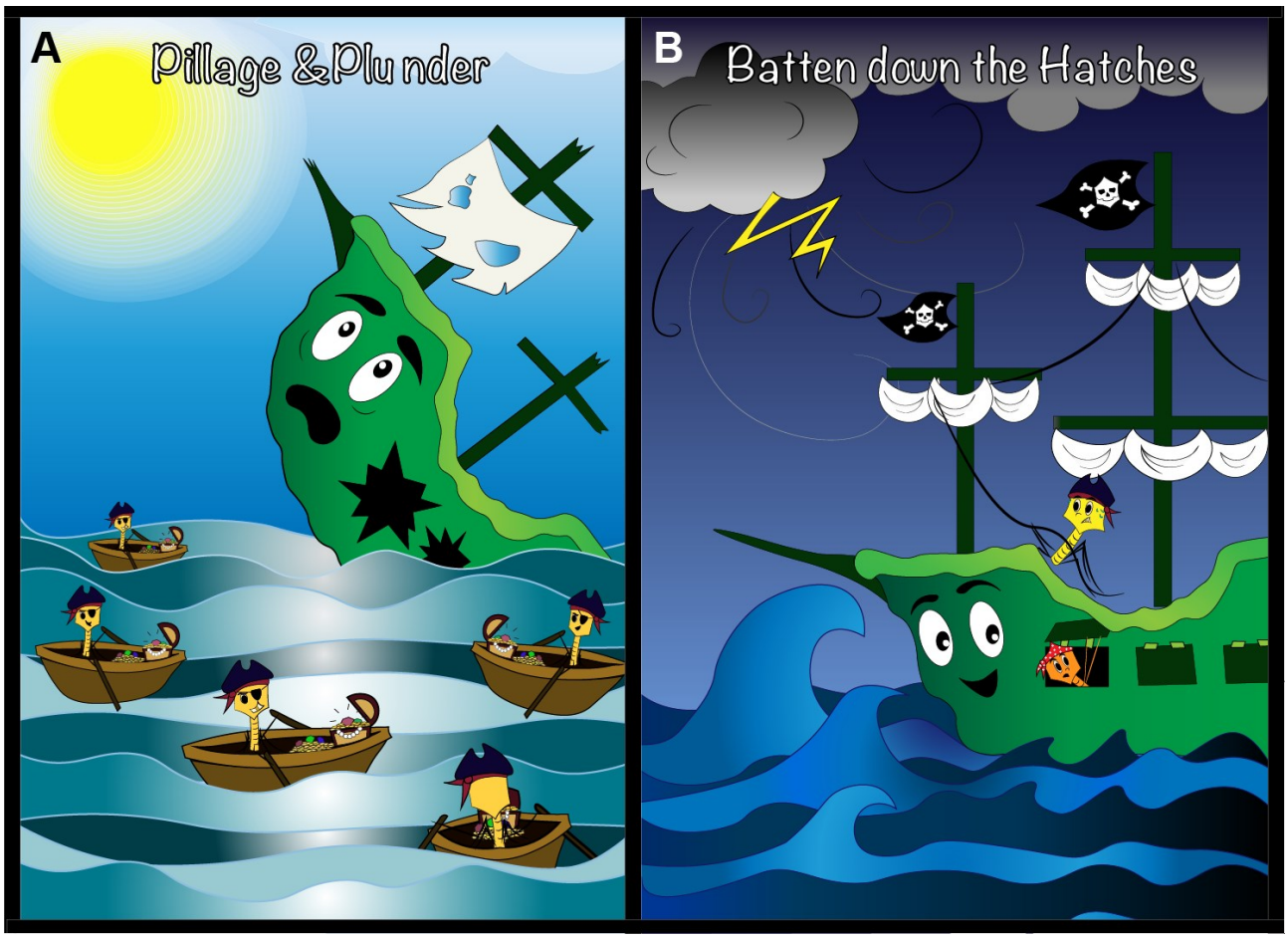
Authors' contributions

JWD, HB and BT wrote the manuscript; MA provided direction and edits for the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The authors would like to thank the anonymous reviewers for their critical and constructive comments. The authors would also like to thank Kema Malki (USF) for providing the artwork for Figure 1.

Figure 1: A cartoonist's depiction of the two types of host-virus interactions in the oceans. Under the 'Pillage and Plunder' model (A), the virus infects its host and redirects energy and substrates towards viral replication before lysing the cell and releasing viral progeny for further infections. Under the 'Batten down the hatches' model (B), viral fitness is improved by increasing host fitness, either by augmenting metabolic flexibility through virally-encoded genes, increasing resistance against other viruses, or by curbing host metabolism to maximise host survival under nutrient limitation.


A
Pillage &Plu nder
B
Batten down the Hatches

438

439

440 **Table 1: Examples of studies that reported/predicted phage-mediated alteration of metabolic function**
441 **in prokaryotic hosts.** Abbreviations not used in the main text: ORF: Open Reading Frame; *dut*: deoxyuridine
442 triphophatase; *radA*: DNA recombination protein; *pseI*: pseudaminic synthase; 2OG: 2-oxoglutarate; 2OG-
443 FeII oxygenase: Fe (II)-dependent oxygenase superfamily; *tctA*: tripartite tricarboxylate transporter; GTA:
444 Gene Transfer Agents.

