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Aquatic virus culture collection: an absent (but necessary) safety net for environmental microbiologists

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ABSTRACT

Viruses are recognised as the most abundant biological entities on the planet. In addition to their role in disease, they are crucial components of co-evolutionary processes, are instrumental in global biogeochemical pathways such as carbon fluxes and nutrient recycling, and in some cases act regionally on climate processes. Importantly, viruses harbour an enormous, as of yet unexplored genetic and metabolic potential. Some viruses infecting microalgae harbour hundreds of genes, including genes involved in cellular metabolic pathways. Collectively, these attributes have given rise to new fields of research: environmental virology and viral ecology. While traditionally the potential of viruses was recognised by isolating novel viruses into culture and subsequent sequencing of their genomes in the laboratory, advancements in next-generation sequencing technologies now allow for direct sequencing of viral genomes from their natural setting, bypassing the need for culturing. Nevertheless, the lack of associated biological reference material with most of these novel environmental genomes is problematic as there are limitations to what can be achieved with sequence data alone. Where aquatic viruses do exist in culture, they are most often kept privately within research institutes and are not available to the wider research community. Many are thus at risk of being lost because research teams rarely have secure long term resources to ensure continued propagation. Culture collections do exist for medically and agriculturally important viruses causing disease, but collections focusing on viruses infecting aquatic algae and bacteria are non-existent. We therefore highlight here the need for a centralised depository for aquatic viruses and present arguments indicating the benefits such a collection would have for the scientific community of environmental virologists.

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Introduction

Microbial culture collections are an essential resource for scientists globally (Smith, 2003). They provide a centralised infrastructure for the deposit and continuous maintenance of a wide taxonomic range of microbial species across the tree of life, they ensure the long term viability of isolates, their associated physiological traits, and metabolic and genetic potential, curate updated bioinformatic, phylogenetic, and physiological data, and offer services and advice to different research sectors (Uruburu, 2003). Collectively, these attributes serve as an invaluable resource to the academic community, which aims to gain basic understanding of microbial interactions and their diversity across different habitats, and to commercially orientated industries, which aim to exploit the biotechnological potential of microbes.

Within microbes, viruses stand out. They are the most abundant biological entities on the planet, with estimates of their global abundance in the ocean being $\sim 10^{30}$

(Suttle, 2005). Beyond viruses which have clear relevance to human, animal and agricultural health, most attention in recent years in environmental virology and ecology has focused on the enormous genetic diversity of viruses in the ocean (Brum et al., 2015; Gregory et al., 2019; Suttle, 2016) and on viruses which display intriguing co-evolutionary dynamics with their microbial host organisms in aquatic environments (Bidle & Vardi, 2011; Breitbart, 2012; Dunigan, Fitzgerald, & Van Etten, 2006; Grimsley et al., 2012; Lindell et al., 2004; Nissimov et al., 2017; Pagarete, Allen, Wilson, Kimmance, & de Vargas, 2009; Van Etten, Lane, & Meints, 1991). For example, recent studies employing next-generation sequencing technologies have revealed nearly 200,000 novel aquatic viral populations globally (Gregory et al., 2019). Other approaches, which include the isolation and full genomic sequencing of viruses infecting marine and freshwater microalgae such as *Emiliania huxleyi* and *Chlorella* sp. respectively, (Fitzgerald et al., 2007a, 2007b, 2007c;

Jeanniard et al., 2013; Nissimov et al., 2017; Pagarete et al., 2013; Wilson et al., 2005), and those infecting amoeba and other protists (Claverie & Abergel, 2018), have elucidated “giant” genomes composed of hundreds of genes. Some of these viral genes have been implicated directly in lipid biosynthesis pathways and carbohydrate metabolism within infected host cells (DeAngelis, Jing, Graves, Burbank, & Van Etten, 1997; Graves et al., 1999; Monier et al., 2009; Nissimov et al., 2019; Rosenwasser et al., 2014; Van Etten et al., 2017; Vardi et al., 2009; Ziv et al., 2016). In addition, virus genes involved in pigment and vitamin B12 biosynthesis, photosystem II (PS-II) and photosystem I (PS-I), and carbon, phosphate and nucleotide metabolism, have been identified in viruses infecting *Prochlorococcus* and *Synechococcus* cyanobacteria (Breitbart, Thompson, Suttle, & Sullivan et al., 2007; Crummett, Puxty, Weihe, Marston, & Martiny, 2016; Enay, Mandel-Gutfreund, & Béjà, 2014; Mann, Cook, Millard, Bailey, & Clokie, 2003); the two most abundant photosynthetic organisms on earth. Perhaps the most fascinating findings in recent years are the discovery that “giant” viruses in the genus Mimivirus are themselves subject to “predation” via associated virophages (Bekliz, Colson, & La Scola, 2016; La Scola et al., 2008) and that some members of this genus contain multiple genes for cytochrome P450 (Lamb et al., 2019, 2009), a type of enzyme that is common in animals, plants and bacteria and may be important in the metabolism of drugs and synthesis of different hormones.

The presence of many of these genes in viruses, now collectively referred to as auxiliary metabolic genes (AMGs), is a result of a co-evolutionary arms-race between hosts and their viruses, and the process of horizontal gene transfer (HGT) during which these genes are “stolen” from the host genomes during viral replication and incorporated into viral genomes. In addition, there is evidence to suggest that these host-virus dynamics and gene transfers are more complicated than this and that some of these genes may have distinct origins independent from the host organism that they currently infect, implying a complex evolutionary history with taxa from across the tree of life (Nissimov et al., 2017). The evolutionary role of viruses is further emphasised by recent discoveries that suggest that up to 8% of the human genome has a viral origin (Wildschutte et al., 2016).

