Viruses manipulate the marine environment

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Marine viruses affect Bacteria, Archaea and eukaryotic organisms and are major components of the marine food web. Most studies have focused on their role as predators and parasites, but many of the interactions between marine viruses and their hosts are much more complicated. A series of recent studies has shown that viruses have the ability to manipulate the life histories and evolution of their hosts in remarkable ways, challenging our understanding of this almost invisible world.

Marine virology has traditionally focused on two areas: viruses as pathogens of aquatic organisms, and phage-driven dynamics of the marine microbial food web. Both of these influence global biogeochemistry and host evolution, and the former also has important economic and conservation implications. For example, two common marine viral diseases, sea-turtle fibropapillomatosis and shrimp white spot syndrome, endanger protected marine species and the financial stability of the aquaculture industry. Although marine virology is about 70 years old (Box 1), it has experienced a recent surge in interest[1,2], largely thanks to methodological advances.

One of the main areas of study in the past few years has been the extent of viral diversity in the marine environment. Diversity has been hard to measure because viruses do not have a universally conserved gene like the ribosomal DNA genes in cellular organisms, and because most viral hosts are difficult to culture. To circumvent these difficulties, whole viral communities have been isolated and analysed using pulsed-field gel electrophoresis or shotgun sequencing[3,4]. Shotgun sequencing led to the rise of marine viral metagenomics, which has shown that viruses are exceptionally diverse: there are more than 5,000 viral genotypes or species in 100 litres of sea water, and up to 1 million species in 1 kg of marine sediment[5,6]. Marine viral metagenomes, or 'viromes', collected from across the world have shown that viral species are globally distributed (everything is everywhere) but that the relative abundance of each species is restricted by local selection[7,8]. These studies have also shown that viral functional diversity, and its potential use for host adaptation, has been vastly underestimated[9].

Marine virology is now poised to move away from bulk measurements of predation and biodiversity towards the detailed analysis of evolution and ecology. In this Review, we show how marine viruses can affect their hosts and environments in startling ways. From the global transfer of niche adaptation genes to modifications of the ontogeny and ecology of marine organisms, it has become clear that the marine virome is a master of manipulation.

Virally encoded host genes

Phage, and to a lesser degree eukaryotic viruses[10], are known to carry and transfer a variety of host genes[11]. Most studies of this phenomenon have focused on the negative effects of viruses modifying their host's physiology. However, viral infections can augment the metabolism, immunity, distribution and evolution of their hosts in many unexpected and potentially positive ways (Fig. 1).

Consider the cyanobacterial genera *Synechococcus* and *Prochlorococcus*, which together account for about 25% of global photosynthesis[12]. Sequencing of the marine viral cyanophages that infect these primary producers showed that genes involved in photosynthesis are commonly carried in phage genomes[13]. These genes include the high-light-inducible (*hli*) gene, as well as *psbA* and *psbD*, which encode the photosystem II (PSII) core reaction-centre proteins D1 and D2, respectively[14] (Table 1). The D1 protein is of particular interest because it is the most labile protein in PSII and the most likely to be rate limiting. During the lytic cycle, most of the host's transcription and translation is shut down by phage. Because phage must maintain the proton motive force if they are to lyse the host, they need to prolong photosynthesis during the infection cycle. The cyanophage-encoded D1 proteins are expressed during the infection cycle, countering the virally induced decline in host gene expression[15]. It is thought that by encoding *psbA* and other genes involved in photosynthesis, phage generate the energy necessary for viral production.

One consequence of cyanophage carrying *psbA* genes is the horizontal gene transfer of photosynthetic genetic elements between hosts (Fig. 1). *Prochlorococcus* has specific ecotypes that live in different parts of the water column[16] and are tuned to the different light and nutrient regimes found there. Given the prevalence of phage-encoded photosynthesis proteins and the occurrence of recombination between phage and host genes, phage populations are expected to serve as gene reservoirs that change the ecological niches of the host[17]. Several lines of evidence support this hypothesis. First, phage *psbA* genes are undergoing independent selection from host *psbA*, and there has clearly been exchange of phage *psbA* between hosts[18]. Second, metagenomic analyses have routinely identified large numbers of *psbA* genes in viral fractions and associated with viral-like open reading frames. It has been estimated that about 60% of the *psbA* genes in the marine environment for which an origin could be identified were actually from phage[19]. A rough calculation suggests that some 10% of total global photosynthesis could be carried out as a result of *psbA* genes originally from phage.