445

Host/s	**Phage/s; cycle (if known)**	**Modification/Phenomena (molecular; physiological; phenotypic)**	**Observed effect (O) or Predicted effect (P) on host metabolism/ host survival**	**References**
Vibrio cholera	VPIΦ and CTXΦ; Lysogenic	Insertion of VPIΦ results in toxin-coregulated pilus (TCP) expression; TCP-facilitated CTXIΦ insertion into host genome	(O) Expression of cholera toxin	[67,68]
Escherichia coli	933W; Lysogenic to lytic switch	Induction of 933W prophages that encode for both shiga toxin (Stx) and a cleavable repressor	(O) Greatly increases *stx* gene expression, and therefore bacterial production and release of Stx.	[65]

Staphylococcus aureus	Φ13; Lysogenic	Integration of Φ13 genome with beta-toxin gene (*hlb*)	(O) Loss of beta-toxin expression (Note: beta-toxin is a sphingomyelinase)	[71]
Escherichia coli	λ ; Lysogenic	λ cI protein expression; cI binds to *pckA* regulatory region preventing transcription	(O) Suppression of phosphoenolpyruvate carboxykinase production & gluconeogenesis; reduced growth rate; predation avoidance	[66]
Vibrio harveyi 645; 20; 45	VHML; Lysogenic	Integration of VHML genome via transposition	(O) Broad suppression of substrate utilization; changes in d-gluconate utilization (625); c-glutamyl transpeptidase activity (20); and sulfatase activity (45)	[69,70]
Listonella	ΦHSIC;	Chromosomal	(O) Reduction in	[21,110]

pelagia	Pseudolysogenic	integration of prophage	substrate utilization	
Cellulophaga baltica MM#3	ΦS_M and ΦS_T; Lytic	On evolution of phage resistance: possible adaptation of amino acid transporters (likely phage receptors) in cell membrane	(O) Reduction in ability to metabolise various carbon sources, including many amino acids	[111]
Synechococcus WH8109	Cyanophage Syn9; lytic	Phage encoded carbon metabolism genes cp12, *talC, psbA, zwf, gnd*, and *nrdA/nrdB*, co-expressed in early infection; two-fold increase in NADPH/NADP ratio	(P) 'light reactions' decoupled from 'dark reactions; ATP & NADPH directed away from the Calvin Cycle. likely towards phage dNTP biosynthesis	[10]
Synechococcus WH8017	S-SM2; lytic	Phage encodes genes for photosynthesis (PSII): *psbA*; *psbD*, and carbon metabolism genes: *gnd*; *tal;*	(P) Photosynthesis augmented during infection; carbon redirected from glucose and amino acid	[42,53]

		zwf; CP12	production to ribose-5P and NAPDH generation (for dNTP synthesis), via PPP-mediated glucose reduction	
Cyanobacteria: various Prochlorococcus and Synechococcus strains	Various: 42 cultured cyanophages	88% of cyanophage genomes include *psbA*; 50% code for both *psbA* and *psbD* (PSII genes)	(P) Boost to phototrophic metabolism during infection.	[33]
Cyanobacteria	Un-cultured cyanophages	Phage-encoded photosystem I genes *psa*A, B, C, D, E, K and JF (from environmental samples)	(P) Channelling of reducing power from respiratory chain towards PSI, possibly for ATP generation	[34]
Prochlorococcus MIT9515	P-TIM68; lytic	Phage encoded photosystem I and II proteins incorporated into host membrane	(O) Photosynthetic capacity maintained; enhanced cyclic electron flow around PSI; (P) Generation	[37]

			additional ATP for phage replication	
Vibrios (including V. parahaemolyticus	KVP40; lytic	Phage ORFs code for: PhoH; putative pyridine nucleotide (NAD^+) salvage pathway, and hydrolysis of NADH	(P) Facilitates cross-membrane transport of NAD^+ precursors, NAD^+ synthesis, and cycling of NADH back to precursors.	[112]
Various (marine metagenomic Assemblages)	Various (marine viral metagenomes)	Most abundant putative viral-encoded enzymes: riboreductases; carboxylyases and transferases; *psbA* genes.	(P) Aids scavenging of host nucleotides (e.g. Riboreductases); supports host metabolism during the infection cycle (e.g., carboxylyases; transferases and D1 protein)	[113]
Various: from 22 'ultra-clean' viromes in POV dataset	Various; classified via protein cluster (PC) generation	35 carbon pathway AMGs, representing a near-full central carbon	(P) In oligotrophic environments, AMGs may redirect host	[53]

		metabolism gene complement.	carbon flux into energy production and the replication of viral DNA.	
Various: from 32 viromes in POV dataset	Various; classified via PC generation	32 new viral AMGs (9 core; 20 photic; 3 aphotic): 9 encode Fe-S cluster proteins and genes associated with DNA replication initiation (*DnaA*), DNA repair (*dut*; *radA*) and motility augmentation (*psel*).	(P) Fe-S cluster modulation may drive phage production (in the photic zone); Genes associated with DNA replication and repair, and motility augmentation could assist high-pressure deep-sea survival.	[30]
Various: 127 SAGs from uncultivated SUP05 bacteria	Various: 69 viral contigs (from SUP05 SAGs)	4 putative AMGs (encoded by 12 viral contigs): *phoH* (on a *bona fide* viral contig); 2OG; 2OG-FeII oxygenase, *tctA* (protein domain only); and *dsrC*.	(P) *dsrC* likely functional in SUP05 sulfur cycling; characterisation of viral DsrC needed to elucidate roles of viruses in modulating	[16]

