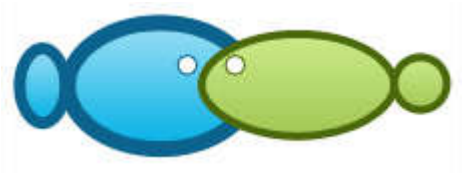

A review on marine viruses in sponges and seawater

[1]Effa S. Jamal, [1]Logajothiswaran Ambalavanan, [1]Shumpei Iehata, [1,2]Sandra C. Zainathan

[1] Faculty of Fisheries and Food Sciences, University Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia; [2] Institute of Marine Biotechnology, University Malaysia Terengganu, 21030 Kuala Nerus, Kuala Terengganu, Malaysia.
Corresponding author: Sandra C. Zainathan, sandra@umt.edu.my

Abstract. In marine environments, viruses play many important roles by affecting biogeochemical cycles, the composition of the microbial population, horizontal gene transfer between cells, and host metabolic reprogramming. Viruses do not self-propel, breathe, or grow outside a host setting, and to survive must infect a host cell. There is a major information gap regarding the occurrences of marine viruses in sponges and seawater, the interaction of sponges with viruses and detection methods used to identify marine viruses in sponges. This review addresses the occurrence of marine virus in sponges and seawater, functions of viruses in marine ecosystems, interaction of viruses with sponges, and detection methods used to identify the presence of the marine viruses. The most prevalent viruses in sponges are from the following families including Siphoviridae, Myoviridae, Podoviridae, Phycodnaviridae, Poxviridae and Mimiviridae. Bacteriophages (families Siphoviridae, Myoviridae and Podoviridae) had the largest proportion in sponges such as *Stylissa carteri*, *Carteriospongia foliascens*, *Lubomirskia baikalensis*, *Amphimedon queenslandica, Xestospongia testudinaria, Rhopaloeide odorabile, Ianthella basta,* and *Hymeniacidon perlevis.* Whereas the most prevalent viruses in marine seawater are algal viruses. As reported in the literature, various detection methods such as PCR amplification, metagenomics analysis, DGGE and TEM (Transmission Electron Microscopy) have been used to detect the presence of viruses in sponges and seawater. To the authors' best konwledge, this is the first review of marine viruses in sponges and seawater.
Key Words: Marine environment, viruses, marine sponges, bacteriophages, algal viruses.

Introduction. The most bountiful and various biological individuals in the earth (Angly et al 2006) are viruses, with 10^6 to 10^9 particles (mL seawater)$^{-1}$ (Kristensen et al 2010) and 10^30 unique viral genotypes (Suttle 2007). Communities of marine viruses are greatly diverse, with over 195,728 documented viral populations (Gregory et al 2019). Viruses are incredibly important life forms in the microbial environment. They are vital to sustain and influence the diversity of all microbial species, both by directly influencing them (by regulating bacterial cell numbers) and by horizontally transmitting genetic material to the host via the transduction process (Lohr et al 2005). Phages can also influence the microbial development based on the 'destroy the winner' hypothesis, by killing certain dominant bacteria enable other similar strains that are immune to the phage to become dominant (Angly et al 2006). The interaction between phages and their bacterial hosts, however has largely remained under review, especially with regard to marine sponge ecosystems (Harrington et al 2012).

Viruses do not self-propel, breathe, or grow outside a host setting and must infect a host cell to survive (Fuhrman 1999; Wommack & Colwell 2000; Danovaro et al 2008; Rohwer & Thurber 2009; Breitbart et al 2018). They have various strategies of infection (Roberts & Compans 1998; Steward et al 2013) including lytic, chronic, and lysogenic life stages (Howard-Varona et al 2017). Viruses inject the nucleic acid into the host cell throughout the lytic cycle, take over periodic cellular processing, and prioritize the production of various virions (infectious particles produced within the host) that are released after the bursting of host cells. Lysogenic viruses will eventually turn to a lytic or

chronic phase that releases virions, either naturally or caused by environmental changes (Hobbs & Abedon 2016). Generated viral copies are extruded from the cell via a non-lethal exclusion in the chronic life cycle. The viral nucleic acid persists within the host cell throughout the lysogenic cycle and reproduces during normal cell division processes as genetic material, without releasing virions. For prolonged periods, viruses will remain in a free virion state (not associated with a host cell) (Allers et al 2013) and drift through environments with considerable stability (Pirtle & Beran 1991).

Viruses directly affect the energy flux in food chains of the marine environment through their regulation of prokaryotic and eukaryotic populations (Fuhrman 1999; Weinbauer 2004; Roux et al 2015). The overview of their variety and benefaction on host ecology, considering the essential role of viruses in marine environments is just a start. Although subsurface maximum of viral profusion has been discovered at several sites, it is mostly detected in euphotic zone (Wommack & Colwell 2000). Furthermore, viruses are one of the major reservoirs of known genes with possible biotechnological applications in the search for new enzymes and compounds (Martínez et al 2014). Sponges are notable taxa to explore the role of viruses in holobiont communities (Claverie et al 2009) because they are ecologically significant, evolutionary important, and host a broad range of macro- and micro-symbionts (Thomas et al 2016). Sponges and their related viruses are likely to affect the ecological function of marine biogeochemical cycling as they both contribute to the cycles of organic and inorganic matters in seawater (Steindler et al 2002). Over the past two decades, direct count methods have progressed from transmission electron microscopy to flow cytometry and epifluorescence microscopy. Thus, viruses were discovered from thousands of samples in the marine seawater (Suttle & Fuhrman 2010). Substantial efforts have been made to improve and standardize the techniques that can be used to describe viruses in marine ecosystems (Weinbauer 2004). While viruses have been recognized as important components of marine ecosystems, they also can regulate the dynamics of the microbial population, play a role in coral bleaching or disease, and mediate biogeochemical cycling of reefs (Thurber et al 2017). However, there is a lack of studies on viruses related to the sponges that are ecologically important even though a few reviews/research exist on the occurrences of marine viruses in seawater.

The first record of viral particles (VLPs) in sponges was detected via transmission electron micrographs (Vacelet & Gallissian 1978). However, the computational tools were not optimized until 2016 to investigate sponge-related viruses using metagenomic sequencing (Laffy et al 2016). A following comparative metagenomic study of coral and sponge-viruses relation revealed a great similarity between four sponge-species intra-species viromes, with order Caudovirales-ordered of dsDNA bacteriophage dominated populations, and a diverse ssDNA virus population of the Microviridae family. Moreover, viruses of order Megavirales, as well as the members of the Phycodnaviridae, Mimiviridae, and Poxviridae families were regularly found. Viromes of these reef sponges have been identified as AMGs involved in herbicide resistance and cobalamin biosynthesis (Laffy et al 2018). In addition, it is essential to understand how sponge viromes respond to the acute anthropogenic effects that are seriously endangering marine environments worldwide, as viruses are key organisms in marine community modulation (Rohwer & Thurber 2009; Mojica & Brussaard 2014).

Thereby, this review addresses the recent knowledge on the diverse virus communities that affect marine sponges. The objective of this review is to provide an update on the occurrence of marine virus in sponges and seawater, functions of viruses in marine ecosystems, interaction of viruses with sponges as well as different detection methods used to detect the presence of the marine viruses.