Attention has also been given to ecologically important viruses in aquatic environments where they are central in dictating the fate of primary production, and to viruses that tap into global biogeochemical cycles of carbon export and nutrient cycling (Fuhrman, 1999; Suttle, 2007; Wilhelm & Suttle, 1999). For instance, the interactions of marine phytoplankton and their viruses extend beyond the traditional two-dimensional view of

host–parasite dynamics and have far-reaching consequences for food web dynamics. While traditionally the flow of energy produced by primary producers in the sunlit part of the ocean has been viewed as unidirectional; i.e., from microalgae to zooplankton grazers, from zooplankton grazers to small fish, and from small fish to larger top predators, we now know that virus infection is at least equally important in controlling microalgal and bacterial primary production in marine environments and the fate of materials post virus lysis (Zhang et al., 2018; Zhang, Wei, & Cai, 2014). Indeed, two additional processes occur during infection of microalgae and photosynthetic bacteria in the ocean. The first is the lysis of host cells, which results in the release of dissolved organic carbon (DOC) into the pool of dissolved organic matter (DOM). Through this release (a process named “the viral shunt”), different metabolites and dissolved substances are remineralized and become readily available via the “microbial loop” to heterotrophic bacteria which utilise these as a food and energy source for growth (Azam, Fandino, Grossart, & Long, 1998; Pomeroy, Williams, le, Azam, & Hobbie, 2007; Wilhelm & Suttle, 1999). The second process is the “virus shuttle” which has a contrasting effect (Sullivan, Weitz, & Wilhelm, 2017; Yamada, Tomaru, Fukuda, & Nagata, 2018), in that the excreted metabolites and substances from the dying cells, which often include sticky polysaccharides and proteinaceous substances (Nissimov & Bidle, 2017; Thornton & Chen, 2017), clump cellular debris and other exuded compounds into larger particles of organic and inorganic material (Laber et al., 2018; Nissimov et al., 2018), which if heavy enough and negatively buoyant, sink through the water column, resulting in a net export of carbon from the surface ocean into the deep.

Finally, a promising development in virus research is the recognition of viruses as potential tools to combat diseases of ecological importance through “phage therapy” (Górski et al., 2019). The idea behind this approach is to utilise the natural properties of viruses as agents of infection and mortality, in order to reduce the host population and its harmful effects. “Phage therapy” (PT) dates back to the beginning of the 20th century after the first discovery of bacteriophages by Frederick Twort (Twort, 1915). An early example of this approach was the application of bacteriophages to patients in France suffering from dysentery in 1919 (Kutter et al., 2010). Subsequently, with the discovery of antibiotics, PT- research was mostly abandoned in the western world and was restricted to research conducted mainly in the Soviet Union. Nowadays however, scientists look back to viruses for answers (Housby & Mann, 2009). They are seen as tools that can combat emerging

antibiotic resistance of pathogens in hospitals and as potential probiotic agents for solving ecological problems. Although still at its infancy, recent examples of research into the probiotic application of viruses include the co-incubations of a coral pathogen (*Vibrio coralliilyticus*) responsible for coral bleaching with its virus (Cohen, Joseph Pollock, Rosenberg, & Bourne, 2013), and the infection of a harmful algal bloom species (*Aureococcus anophagefferens*) by several different virus isolates (Gastrich et al., 2004; Gobler, Anderson, Gastrich, & Wilhelm, 2007). Although these were only controlled laboratory experiments, in both cases, co-incubation of host and virus resulted in a positive effect; i.e., reduced coral bleaching due to *V. coralliilyticus* and reduced growth rates of *A. anophagefferens* when infected by some virus-specific isolates (Cohen et al., 2013; Gobler et al., 2007).

Isolation of new aquatic viruses involves screening suitable susceptible cultured hosts (or in some cases, isolation of new susceptible hosts) with water samples thought to contain viruses. Upon fulfilment of Koch's postulates; a process and a methodology that leads to the clonal isolation and purification of a new disease-causing microbe (Rivers, 1937), the virus should be propagated on a regular basis (i.e., its long term viability is not necessary guaranteed), or ideally cryopreserved to maintain the genetic integrity of the virus isolate. This requires dedicated and skilled personnel and appropriate facilities and infrastructure, including specific growth media for the host organism, controlled environment growth chambers, and above all, some biological knowledge of the host-virus system. Virus propagation, even when hosts and viruses are in culture, includes knowledge of the infection strategy of the virus, its viral titre, its latent period, and the storage conditions that will allow its stability and viability over time. Developing the infrastructure and necessary skills in today's competitive funding climate is not trivial. It is easy to envision a scenario whereby lack of funding for even a short period may result in the loss of culture isolates due to changing personnel and/or poorly maintained infrastructure.

It is notable that the incredibly fast pace with which next-generation sequencing technologies have developed in recent years now allow for the sequencing and assembly of virus genomes directly from their natural setting (without the need for culturing). Consequently, this generates an enormous amount of novel, publishable biodiversity and functional data, quickly and effectively. Such studies tend to rely on large collaborative consortiums, where individual research groups contribute various aspects to the project, from ship time to collect the initial samples to molecular biology facilities and cloud computing for sequencing and bioinformatics. This results in a

relatively quick project turnover rate from initial investment and start of a project to its outputs and its conclusion, an aspect which is perhaps lacking in the traditional approach of virus isolation and purification outlined above.

Regardless of the type of approach employed in virus ecology, the need for cultivating viruses and making them readily-available to the scientific community (as other culture collections already do) is essential. Here, we outline some of the main existing virus culture collections and briefly summarise their contributions to the community, provide examples of aquatic microalgal and bacterial host-virus model systems where detailed study of laboratory virus isolates have allowed the virus ecology research field to go forward, discuss the importance of a virus depository and a bio-bank specific to the aquatic virus research community (aquatic viruses are defined here as those infecting algae and bacteria, not aquatic animals), outline the challenges this may pose, and highlight the research opportunities that an Aquatic Virus Culture Collection (AVCC) may be able to exploit.

Why do we need an aquatic virus culture collection?

There are numerous potential benefits for an AVCC and several reasons why it may even be considered vital. These include a rapidly growing number of aquatic virus ecologists on a global scale (Fig 1), the need to preserve virus isolates currently in culture (Table 1), and improve experimental reproducibility, quality, and traceability; aspects that are critical to progress the virus ecology field in the age of metagenomics.

The aquatic virus ecology research field is expanding

The need for a centralised depository for viruses infecting aquatic microbes (i.e., microalgae and bacteria) is highlighted by the increase in the number of institutions and individual research groups in recent years engaged in virus ecology research. For example, an initiative in 2015 funded by the Gordon and Betty Moore Foundation for the creation of a web-based resource to enable discussions and sharing of research methods, and facilitate interactions among aquatic virus ecologists, enabled researchers from all over the world to collaborate in a virtual space, share laboratory, field and bioinformatics protocols, and promote important events and research highlights relevant to the field (Kindler et al., 2016). The fact that this platform, called the Viral Ecology Research and Virtual Exchange Network (VERVENet), has more than 300 current registered members from institutions scattered all