			electron transfer during viral infection.	
Various: Actinobacteria, proteobacteria (α; δ; γ) Bacteroidetes, Cyanobacteria, Deferribacteres	Various, inc. members of T4 (superfamily) and T7 (genus)	243 putative AMGs (95 previously known [6]) including *dsr*C (11 genes), *sox*YZ (4 genes), both originating from T4 superfamily; *P-II* (encodes a nitrogen metabolism regulator) and *amo*C (encodes ammonia monooxygenase sub-unit)	(P) Viral roles in: Sulfur oxidation, via Dsr and Sox pathways; Nitrogen cycling (influenced by *P-II*), with potential for alternative pathways of N and NH_3 uptake during N starvation, and NH_3 oxidation via *amo*C).	[47]
Various: 113 genomes (marine bacteria)	Various: 64 pro-phage-like elements (21 GTAs)	High relative incidence of transcriptional regulatory and repressor-like proteins in putative prophages (comparison: lytic phages)	(P) Suppresses non-essential host metabolic activities in unfavourable environments/periods	[21]

Listeria monocytogenes	'A118-like prophage' (reversible excision)	*comK* gene, encoding *L. monocytogenes* competence system master regulator, is activated by the excision of A118-like prophage	(O) A118-like prophage is excised only when a *L. monocytogenes* cell is engulfed by a phagosome: the host's activated competence system facilitates escape, after which prophage reintegrate with host *comK* gene, deactivating host's competence system	[74]
Anabaena spp.; Nostoc spp.	Non-infective 'prophages' (x3; non-reversible excision)	Recombinases (prophage-encoded) act to excise prophages from 3 host genes that are involved in nitrogen fixation (*nifD*; *fdxN*; *hupL*)	(O) In low nitrogen environments, excision of prophages from host N-fixation genes enables conversion of host cell to form nitrogen-fixing heterocysts	[74]

Synechococcus elongatus	Cyanophage AS-1	Prevents normal ppGpp accumulation under nutrient limitation, and the corresponding expression of genes for starvation survival	(O) Inhibits the host's natural starvation response under nutrient limitation; (P) promotes metabolic activity otherwise undertaken only when food is plentiful, facilitating phage production in low nutrient conditions	[14,114]

446

447

448

449 **References**

450 1. Haldane JBS. What is Life? Lindsay Drummond; 1945.

451 2. Suttle CA. Viruses in the sea. *Nature*. 2005;437:356–61.

452 3. Falkowski PG, Fenchel T, Delong EF. The microbial engines that drive Earth's biogeochemical cycles. *Science*.
453 2008;320:1034–9.

454 4. Suttle CA. Marine viruses — major players in the global ecosystem. *Nat Rev Microbiol*. 2007;5:801–12.

455 5. Noble RT, Fuhrman JA. Rapid virus production and removal as measured with fluorescently labelled viruses as
456 tracers. *Appl Environ Microbiol*. 2000;66:3790–7.

457 6. Breitbart M. Marine Viruses: Truth or Dare. *Ann Rev Mar Sci*. 2012;4:425–48.

458 7. Middelboe M, Jorgensen NOG, Kroer N. Effects of viruses on nutrient turnover and growth efficiency of noninfected
459 marine bacterioplankton. *Appl Environ Microbiol*. 1996;62:1991–7.

460 8. Middelboe M, Jorgensen NOG. Viral lysis of bacteria: an important source of dissolved amino acids and cell wall
461 compounds. *J Mar Biol Assoc U K*. 2006;86:605–12.

462 9. Weitz JS, Stock CA, Wilhelm SW, Bourouiba L, Coleman ML, Buchan A, et al. A multitrophic model to quantify the
463 effects of marine viruses on microbial food webs and ecosystem processes. *ISME J.* 2015;9:1352–64.

464 10. Thompson LR, Zeng Q, Kelly L, Huang KH, Singer AU, Stubbe J, et al. Phage auxiliary metabolic genes and the
465 redirection of cyanobacterial host carbon metabolism. *Proc Natl Acad Sci USA*. 2011;108:16147–8.

466 11. Hurwitz BL, U'Ren JM. Viral metabolic reprogramming in marine ecosystems. *Curr Opin Microbiol*. 2016;31:161–
467 8.

468 12. De Smet J, Zimmermann M, Kogadeeva M, Ceyssens P-J, Vermaelen W, Blasdel B, et al. High coverage
469 metabolomics analysis reveals phage-specific alterations to *Pseudomonas aeruginosa* physiology during infection.
470 *ISME J*.; 2016;10:1823–35.

471 13. Howard-Varona C, Hargreaves KR, Solonenko NE, Markillie LM, White RA 3rd, Brewer HM, et al. Multiple
472 mechanisms drive phage infection efficiency in nearly identical hosts. *ISME J*. 2018;12:1605-1618.