Methodology. Databases such as Web of Science, CABI, Google Scholar, Science Direct and PubMed were searched using the following terms:

1. Marine virus *
2. Sponges*
3. Detection*or diagnosis*
4. Seawater

5. Molecular methods* or detection method* or extraction method*
6. And combinations of the above terms.

Marine sponge ecosystem. Sponges form an essential and functional part of the coral reef system (Diaz & Rützler 2001; Bell 2008). They can be found in various environments including intertidal zones, shallow, mesophotic and deep reefs (Roberts & Davis 1996; Barnes 1999; Olson & Mccarthy 2005). They are also immune to serious temperature, salinity, current, radiation and pollutant conditions (Richelle-Maurer et al 1994; Barnes 1999; Taylor et al 2007). Several sponge biological roles can be partially attributed to their associated microorganisms, including feeding, protection and growth, which can affect the way sponges react to environmental changes (Richelle-Maurer et al 1994; Shieh & Lin 1994; Walters & Pawlik 2005; Taylor et al 2007; Webster & Taylor 2012). In marine sponges, the functions prokaryotes play have been broadly reviewed (Thomas et al 2010; Webster & Thomas 2016; Lurgi et al 2019).

Sponges in marine seawater are significant organisms of the world's benthic habitats, which influence benthic or pelagic processes in terms of both biomass and their ability (Dayton et al 1974; Dayton 1989; Gili & Coma 1998; Maldonado et al 2005). Due to their longevity, abundance, and exceptional survival, marine sponges of the benthic population are essential species. Despite the lack of specialized organs and behaviour, sponges in balmy, tropical and polar environments are essential and omnipresent. Many sponges that have silica spicules are threatened to ocean acidification and global warming, offering a cutting edge over other calcareous species (Bell et al 2013). Most sponge species are suspension filter feeders with a complex aquifer system composed of channels and chambers that allow large amounts of seawater to be filtered. In some cases, sponge species may filter more than a thousand times their own volume every day (Patterson et al 1997). Flagellated choanocyte cells are driven by a unidirectional water flow (ostia-chamber-atrium-oscula) which is also responsible for collecting and maintaining small particles of high efficiency (<0.1 μm) (Thomassen & Riisgård 1995).

Sponges are ancient aquatic animals which lack organs but have a unique matrix of collagen and cells. Their frames are structured around a simple or complex water system that makes them extremely efficient filter feeders (Campbell et al 2009). Marine sponges are classified as spongy (Demospongiae), glass (Haxactinellida), and bony (Calcarea), except for the Archeocyatha (primitive class of sponges fossils during cambrian period). A wide variety of textures, colors, shapes, and sizes are available for sponges. Most of them are bright in color (shaded in orange, yellow, red or green) with highly differentiated mesophyll consist of spicules of various shapes and many unique functional cells. Marine sponges have captivated remarkable recognition from diverse scientific disciplines due to the control of proteins and chemical molecules such as collagen especially in Demospongiae. They produce various novel chemical compounds and attract incredibly interesting chemical compounds. Sponges serve as microbial fermenters in marine microbiology and biotechnology that create new approaches by accomodating symbionts such as nematodes, bacteria, fungi, worms, clams, dinoflagellates, diatoms, green algae, red algae and cyanobacteria (Webster 2007; Pallela & Kim 2011).

De Goeij et al (2013) suggested that in a process known as the "sponge-loop," sponges can make dissolved organic matter accessible to other reef species through rapid cellular turnover and detritus expulsion. Although this energy and nutrient flux has been experimentally demonstrated, the rapid cell turnover mechanisms have not been clarified, although sponge symbionts were involved in this process. These filter feeders are also noteworthy for their excellent efficacy in extracting microbial and viral particles from seawater (Hadas et al 2006).

In 1993, the scientist Lynn Margulis coined the word "holobiont" to describe the entire organism consisting of different bionts living in symbiogenesis (Margulis 1981). Holobiont has been widely used to refer to coral reef species, including marine sponges, since in their mesohylene eukaryotes they frequently host a wide variety and abundance of bacteria, archaea and unicellular eukaryotes (collagenous matrix within the sponge). Up to half of the sponge biomass can consist of these microorganisms (Cuvelier et al

2014), and in marine sponges symbiotic relationships from mutualism to commensalism and parasitism are proven (Webster & Taylor 2012). Nutritional functions, such as contributions by nitrogen-fixing bacteria and autotrophic symbionts (Wilkinson & Fay 1979; Shieh & Lin 1994), are especially significant among those interactions. 80% of intertidal sponge species are estimated to benefit nutritionally from compounds directly produced by photosynthetic symbionts (Steindler et al 2002). Furthermore, sponge symbionts provide UV defense, protein recycling and photosynthate development (Wilkinson 1980; Arillo et al 1993; Steindler et al 2002; Yahel et al 2003; Taylor et al 2007).

For the development of secondary metabolites, sponges often rely on microbes which are often used in predatory and competitive pressure defense mechanisms (Walters & Pawlik 2005), and they are therefore a major focus of bioactivity studies (Taylor et al 2007; Perdicaris et al 2013). Symbiotic interactions are so intrinsically related that the holobiont genomic repertoire (or hologenome) has been indicated to be a unit of natural selection and evolution (Rosenberg et al 2007). According to their potential importance as new sources of pharmaceutical drugs, sponges have been discovered as useful, changing our outlook on and understanding of these basic metazoans' biology and biodiversity. The influence of reckless human activity has altered their fragile mechanism as they reside mainly in coral reefs (Van Soest et al 2012).

Viruses in marine ecosystems. Viruses were recognised over 70 years ago as natural components of aquatic ecosystems (Kriss & Rukina 1947), but their widespread effect was not recognized until the early 1990s, when their high abundance and key roles were discovered in structuring marine planktonic ecosystems (Borsheim 1993; Bratbak & Heldal 1990). Studies in this region have since been attempted to describe marine viral diversity, distribution, and interactions, focusing primarily on oceanic seawater (Jover et al 2014; Danovaro et al 2008; Gregory et al 2019). Communities of marine viruses are especially diverse, with over 195,728 recorded viral populations (Gregory et al 2019). Viruses are small intracellular binding parasites (20-100 nm), consisting of genetic material based on DNA or RNA (single, double stranded or both) that are covered by a protein coat (Gelderblom 1996).

In marine environments, viruses play many important roles affecting biogeochemical cycles, the composition of the microbial population, horizontal gene transfer between cells, and host metabolic reprogramming. Viral infections are responsible for killing 20% of the total daily marine microbial biomass (Suttle 2007) in a process known as "viral shunt", where the biomass is converted and trapped into particulate and dissolved organic carbon and inorganic nutrients. This process is aimed at the cycling of these organic and inorganic nutrients, supplying other organisms with this matter (Middelboe & Lyck 2002; Shelford et al 2012). Viruses are therefore essential species in the microbial loop that directly influence the flow of matter and energy in the webs of marine food (Azam et al 1983; Bratbak et al 1994). This process is essential in marine oligotrophic environments, such as coral reefs, as a major source of organic compounds (Dell'Anno et al 2015; Thurber et al 2017). In addition, the induced mortality of virus can be selective through the community of host distribution determination and acting as an essential bottom-up environmental operator of the microbial community composition (Hewson & Fuhrman 2007). Lehahn et al (2014) provided an illustration of how viruses can influence the composition of the microbial population, showing that viruses are likely to monitor algal blooms by infecting coccolithophore cells and highlighting the environmental importance of viruses as agents of mortality.