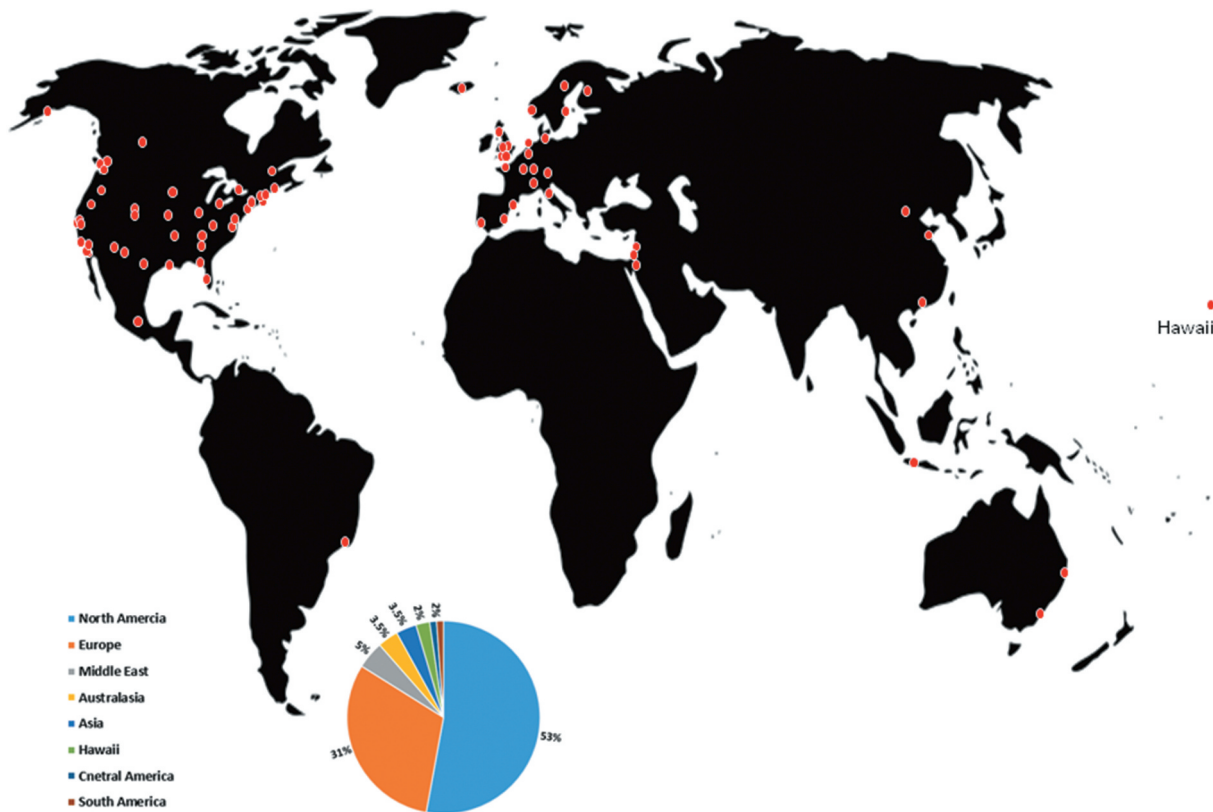


Figure 1. The aquatic virus ecology is a widespread, global research field. Although this map is not inclusive of all the research groups and institutions engaged in aquatic virology and ecology, the red dots represent a subset of geographical locations (pie chart) where some institutions and researchers engaged in aquatic algal-virus and/or aquatic bacterial-virus research are located. The figure was adapted and modified from the “collaboration map” within the VERVENet platform (www.protocols.io/groups/verve-net), with additional information added based on our current knowledge of where some of the research is presently done.

over the world (Fig 1) emphasises the growth of the aquatic virus ecology research field. This number is undoubtedly not inclusive of the overall environmental virology community as it mainly includes researchers who focus on viruses infecting environmentally relevant algae and bacteria in coastal, open ocean and freshwater pond, and river environments.

Aquatic viruses are held in private culture collections, but ...

Many of the research groups engaged in aquatic virus ecology (Fig 1) host private collections of virus isolates (Table 1). These include dsDNA, ssDNA and ssRNA viruses infecting different species of microalgae, and dsDNA viruses infecting cyanobacteria (note that microalgae-infecting dsRNA viruses have been also identified but are not included in Table 1). Most of these viruses (and associated hosts) were isolated during specific research projects in the last 20–30 years and have yielded numerous high-impact publications. However, the long term preservation of some of these isolates is not guaranteed, as their

propagation is often necessary on a monthly to yearly basis, whereby hosts are infected with viable virus lysates to renew the viral stock. With the exception of a few examples where cryopreservation of some aquatic viruses has been employed successfully (Coy, Alsante, Van Etten, & Wilhelm, 2019; Nagasaki & Yamaguchi, 1999), most aquatic virus isolates are currently kept in the dark at near freezing temperatures (i.e., $\sim 4^{\circ}\text{C}$). The semi-continuous propagation of viruses is laborious and, regrettably, the loss of virus isolates is possible even if these are maintained within the framework of an existing algal culture collection. For example, an online strain availability catalogue search shows that out of a total number of 204 *Micromonas*-infecting virus strains, 159 have been now lost at the Roscoff Culture Collection and are no longer available to the scientific community.

It is also worth noting that serial passage of viruses (rather than cryopreservation) can compromise their genetic integrity through genetic drift and accumulation of mutations from generation to generation. In that respect, a centralised AVCC will benefit the community enormously. It can serve as a centralised depository for aquatic

Table 1. A subset of some of the private aquatic virus culture collections and the host species that these virus isolates infect. Note that the list is partial and does not represent a comprehensive summary of all the different aquatic viruses currently in culture or all the different institutions that engage in aquatic algal and/or bacterial research. Rather, it emphasises that there are many aquatic virus isolates within the research community, but not in a centralised, purposely - orientated depository. The information in this table was supplied by colleagues and our current knowledge of where some of the work is currently being done.