473 14. Bryan MJ, Burroughs NJ, Spence EM, Clokie MR, Mann NH, Bryan SJ. Evidence for the intense exchange of
474 MazG in marine cyanophages by horizontal gene transfer. *PLoS One*. 2008;3:e2048.

475 15. Zeng Q, Chisholm SW. Marine Viruses Exploit Their Host's Two-Component Regulatory System in Response to
476 Resource Limitation. *Curr Biol*. 2012;22:124–8.

477 16. Roux S, Hawley AK, Beltran MT, Scofield M, Schwientek P, Stepanauskas R, et al. Ecology and evolution of
478 viruses infecting uncultivated SUP05 bacteria as revealed by single-cell- and meta-genomics. *Elife*. 2014;3:e03125.

479 17. Labonté JM, Swan BK, Poulos B, Luo H, Koren S, Hallam SJ, et al. Single-cell genomics-based analysis of virus–
480 host interactions in marine surface bacterioplankton. *ISME J*. 2015; 9(11):2386-2399.

481 18. Jiang SC, Paul JH. Significance of Lysogeny in the Marine Environment: Studies with Isolates and a Model of
482 Lysogenic Phage Production. *Microb Ecol*. 1998;35:235–43.

483 19. Stopar D, Cerne A, Zigman M, Poljsak-Prijatelj M, Turk V. Viral abundance and a high proportion of lysogens

484 suggest that viruses are important members of the microbial community in the Gulf of Trieste. *Microb Ecol*. 2004;47:1–
485 8.

486 20. Leitet C, Riemann L, Hagström Å. Plasmids and prophages in Baltic Sea bacterioplankton isolates. *J Mar Biol*
487 *Assoc U K. Cambridge University Press*; 2006;86:567–75.

488 21. Paul JH. Prophages in marine bacteria: dangerous molecular time bombs or the key to survival in the seas? *ISME J*.
489 2008;2:579–89.

490 22. Munson-McGee JH, Peng S, Dewerff S, Stepanauskas R, Whitaker RJ, Weitz JS, et al. A virus or more in (nearly)
491 every cell: ubiquitous networks of virus–host interactions in extreme environments. *ISME J*. 2018; 12:1706-1714.

492 23. Williamson SJ, McLaughlin MR, Paul JH. Interaction of the PhiHSIC virus with its host: lysogeny or
493 pseudolysogeny? *Appl Environ Microbiol*. 2001;67:1682–8.

494 24. Howard-Varona C, Hargreaves KR, Abedon ST, Sullivan MB. Lysogeny in nature: mechanisms, impact and
495 ecology of temperate phages. *ISME J*. 2017; 1511-1520.

496 25. Doron S, Fedida A, Hernández-Prieto MA, Sabehi G, Karunker I, Stazic D, et al. Transcriptome dynamics of a
497 broad host-range cyanophage and its hosts. *ISME J*. 2016;10:1437–55.

498 26. Horvitz HR. Bacteriophage T4 mutants deficient in alteration and modification of the *Escherichia coli* RNA
499 polymerase. *J Mol Biol.* 1974;90:739–50.

500 27. Koch T, Raudonikiene A, Wilkens K, Rüger W. Over expression, Purification, and Characterization of the ADP-
501 Ribosyltransferase (gpAlt) of Bacteriophage T4: ADP-Ribosylation of *E. coli* RNA Polymerase Modulates T4 "Early"
502 Transcription. *Gene Expr*. 1995;4:253–64.

503 28. De Smet J, Hendrix H, Blasdel BG, Danis-Wlodarczyk K, Lavigne R. *Pseudomonas* predators: understanding and
504 exploiting phage-host interactions. *Nat Rev Microbiol*. 2017; 15:517-530

505 29. Breitbart M, Thompson LR, Suttle CA, Sullivan MB. Exploring the Vast Diversity of Marine Viruses.
506 Oceanography . *Oceanography Society*; 2007;20:135–9.

507 30. Hurwitz BL, Brum JR, Sullivan MB. Depth-stratified functional and taxonomic niche specialization in the "core"
508 and "flexible" Pacific Ocean Virome. *ISME J*. 2015;9:472–84.

509 31. Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res*. 2000;28:27–30.

510 32. Brum JR, Sullivan MB. Rising to the challenge: accelerated pace of discovery transforms marine virology. *Nat Rev*
511 *Microbiol*. 2015;13:147–59.

512 33. Sullivan MB, Lindell D, Lee JA, Thompson LR, Bielawski JP, Chisholm SW. Prevalence and evolution of core
513 photosystem II genes in marine cyanobacterial viruses and their hosts. *PLoS Biol*. 2006;4:e234.

514 34. Sharon I, Alperovitch A, Rohwer F, Haynes M, Glaser F, Atamna-Ismaeel N, et al. Photosystem I gene cassettes are
515 present in marine virus genomes. *Nature*. 2009;461:258–62.

516 35. Bragg JG, Chisholm SW. Modeling the fitness consequences of a cyanophage-encoded photosynthesis gene. *PLoS*
517 *One*. 2008;3:e3550.

518 36. Hellweger FL. Carrying photosynthesis genes increases ecological fitness of cyanophage *in silico*. *Environ*
519 *Microbiol*. Wiley Online Library; 2009;11:1386–94.