Another function of viruses is their ability to reprogram their host's metabolism by adding auxiliary metabolic genes (AMGs) that are virally encoded (Jiang & Paul 1998; Hurwitz et al 2015). Gene acquisition from their hosts may affect how host genes are expressed, change the metabolism of the host, sometimes redirect host energy and resources to maximize viral output (Thompson et al 2011). For example, it has been suggested that "bacterial-like" genes found in cyanophages generate the energy required for replication of cyanophage's DNA (Lindell et al 2007). However, it has been suggested that the combination of viral and host genes during infection can simultaneously benefit

the hosts by increasing their metabolism (Breitbart et al 2007; Paul 2008; Rosario & Breitbart 2012; Crummett et al 2016). For instance, phages (i.e. microbe-infecting viruses) infecting *Synechococcus* and *Prochlorococcus* bacteria have been proposed to help sustain the photosynthetic activity of the host during infection through genes encoding photosystem proteins (psbA, hliP) (Lindell et al 2004; Sullivan et al 2005). Likewise, within the 452 viral Global Ocean Survey assembled viral genomic scaffolds, 34 microbial gene families encoding distinct energy of metabolism pathways (electron transport, photosystem, and carbohydrate metabolism genes) were detected, indicating that marine phages may affect their host fitness through translation and post-translation control (Sharon et al 2011). A further detailed research on global ocean viromes (viral genome) reported 243 viral coded AMGs, indicating that nitrogen and sulfur cycling in the epipelagic ocean can be manipulated by viruses (Roux et al 2016). The expression of viral genes in lysogenic cells has been shown to promote host fitness, allowing antibiotic resistance, toxin development, and immune responses (Williamson et al 2001; Howard-Varona et al 2017). Therefore, several guidelines for the viral effect on host metabolism also lack validation by scientific research (Warwick-Dugdale et al 2019).

Viruses are present everywhere, and a recent study separated viral populations into five ecological zones [(Arctic, Antarctic, Temperate and Tropical Epipelagic (1-150 m), Mesopelagic (150-1000 m), and Bathypelagic (> 2000 m)], each forming a separate genotypic cluster, with tropical surface waters being a viral diversity hotspot (Gregory et al 2019). Clusters of viral and microbial dissemination have also been shown, suggesting that the primary factor structuring marine viral communities is the physicochemical structuring of marine microbial communities (Gregory et al 2019). In addition, viral assemblages have been proposed to exhibit greater richness at lower latitudes, caused by the proportions of different taxa present in these locations rather than by their presence or absence at specific locations (Angly et al 2006). Given that coral reefs are highly diverse ecosystems, by influencing the structure of prokaryotic and eukaryotic populations, controlling the composition, diversity, and functions of these groups, modulating their interactions, viruses may play an important role in preserving their complexity (Rohwer & Thurber 2009; Thurber & Correa 2011; Sweet & Bythell 2017; Weynberg 2018). The high diversity of symbionts in sponges makes them an ideal model for characterizing the role of viruses in marine holobionts, given that viruses theoretically infect all living cells (Fuhrman 1999).

Viruses in sponges. Viruses and virus-like particles are widespread in habitats of coral reefs including sponges and mostly detected in the water environment neighboring to reefs in the coral surface microlayer within their zooxanthellae endosymbionts and coral tissues (Wilson et al 2001; Seymour et al 2005; Davy et al 2006; Patten et al 2006, 2008; Davy & Patten 2007; Lohr et al 2007). While transmission electron microscopy (TEM) in sponges has detected VLPs (Vacelet & Gallissian 1978), no confirmed molecular reports are known to our knowledge of viruses affecting a marine sponge. Claverie et al (2009) stated that analysis of Mimiviridae, that was recently done using TEM micrographs (originally produced by Vacelet), concluded that the pictures of results showed infection of sponge phagocytes with a giant Mimiviridae-related virus, where sponge cells were classified as 'a fully active virus factory'. Moreover, another analysis under experimental conditions is the infection of an alphaproteobacterium symbiont from marine sponge *Ircinia strobilina* by bacteriophage FJL001. However, the detail of this interaction in nature was not recorded (Lohr et al 2005). Latest metaviromic (viral metagenomic) studies have shown that viruses are significant components of coral reef species that may interfere with their host functions via AMGs (Levin et al 2017; Weynberg et al 2017a, b; Laffy et al 2018). However, only a few studies have identified sponge viruses. Forty years ago, the first study on sponge-associated viruses was reported, demonstrating isometric particles similar to adenoviruses in *Verongia cavernicola* sponge cells by means of TEM examination (Vacelet & Gallissian 1978). Recent metagenomic studies have provided compelling evidence that viruses are important components of the holobiont sponge (Laffy et al 2019). The sponge virome has been shown to be substantially complex, species-specific, and widely expressed by groups of bacteriophages (Laffy et al 2018;

Jahn et al 2019). In addition, sponge viruses have genes that encode host auxiliary metabolic functions, such as genes for herbicide resistance (Laffy et al 2018) and ankyrin repeat protein, which have been suggested to help bacterial hosts escape eukaryotic immune responses within sponge holobionts (Jahn et al 2019).

Viruses are recognized to be pathogens of many aquatic species (Munn 2006), but very little attention has been given to their position as symbionts. This is despite the fact that a number of taxonomically diverse marine hosts have been found to benefit from many unrelated viruses (van Oppen et al 2009). Viruses, primarily by lateral gene transfer, may also serve as agents of evolution (Villarreal 2005). Sponges are notable taxa for investigating the role of viruses in communities of holobionts (Claverie et al 2009) because they are ecologically significant, evolutionary important and host a variety of macro- and micro-symbionts (Thomas et al 2016). The possible importance of viruses in sponges has been stated in previous publications (Claverie et al 2009; Webster & Taylor 2012; Laffy et al 2016, 2018), including sponge disease (Luter et al 2010). Sponges and their related viruses are likely to affect the ecological function of marine biogeochemical cycling as they are both characterized as contributing to the cycling of organic and inorganic matter in seawater (Wilkinson & Fay 1979; Shieh & Lin 1994; Fuhrman 1999; Steindler et al 2002). Although the first findings on the functions of the sponge virome have been revealed, some aspects of the relationship are still unknown.

Table 2 states that viruses are the most common components of the holobiont sponge with the following families: Siphoviridae, Myoviridae, Podoviridae, Phycodnaviridae, Poxviridae and Mimiviridae. Bacteriophages (the families Siphoviridae, Myoviridae and Podoviridae) had the largest proportion in sponges such as *Stylissa carteri*, *Carteriospongia foliascens* (Pascelli et al 2018), *Lubomirskia baikalensis* (Butina et al 2015, 2019), *Amphimedon queenslandica*, *Xestospongia testudinaria*, *Rhopaloeide odorabile*, *Ianthella basta* (Laffy et al 2018) and *Hymeniacidon perlevis* (Harrington et al 2012). Considering the high abundance and diversity of their bacterial hosts in the associated populations, this was predictable. Normally, these viruses predominate in aquatic biome viromes. Metagenomic studies of marine sponges have also shown that tailed bacteriophages predominate (Laffy et al 2016, 2018).

At death, viruses modulate microbial-driven processes through transfer genes and reprogram metabolic via viral-coded auxiliary metabolic genes (Bergh et al 1989; Rohwer & Thurber 2009; Danovaro et al 2011; Hurwitz et al 2014; Breitbart et al 2018). In recent years, the diversity and function of reef invertebrate related viruses, including sea anemones, starfish (Hewson et al 2014); scleractinian corals and their associated microbial communities (Wilson & Chapman 2001) have increased (Wilson & Chapman 2001; Patten et al 2008; Weynberg et al 2014, 2017a, b; Laffy et al 2018). However, while viruses have been recognized as important components of coral reef ecosystems, they can regulate the dynamics of the microbial population, play a role in coral bleaching or disease, and mediate biogeochemical cycling of reefs (Thurber et al 2017). Despite this, there is a lack of studies studying viruses related to coral sponges that are ecologically important.