host name ¹	virus name ²	common name ³	# of different virus strains ⁴	location ⁵	
<i>Emiliania huxleyi</i>	EhV	coccolithovirus	15	Scottish Association for Marine Science, UK	
			14	Plymouth Marine Laboratory, UK	
			14	Bigelow Laboratory for Ocean Sciences, USA	
			15	Rutgers University, USA	
			3	University of Bergen, Norway	
<i>Aureococcus anophagefferens</i>	AaV	mimivirus	1	The University of Tennessee- Knoxville, USA	
<i>Chaetoceros tenuissimus</i>	CtenDNAV	diatom virus	>50	National Research Institute of Fisheries and Environment of Inland Sea (FEIS), Japan Fisheries Research and Education Agency (FRA), Japan	
<i>Chaetoceros tenuissimus</i>	CtenRNAV	diatom virus	>50		
<i>Chaetoceros lorenzianus</i>	ClorDNAV	diatom virus	1		
<i>Chaetoceros setoensis</i>	CsetDNAV	diatom virus	1		
<i>Chaetoceros debilis</i>	CdebDNAV	diatom virus	1		
<i>Chaetoceros sp. strain TG07-C28</i>	Csp05DNAV	diatom virus	1		
<i>Chaetoceros sp. strain SS628-11</i>	Csp07DNAV	diatom virus	1		
<i>Chaetoceros socialis f. radians</i>	CsfrRNAV	diatom virus	1		
<i>Chaetoceros sp. strain SS08-C03</i>	Csp03RNAV	diatom virus	1		
<i>Heterocapsa circularisquama</i>	HcRNAV	diatom virus	1		
<i>Heterocapsa circularisquama</i>	HcDNAV	diatom virus	1		
<i>Chlorella variabilis</i> (NC64A)	NC64A virus	chlorovirus	49		University of Nebraska- Lincoln, USA
<i>Chlorella variabilis</i> (Syngen)	Syngen virus	chlorovirus	20		
<i>Chlorella variabilis</i> (SAG 3.83)	SAG virus	chlorovirus	19		
<i>Chlorella variabilis</i> (NIES 2541)	NIES 2541 virus	chlorovirus	3		
<i>Chlorella variabilis</i> (NIES 2540)	NIES 2540 virus	chlorovirus	2		
<i>Micractinium conductrix</i> (Pbi)	Pbi virus	chlorovirus	18		
<i>Micromonas pusilla</i>	MpV	prasinovirus	45	Roscoff Culture Collection, France	
			2	Bigelow Laboratory for Ocean Sciences, USA	
			19	Royal Netherlands Institute for Sea Research (NIOZ), Netherlands	
	MpRV	reovirus	1	Bigelow Laboratory for Ocean Sciences, USA	
<i>Micromonas polaris</i>	MpoV	prasinovirus	4	Royal Netherlands Institute for Sea Research (NIOZ), Netherlands	
<i>Phaeocystis globosa</i>	PgV	megavirus	8	Royal Netherlands Institute for Sea Research (NIOZ), Netherlands	
<i>Tetraselmis striata</i>	TsV		1	Bigelow Laboratory for Ocean Sciences, USA	
			5	University of Bergen, Norway	
<i>Prymnesium kappa</i>	PkV	mimivirus	2	University of Bergen, Norway	
<i>Haptolina ericina</i>	HeV	mimivirus	1	University of Bergen, Norway	
	CeV		1		
<i>Cylindrospermopsis raciborskii</i>	CrV	cyanophage	1	Royal Netherlands Institute for Sea Research (NIOZ), Netherlands	

¹For simplicity purposes, most of the host names are provided as on a species level. Note that most of the hosts outlined here are microalgae, but the cyanobacterium *Cylindrospermopsis raciborskii* is also indicated. There are numerous other research groups and institutions that also work with *Prochlorococcus*- and *Synechococcus* - infecting viruses, but these are not indicated here.

²These are the names of the specific viruses infecting a given host species and/or a host strains.

³The common name represents either the genus to which each virus belongs, or a description that indicates the group of host organisms that the virus infects.

⁴Note that these numbers refer only to the number of different virus strains currently held at a particular location. They do not necessarily indicate that they are different than those held at a different location. For example, there are only a total of 15 different coccolithovirus isolates to date, with some institutions having only a subset of this total.

⁵These are only a subset of the institutes at which these viruses and their hosts are currently held and does not represent a comprehensive list.

algal and bacterial viruses, act as a safe-keeping space for the community's many viral isolates, and a place where various studies to determine the optimal cryopreservation methods (ensuring high viral titres) for long term virus preservation are conducted. Optimised cryopreservation methods will consequently eliminate the need for serial

virus cultivation and continuous propagation of viable lysates, and at the same time, cut the costs associated with continuous culturing on a live host. Crucially, the AVCC may allow for some freedom and a degree of confidence in experimental reproducibility, as virus propagation will be minimised to when experimental procedures are required.

Many microbial culture collections, already employ such an approach. For instance, in addition to supplying thousands of algal strains to the scientific community, and providing taxonomic services and engaging in consultancy activities, the Culture Collection of Algae and Protozoa (CCAP) in Scotland (UK) also engages in the development and optimisation of cryopreservation methods and techniques. Similarly, the National Collection of Pathogenic Viruses (NCPV) in the UK and the American Type Culture Collection (ATCC) in the USA provide cryopreservation and lyophilisation services to new deposits, supply virus strains at temperatures below -80°C (typically in combination with cryoprotectants such as dimethylsulfoxide [DMSO] and fetal bovine serum [FBS]), and provide specific guidelines and support to the community via detailed protocols on virus maintenance, propagation and storage. Collectively, these services provide additional tools to the research community in the form of optimised methods for preserving microbes, and, in the case of viruses, preserve and maintain high levels of infectivity and viability.

The application of cryopreservation to aquatic viruses is not trivial as it relies heavily on existing infrastructure such as freeze-dryers, extremely low temperature storage facilities, and self-contained liquid nitrogen generation capabilities. Hence we suggest that an Aquatic Virus Culture Collection (AVCC) focusing on algal and bacterial viruses from aquatic environments should be at the onset, established in parallel and in close proximity to an existing culture collection that already has such infrastructure in place (e.g., CCAP in Scotland; CPCC in Canada; RCC in France; NCMA in Bigelow, USA; UTEX in Austin Texas, USA; etc.). Importantly, cryopreservation development efforts within the framework of an AVCC can benefit from progress already made by others in the field of environmental virology. Cryopreservation of viruses infecting the red tide-forming dinoflagellate *Heterosigma akashiwo* (i.e., HaVs) was shown to be successful at -196°C in the presence of cryoprotectants such as DMSO (Nagasaki & Yamaguchi, 1999). Nevertheless, even at the optimum conditions, the highest recovery of HaV infectious virus particles was only 8.3%. The low recovery of infectious viruses here and in other similar efforts is likely due to the unknown as of yet toxic effects of osmoprotectants on viruses, and the effects this may have on their viability. To that end, a recent study employed an innovative approach, whereby a very high percentage of PBCV-1 chlorovirus particles was maintained while cryopreservation was performed during active host infection (Coy et al., 2019). The approach utilised the addition of DMSO, ethylene glycol and proline, and storage at -150°C . It was hypothesized that this may be an approach worth considering for other algal

viruses, which may be benefiting from naturally occurring cryoprotectants of their hosts during infection. Indeed, an AVCC that will have at its disposal a range of host organisms and their viruses, and the appropriate infrastructure for cryopreservation methods development, will be the perfect place to develop similar to the aforementioned approaches with other host-virus model systems.