Transformation events also mediate one of the most dramatic effects of phage on their hosts: the switch from symbiont or benign microorganism to pathogen. The best-known marine example occurs in *Vibrio cholerae*, a common near-shore bacterium that is normally harmless but becomes one of humanity's greatest scourges by incorporating phage cholera toxin (CTX) genes[20]. Large-scale metagenomics has shown that viruses contain high numbers of virulence genes (Table 1), including some that facilitate antibiotic resistance, toxicity, host adhesion and host invasion. Bacteria that take up these genes extend their ecological niches, although this ultimately has a negative impact on humans.

In addition to virulence genes, marine viromes contain many genes that are involved in unanticipated metabolic and functional pathways. Comparisons of paired microbial and viral fractions (microbiomes and

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viromes, respectively) show that the relative frequency of respiration genes is lower in the viromes, whereas genes involved in nucleic-acid metabolism are more abundant[9]. Less expected was the observation that microbiomes and viromes carry almost equal frequencies of metabolic genes involved in carbohydrate and protein metabolism. Totally unexpected was the finding that genes involved in vitamin and cofactor synthesis, stress-response genes such as those encoding chaperones, and genes associated with bacterial motility and chemotaxis were more common in viromes than in their corresponding microbiomes[9].

Viromes as novel gene banks

Viromes are good hunting grounds for unique host-adaptation genes, as shown in a recent metagenomic study of phage from deep-sea hydrothermal vents. The abundance of viral particles was found to be higher in the diffuse flow, a region where cold sea water mixes with warm fluids from hydrothermal vents, than in the surrounding sea water[21]. Both this observation and the taxonomic make-up of the viromes suggest that temperate prophages are being induced in the diffuse flow. Only about 25% of sequences from the vent viromes had any significant similarity to sequences in the GenBank database. This high abundance of novel sequence suggests that these deep-sea viral communities could be a store of genes that may be involved in microbial adaptation to the high pressures, high temperatures and high concentrations of inorganic chemicals (such as sulphides, iron, salt and calcium) found in vent systems.

Generalized transducing agents

If viromes serve as reservoirs of genes, then determining the rate of exchange between viruses and their hosts is important. One study found a high rate of transduction in the marine environment[22]. Extrapolation of these data suggests that as many as 10^{24} genes are moved by transduction from virus to host each year in the world's oceans. However, the actual amount of transduction by marine viruses and viral-like entities may be much greater than previously thought because of the action of generalized transducing agents (GTAs)[23]. GTA particles are similar in morphology to phage, but they are smaller (with a head diameter of 30–50 nm) and contain a smaller amount (about 4 kilobases, kb) of DNA. What makes GTAs unique is that they only carry host DNA, which is injected into a recipient[24], providing an efficient form of transduction.

GTAs were originally identified in the bacterium *Rhodobacter capsulatus* but have now been found in a variety of bacteria (including Spirochaetaceae and Proteobacteria) and archaeal organisms (including *Methanococcus*). The GTAs found in α-proteobacteria, such as the Rhodobacterales, have been shown to be vertically transmitted and to have evolved from a single common ancestor, and they probably arose before the diversification of bacterial phyla[24]. Because Rhodobacterales are extremely abundant in the ocean, the transmission of such genetic agents is likely to have significant consequences for marine microbial ecology. A recent study has shown that genes encoding GTAs are found in most marine systems[25]. The same study also showed that these GTAs are produced by marine bacteria and move genes between species of α-proteobacteria. These observations suggest that GTA-related gene swapping may contribute to the niche partitioning of closely related species in the ocean.