Viruses in seawater. In the early 1960s, viruses were identified as the etiologic agents of fish diseases including Oregon sockeye disease and infectious pancreatic necrosis (Crane & Hyatt 2011). Since then, it has been shown that the virus is important to all marine life for disease infection from protists to bacteria, molluscs, crustaceans, fish, and mammals (Munn 2006). In the early 1990s, it was found that viral infections affect the aquatic ecosystems including plant and animal pathogens, viruses, and single-celled organisms such as bacteria (bacteriophages) and phytoplankton, which lead to major effect on the ecosystem cycle. Karl-Heinz Moebus discovered that infection and isolation patterns of bacteriophages acquired during a crosscut across the North Atlantic (Moebus 1980; Moebus & Nattkemper 1981). Hence, research into marine viruses has become a highly relevant and independent area of marine biology research, prompted by the increasing realization of the importantance and diverse functions of viruses in marine biology (Brussard et al 2008; Rohwer & Thurber 2009).

Table 1

Detection of viruses in sponges since 2005

Location/Country	*Sponge*	*Virus*	*References*
Tennessee Reef, Florida Keys, United States	*Ircinia strobilina*	Alphaproteobacteria virus phiJl001	Lohr et al (2005)
Ballyhenry Island, United Kingdom	*Hymeniacidon perlevis*	Myoviridae Siphoviridae Podoviridae	Harrington et al (2012)
Lake Baikal, Russia	*Lubomirskia baikalensis*	Myoviridae cyanophages	Butina et al (2015)
Al Fahal, Red Sea, Saudi Arabia & Orpheus Island, Great Barrier Reef, Australia	*Stylissa carteri* & *Carteriospongia foliascens*	Podoviridae Caudovirales Siphoviridae Myoviridae Inoviridae Geminiviridae	Pascelli et al (2018)
Papua New Guinea	*A. queenslandica*	Herpesviridae Myoviridae Iridoviridae Poxviridae Siphoviridae Podoviridae Phycodnavirirdae	Laffy et al (2018)
	X. testudinaria	Siphoviridae Myoviridae Podoviridae	
	R. odorabile	Podoviridae Myoviridae Siphoviridae Parvoviviridae Poxviridae Bidnaviridae	
	I. basta	Microviridae Myoviridae Siphoviridae Podoviridae Poxviridae Bidnaviridae Parvoviviridae Phycodnaviridae	
Maloye More Strait of Lake Baikal, Russia	*Lubomirskia baikalensis*	Siphoviridae Myoviridae Podoviridae Phycodnaviridae; *Chrysochromulina ericina* virus & Emiliania huxleyi virus 86 Poxviridae; BeAn 58058 virus Mimiviridae; Yellowstone lake mimivirus & Megavirus chiliensis Yellowstone lake mimivirus Nimaviridae Adenoviridae Polydnaviridae Alloherpesviridae	Butina et al (2019)

It was then discovered that the most prevalent biological entities in oceanic marine environments were viruses (Bergh et al 1989) at 10^8 viruses (mL^{-1}). Scientific method developments in marine virus identification and enumeration (Brussard et al 2000) facilitated advances in more comprehensive studies of high spatial and temporal resolution viral abundance, and diversity.

Viruses play important roles in aquatic ecosystems, including the regulation of host diversity and nutrient and carbon flow via viral shunt (Willhelm & Suttle 1999). They are highly abundant, typically ranging from 10^6 mL^{-1} to 10^8 mL^{-1} across different environments (Ortmann & Suttle 2005; Paul et al 1993; Proctor & Fuhrman 1990), with usually lower abundances in deep sea and higher abundances in coastal development. Since contact rates are proportional to viral abundance between viruses and their potential hosts, higher virus densities usually have a greater impact on microbial host populations (Murray & Jackson 1992; Mann 2003). Subsequently, the expansion of coral reef virus research (Thurber et al 2017), sediments (Danovaro et al 2008; Middelboe & Glud 2006), the deep biosphere (Engelhard et al 2013), and freshwater environments (Middelboe et al 2008) emphasized that viruses are all aquatic environments' integrated inhabitants. The virus detection in marine seawater are shown in Table 1 with algal viruses as the most common of all viruses in the oceans.

Algae are the engines of aquatic ecosystems that turn organic matter and power aquatic food chains into inorganic matter. Their growth affects the productivity of aquatic ecosystems, as well as the quality of the ecosystem, either by encouraging or hindering beneficial human use such as by maintaining fisheries, or by producing harmful blooms. A few decades ago, scientists found that in almost all aquatic ecosystems, viruses infecting eukaryotic algae are present, and that most of these pathogens are both species-specific and cause the lysis or death of their hosts. As an aside, the word 'algae' will be used exclusively to refer to eukaryotic algae in this review; cyanobacteria are also included under the umbrella term algae in some descriptions of aquatic primary development, such as in the first two sentences of this paragraph, but in this study, the authors have focused only on eukaryotes. The discovery of ubiquitous algal viruses in aquatic environments raised many questions about the impact of viruses on primary aquatic production and their role in the ecology and evolution of algal viruses, and stimulated the development of the ecology of algal viruses as a field of study. Study on this subject has taught a great deal about the significance of algal viruses, and many scientific papers have summarized this work over the years (Brussaard et al 2004; Brussaard & Martínez 2008; Dunigan et al 2006; Kang et al 2005; Nagasaki 2008; Short 2012; Suttle 2000; Van Etten et al 2002; Wilson et al 2009; Van Etten & Meints 1999; Yamada et al 2006). But there is still a lack a profound understanding of the diversity of algal viruses and the variety of algae susceptible to viral infections.

Molecular methods used to detect viruses in sponges and seawater. Significant efforts have been made to improve and standardize the methods to describe viruses in marine ecosystems (Weinbauer 2004). There are still many technical problems that disrupt our ability to get a comprehensive picture of sponge-viral communities (Hurwitz & Sullivan 2013). All have inherent strengths and limitations in the various approaches to characterize viral communities and can often be complementary. Using three complementary techniques, which are metagenomics analysis, TEM and PCR amplification, sponge-virus interactions are currently observed.

Metagenomic analysis. Metaviromics is the analysis of viral genome communities collected by viral purification and subsequent shotgun sequencing directly from environmental samples (Kim et al 2013). The metaviromic approach is an important tool for virus description, regarding that many viral hosts are not suitable for cultivation (Edwards & Rohwer 2005) and that viruses do not have a universal marker gene to enable identification (Rohwer & Edwards 2002; Rohwer & Thurber 2009). In conjunction with development of Next Generation Sequencing (NGS) technologies, the use of molecular approaches has significantly reduced the cost of collecting genomic data, accelerating the development of sequence-based viral community studies (Wommack et al 2008). Metaviromics was first used in 2002 (Breitbart et al 2002) to classify marine viruses and had a major effect on the study of viral diversity, quadruplicating the number of reported sequenced viral genomes (Roux 2019). The analysis and interpretation of metaviromic data can however be difficult due to the high diversity of viruses in natural environments and the small number of viral genomes present in reference databases

(Kim et al 2013). The use of suitable bioinformatic technologies is therefore an important component in the analysis of datasets of metaviromic sequencing.