520 37. Fridman S, Flores-Uribe J, Larom S, Alalouf O, Liran O, Yacoby I, Salama F, BailleuI B, Rappaport F, Ziv T,
521 Sharon I, Cornejo-Castillo FM, Philosof A, Dupont CL, Sánchez P, Acinas SG, Rohwer FL, Lindell D, Béjà O. A
522 myovirus encoding both photosystem I and II proteins enhances cyclic electron flow in infected *Prochlorococcus* cells.
523 *Nat Microbiol*. 2017; 2(10): 1350-1357.

524 38. Tyrrell T. The relative influences of nitrogen and phosphorus on oceanic primary production. *Nature*.
525 1999;400:525–31.

526 39. Benitez-Nelson CR. The biogeochemical cycling of phosphorus in marine systems. *Earth-Sci Rev*. 2000;51:109–35.

527 40. Behrenfeld MJ, O'Malley RT, Siegel DA, McClain CR, Sarmiento JL, Feldman GC, et al. Climate-driven trends in
528 contemporary ocean productivity. *Nature*. 2006;444:752–5.

529 41. Kelly L, Ding H, Huang KH, Osburne MS, Chisholm SW. Genetic diversity in cultured and wild marine
530 cyanomyoviruses reveals phosphorus stress as a strong selective agent. *ISME J*. 2013;7:1827–41.

531 42. Sullivan MB, Huang KH, Ignacio-Espinoza JC, Berlin AM, Kelly L, Weigele PR, DeFrancesco AS, Kern SE,
532 Thompson LR, Young S, Yandava C, Fu R, Krastins B, Chase M, Sarrachino D, Osburne MS, Henn MR, Chisholm
533 SW. Genomic analysis of oceanic cyanobacterial myoviruses compared with T4-like myoviruses from diverse hosts and
534 environments. *Environ Microbiol.* 2010;12:3035–56.

535 43. Sowell SM, Wilhelm LJ, Norbeck AD, Lipton MS, Nicora CD, Barofsky DF, Carlson CA, Smith RD, Giovannoni
536 SJ. Transport functions dominate the SAR11 metaproteome at low-nutrient extremes in the Sargasso Sea. *ISME J.*

2009;3:93–105.

44. Goldsmith DB, Crosti G, Dwivedi B, McDaniel LD, Varsani A, Suttle CA, Weinbauer MG, Sandaa RA, Breitbart M. Development of *phoH* as a Novel Signature Gene for Assessing Marine Phage Diversity. *Appl Environ Microbiol*. 2011;77:7730–9.

45. Lindell D, Jaffe JD, Coleman ML, Futschik ME, Axmann IM, Rector T, Kettler G, Sullivan MB, Steen R, Hess WR, Church GM, Chisholm SW. Genome-wide expression dynamics of a marine virus and host reveal features of co-evolution. *Nature*. 2007;449:83–6.

46. Tetu SG, Brahamsha B, Johnson DA, Tai V, Phillippy K, Palenik B, Paulsen IT. Microarray analysis of phosphate regulation in the marine cyanobacterium *Synechococcus sp*. WH8102. *ISME J*. 2009;3:835–49.

47. Roux S, Brum JR, Dutilh BE, Sunagawa S, Duhaime MB, Loy A, Poulos BT, Solonenko N, Lara E, Poulain J, Pesant S, Kandels-Lewis S, Dimier C, Picheral M, Searson S, Cruaud C, Alberti A, Duarte CM, Gasol JM, Vaqué D, Tara Ocean Coordinators, Bork P, Acinas SG, Wincker P, Sullivan MB. Ecogenomics and potential biogeochemical impacts of globally abundant ocean viruses. *Nature*. 2016;537:689–93.

48. Ahlgren NA, Fuchsman CA, Rocap G, Fuhrman JA. Discovery of several novel, widespread, and ecologically distinct marine Thaumarchaeota viruses that encode *amoC* nitrification genes. *ISME J*. 2018; doi: 10.1038/s41396-018-0289-4

49. He T, Li H, Zhang X. Deep-Sea Hydrothermal Vent Viruses Compensate for Microbial Metabolism in Virus-Host Interactions. *MBio*. 2017;8. doi: 10.1128/mBio.00893-17

50. Anantharaman K, Duhaime MB, Breier JA, Wendt KA, Toner BM, Dick GJ. Sulfur oxidation genes in diverse deep-sea viruses. *Science*. 2014;344:757–60.

51. Chow CET, Winget DM, White RA, Hallam SJ, Suttle CA. Combining genomic sequencing methods to explore viral diversity and reveal potential virus-host interactions. *Front Microbiol*. 2015;6(265): doi: 10.3389/fmicb.2015.00265

52. Wright JJ, Konwar KM, Hallam SJ. Microbial ecology of expanding oxygen minimum zones. *Nat Rev Microbiol*. 2012;10:381–94.

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

564 54. Dekel-Bird NP, Sabehi G, Mosevitzky B, Lindell D. Host-dependent differences in abundance, composition and
565 host range of cyanophages from the Red Sea. *Environ Microbiol*. 2015;17:1286–99.