Gene swapping between domains and ecosystems

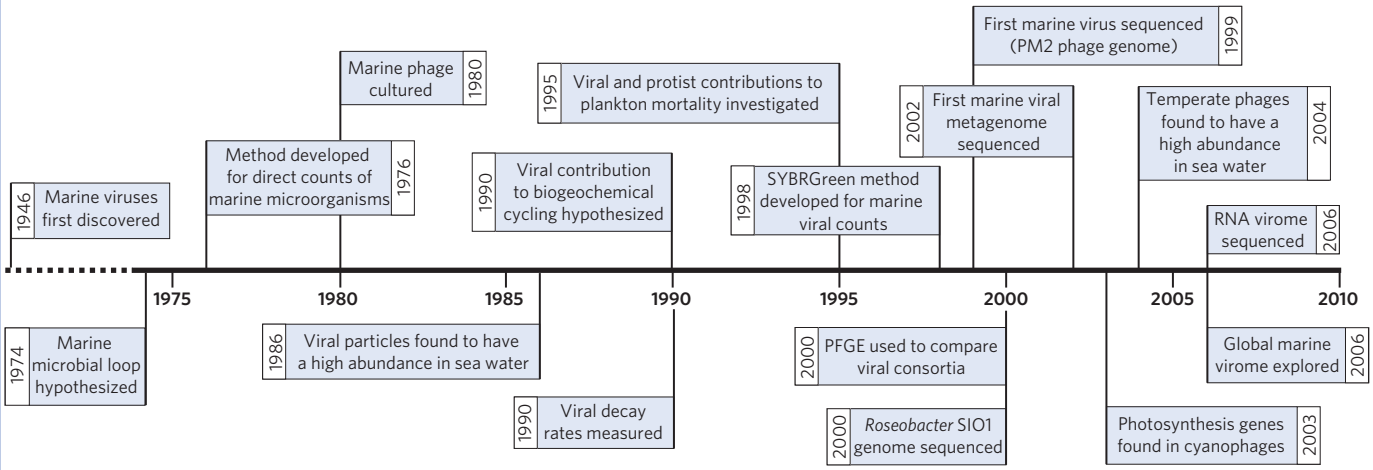
Known phage and GTAs have relatively restricted host ranges, limiting the rates by which genes can move from one host to another by these mechanisms. No known virus routinely moves between the three domains of life. However, viral-like particles in sea water and hot springs have been shown to transfer genes between Archaea, Bacteria and Eukarya[26,27]. The exact nature of these particles is still being analysed, but this finding opens up a new realm of horizontal gene transfer.

Viruses not only move genetic material from one organism to another, but from one ecosystem to another. Several phage sequences have been found to be spread ubiquitously through the biosphere[28,29]. There is also evidence that phage from one environment can successfully infect and replicate in marine microorganisms from unrelated environments[30]. These results support the hypothesis that viruses can move throughout the world and contribute to a global genetic pool. It may be the case that although local viral diversity is very high, total global diversity is limited by the worldwide movement of virions.

Viral manipulation of viruses

Acanthamoeba spp. are common protists found in soil and fresh and salt water[31]. They primarily graze on microorganisms, and some species

Box 1 | Highlights in marine virology research



Bacterial viruses (phage) in sea water were first observed in the first half of the last century[59,60], although their presence remained unexplained until Lawrence Pomeroy hypothesized the 'marine microbial loop'[61] in 1974. In 1979, Francisco Torrella and Richard Morita discovered that marine viral particles were particularly abundant (10^4 per millilitre) and morphologically similar to phage[62], and phage from marine bacteria were soon cultured[63]. In the 1990s, much was learned about the genetic diversity of marine phage and eukaryotic viruses and their importance to the ecology of the marine plankton community. Numerous studies demonstrated the contribution of viruses and protists to global biogeochemical cycling arising from the lysis of plankton[4,39,64–66]. The first marine viral genomes were then sequenced[57,67], and genomics and metagenomics have since been used to characterize the diversity of both RNA viruses[68,69] and DNA viruses[7,70] in sea water, along with their effects on host physiology and ecology[71–73]. The timeline shown here (not to scale) lists the main events in marine virology research. PFGE, pulsed-field gel electrophoresis.

