The potential of nature's unseen industrious heroes: marine viruses

Michael J. Allen (University of Exeter, UK)
Félix Cicéron (Plymouth Marine Laboratory, UK)
Adam Monier (University of Exeter, UK)

The last two years have been a crash course in educating the world about viruses, virology and infectious diseases. Unsurprisingly, viruses have emerged as the harbingers of doom. However, with their newly acquired knowledge, the new armchair virologists fail to grasp the importance of viruses in the living world. They may cause us harm, but without their relentless activity, we'd all be dead anyway. In addition to owing them our lives, they also have the potential to improve our lives with novel biotechnological applications.

Had enough of viruses yet? It's easy to recognize that the past two years have created a lot of armchair virologists who now know a staggering amount about one particular virus. The general public have been 'educated' about the language of replication rates, evolution, selection pressures, antigens, sequencing, mRNA, PCR…. Never was the world so primed and receptive to information about viruses and their study. Government briefings, YouTube and Twitter became esteemed sources of learning as a myriad of newly bred virology 'experts' self-educated in the confines of covid restrictions.

As a bruised and battered world emerges from the covid pandemic and viral fatigue begins to set in, it will be hard for the six to seven billion new viral experts to believe anything other than 'viruses are bad to the bone' as they move on with their lives. Yet, they are so very wrong.

Think how many viruses even the average armchair virologist can name, aside from the indomitable new kid on the block, SARS-CoV-2: 10? 20? 30? Try it, you might surprise yourself. We rightly obsess about them; they directly impact our lives. They make us feel poorly, restrict us to bed and sadly sometimes end the lives of our loved ones prematurely. Remember though: you're likely only thinking about human viruses. Every living organism you have ever seen will have viruses that infect it. And like humans, there will be *a lot* of different viruses that infect them. Humans are not special or particularly vulnerable to viruses; it's just human viruses are particularly relevant to us for study. We have skin in their game. What about the other viruses? The viruses of bacteria, squirrels, jellyfish, leeches, tardigrades, orchids, octopuses and koala bears?!?! Everything alive is most likely susceptible to viral infection and most likely by far more than one single type of virus. Viruses have been around since life evolved, perhaps even before the first cell (see Further Reading). The impact of viruses is incredible, but it is unseen and unnoticed for the most part. They are the oil that keeps the planetary ecosystem moving. The constant death of microbial

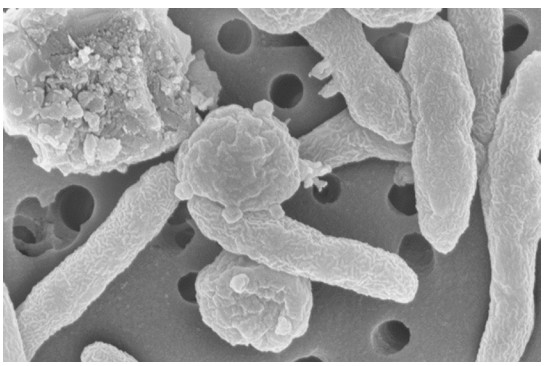

Figure 1. OtV binding to *Ostreococcus tauri*, the smallest free-living eukaryotic cell.

cells provides the resources for the growth of the living survivors. It is the circle of life, and viruses drive it and keep it turning. It is perhaps one of science's ironies that the evolutionary tree of life has no real place for viruses when without them, most likely, there would be no life at all. The simple truth is, without their relentless activity, we would all be dead. Not some of us like with human viruses, but all of us. Global biogeochemical cycles would grind to halt. The world would stagnate. Life would come to a standstill.

Let's use the oceans as our prime example: there are an estimated ~10^{31} viruses in the ocean, with a whopping ~10^{21} infections predicted to occur every second. That's around 1,000,000,000,000,000,000,000 infections every second. Compare that with the daily covid briefing numbers we have become so familiar with. As we write this article (late December 2021) there are ~10^{6} new confirmed cases worldwide (a mere 12 infections per second). New viruses are being made all the time, but the number of viruses in the oceans doesn't change much at all. Applying the *R* number concept ('greater than 1 = increasing, less than 1 = decreasing') to the total global marine viral population perspective, this number would appear surprisingly stable, despite the fluctuations and oscillations

in the living populations with which they are associated. Even Facebook-educated virologists will quickly recognize the global viral '*R* number' remains at 1 as a stable equilibrium between life and death is maintained. They're infecting the charismatic macrofauna (such as the whales, turtles and dolphins) we all know and love, but also, far more importantly, they regulate the microbial population (bacteria and microalgae in particular, Figure 1) that is the real planetary machinery that keeps global biogeochemical cycles running. The sad thing is these silent killers are utterly unappreciated and poorly studied. Most of our understanding comes from metagenomic studies and a handful of model systems.