Table 2

Detection of viruses in seawater since 1998

Location	*Viruses*	*References*
Mamala Bay, Oahu, Hawaii.	T7-like Podoviridae	Jiang and Paul (1998)
Marine Biological Field Station, Espeland, south of Bergen, western Norway	*Pyramimonas orientalis* virus (PoV)	Larsen et al (2001)
	Chrysochromulina ericina virus (CeV)	
	Phaeocystis pouchetii virus (PpV)	
Norwegian coastal waters	*Chrysochromulina ericina* virus (CeV)	Sandaa et al (2001)
	Pyramimonas orientalis virus (PoV)	
Enoshima Beach (east beach and west beach), Tokyo Bay (Daiba Kaihin Park and Kasai Kaihin Park), Japan	Enterovirus Norwalk virus	Katayama et al (2002)
Pacific Ocean in British Columbia, Canada & Southern Ocean near the Antarctic peninsula	Algal Virus (Phycodnaviridae)	Short & Suttle (2002)
Uwa Sea, Ehime Prefecture, Japan	Marine birnavirus (MABV)	Kitamura et al (2002)
Tijuana River mouth in San Diego, California)	Hepatitis A virus (HAV)	Brooks et al (2005)
Raunefjorde, south of Bergen, Norway	Cyanophage	Sandaa et al (2008)
Yaquina Bay in Newport, Oregon, United States	*Cafeteria roenbergensis* virus (CroV)	Fischer et al (2010)
Malibu Lagoon, Malibu Tijuana River, Imperial Beach Baja Malibu, Baja California Punta Bandera, Baja California	Enterovirus Adenovirus	Sassoubre et al (2012)
Bohai Bay, China	Rotavirus	Ming et al (2014)
Kane'ohe Bay, Hawai'i	Picornavirales	Culley et al (2014)
Norwegian coastal waters	Cyanophages	Sandaa & Larsen (2006)
Georges River estuary, NSW, Australia	Ostreid herpesvirus-1 (OsHV-1)	Evans et al (2017)
Southwest coast of Canada, Canadian Arctic, Nunavut coastal ocean, Bering Sea, Gulf of Mexico, Tropical coastal Peru, Southern Chile, Coastal warm Indian Ocean Aghulas & Southern Atlantic Benguela	Picornavirales	Vlok et al (2019)
Qingdao, Yellow Sea, China	Alteromonas phages P24	Zhang et al (2020)

Holovir is a reliable pipeline for the assessment of metaviromic data taxonomic composition and function, specifically designed to classify viral metagenomes from host-associated holobiont communities (Laffy et al 2016). As part of this computational workflow, the taxonomic assignment of the metaviromic data is carried out using BLAST analysis to analyze the homology within the NCBI RefSeq database between anticipated gene data and a common viral reference sequence (Pruitt et al 2007). Taxonomic assignment is then performed using the basic ancestor scoring system based on the best important matches to the viral reference database. HoloVir performs pairwise comparisons of single read and predicted gene datasets against the viral RefSeq database to assign taxonomy and additional comparison to phage-specific and cellular markers. These are undertaken to support the taxonomic assignments and identify potential cellular contamination. Broad functional classification of the predicted genes is provided by assignment of COG (Cluster of Orthologous Groups) microbial functional

category classifications using EggNOG and higher resolution functional analysis is achieved by searching for enrichment of specific Swiss-Prot keywords within the viral metagenome (Laffy et al 2016). Several studies verified the use of these viral purification techniques and the Holovir protocol was used to identify the various and role of viruses within marine holobionts (Weynberg et al 2017a, b; Weynberg 2018; Laffy et al 2019).

Metaviromics has greatly increased the understanding on variety and roles of viruses linked to marine hosts (Willner & Hugenholtz 2013; Laffy et al 2016). In addition, the generation of high quality viromes associated with marine holobionts, the development of techniques used to isolate and purify viral nucleic acid are fundamental (Weynberg et al 2014; Wood-Charlson et al 2015). The ability to isolate viromes associated with both eukaryotic and prokaryotic components of marine holobionts and expanding the understanding of the viral roles within these host-associated communities, is a key advantage of using this methodology. In attempts to classify viruses and their functions in coral reef sponges, these methodological methods will greatly complement TEM analysis.

Transmission electron microscopy (TEM). In general, viruses are structurally simple, consisting primarily of genetic material surrounded by a protein coat and the lack of distinct morphological variations between viruses means that these biological entities are often difficult to characterize (Perez-Sepulveda et al 2016). The morphology of sponge viruses has traditionally been characterized by the most prevalent and time-consuming method of examining viral diversity in aquatic ecosystems, which is TEM (Wommack & Colwell 2000). Viral classification is primarily based on capsid morphology and size, and TEM is a powerful tool for virus visualization and putative identification, with TEM presenting the first proof of high viral abundance and revealing in marine hosts the morphological diversity (Bergh et al 1989) and relative abundance of virus-like particles (Borsheim 1993; Bratbak & Heldal 1990; Wilson & Chapman 2001; Patten et al 2008; Pollock et al 2014; Hewson et al 2014). Technical problems such as the frequent low representation of samples, uneven staining, virus removal during washing steps, low detection limits, and lack of identification of non-typical viruses can obstruct the analysis of TEM analyses (Weinbauer & Suttle 1997; Bettarel et al 2000).

Analysis of histological parts is the most powerful way to recognize direct viral-host interactions (including host specificity and reproductive mode) (Wilson et al 2005). To have a broad description of host virus relationships, it is important to use multiple isolation methods. However, while the detection of VLPs in TEM images may well indicate signs of viral infection, caution is important when trying to interpret the pathogenesis due to the processing of non-viral artifacts and particles often associated with infection in thin tissue pieces (Sweet & Bythell 2017).

PCR amplification. DNA polymerases are the mechanisms that replicate genomes and protect their integrity to ensure that genetic information is transmitted faithfully from one generation to the next and are the main players in the repair and replication of DNA (Bebenek & Kunkel 2004). To analyze the diversity of viruses, numerous molecular instruments have been used. In comparison to prokaryotes or eukaryotes in which the 16S rDNA or 18S rDNA gene is retained, there is no specific universal genetic marker in viruses. The evaluation of virus diversity using PCR or PCR-methods must resort to group-specific gene markers (Short et al 2010). The development of PCR-based detection methods has provided the basis for the fast and reliable detection in the clinical setting of viral nucleic acids (Whalley et al 2001; Humar et al 2002; Snijders et al 2003; Biedermann et al 2004; Wagner et al 2004; Watzinger et al 2004). PCR transfers some of the gold standard culture, anti-genaemia and serological assay of the cells (Niubo et al 1994). To achieve quantitative data with promising results, a validated combination of PCR and detection assays (called 'conventional PCR') is used. However, the laborious post-PCR handling measures needed to validate the amplicon suffered from these techniques (Guatelli et al 1989). PCR assays have been used in clinical trials, to identify

nucleic acid enterovirus sequences (Holland et al 1991; Lomeli et al 1989) and environmental samples (Freymuth et al 1995; Niubo et al 1994; Watzinger et al 2001).

A welcome one was the possibility that amplicon detection could be visualized compared to conventional assays as the amplification proceeded (Lomeli et al 1989). This technique has provided a great deal of insight into the kinetics of the reaction and is the basis of the kinetic or 'real-time' PCR (Holland et al 1991; Lee et al 1993; Gibson et al 1996). Real time PCR is one of the most used method in laboratories around the world that able to build immense amount of data produced by traditional PCR assays. Compared to traditional PCR, there are disadvantages of using real-time PCR, such as the failure to observe the amplicon size without opening the device, the relatively restricted multiplex capability of current implementations, and the inadaptability of some fluorogenic chemistry platforms. When used in low-throughput laboratories, the start-up costs of real time PCR can also be restricted. These drawbacks are mostly due to the control of mechanical machinery or the available fluorogenic dyes of 'fluorophores' (Mackay et al 2002).