An aquatic virus culture collection will allow for increased experimental reproducibility and traceability

With the increased use of state-of-the-art single-cell and single-virus genomics approaches in virus ecology, use of single-cell viral infection-dynamics visualisation methods, and efforts to link mechanistic understanding from laboratory experiments to larger scale field observations of host-virus interactions (Allers et al., 2013; Ku et al., 2020; Nissimov et al., 2019), it is likely that the number of studies using virus isolates from private collections (Table 1) will increase. At the same time, the microbiology field in general is also recognising that many studies across the different sub-disciplines can benefit from standardisation of protocols and common analytical frameworks (Thompson et al., 2017), improving the reproducibility of studies, and the way key findings are being reported. Indeed, some peer-reviewed journals now request authors to identify within their manuscripts the strain number of their experimental subjects (i.e., microbe of interest), the culture collection(s) that these subjects have been obtained from (or deposited in), and the NCBI accession numbers and metadata if available. For instance, journals such as the *Journal of Virology*, *Viruses*, the *Journal of General Virology*, the *Virology Journal*, and *Virology*, expect that authors adhere to the updated international standards of virus taxonomy for virus nomenclature and naming (which is overseen by the International Committee on Taxonomy of Viruses – ICTV), and ensure that where possible, novel strains and those used in experiments are deposited in recognised culture collections, and referred to, so that other researchers may have access to them. An even stricter requirement is employed by the *International Journal of Systematic and Evolutionary Microbiology* where authors have to provide evidence that their microbial strains are deposited in at least two different and recognised culture collections, residing in separate countries. In addition, existing culture collections also insist that their efforts are acknowledged when strains are used and mentioned in publications. Simply put, nowadays, a microbial species name is no longer sufficient, as mistakes can be made and taxonomic names may change.

These requirements have been put in place in order to enable scientists to use authenticated microbial strains, and where possible, eliminate biases arising from contaminated or non-axenic cultures. For example, a recent peer-reviewed study focusing on the design of a vaccine against the Dengue virus issued a retraction when the authors discovered that their experimental system was contaminated, and contained a wild-type virus (Messer et al., 2015). It is likely that similar instances of cross-contamination or the use of an unauthenticated virus strain in experiments also occur in viral ecology studies. It is easy to imagine that due to the currently unofficial and uncontrolled manner in which viruses within the aquatic virology community change hands, mismatches and methodological mishandling of strains during experiments may occur. For example, the majority of coccolithoviruses currently in culture (Table 1) were isolated between the years 1999–2001 from the English Channel. In subsequent years, these viruses have been sent to laboratories across the world, including researchers in Norway, the USA, Israel, and Scotland, where the collective research has focussed on elucidating various aspects of their ecology, biology and role in ocean biogeochemistry. Nevertheless, there have been numerous inconsistencies with the way different researchers propagate these viruses prior to experiments, specifically as it relates to the way new virus stocks are being propagated (i.e., the timing of new virus harvesting and the type of host used for virus propagation is not consistent across different research groups or different experiments). This is an aspect that may fundamentally affect the number of infectious viruses available and the subsequent infection dynamics one is interested in studying. Hence, one can envisage how the field may benefit from depositing their strains in a designated AVCC, which will employ standardised protocols, and provide quality control assurances as it relates to virus stocks, their viability, their exact identity, and propagation history.

And although the outlined above requirements cannot be applicable to studies where viruses have not been isolated, studies where virus genomes have been assembled *de novo* directly from metagenomic datasets have now also implemented modifications and improvements to the way their data is being reported. This includes guidelines for the Minimum Information about an Uncultivated Virus Genome (MIUViG), comprising of the origin of isolation, the quality of the genome and its annotation, associated biogeographic distribution of the viral genome, and the potential host organism the virus may be infecting (Roux et al., 2019). Hence, reproducibility, traceability, standardisation and reporting are important evolving issues in viral ecology.

Preservation of reference biological material that may otherwise be lost

A recent study showed nearly 200,000 novel virus populations as part of the TARA ocean expedition, with many virus groups clustered temporarily and spatially throughout the global ocean (Gregory et al., 2019). It was also revealed that the Arctic, which is the fastest warming ecosystem on Earth (Stocker et al., 2013), is currently a hot spot of virus biodiversity. Although we do not know the exact effects of climate change on many microbial constituents, there is some evidence that viruses are subject to environmental change (Danovaro et al., 2011). Hence, it is likely that viruses in hot spot zones such as the Arctic may be lost at some point due to environmental change, or at least have their current community structure and associated genetic diversity altered. This is especially true for some viruses, which are cold-adapted (or cold-active) and are evolutionary optimised to exploit and infect their hosts only at lower temperatures (Wells, 2008). Indeed, a recent “consensus statement” by researchers from 35 different institutions emphasised the global importance of microorganisms (including viruses) in climate change biology, and the importance of including microorganisms and what they represent as crucial ecological building blocks for an environmentally sustainable global ecosystem (Cavicchioli et al., 2019).

Despite the diversity of viruses in the field, and the previously outlined metabolic diversity and potential hidden within cultured virus isolates, efforts to preserve this diversity are so far inadequate in our view. The societal benefits of many of the outlined aquatic viruses already in culture (Table 1), and that of viruses that are still in their natural setting are so far unknown. Yet we believe that efforts should be made to preserve it, and novel approaches to obtain additional viral isolates and their hosts should be developed. As with most microorganisms, most viruses are uncultured, which means that even when we discover via direct sequencing novel viruses and potential functions, the information obtained may remain meaningless in the absence of relevant reference biological material. Further, if we are deterred by the isolation of novel viruses as model systems, the accurate annotation of virus genes from the environment will remain at its infancy, and will be at the mercy of mainly advances made in research that focuses on viruses important in human and animal health.

Classifying, cataloguing and preserving viruses has an unexplored potential

The importance of classifying and cataloguing viruses was highlighted in a recent note by Kuhn et al. (2019)

which suggests that viruses, and in particular those that can be useful in “phage therapy”, may be a crucial milestone in treating multi-drug resistant bacteria causing disease, and in the potential discovery of new enzymes useful to the pharmaceutical industry. In addition, these authors note that although environmentally important viruses may not be pathogenically relevant and may not have an immediate application and an obvious societal benefit, their long term preservation and the cataloguing of additional isolates may yield unexpected benefits in the future, just as has previously occurred with seemingly harmless bacterial isolates which provided to the scientific community novel antibacterial agents, heat resistant proteases, and restriction enzymes (Kuhn et al., 2019).

One of the major road-blocks to our understanding of the roles of putative virus genes or those with no assigned function in large metagenomics datasets is the poor availability of isolates in culture that hinders accurate annotation and the ability to link the metagenome information to gene products and subsequent functions. In other words, metagenomics data alone does not allow for testing the expression profile of genes of interest and confirming the identity and subsequently the role of some of these genes as it relates to various metabolic and phenotypic characteristics. This includes rates of substrate uptake and preference, the morphology of viruses which can be important during infection, and the optimum conditions (i.e., temperature, pH, etc.) at which viruses exhibit maximum activity. In addition, *de-novo* sequenced and assembled genomes from large datasets do not reveal much about the intra-strain differences of closely - related but different virus strains, as it relates to their virulence and the host responses and defence mechanisms they may trigger during infection.