566 55. Sullivan MB, Waterbury JB, Chisholm SW. Cyanophages infecting the oceanic cyanobacterium *Prochlorococcus*.
567 *Nature*. 2003;426:584–584.

568 56. Landry MR, Kirshtein J, Constantinou J. Abundances and distributions of picoplankton populations in the central
569 equatorial Pacific from 12 N to 12 S, 140 W. *Deep Sea Res Part 2 Top Stud Oceanogr*. Elsevier; 1996;43:871–90.

570 57. Treusch AH, Vergin KL, Finlay LA, Donatz MG, Burton RM, Carlson CA, Giovannoni SJ. Seasonality and vertical
571 structure of microbial communities in an ocean gyre. *ISME J.* 2009;3:1148–63.

572 58. Giovannoni SJ, Vergin KL. Seasonality in ocean microbial communities. *Science*. 2012;335:671–6.

573 59. Enav H, Kirzner S, Lindell D, Mandel-Gutfreund Y, Béjà O. Adaptation to sub-optimal hosts is a driver of viral
574 diversification in the ocean. *Nat Commun*. 2018;9(1): doi: 10.1038/s41467-018-07164-3

575 60. Jiang SC, Paul JH. Seasonal and diel abundance of viruses and occurrence of lysogeny/bacteriocinogeny in the
576 marine environment. *Marine ecology progress series Oldendorf*. 1994;104:163–72.

577 61. Wommack KE, Colwell RR. Virioplankton: viruses in aquatic ecosystems. *Microbiol Mol Biol Rev*. 2000;64:69–
578 114.

579 62. Weinbauer MG, Suttle CA. Potential significance of lysogeny to bacteriophage production and bacterial mortality in
580 coastal waters of the Gulf of Mexico. *Appl Environ Microbiol*. 1996;62:4374–80.

581 63. De Corte D, Sintes E, Yokokawa T, Reinthaler T, Herndl GJ. Links between viruses and prokaryotes throughout the
582 water column along a North Atlantic latitudinal transect. *ISME J*. 2012;6:1566–77.

583 64. Wigington CH, Sonderegger D, Brussaard CPD, Buchan A, Finke JF, Fuhrman JA, Lennon JT, Middelboe M,
584 Suttle CA, Stock C, Wilson WH, Wommack KE, Wilhelm SW, Weitz JS. Re-examination of the relationship between
585 marine virus and microbial cell abundances. *Nature Microbiol*. 2016;15024.

586 65. Tyler JS, Mills MJ, Friedman DI. The operator and early promoter region of the Shiga toxin type 2-encoding
587 bacteriophage 933W and control of toxin expression. *J Bacteriol*. 2004;186:7670–9.

588 66. Chen Y, Golding I, Sawai S, Guo L, Cox EC. Population fitness and the regulation of *Escherichia coli* genes by
589 bacterial viruses. *PLoS Biol*. 2005;3:e229.

590 67. Waldor MK, Mekalanos JJ. Lysogenic conversion by a filamentous phage encoding cholera toxin. *Science*.
591 1996;272:1910–4.

592 68. Karaolis DK, Somara S, Maneval DR Jr, Johnson JA, Kaper JB. A bacteriophage encoding a pathogenicity island, a
593 type-IV pilus and a phage receptor in cholera bacteria. *Nature*. 1999;399:375–9.

594 69. Oakey HJ, Cullen BR, Owens L. A hypothetical model for VHML phage conversion of *Vibrio harveyi*. *Diseases in*
595 *Asian Aquaculture*. 2005;457–64.

596 70. Vidgen M, Carson J, Higgins M, Owens L. Changes to the phenotypic profile of *Vibrio harveyi* when infected with
597 the *Vibrio harveyi* myovirus-like (VHML) bacteriophage. *J Appl Microbiol*. 2006;100:481–7.

598 71. Coleman D, Knights J, Russell R, Shanley D, Birkbeck TH, Dougan G, Charles I. Insertional inactivation of the
599 *Staphylococcus aureus* β-toxin by bacteriophage φ13 occurs by site-and orientation-specific integration of the φ 13
600 genome. *Mol Microbiol*. 1991;5:933–9.

601 72. Rabinovich L, Sigal N, Borovok I, Nir-Paz R, Herskovits AA. Prophage excision activates *Listeria* competence
602 genes that promote phagosomal escape and virulence. *Cell*. 2012;150:792–802.

603 73. Brüssow H, Canchaya C, Hardt W-D. Phages and the evolution of bacterial pathogens: from genomic
604 rearrangements to lysogenic conversion. *Microbiol Mol Biol Rev*. 2004;68:560–602

605 74. Feiner R, Argov T, Rabinovich L, Sigal N, Borovok I, Herskovits AA. A new perspective on lysogeny: prophages
606 as active regulatory switches of bacteria. *Nat Rev Microbiol*. 2015;13:641–50.

607 75. Yu ZC, Chen XL, Shen QT, Zhao DL, Tang BL, Su HN, Wu ZY, Qin QL, Xie BB, Zhang XY, Yu Y, Zhou BC,
608 Chen B, Zhang YZ. Filamentous phages prevalent in *Pseudoalteromonas spp*. confer properties advantageous to host
609 survival in Arctic sea ice. *ISME J*. 2015;9:871–81.