To investigate the diversity of phycodnavirus in both marine and freshwater environments, the polB and mcp markers encoding DNA polymerase and the main capsid protein were used respectively (Chen et al 1996; Short & Suttle 2002, 2003; Short & Short 2008; Larsen et al 2008; Clasen & Suttle 2009; Park et al 2011; Gimenes et al 2012). The T4-like portal protein coding gene g20 has been used extensively to detect the presence of cyanomyovirus populations in a number of both marine and freshwater habitats (Fuller et al 1998; Wilson et al 1999; Zhong et al 2002; Marston & Sallee 2003; Frederickson et al 2003; Dorigo et al 2004; Wang & Chen 2008 Short & Suttle 2005; Mühling et al 2005; Wilhelm et al 2006; Wang et al 2010; Parvathi et al 2012; Clasen et al 2013; Zhong & Jacquet 2013). Table 3 shows that the PCR analysis is the most common method used in detection viruses in sponges and seawater.

Table 3

Detection methods of viruses in sponges and seawater

Method	*Location/ Country*	*Virus*	*References*
PCR	Gulf of Mexico	*M. pusilla* virus	Chen et al (1996)
Transmission electron microscopy (TEM)	Norwegian coastal waters	Chrysochromulina ericina (Prymnesiophyceae) *Pyramimonas orientalis* (Prasinophyceae)	Sandaa et al (2001)
Transmission electron microscopy (TEM)	Bay by the Marine Biological Field Station of the University of Bergen, western Norway	*Chrysochromulina ericina* virus (CeV) *Pyramimonas orientalis* virus (PoV)	Sandaa et al (2001)
Transmission electron microscopy (TEM), light microscopy (LM), PCR denaturing gradient gel electrophoresis (PCR-DGGE), flow cytometry (FCM), pulsed-field gel electrophoresis (PFGE)	Espeland, south of Bergen, western Norway	*Pyramimonas orientalis* virus (PoV) *Chrysochromulina ericina* virus (CeV) *Phaeocystis pouchetii* virus (PpV)	Larsen et al (2001)
PCR	Uwa Sea, Ehime Prefecture, Japan	Marine birnavirus *(MABV)*	Kitamura et al (2002)
PCR	Norwegian and English coastal waters	*Emiliania huxleyi* virus (EhV)	Castberg et al (2002)
RT-PCR	Enoshima Beach and Tokyo Bay in Japan	Enterovirus Norwalk virus	Katayama et al (2002)
PCR & DGGE	Pacific Ocean in British Columbia, Canada & Southern Ocean near the Antarctic peninsula	Algal Virus (Phycodnaviridae)	Short & Suttle (2002)
Transmission electron microscopy (TEM)	Tennessee Reef, Florida Keys, United States	Alphaproteobacteria virus phiJl001	Lohr et al (2005)

Conventional RT-PCR	Tijuana River mouth in San Diego, California)	Hepatitis A virus (HAV)	Brooks et al (2005)
PCR	Raunefjorde, south of Bergen, Norway	Cyanophage	Sandaa et al (2008)
Genome sequencing analysis	Yaquina Bay in Newport, Oregon, United States	*Cafeteria roenbergensis* virus (CroV)	Fischer et al (2010)
Transmission electron microscopy (TEM)	Ballyhenry Island, United Kingdom	Myoviridae phages, Siphoviridae phages and Podoviridae phages	Harrington et al (2012)
RT-PCR, RT-QPCR, nested PCR & QPCR	Malibu Lagoon in Malibu, Tijuana River in Imperial Beach, Baja Malibu in Baja California, Punta Bandera in Baja California	Enterovirus Adenovirus	Sassoubre et al (2012)
RT-qPCR & metagenomics analysis	Kane'ohe Bay, Hawai'i	Picornavirales	Culley et al (2014)
Integrated cell culture and real-time quantitative polymerase chain reaction (ICC-qPCR)	Bohai Bay, China	Rotavirus	Ming et al (2014)
PCR	Southern basin of the Lake Baikal, Russia	Cyanophages	Butina et al (2015)
Genome sequencing analysis	Papua New Guinea	Herpesviridae Myoviridae Iridoviridae Poxviridae Siphoviridae Podoviridae Phycodnavirirdae Parvoviviridae Bidnaviridae Microviridae	Laffy et al (2018)
Transmission electron microscopy (TEM)	Orpheus Island, Great Barrier Reef, Australia & Al Fahal, Red Sea, Saudi Arabia	Podoviridae Caudovirales Siphoviridae Myoviridae Inoviridae Geminiviridae	Pascelli et al (2018)
Metagenomics analysis	Maloye More Strait of Lake Baikal in southern Siberia, Russia	Double-stranded DNA (dsDNA) viruses	Butina et al (2019)
Metagenomic analysis	Southwest coast of Canada, Canadian Arctic, Nunavut coastal ocean, Bering Sea, Gulf of Mexico, Tropical coastal Peru, Southern Chile, Coastal warm Indian Ocean Aghulas &Southern Atlantic Benguela	Picornavirales	Vlok et al (2019)
Transmission electron microscopy (TEM) & Genome sequencing	Qingdao, Yellow Sea, China	Alteromonas phages P24	Zhang et al (2020)

Different extraction methods for the detection of viruses in sponge and seawater. Most of the marine sponges have complex and diverse microbial communities that affects the biology of the host and have immense biotechnological capability due to the production of active metabolites biologically (Hentschel et al 2006; Taylor et al 2007; Kamke et al 2010). Previous studies primarily depended on the accuracy of the DNA and RNA sample extraction while relying on the standard of preservation of the sample (Thakuria et al 2009; Triant & Whitehead 2009) for all undoubted strengths of the methods such as functional gene analysis, 16S rRNA gene cloning, and metagenomics analysis (Schmitt et al 2007; Mohamed et al 2008; Steger et al 2008; Hoffmann et al

2009). The recovery of RNA is often still troublesome due to the great amount and perseverance of RNases in the environment and the short life period of bacterial mRNA. Meanwhile, DNA extraction depends on various tissue types (Costa et al 2004; Milling et al 2005; Chomczynski & Sacchi 2006). For the sponge extraction method, freezing is used occasionally to conserve the nucleic acid samples for analysis (Hentschel et al 2002; Taylor et al 2004, 2005; Bayer et al 2008; Webster et al 2008; Webster et al 2011). However, it is not optionally used at cruise locations or remote field locations (Gorokhova 2005). Equally relevant is the following steps to remove nucleic acids. This should produce as many microorganisms with DNA and RNA as possible, mitigating bias in the sample or remove specific organisms (von Wintzingerode et al 1997; Weinbauer et al 2002; Bisanti et al 2009). In the studies of sponge-microbes association, different extraction methods are used including chemical, mechanical (e.g., bead beating) and enzymatic application and (Taylor et al 2004; Schmitt et al 2007; Mohamed et al 2008; Webster et al 2008). It was inferred that simultaneous extraction, which is the capability to co-extract DNA and RNA samples, could be effective to obtain high quality extraction samples (Triant & Whitehead 2009).

Qiagen kit. The QIAamp DNA Investigator Kit protocol has four main steps which are degradation of cellular membranes using a combination of enzymatic action and mechanical lysis (heating and shaking), binding of DNA to the silica-based membrane of the QIAamp spin column, washing of contaminants through the membrane using buffers and elution of DNA. In this package, an operator can use the QIAamp spin columns manually, or the instructions can be set to auto by using the QIAcube robot (QIAGEN, QIAamp1 DNA Investigator Handbook 2010). The silica-based approach is a robust DNA extraction method, deleting inhibitors from the sample while preserving a high production of good quality DNA (Phillips et al 2012).