For instance, it is work with virus isolates in the laboratory that elucidated the role of virus-derived glycosphingolipids in terminating coccolithophore blooms and the rate-limiting biochemical steps in this process (Han et al., 2006; Vardi et al., 2012, 2009), the competitive interactions between different coccolithoviruses (i.e., viruses infecting the *E. huxleyi* microalga) over the host for infection and the use of glycosphingolipid as virulence factors affecting viral success (Nissimov, Napier, Allen, & Kimmance, 2016; Nissimov et al., 2019), autophagy pathways in *E. huxleyi* during infection (Schatz et al., 2014), the virus strain-specific variations with regards to the induction of polysaccharide production in infected *E. huxleyi* cells (Nissimov et al., 2018), and the role of diatom-infecting viruses in aggregating material into sinking particles, important to the carbon biogeochemistry of the ocean (Yamada et al., 2018). Other notable examples where aquatic virus

isolates were used to shed light on putative functions include the discovery of auxiliary metabolic genes (AMGs) in marine cyanophages (Breitbart, 2012), chlorovirus genes involved in carbohydrate metabolism (Van Etten et al., 2017), and genes involved in the production of fibers in Mimiviruses (Sobhy, La Scola, Pagnier, Raoult, & Colson, 2015).

A complementary approach to the above which also requires virus isolates is visualising the morphology of viruses and empirically testing the physicochemical conditions that affect viruses and their infectivity. Examples of discoveries made based on visualisation and experimental work of isolates include the use of cryo-scanning electron microscopy and electron tomography of the *Acanthamoeba polyphaga* virus capsid which elucidated the manner in which its large genome is released into the host cell (Zauberman et al., 2008), the budding release of coccolithoviruses from infected cells visualised by TEM (Mackinder et al., 2009), and the effects of elevated temperatures, pCO₂ concentrations, and nutrient availability on the infectivity and the lytic cycle of *E. huxleyi*- and *Micromonas pusilla*-infecting viruses (Kendrick et al., 2014; Maat, Crawford, Timmermans, & Brussaard, 2014).

The deposit of aquatic viruses into a virus culture collection and their distribution, will with no doubt require a careful and thoughtful approach. It will have to ensure that the viruses are classified and integrated within their correct grouping within the collection, and that novel cataloguing approaches are considered. Indeed, existing cataloguing systems in already established non-viral culture collections may need to be modified. This will require that the depositors submit the maximum amount of information and metadata at their disposal and making sure that the ICTV guidelines for the taxonomy, classification, and nomenclatures for the viruses they deposit are adhered to. A possible outline of the type of information which may be required by an AVCC for a new deposit for cataloguing is provided in Table 2.

The concept of a virus culture collection is not new

The World Data Center for Microorganisms (WDCM) and the World Federation for Culture Collections (WFCC) collate information on more than 700 culture collections globally, with collective deposits of > 3 million microbial strains (www.wfcc.info/ccinfo/home/). Of these, only ~1% are virus isolates, consisting predominantly of human pathogenic viruses, animal viruses, plant viruses, and bacterial viruses (phages). In addition, only ~5% of culture collections registered at the WFCC contain algal cultures. With the exception of the Roscoff

Table 2. A potential virus information sheet at an AVCC should provide information to users about the viruses within the collection and their host organism. Initial details should be provided by the depositors of the viral strains with as much information as possible, while adhering to the taxonomic guidelines of The International Committee on Taxonomy of Viruses (ICTV). Note that the accompanying information listed under the host organism is not as detailed as that usually provided by an existing algal or bacterial culture collection. This information (and the extent to which it goes into detail) may vary depending on the type of information available to the AVCC about the specific host organism.

	Catalogue field	Details
Virus	Family:	Taxonomic details as per the ICTV guidelines (https://talk.ictvonline.org/)
	Order:	
	Genus:	
	Species:	
	Previous known names:	Other designations by which the virus is known / synonyms
	Genome:	Nucleic acids- dsDNA/ssDNA/dsRNA/ssRNA
		Shape- linear, circular, segmented (if information available)
		Genome size (if information available)
	Size:	The approximate size of the virus particle (nm)
	Infection mode:	Lytic/lysogenic (if known)
	Isolated by:	Name of person and institute
	Isolation year:	Year the virus was isolated
	Origin of isolation	Marine/freshwater/brackish & the geographical location if known
	Sequenced by:	Name of the person/research group that sequenced the virus (if applicable)
	Sequencing year:	Year the virus was sequenced and information on the sequencing platform and center (if known)
	NCBI accession #	Of draft genome, full genome, or partial coding sequence of a marker gene (if available)
	Deposited at the AVCC:	The date when the virus was first deposited at the AVCC
Designated # at AVCC:	The culture collection number given for the virus/strain	
Culture media:	The medium in which the virus is stored	
Storage temperature:	4°C, -20°C, -80°C, or -196°C	
Filter purification:	0.2 µm/ 0.45 µm purification of virus particles from cell debris prior to storage (yes/no)	
Cryopreserved:	Yes/no	
Viability/stability	The time intervals the virus should be propagated to prevent a significant decrease in its infectious titre	
Images:	Availability of SEM/TEM images of the virus particle (yes/no)	
Other information	Availability of virus propagation/ maintenance methods and associated health and safety information	
References	Relevant literature for the virus genome, biology, and its ecology (if available)	
Host Organism	Genus/Species:	The host organism that the virus infects and is propagated in at the AVCC
	Type of organism:	Microalgae/bacteria
	Cell features:	Any distinctive features of the host cell (e.g. calcification, silification, flagellum, etc.)
	Strain designation #:	The designation of the host strain at the AVCC or its name if acquired from a different culture collection
	Availability of host:	Is the host organism available at the AVCC for purchase? (yes/no)
	Origin:	The environment from which the strain was isolated (freshwater/marine/brackish)
	Culture:	Details of the medium used to culture the strain at the AVCC
	Light intensity:	The average light intensity for optimal growth
	Temperature:	Maximum and minimum temperature for growth
	Diel cycle:	The light:dark cycle for growth
	Other information:	Availability of methods for host culturing and maintenance
References:	Relevant literature for the host organism that the aforementioned virus infects (if available)	

Culture Collection (RCC) in France, which has integrated a number of marine virus isolates within their algal culture collection (i.e., predominantly prasinoviruses infecting *Micromonas* sp.), a detailed survey reveals no substantial move by the community towards the establishment of a more centralised depository and a “biobank” for viruses infecting aquatic algae and/or bacteria.