610 76. Brum JR, Hurwitz BL, Schofield O, Ducklow HW, Sullivan MB. Seasonal time bombs: dominant temperate viruses
611 affect Southern Ocean microbial dynamics. *ISME J*. 2016;10:437–49.

612 77. Cordingly D. Under the black flag: The romance and the reality of life among the pirates. *Random House*
613 *Incorporated*; 2006.

614 78. Chatterji D, Ojha AK. Revisiting the stringent response, ppGpp and starvation signaling. *Curr Opin Microbiol*.
615 2001;4:160–5.

616 79. Gross M, Marianovsky I, Glaser G. MazG -- a regulator of programmed cell death in *Escherichia coli*. *Mol*
617 *Microbiol*. 2006;59:590–601.

618 80. Amitai S, Kolodkin-Gal I, Hananya-Meltabashi M, Sacher A, Engelberg-Kulka H. *Escherichia coli* MazF Leads to
619 the Simultaneous Selective Synthesis of Both "Death Proteins" and "Survival Proteins." *PLoS Genet*. 2009;5:e1000390.

620 81. Hazan R, Engelberg-Kulka H. *Escherichia coli* mazEF-mediated cell death as a defense mechanism that inhibits the
621 spread of phage P1. *Mol Genet Genomics*. 2004;272:227–34.

622 82. Fineran PC, Blower TR, Foulds IJ, Humphreys DP, Lilley KS, Salmond GPC. The phage abortive infection system,
623 ToxIN, functions as a protein-RNA toxin-antitoxin pair. *Proc Natl Acad Sci U S A*. 2009;106:894–9.

624 83. Bryan D, El-Shibiny A, Hobbs Z, Porter J, Kutter EM. Bacteriophage T4 Infection of Stationary Phase *E. coli*: Life
625 after Log from a Phage Perspective. *Front Microbiol*. 2016;7:1391.

626 84. Kang I, Oh HM, Kang D, Cho JC. Genome of a SAR116 bacteriophage shows the prevalence of this phage type in
627 the oceans. *Proc Natl Acad Sci U S A*. 2013;110:12343–8.

628 85. Duhaime MB, Wichels A, Waldmann J, Teeling H, Glöckner FO. Ecogenomics and genome landscapes of marine
629 *Pseudoalteromonas phage* H105/1. *ISME J*. 2011;5:107–21.

630 86. Sullivan MB, Coleman ML, Weigele P, Rohwer F, Chisholm SW. Three *Prochlorococcus* cyanophage genomes:
631 signature features and ecological interpretations. *PLoS Biol*. 2005;3:e144.

632 87. Clokie MRJ, Mann NH. Marine cyanophages and light. *Environ Microbiol*. 2006;8:2074–82.

633 88. Erez Z, Steinberger-Levy I, Shamir M, Doron S, Stokar-Avihail A, Peleg Y, et al. Communication between viruses
634 guides lysis-lysogeny decisions. *Nature*. 2017;541:488–93.

635 89. Kumar S, Kolodkin-Gal I, Engelberg-Kulka H. Novel quorum-sensing peptides mediating interspecies bacterial cell
636 death. *MBio*. 2013;4:e00314–13.

637 90. Howard-Varona C, Roux S, Dore H, Solonenko NE, Holmfeldt K, Markillie LM, Orr G, Sullivan MB. Regulation
638 of infection efficiency in a globally abundant marine *Bacteriodetes* virus. ISME J. 2017;11(1): doi:
639 10.1038/ismej.2016.81

640 91. Rappé MS, Giovannoni SJ. The uncultured microbial majority. *Annu Rev Microbiol*. 2003;57:369–94.

641 92. Lang AS, Beatty JT. Importance of widespread gene transfer agent genes in alpha-proteobacteria. *Trends Microbiol*.

642 2007;15:54–62.

643 93. Roux S, Krupovic M, Debroas D, Forterre P, Enault F. Assessment of viral community functional potential from
644 viral metagenomes may be hampered by contamination with cellular sequences. *Open Biol*. 2013;3:130160.

645 94. Enault F, Briet A, Bouteille L, Roux S, Sullivan MB, Petit MA. Phages rarely encode antibiotic resistance genes: a
646 cautionary tale for virome analyses. *ISME J*. 2017;11:237–47.

647 95. Roux S, Emerson JB, Eloe-Fadrosh EA, Sullivan MB. Benchmarking viromics: An *in silico* evaluation of
648 metagenome-enabled estimates of viral community composition and diversity. *PeerJ*; 2017: 5:e3817. doi:
649 10.7717/peerj.3817

650 96. Martinez-Hernandez F, Fornas O, Lluesma Gomez M, Bolduc B, de la Cruz Peña MJ, Martínez JM, Anton J, Gasol
651 JM, Rosselli R, Rodriguez-Valera F, Sullivan MB, Acinas SG, Martinez-Garcia M. Single-virus genomics reveals
652 hidden cosmopolitan and abundant viruses. *Nat Commun*. 2017;8:15892. doi: 10.1038/ncomms15892

653 97. Warwick-Dugdale J, Solonenko N, Moore K, Chittick L, Gregory AC, Allen MJ, Sullivan MB, Temperton B. Long-
654 read metagenomics reveals cryptic and abundant marine viruses. *bioRxiv*. 2018 [cited 2018 Jun 26]. p. 345041.
655 Available from: https://www.biorxiv.org/content/early/2018/06/12/345041

656 98. Kupczok A, Neve H, Huang KD, Hoeppner MP, Heller KJ, Franz CMAP, Dagan T. Rates of Mutation and
657 Recombination in Siphoviridae Phage Genome Evolution over Three Decades. *Mol Biol Evol*. 2018;35:1147–59.