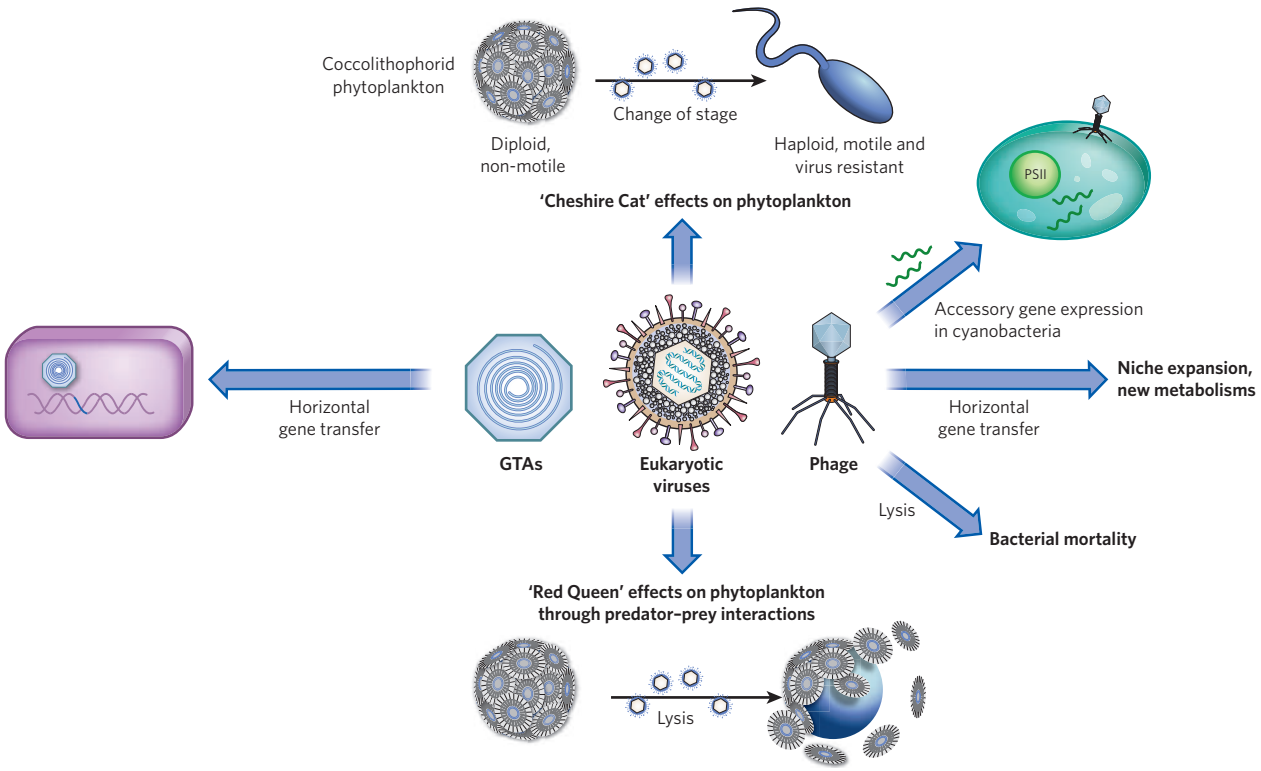


Figure 1 | Effects of marine viruses on their hosts. Marine viruses and viral-like entities, including eukaryotic viruses, phage and generalized transducing agents (GTAs), can have various effects on host cells. When a phage infects its bacterial host cell, it can either kill the cell (lysis), or transfer genetic material obtained from a previous host (horizontal gene transfer) or from its own genome (accessory genes expression). The transferred genes can allow a cell to expand into different niches (for example, through the activation of photosynthetic genes, changing the life cycle of biogeochemically important phytoplankton such as cyanobacteria and coccolithophorids). Similarly, small viral-like particles known as GTAs can transfer genes between marine organisms. Two possible scenarios have been proposed for viral effects on cells. In the 'Red Queen' effect, the virus and cell are locked in an evolutionary 'arms race', such that they continue to evolve mechanisms of resistance to each other until eventually the virus causes the host cell (illustrated here by a coccolithophorid) to die. In the 'Cheshire Cat' hypothesis, however, the coccolithophorid simply moves from its diploid, non-mobile stage to a motile, haploid stage, thereby evading the virus. PSII, photosystem II.

are pathogens of immunocompromised humans. A virus that infects *Acanthamoeba polyphaga* was isolated and sequenced five years ago[32]. Mimivirus, as it was called, has a very large virion (more than 700 nm) and a genome of 1.2 megabases (Mb)[33], so it is larger and more genetically complex then many cellular organisms. Its large genome may be simply a by-product of having a large capsid (the genome's protein shell), in which case most of the genes may not be important for viral reproduction. If this is the case, these viruses serve as gigantic gene reservoirs. Evidence in favour of this hypothesis comes from the fact that the mimivirus genome is highly chimaeric and contains many genes related to the host[34]. However, nucleotide-composition studies suggest that horizontal gene transfer from the host is less common in large eukaryotic viruses than in phage[10].