Viral diversity is truly amazing. However, it is this diversity that makes viruses so difficult to study and classify: where do you even start? Perhaps instead of focusing, as we have done, on the viruses of relevant and justifiable academic model systems, it is time to spread the net wider and look at the bigger picture. Viruses have a proud history of industrial exploitation: from vaccine production, cellular transformation, expression systems, phage therapies and novel enzymes; viruses have already provided us with a wealth of biotechnological options. What of the mysterious environmental viruses of which we know so little? The tiny glimpse we have taken so far shows a tantalizing genomic landscape ripe for exploitation.

Algal viruses, in particular, are showing great promise in multiple biotechnological settings. Algal biotechnology is in its ascendancy, as sustainable sources of biomass for commodity chemical precursors, biofuels, plastics, food supplements, as well as carbon capture and sequestration, become increasingly popular. The integration of viruses into cultivation practices may be a natural next step. The potential use of viruses as an energetically efficient way to induce cell lysis, aiding the downstream processing of bulk biomass, is certainly a captivating notion. After all, viruses have been breaking cells apart for billions of years, so why not take advantage of this property? Pushing this idea further still, viruses can potentially be used to enhance the biochemical or metabolic properties (and therefore value) of such biomasses beyond simply making their components more readily accessible. Indeed, work over the past decades has shown increasingly larger, complex virus genomes packed to the rafters with genes associated with auxiliary metabolic functions. Sometimes these extra genes have been stolen from hosts (by a process known as horizontal gene transfer) and have been tweaked or enhanced for optimum impact; but more often than not we have no idea how they have come to be there or what they are doing. The concept of viruses as minimalist pocket bags of genes has long since been denounced: there is a metabolic complexity that we have barely scratched the surface of.

Viral genomes are a fantastic source of novel genes. While most viral genomes suffer from frustratingly poor

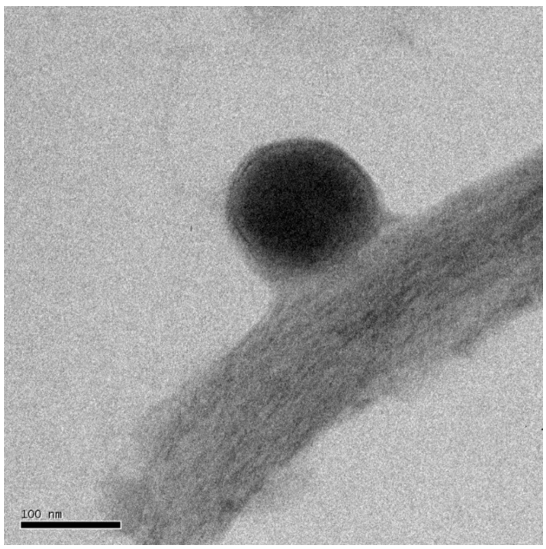

Figure 2. Coccolithovirus binding a coccolithophore membrane.

functional annotation, the genes we can identify putative functions for give us a glimpse of the future possibilities. Take the coccolithoviruses (Figure 2) (which infect the cosmopolitan, calcifying microalgae *Emiliania huxleyi*, Figure 3) as an example; they have been shown to encode a near complete biosynthetic pathway for a novel ceramide – a type of sphingolipid used in various cosmetics for skin and hair care, with a global market of around $350m last year, and strong potentials in medicine for applications related to cell differentiation and tumour treatments. The distantly related Chloroviruses (which infect *Chlorella* spp., one of the most widely cultivated microalgae globally, with an annual market expected to reach around $400m by 2028) have the required genes to make hyaluronic acid, a sugar polymer with a global market of $8.5bn in 2020. Both of these platforms could be utilized to produce sustainable, vegan-friendly products employing viruses to naturally manipulate metabolites in a non-GMO framework. Coming from the cold waters of the world's oceans, the potential for utilizing marine enzymes in, e.g., biological washing detergents for use at ambient temperatures is an exciting proposition. True to form, functional lipases and esterases have been shown to be encoded in coccolithovirus genomes. Novel restriction endonucleases, methylases and glycosyltransferases are also found there and have potential uses in the biotechnology toolbox.