As studied by Simister et al (2011), the Qiagen All Prep DNA/RNA Mini kit (Cat. #80204) was conducted to the existing protocols. Both DNA and RNA were eluted two times to acquire the utmost production of nucleic acids of the sample. To ensure the purity of RNA for downstream applications (DNase stop solution was incubated for a maximum of 5 min), 3U RQ1 RNase-free DNase (Promega) was treated with RNA as described by the manufacturer's protocol. The Qiagen kit is preferred if time is the main concern. Although RNA yields are reduced, dangerous reagents such as chloroform and phenol were also eluded for further investigations. As indicated by Mohamed et al (2008), the study of nifH gene expression in sponges has already been successfully applied to this extraction protocol. However, the Qiagen package is quite expensive compared to Griffiths (Griffiths et al 2000) which is more recommended if considerable amounts of RNA are required.

Griffiths method. Griffiths method was invented originally for soil samples extraction to extract complete DNA and RNA with slight modifications (Griffiths et al 2000). The first step of this method is to freeze the samples in a 600 µL buffer. Then, the samples were thawed on ice and phenolchloroform-isoamyl alcohol was added (25:24:1, pH 8.0) for about 600 µL. During the aqueous phase, it was isolated through centrifugation process at 13,000 g for 5 minutes as well as the nucleic acids (room temperature). Through these improvements, the amount of supernatant containing nucleic acids was increased. With 0.1 x 3 M sodium acetate supernatant volume (pH 5.2) of total nucleic acids and 0.6 x isopropanol supernatant volume were precipitated for 1 h from the collected aqueous sheet, followed by 10-min centrifugation (13,000 x g at 4° C). Pelleted nucleic acids in ice-cold 70 percent (vol/vol) ethanol were then washed, air dried and re-suspended in 50 µL of RNase-free water. By incubation of half the sample (25 µL aliquot) with RNase A (Sigma) at a final concentration of 100 μgmL^{-1} for 10min at 37° C, pure DNA was obtained. Pure RNA was obtained by treating the other 25 µL of the sample with 3U RQ1 RNase-free DNase (Promega).

Hexadecyltrimethylammonium bromide (CTAB)-based DNA extraction. In many molecular biology applications, isolation of high-quality genomic DNA is essential. The

traditional hexadecyltrimethylammonium bromide (CTAB) method is cost-effective, but this protocol takes time (Doyle & Doyle 1987; Allen et al 2006). However, high-quality DNA can be quickly separated using commercial DNA extraction kits with simple protocols before the column been discarded (Deavours & Dixon 2005; Tesniere et al 2006). When large numbers of DNA samples are required, which implies time and costs, the use of either the CTAB method or the use of commercial kits may be limited. It has been suggested that silica columns can be reused to remove any DNA transferred to the binding matrix after acid treatment since silica matrices are extremely stable over long periods under mild acid conditions (Siddappa et al 2007). As indicated earlier, DNA extraction from the tissues of sponge was obtained by agitating rapidly the sample with grinding media which is beads or balls (bead beating method) in an ammonium acetate buffer that contained CTAB (Taylor et al 2004).

TRIzol. The TRIzol reagent is an easy and highly effective technique for RNA exaction (Invitrogen, Carlsbad, CA, USA). Trizol is a monophasic solution of phenol and guanidine isothiocyanate that can be used to separate the three biomolecules at the same time (Chomczynski 1993). It has been commonly used and recorded for nucleic acid extraction, but is not popular for the extraction of proteins. For protein recovery, a lysis buffer is recommended, as functional studies are often needed (Butt et al 2007) and the yield is greater with a bigger protein repertoire (Sun et al 2009). Trizol preserves the integrity of RNA, DNA, and proteins through sample homogenization or lysis, while destroying cells and dissolving cell components (Kirkland et al 2006; MacIntyre et al 2006). The solution is divided into an aqueous stage and an organic phase with the addition of chloroform preceded by centrifugation. RNA remains in the aqueous phase exclusively. RNA is recovered by precipitation with isopropyl alcohol after the aqueous phase transition; the sequential precipitation producing interphase DNA with ethanol will recover the DNA and proteins in the sample, and organic phase proteins are formed by additional precipitation with isopropyl alcohol. However, when mere presence has to be demonstrated and the amount of samples is a restriction, for all experiments performed on the same cell mass, a single extraction agent is necessary (Man et al 2006). This reagent is an enhancement of the single-step RNA isolation method of Chomczynski & Sacchi (1987).

Conclusions. Marine viruses in sponge communities are mostly dominated by bacteriophages (Siphoviridae, Myoviridae and Podoviridae). Within the sponge holobiont, numerous eukaryotic viral groups were identified and possibly affecting sponge cells or in the holobiont. This review has elaborated considerably on previous findings that emphasize the species specificity of the sponge-viral community and the role of marine viruses and sponges in the marine ecosystem. The composition of the sponge viral community is also caused by environmental factors, including geographic area and seawater temperature. The best molecular approach used to detect marine viruses in sponges is PCR analysis, indicating this tool for future research. The findings have highlighted the important roles of viruses in sponges and marine environment. The present review has greatly enhanced the understanding of sponge-viral interactions and enriched the understanding of sponge ecology.

Acknowledgements. The authors would like to thank the Faculty of Fisheries and Food Sciences, Universiti Malaysia Terengganu for their immense support. This research was funded by Fundamental Research Grant Scheme, Ministry of Higher Education, Malaysia (FRGS vot. no. 59535). The authors also would like to thank the anonymous reviewers and editors for their helpful and constructive comments.

References

Allers E., Moraru C., Duhaime M. B., Beneze E., Solonenko N., Barrero-Canosa J., Sullivan M. B., 2013 Single-cell and population level viral infection dynamics revealed by

phage FISH, a method to visualize intracellular and free viruses. Environmental Microbiology 15(8):2306-2318.
Allen G. C., Flores-vergara M. A., Krasnyanski S., Kumar S., Thompson W. F., 2006 A modified protocol for rapid DNA isolation from plant tissues using cetyltrimethylammonium bromide. Nature Protocols 1:2320–2325.
Angly F. E., Felts B., Breitbart M., Salamon P., Edwards R. A., Carlson C., Chan A. M., Haynes M., Kelley S., Liu H., Mahaffy J. M., 2006 The marine viromes of four oceanic regions. PLoS Biol 4(11):e368.
Arillo A., Bavestrello G., Burlando B., Sara M., 1993 Metabolic integration between symbiotic cyanobacteria and sponges a possible mechanism. Mar Bio 117:159-162.
Azam F., Fenchel T., Field J. G., Gray J. S., Meyer-Reil L. A., Thingstad F., 1983 The ecological role of water-column microbes in the sea. Marine Ecology Progress Series 10:257–263.
Barnes D. K., 1999 High diversity of tropical intertidal zone sponges in temperature, salinity and current extremes. African Journal of Ecology 37(4):424-434.
Bayer K., Schmitt S., Hentschel U., 2007 Microbial nitrification in Mediterranean sponges: Possible involvement of ammonia-oxidizing Betaproteobacteria. Porifera Research: Biodiversity, Innovation and Sustainability 28:165-171.
Bebenek K., Kunkel T., 2004 Functions of DNA polymerases. Advances in Protein Chemistry 69:137-165.
Bell J., 2008 Functional roles of sponges. Estuarine, Coastal and Shelf Science 79:341-353.
Bell J. J., Davy S. K., Jones T., Taylor M. W., Webster N. S., 2013 Could some coral reefs become sponge reefs as our climate changes? Global Change Biology 19(9):2613-2624.
Bergh Ø., BØrsheim K. Y., Bratbak G., Heldal M., 1989 High abundance of viruses found in aquatic environments. Nature 340:467-468.
Bettarel Y., Sime-Ngando T., Amblard C., Laveran H., 2000 A comparison of methods for counting viruses in aquatic systems. Applied and Environmental Microbiology 66:2283–2289.
Bisanti M., Ganassi S., Mandrioli M., 2009 Comparative analysis of various fixative solutions on insect preservation for molecular studies. Entomologia Experimentalis et Applicata 130:290-296.
Biedermann K., Dandachi N., Trattner M., Vogl G., Doppelmayr H., More E., Staudach A., Dietze O., Hauser-Kronberger C., 2004 Comparison of real-time PCR signal-amplified in situ hybridizationand conventional PCR for detection and quantification of human papillomavirus in archival cervicalcancer tissue. J Clin Microbiol 42(8): 3758–3765.
Borsheim K. Y., 1993 Native marine bacteriophages. FEMS Microbiol Ecol 102:141-159.
Bratbak G., Heldal H., 1990 Enumeration and biomass estimation of planktonic bacteria and viruses by transmission electron microscopy. Appl Environ Microbiol 56:352-366.
Bratbak G., Thingstad T. F., Heldal M., 1994 Viruses and the microbial loop. Microbial Ecology 28:209-221.
Breitbart M., 2012 Marine viruses: truth or dare. Ann Rev Mar Sci 4:425-448.
Breitbart M., Bonnain C., Malki K., Sawaya N. A., 2018 Phage puppet masters of the marine microbial realm. Nature Microbiology 3(7):754-766.
Breitbart M., Thompson L., Suttle C., Sullivan M., 2007 Exploring the vast diversity of marine viruses. Oceanography 20:135–139.
Breitbart M., Salamon P., Andresen B., Mahaffy J. M., Segall A. M., Mead D., Azam F., Rohwer F., 2002 Genomic analysis of uncultured marine viral communities. Proc Natl Acad Sci 99:14250–14255.
Brussaard C. P. D., Martinez J. M., 2008 Algal bloom viruses. Plant Viruses 2(1):1-13.
Brussaard C. P. D., Marie D., Bratbak G., 2000 Flow cytometric detection of viruses. J Virol Methods 85:175–182.