A closely related strain of mimivirus, called mamavirus, adds another twist to the viral manipulation story[35]. Mamavirus (Fig. 2a) is infected by a satellite-phage-like entity, or 'virophage', called Sputnik (Fig. 2b). This 'virus of a virus' has an 18-kb genome. Inoculation of the host with both mamavirus and Sputnik increases the production of Sputnik and negatively affects mamavirus production. This is reminiscent of coliphages, in which P4 parasitizes the larger P2 phage. Recent mining of marine microbiomes has shown that viruses similar to large eukaryotic viruses are common and widely distributed in marine ecosystems[36,37].

Viral manipulation of protists

Coccolithophores are an abundant group of eukaryotic phytoplankton that are characterized by their intricate calcium carbonate scales, which are known as coccoliths (Fig. 2c). Blooms of coccolithophores influence global temperatures by increasing Earth's albedo (that is, more sunlight is reflected). Additionally, the sinking of the coccoliths and associated organic matter is one of the main mechanisms by which the ocean's biological pump draws down atmospheric carbon dioxide[38]. *Emiliania huxleyi*, named after Charles Darwin's advocate Thomas Huxley, is the most abundant species of coccolithophorid. It undergoes massive blooms that turn the sea a milky blue that is observable from satellites, but these blooms rapidly disappear. The main mechanism for these boom-and-bust cycles was thought to be infection and lysis by *E. huxleyi*-specific viruses. This hypothesis was first presented when large viral-like particles were found to co-occur with *E. huxleyi* and other phytoplankton in nutrient-augmented mesocosm experiments[39]. To test this hypothesis, viruses and microorganisms were sampled off the coast of Plymouth, UK, during a coccolithophore bloom[40]. Satellite images showed decreases in the light reflected from the coccolithophores at one of the sites. This area had lower concentrations of *E. huxleyi* cells but higher concentrations of viruses and free coccoliths. These data suggested that a viral lysis event had blown the coccolithophores apart. In support of this conclusion, two coccolithoviruses, EhV84 and EhV86, were isolated and sequenced from this bloom[41]. When the genome of EhV86 was sequenced, it turned out to be one of the largest known marine viral genomes (about 400 kb)[42].

The EhV86 genome is also notable for the presence of genes similar to those involved in ceramide production. Ceramides are involved in apoptosis (programmed cell death) and cell-cycle arrest. Ceramide production initiates apoptosis through the activation of caspases, the proteases that sit at the centre of the apoptotic pathway. Inhibiting the activity of metacaspase (a protein similar to caspases) in *E. huxleyi* effectively stops EhV1 production[43]. Furthermore, bioinformatic analysis has shown that many of

Table 1 | Some virally encoded proteins thought to modify the phenotypes of their marine hosts

Gene/Protein	Function	Host genus	Virus/Virome	Reference
PsbA	Photosynthesis	*Prochlorococcus* *Synechococcus*	P-SSP7, P-SSM2, P-SSM4 S-PM2	15 13
PsbD	Photosynthesis	*Prochlorococcus*	P-SSM4	17
Hli	Protection from photo-inhibition	*Prochlorococcus*	P-SSP7, P-SSM2, P-SSM4	14
PetE	Photosynthesis	*Prochlorococcus*	P-SSM2	14
PetF	Photosynthesis	*Prochlorococcus*	P-SSM2	14
TalC	Carbon metabolism	*Prochlorococcus*	P-SSM2	14
PstS	Phosphate recycling	*Roseobacter*	Roseophage SIO1	57
PhoH	Phosphate recycling	*Roseobacter*	Roseophage SIO1	57
Ceramide	Apoptosis	*Emiliania*	EhV86	43
CTX	Pathogenesis	*Vibrio*	CTXΦ	20

the viral proteins have caspase recognition sites, and cleavage of these sites by caspases is presumably necessary for viral production[44,45]. Although speculative, this viral manipulation of the host is interesting because the apoptotic pathway was probably once a mechanism to prevent the spread of viruses in coccolithophore blooms, but the pathway has been subverted to increase viral spread. As noted by the authors, this is a great example of the 'Red Queen' hypothesis in action[43]. As *E. huxleyi* develops resistance, the virus finds a way around it. They are in an arms race, where they need to 'run' (evolve) just to maintain their position (Fig. 1).