We haven't even mentioned the gigantic number of genes of unknown function that dominates our viral data sets: what are all these viral proteins doing? It's difficult to comprehend the true extent of discoveries that are yet to made out there. And it's not just viral genes for which uses can be found: indeed, attenuated (trimmed) viruses can be used as vectors for cell transformation (as is the case of the AstraZeneca vaccine, or the baculovirus-insect cell system),

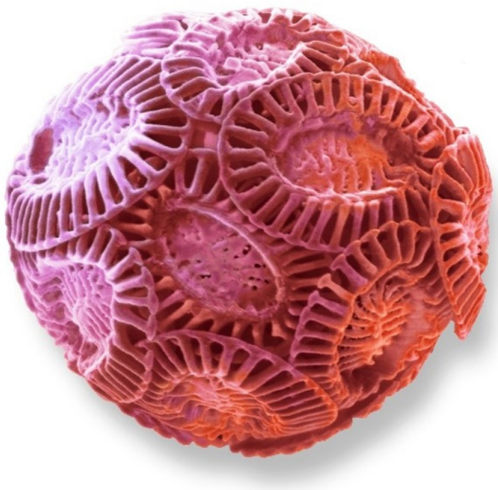

Figure 3. False colour SEM of the coccolithophore *Emiliania huxleyi (Image by Mike Allen & Steve Gschmeissner).*

and novel high-activity viral promoters can be used in expression cassettes (akin to the widely utilized T7 phage promoters) along with terminator sequences. A developing tool derived from a cunning viral lifestyle trick is the 2A self-cleaving peptide, which induces ribosome skipping during translation, allowing the production of two proteins in a similar ratio out of one single mRNA. Inteins (the self-splicing protein equivalent to introns in genes) have been called 'Nature's gift to protein chemists' for their wide application in protein synthesis and labelling. Guess what? Novel inteins have been identified in the coccolithoviruses, which further extend the library of available sequences to biotechnologists. The coccolithoviruses represent just one virus family and provide all these leads; it is astounding when you think of the genomic diversity contained within the rest of the viral community and the potential biotechnological exploitation. So, why aren't we inundated with success stories extolling and confirming the virtues of marine viruses? Perhaps the simplest answer is: with so much opportunity and so few researchers to get momentum going, where do you even begin to start? The might of 'academic push' has, and always will, require the sharp focus of 'industrial pull' to ensure success. While academia has become frozen in the glorious spotlight as it marvels at the potential possibilities, it has failed to capture the imagination of the biotechnological realms. Industry needs to take a good look and see for itself what it is missing out on; once that happens, there'll be no limits to the possibilities. Perhaps then the general public will start to think of viruses as the good guys for once. ■

Further Reading

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Professor Michael J. Allen is an Associate Professor of Single Cell Genomics in the College of Life and Environmental Sciences at University of Exeter. His interests are varied and encompass both blue skies and applied research topics. Blue skies research focuses mainly on understanding the role of viruses in the ocean using genomic, proteomic, transcriptomic and metabolomic approaches. Applied research focuses on biocatalysis, bioremediation, biotransformation, bioprocessing and technology development. For more information on Mike and his research, follow him @Mike_J_Allen on twitter and check out his website www.bluemicrobe.co.uk

Dr Félix Cicéron completed his PhD on the study of a plant glycosyltransferase involved in the synthesis of the polysaccharide xyloglucan. He then worked on bacterial strain engineering, bioprocessing and polymer characterization, before diving into the world of microalgae and their multiple applications in the biotech industry. He started studying diatom lipid metabolism within Eric Maréchal's team, LPCV Grenoble, France, where he used the CRISPR/Cas9 technique to inactivate genes of interest and analyse the effects on corresponding lipid profiles. Dr Cicéron is currently running multiple projects related to gene editing and recombinant production of antibodies in microalgae.

Dr Adam Monier is a marine microbiologist interested in the ecology and evolution of microbial eukaryotes and associated viruses and their interplay in oceans. Adam received his MSc and PhD in bioinformatics from Aix-Marseille Université (France), and his doctoral work was followed by postdoctoral research at the Monterey Bay Aquarium Research (USA) Institute, which focused on the development of metagenomic tools to study marine microbial eukaryotes, and at Université Laval (Canada) on the effect of sea ice loss on the diversity and function of Arctic Ocean microbial communities. In 2014, Adam was awarded a Royal Society Newton Fellowship to work at the University of Exeter on the impact of horizontal gene transfer events in phytoplankton–virus interactions. Since 2018, he is a Senior Research Fellow and a Royal Society University Research Fellow at the Living Systems Institute where his group uses 'omics' tools to determine the influence of viral infection on phytoplankton physiology and its repercussions on marine ecosystems.