Some existing microbial culture collections have a long history of maintaining bacterial viruses with recognised potential and applications in human health and agricultural research. For instance, the Felix d’Hérelle Reference Center for Bacterial Viruses in Quebec (Canada) was founded in 1982 and maintains >400 bacteriophage isolates. Other such collections include the German Collection of Microorganisms and Cell Cultures (i.e., the DSMZ at the Leibniz Institute) which was established

in 1969 and has around 300 bacteriophage isolates, the National Collection of Type Cultures (NCTC) in Salisbury (UK), which was established in 1920 and has around 100 bacteriophage isolates, and the Bacteriophage Bank of Korea in Yongin (South Korea), which was established in 2010 and has >1000 bacteriophage isolates that target pathogens such as *Salmonella enterica*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Acinetobacter baumannii*, *Enterococcus faecalis*, *Serratia marsescens*, and *Bacillus cereus*.

Broadly, the collective aim of these virus collections is to collect, conserve and distribute reference virus isolates (and associated information), expand the range of available isolates for biodiversity-focussed research, curate viruses that target other pathogens, conduct research for standardising virus-related methodology, participate in various external education and research activities, and

to generally ensure reproducibility in virus research. Crucially, these collections provide additional services to the research community, including research services such as electron microscopy for virus morphology, analysis of virion protein composition, genome-wide bioinformatics analysis, strain screening for lysogeny, host-range analysis, viability testing, and development of methods for long term virus preservation.

The collection and maintenance of viruses goes beyond that of bacteriophage isolates. For instance, in addition to a bacteriophage collection, the American Type Culture Collection (ATCC) in Manassas (Virginia, USA), which was established in 1925 and has > 350 bacteriophage isolates, also serves as a depository for more than 3000 animal and plant viruses. In addition to numerous human viruses, these include viruses infecting livestock and various crops. The National Collection of Pathogenic Viruses (NCPV) in the UK was established in 1999 and provides numerous clinically important viruses. While the collective aim of the ATCC and NCPV collections is the same as that outlined above for bacteriophage culture collections, they also provide additional services such as virus purification, virus nucleic acid extraction, and supply of antibodies and synthetic viral nucleic acids. Importantly, these collections

have laboratory infrastructure at a high biosafety level necessary for work conducted with extremely infectious pathogens, with which private institutions and some universities may not be equipped.

To summarise, virus culture collections provide important services that may not otherwise be available to the community. While important lessons can be learned from the existing virus culture collections outlined here, we posit that these are likely not suitable for the maintenance and provision of aquatic viruses and their hosts. Rather, we suggest that an AVCC should take advantage of existing expertise on marine and freshwater research (and of existing infrastructure) in a culture collection that has a focus on aquatic microorganisms.

Challenges and ways forward

The creation of an AVCC will involve multiple challenges. Its initial objective should be simple: *to be an aquatic virus culture collection where the community can deposit their viral strains for long term preservation, obtain viral isolates and their hosts, and to supply support through streamlined, reproducible protocols (Fig 2(A)).* This, however, will require the active involvement of

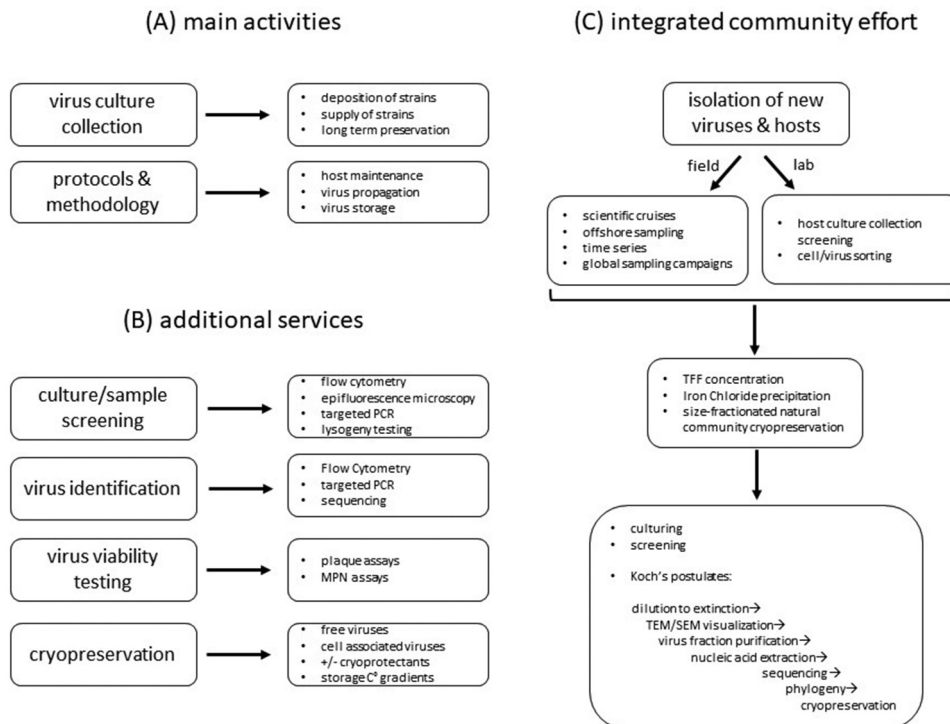


Figure 2. Schematic representation of the type of services an Aquatic Virus Culture Collection (AVCC) may be able to provide, and a road map for the isolation of novel host-virus model systems. **(A)** Main services are predominantly deposition and supply of viral strains and their long term preservation in culture or a cryopreserved state. **(B)** Additional services which will allow for the collection to be financially sustainable include sample screening for viruses, identification of viruses, viability testing and optimisation of cryopreservation methods. **(C)** The road map for isolating novel hosts and their viruses from aquatic include field- and lab based approaches with the ultimate goal being fulfilment of Koch's postulates of new viruses and their hosts, and their subsequent long term preservation at the AVCC.

researchers within the community on more than one level. Firstly, a communal agreement and a push towards funding for setting up this collection will be essential. From the onset, this could be achieved by a detailed “mission statement” for the AVCC for which members of the community provide formal expressions of support. These would strengthen subsequent proposals for national and international funding. Once initial funding is in place, a pilot collection could be established which will include a subset of aquatic viruses and their hosts. This will demonstrate the concept and its benefits to the community, and will make a case for larger subsequent proposals to expand the collection. An AVCC can be established in parallel to an existing culture collection that specialises in culturing algae (e.g., the CCAP in Scotland, the RCC in France, or the CPCC in Canada). Such an approach will benefit the new initiative in that it will take advantage of already existing scientific expertise and infrastructure, and established laboratory microbiology practice developed in these culture collections for the mitigation of potential health and safety risks through established bio-safety standard operational procedures.