658 99. Hurwitz BL, Westveld AH, Brum JR, Sullivan MB. Modeling ecological drivers in marine viral communities using
659 comparative metagenomics and network analyses. *Proc Natl Acad Sci U S A*. 2014;111:10714–9.

660 100. Hurwitz BL, Sullivan MB. The Pacific Ocean Virome (POV): A Marine Viral Metagenomic Dataset and
661 Associated Protein Clusters for Quantitative Viral Ecology. *PLoS One*. 2013;8:e57355.

662 101. Brum JR, Ignacio-Espinoza JC, Roux S, Doulcier G, Acinas SG, Alberti A, Chaffron S, Cruaud C, de Vargas C,
663 Gasol JM, Gorsky G, Gregory AC, Guidi L, Hingamp P, Iudicone D, Not F, Ogata H, Pesant S, Poulos BT, Schwenck
664 SM, Speich S, Dimier C, Kandels-Lewis S, Picheral M, Searson S, Tara Oceans Coordinators, Bork P, Bowler C,
665 Sunagawa S, Wincker P, Karsenti E, Sullivan MB. Ocean plankton. Patterns and ecological drivers of ocean viral
666 communities. *Science*. 2015;348:1261498.

667 102. Needham DM, Sachdeva R, Fuhrman JA. Ecological dynamics and co-occurrence among marine phytoplankton,
668 bacteria and myoviruses shows microdiversity matters. *ISME J.* 2017; 11(7): 1614-1629.

669 103. Ahlgren NA, Ren J, Lu YY, Fuhrman JA, Sun F. Alignment-free oligonucleotide frequency dissimilarity measure
670 improves prediction of hosts from metagenomically-derived viral sequences. *Nucleic Acids Res*. 2016;45:39–53.

671 104. Galiez C, Siebert M, Enault F, Vincent J, Söding J. WIsH: who is the host? Predicting prokaryotic hosts from
672 metagenomic phage contigs. *Bioinformatics*. 2017;33:3113–4.

673 105. Coenen AR, Weitz JS. Limitations of Correlation-Based Inference in Complex Virus-Microbe Communities.
674 *mSystems*. 2018;3(4). doi: 10.1128/mSystems.00084-18

675 106. Mirzaei MK & Maurice CF. Ménage à trois in the human gut: interactions between host, bacteria and phages. *Nat*
676 *Rev Microbiol*. 2017; 15; 397-408.
677
678 107. Defoe D, Johnson C. A General History of the Robberies and Murders of the Most Notorious Pyrates, and Also
679 Their Policies, Discipline and Government, from Their First Rise and Settlement in the Island of Providence, in 1717, to
680 the Present Year 1724. 1724.

681 108. Henson MW, Pitre DM, Weckhorst JL, Lanclos VC, Webber AT, Thrash JC. Artificial Seawater Media Facilitate
682 Cultivating Members of the Microbial Majority from the Gulf of Mexico. *mSphere*. 2016;1(3): doi:
683 10.1128/mSphere.00124-16

684 109. Kang I, Kim S, Islam MR, Cho J-C. The first complete genome sequences of the acI lineage, the most abundant
685 freshwater Actinobacteria, obtained by whole-genome-amplification of dilution-to-extinction cultures. *Sci Rep*.
686 2017;7:42252.

687 110. Williamson SJ, Paul JH. Environmental factors that influence the transition from lysogenic to lytic existence in the
688 phiHSIC/*Listonella pelagia* marine phage-host system. *Microb Ecol*. 2006;52:217–25.

689 111. Middelboe M, Holmfeldt K, Riemann L, Nybroe O, Haaber J. Bacteriophages drive strain diversification in a
690 marine *Flavobacterium*: implications for phage resistance and physiological properties. *Environ Microbiol*.
691 2009;11:1971–82.

692 112. Miller ES, Heidelberg JF, Eisen JA, Nelson WC, Durkin AS, Ciecko A, Feldblyum TV, White O, Paulsen IT,
693 Nierman WC, Lee J, Szczypinksi B, Fraser CM. Complete genome sequence of the broad-host-range vibriophage
694 KVP40: comparative genomics of a T4-related bacteriophage. *J Bacteriol.* 2003;185:5220–33.

695 113. Angly FE, Felts B, Breitbart M, Salamon P, Edwards RA, Carlson C, et al. The marine viromes of four oceanic
696 regions. *PLoS Biol*. 2006;4:e368.

114. Borbély G, Kaki C, Gulyás A, Farkas GL. Bacteriophage infection interferes with guanosine 3’-diphosphate-5'-diphosphate accumulation induced by energy and nitrogen starvation in the cyanobacterium *Anacystis nidulans*. *J Bacteriol*. 1980;144:859–64.