A wonderful twist to the tale of the coccolithophore and its virus comes from the study of an alternative escape route from the viral predators[46]. There are two distinct life stages in *E. huxleyi*[47]: the first is the diploid coccolith-bearing cell, which is the form most commonly studied; the second, haploid, stage exists as naked cells that can be motile or non-motile. It turns out that the haploid sexual stage of *E. huxleyi* is resistant to the viruses isolated from diploid cells[46], providing a way of avoiding viral infection and colony collapse. On the basis of these results, the authors proposed that the Red Queen hypothesis should be supplemented with the 'Cheshire Cat' model, in which there is a disappearing act rather than a running in place (Fig. 1).

Viral manipulation of metazoans

Marine viruses also infect and manipulate their metazoan hosts. One interesting example is the solar-powered sea slug[48]. These molluscs are some of the most fascinating creatures in the world, eating algae and harvesting chloroplasts through specialized epithelial cells in the gut (Fig. 3). Once phagocytosed, the chloroplasts are maintained within the animal's cells for months at a time, during which the slug gains energy directly from photosynthesis[49]. This process, which is effectively theft of the chloroplasts, is called kleptoplasty. However, plastid genomes only encode 10–20% of the genes necessary for photosynthesis, so where do the rest of the proteins for chloroplast function come from? To address this question, chloramphenicol was used to block protein synthesis by the chloroplast, and cycloheximide was used to inhibit eukaryotic ribosomes[50,51]. These experiments showed that some nuclear-encoded chloroplast proteins are synthesized by the slug cells and suggested that horizontal gene transfer occurred between the algal nuclear genome and the slug genome (plant-to-animal gene transfer)[52]. Recent genomic data show that this is indeed the case, and the most likely mechanism for that transfer is by way of a eukaryotic virus[53].

The best studied solar-powered slug is *Elysia chlorotica*, which eats only the alga *Vaucheria litorea* (Fig. 3c). The chloroplasts are absorbed by the slug's gut and are maintained for many months until the slugs lay eggs and suddenly die. This synchronous death is tightly correlated with the appearance of viral particles[53,54] (Fig. 3a, b), suggesting that the slug's annual life cycle is brought about by endogenous viruses. Supporting this hypothesis are the observations that there are no records of viral infection in juveniles and that animals maintained for months in the absence of food and other slugs still undergo this synchronous death.

Viral particles and crystalline arrays (Fig. 3b) have also been identified in the stolen chloroplasts and in the nuclei and cytoplasm of the host slug (Fig. 3a). Although the identity of these viruses remains unknown, reverse transcriptase activity has been detected during viral production stages, suggesting that vertically transmitted retroviruses are partly responsible for the deaths. The presence of viruses in both the chloroplast and the nucleus provides a hypothetical mechanism for the horizontal gene transfer of photosynthetic genes to the host[53]. Viruses, then, have two unexpected roles: first, they dramatically alter the slug's