Perhaps equally important will be the willingness of key institutions and their members to provide specific protocols on the different host-virus systems they currently work with. There is no universal method for aquatic virus/host propagation and storage. For example, coccolithoviruses are currently stored by separating the viral particles from the cell debris post infection via 0.45 µm filtration. At 4°C, their infectivity is maintained for only between 6–12 months and the infectious titre reduces quickly. In contrast, chloroviruses are not filtered post cell lysis and can remain highly infectious at 4°C for more than a year. Other smaller ssDNA and ssRNA viruses such as those infecting the diatom *Chaetoceros tenuissimus* are separated from cellular debris via a 0.2 µm pore size filtration. Their infectivity at 4°C may be even more short-lived than that of coccolithoviruses. Other parameters such as optimal temperature for host growth, the light:dark cycle, and the cellular growth phase at which infections are conducted to ensure maximal production of viral particles also differ among systems. Clearly, these different approaches require detailed knowledge of the host-virus systems (i.e., scientific input from those familiar with the different model systems will be essential).

While members in the community are unlikely to object to sharing their protocols publicly (note that many protocols used by the aquatic virus research community are already shared publically via the online protocols.io VERVENet platform, and special issue publications such as the ASLO Manual of Aquatic

Viral Ecology from 2010), a more challenging aspect will be putting in place agreements for research groups to supply virus isolates to a collection which will ultimately supply these to third parties. Researchers may be reluctant to share with the wider community viral isolates that they have worked intensively on and which have yielded successful grant applications, research projects, and publications. However, depositing in a centralised repository will eliminate the time currently spent by research groups propagating fresh viral stocks whenever potential colleagues wish to obtain one of their isolates, and will serve as a back up to their own private collection. Coupled with research into cryopreservation, the AVCC will also minimise the risk of virus stock contamination and genetic drift. Perhaps the biggest incentive to research groups supporting this initiative will be the added international exposure this will generate, and their global recognition as the first port of call for science-related queries by the community with respect to their particular host-virus system. Moreover, such an exposure may result in unexpected, novel collaborations and research opportunities.

An additional challenge that most culture collections face (and the AVCC will be no exception) is to ensure long term financial sustainability. In addition to charging for strain provision, culture collections take advantage of the expertise and infrastructure at their disposal to provide additional services for a fee. For an AVCC, these services could include the screening of environmental samples or cultures for the presence of viruses using flow cytometry (FC), epifluorescence microscopy, and polymerase chain reaction (PCR) fingerprinting; testing samples for lysogenic viruses by exposing host cells to mitomycin C and UV-irradiance; the identification of virus groups and families via FC, targeted PCR, and sequencing; testing the viability of deposited viruses using plaque and most probable number (MPN) assays; and optimising protocols for cryopreservation of marine and freshwater algal and bacterial viral isolates (Fig 2 (b)). Consumer-fees would support costs associated with purchase of consumables and chemicals, and the maintenance of necessary instrumentation.

An AVCC can be also seen as a research opportunity and a prospect to advance the viral ecology research field. As previously mentioned, it is known from sequencing of environmental samples that viral diversity is much higher than previously thought. It is thus likely that the viral isolates currently in culture (Table 1) cover only a small fraction of their real genetic, metabolic and phenotypic diversity. This is particularly true for viral functional genes important during infection and replication, and those that may have as of yet unexplored biotechnological applications. Therefore,

in our view, increased efforts into isolation of new viruses and their hosts are essential (Fig 2(c)). These efforts should be collaborative and take advantage of already existing or planned field sampling campaigns of lakes, rivers, coastal areas, and open oceans. There are previous examples where a research community approach combined its resources in a cross-disciplinary collaborative manner to sample various habitats. These include campaigns such as the TARA Ocean expeditions (Karsenti et al., 2011), the Sorcerer II Global Ocean Sampling Expedition (Rusch et al., 2007), and the Malaspina 2010 Circumnavigation Expedition (Duarte, 2015). Their collective goal was to utilise state of the art next-generation sequencing to map and elucidate the global diversity of marine life at different temporal and spatial scales and couple this diversity with oceanographic measurements. However, the isolation of novel hosts and their viruses was not one of their aims.

A possible approach and an expansion to the above may therefore be a commitment by the community to capture and isolate novel hosts and their viruses during similar ongoing and planned research expeditions and work alongside a designated AVCC. This may be achieved through the use of standardised field protocols for the size fractionation of microbial groups and their *in situ* cryopreservation (for subsequent growth on various selective media at an AVCC), concentrating virus fractions in the field via tangential flow filtration (TFF) and iron chloride precipitation (Grzenia, Carlson, & Wickramasinghe, 2008; John et al., 2011), screening of potential field and culture collection samples for the presence of different viral groups, and subsequently (at the conclusion of a field campaign), testing and isolating novel viruses from the aforementioned samples on host strains, which are either already in culture, or on newly isolated hosts, at the AVCC facility. Upon the isolation of new viruses, additional procedures which take advantage of expertise and availability of low temperature storage facilities at an AVCC, should focus on virus purification, sequencing, viability, lysogeny testing and cryopreservation optimisation for long term storage. As a starting point, appropriate candidates to undertake the aforementioned approach in conjunction with a virus culture collection pilot project may be ongoing global and regional campaigns such as The Ocean Sampling Day (OSD) initiative (Kopf et al., 2015), the Atlantic Meridional Transect programme (Robinson, Holligan, Jickells, & Lavender, 2009), and routine off-shore sampling campaigns such as the L4 time series off the coast of Plymouth (Harris, 2010) and the Hawaii Ocean Time-series at station Aloha (Karl & Lukas, 1996).

Concluding remarks

Despite the existence of culture collections dedicated to agriculturally relevant and/or pathogenic viruses, a centralised “bank” or depository for the growing aquatic virus research community is absent. This is despite the recognition of aquatic viruses as important players in evolutionary processes, their environmental role in global food web dynamics and biogeochemistry, and their industrial, unexplored as of yet potential. A vision was thus presented here with arguments in support for the establishment of the first culture collection dedicated for viruses infecting aquatic microorganisms. The arguments presented here highlighted not only the needs and the benefits of such a resource, but also its challenges. Importantly, we have argued that an aquatic virus culture collection should be a research community-backed effort, an effort that complements and builds upon past and ongoing research, and takes advantage of existing infrastructure and expertise of culture collections which are already dedicated to the storage of microalgae and bacteria.

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