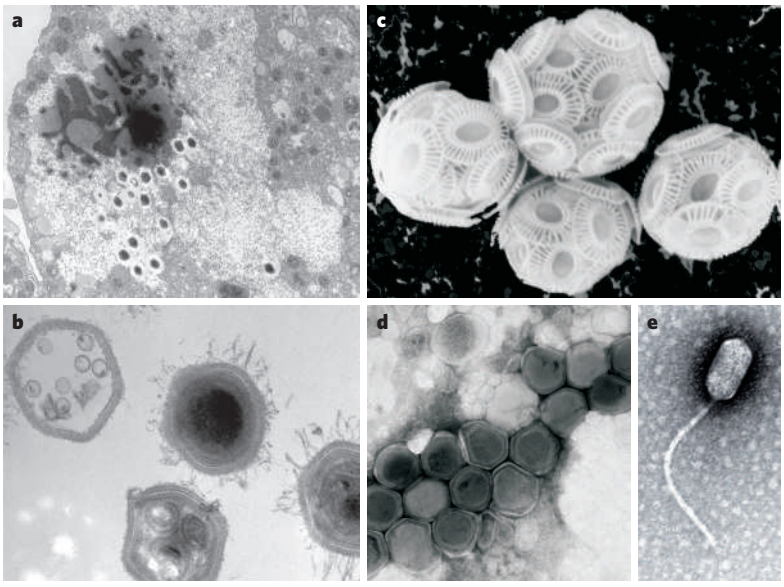


Figure 2 | Library of recently discovered marine viruses. Electron micrographs of mamavirus (**a**) and the virophage Sputnik (**b**). Mamavirus is a large icosahedral virus with a 1.2-Mb genome that infects the protist *Acanthamoeba*. Sputnik is a virus that infects mamavirus and lowers its fitness. *Emiliania huxleyi* (**c**), a coccolithophore important for marine primary production and nutrient recycling, and the *E. huxleyi*-like virus that causes boom-and-bust cycles and alters the life stages of its host (**d**). Phage of the cosmopolitan cyanobacterium *Prochlorococcus* (**e**). (Panels **a** and **b** courtesy of D. Raoult, Centre National de la Recherche Scientifique, Marseille, France. Panels **c** and **d** courtesy of W. Wilson, Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, Maine. Panel **e** reproduced, with permission, from ref. 58.)

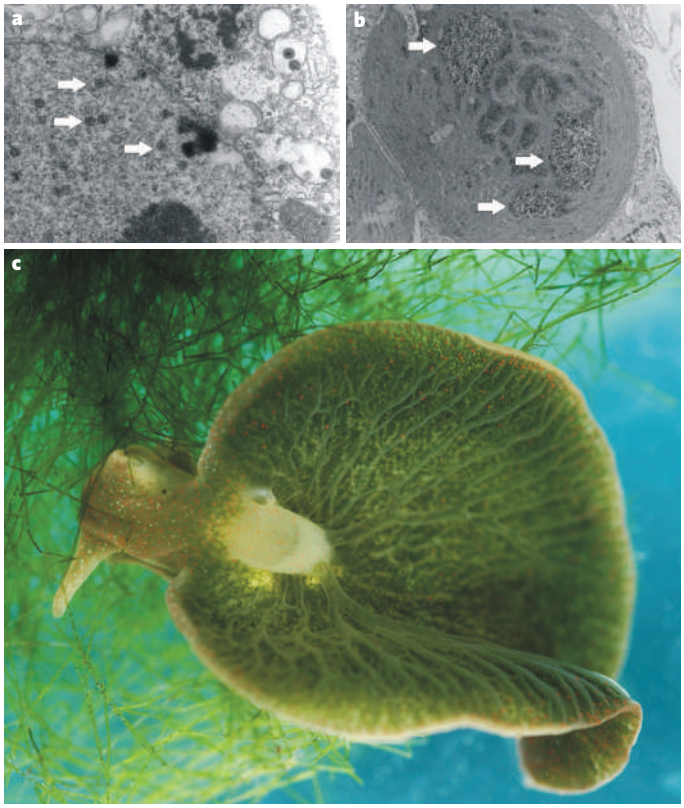


Figure 3 | Viruses that alter the life cycle of solar-powered slugs. Transmission electron micrograph of viral-like particles (arrows) in the nuclei and the cytoplasm (**a**) and within the stolen and incorporated algal chloroplasts (**b**) of the sea slug *Elysia chlorotica* (**c**), which uses solar energy. (Images courtesy of S. Piece, University of South Florida, Tampa.)

life history; and second, they are probably the vector for this horizontal gene transfer between an animal and a plant.

The future of marine virology

So far, the study of marine viruses has been dominated by the search for pathogens, but this will need to change if we are to appreciate the diverse ways that viruses affect life on Earth. We hope that future marine viral work will focus on three major areas of research. First, there needs to be a push to discover and evaluate viruses that infect marine archaeal organisms. Studies of these archaeal viruses — especially in the context of archaeal species that produce or consume greenhouse gases such as methane, or that help recycle limiting nutrients such as inorganic iron and nitrogen in the ocean — will yield interesting and important results. Second, we need to study the effects of viruses on the structure and function of zooplankton communities. Only a few studies have investigated the direct or indirect roles of viruses on zooplankton in the oceans. Culture studies have shown that viruses can contribute to boom-and-bust cycles in many metazoans, but no one has explored how the top-down forcing of metazoans by viral infections will affect zooplankton communities. Finally, recent work in the field of entomology has revealed remarkable tripartite symbioses between insects, bacteria and phage[55,56]. In these systems, the abundance of bacterial symbionts is controlled by viruses. This, in effect, changes the physiology and ecology of the host. These tripartite symbioses between eukaryote, microorganism and phage exemplify the intricacies of viral ecology that have so far been overlooked. We expect to find similarly complex interactions between marine organisms, their symbionts and the viruses that affect one or both. There is much exciting biology waiting to be discovered. ■

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