Brussaard C. P. D., Noordeloos A. A. M., Sandaa R. A., Heldal M., Bratbak G., 2004 Discovery of a dsRNA virus infecting the marine photosynthetic protist *Micromonas pusilla*. Virology 319:280–291.
Brooks H. A., Gersberg R. M., Dhar A. K., 2005 Detection and quantification of hepatitis A virus in seawater via real-time RT-PCR. Journal of Virological Methods 127(2):109-118.
Butina T. V., Potapov S. A., Belykh O. I., Belikov S. I., 2015 Genetic diversity of cyanophages of the myoviridae family as a constituent of the associated community of the Baikal sponge *Lubomirskia baicalensis*. Russian Journal of Genetics 51(3): 313-317.
Butina T. V., Bukin Y. S., Khanaev I. V., Kravtsova L. S., Maikova O. O., Tupikin A. E., Kabilov M. R., Belikov S. I., 2019 Metagenomic analysis of viral communities in diseased Baikal sponge *Lubomirskia baikalensis*. Limnology and Freshwater Biology 1:155-162.
Butt R. H., Pfeifer T. A., Delaney A., Grigliatti T. A., Tetzlaff W. G., Coorssen J. R., 2007 Enabling coupled quantitative genomics and proteomics analyses from rat spinal cord samples. Mol Cell Proteomics 6:1574–1588.
Campbell J. S., Simms J. M., 2009 Status Report on Coral and Sponge Conservation in Canada. Department of Fisheries and Oceans, Ottawa, Canada. 87 pp.
Castberg T., Thyrhaug R., Larsen A., Sandaa R. A., Heldal M., Van Etten J. L., Bratbak G., 2002 Isolation and characterization of a virus that infects *Emiliania huxleyi* (Haptophyta). Journal of Phycology 38(4):767-774.
diversity in marine algal virus communities as revealed by sequence analysis of DNA polymerase genes. Applied and Environmental Microbiology 62(8):2869-2874.
Chomczynski P., 1993 A reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples. Biotechniques 15(3):532-534.
Chomczynski P., Sacchi N., 1987 Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162(1):156-159.
Chomczynski P., Sacchi N., 2006 The single-stepmethod of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction: twenty-something years on. Nature Protocols 1(2):581–585.
Clasen J. L., Suttle C. A., 2009 Identification of freshwater Phycodnaviridae and their potential phytoplankton hosts, using DNA pol sequence fragments and a genetic-distance analysis. Applied and Environmental Microbiology 75(4):991-997.
Clasen J. L., Hanson C. A., Ibrahim Y., Weihe C., Marston M. F., Martiny J. B. H., 2013 Diversity and temporal dynamics of Southern California coastal marine cyanophage isolates. Aquat Microb Ecol 69:17–31.
Claverie J. M., Grzela R., Lartigue A., Bernadac A., Nitsche S., Vacelet J., Ogata H., Abergel C., 2009 Mimivirus and Mimiviridae: giant viruses with anincreasing number of potential hosts, including corals and sponges. Journal of Invertebrate Pathology 101(3):172-180.
Costa R., Gomes N. C. M., Milling A., Smalla K., 2004 An optmized protocol for simultaneous extraction of DNA and RNA from soils. Brazilian Journal of Microbiology 35(3):230–234.
Crane M., Hyatt A., 2011 Viruses of fish: an overview of significant pathogens. Viruses 3(11):2025-2046.
Crummett L. T., Puxty R. J., Weihe C., Marston M. F., Martiny J. B. H., 2016 The genomic content and context of auxiliary metabolic genes in marine cyanomyoviruses. Virology 499:219–229.
Culley A. I., Mueller J. A., Belcaid M., Wood-Charlson E. M., Poisson G., Steward G. F., 2014 The characterization of RNA viruses in tropical seawater using targeted PCR and metagenomics. MBio 5(3): e01210-01214.
Cuvelier M. L., Blake E., Mulheron R., McCarthy P. J., Blackwelder P., Thurber R. L. V., Lopez J. V., 2014 Two distinct microbial communities revealed in the sponge Cinachyrella. Frontiers in Microbiology 5:581.

Danovaro R., Corinaldesi C., Filippini M., Fischer U. R., Gessner M. O., Jacquet S., Magagnini M., Velimirov B., 2008 Viriobenthos in freshwater and marine sediments: a review. Freshwater Biology 53(6):1186-1213.
Danovaro R., Corinaldesi C., Dell'Anno A., Fuhrman J. A., Middelburg J. J., Noble R. T., Suttle C.A., 2011 Marine viruses and global climate change. FEMS Microbiology Reviews 35:993–1034.
Davy S., Burchett S., Dale A., Davies P., Davy J., Muncke C., Hoegh-Guldberg O., Wilson W., 2006 Viruses: agents of coral disease? Diseases of Aquatic Organisms 69:101–110.
Davy J. E., Patten N. L., 2007 Morphological diversity of virus-like particles within the surface microlayer of scleractinian corals. Aquat Microb Ecol 47:37–44.
Dayton P. K., Robilliard G. A., Paine R. T., Dayton L. B., 1974 Biological accommodation in the benthic community at the McMurdo Sound, Antarctica. Ecological Monographs 44:105–128.
Dayton P. K., 1989 Interdecadal variation in an Antarctic sponge and its predators from oceanographic climate shifts. Science 245(4925):1484-1486.
Deavours B. E., Dixon R. A., 2005 Metabolic engineering of isoflavonoid biosynthesis in alfalfa. Plant Physiology 138(4):2245-2259.
De Goeij J. M., Van Oevelen D., Vermeij M. J., Osinga R., Middelburg J. J., De Goeij A. F., Admiraal W., 2013 Surviving in a marine desert: the sponge loop retains resources within coral reefs. Science 342(6154):108-110.
Dell'Anno A., Corinaldesi C., Danovaro R., 2015 Virus decomposition provides an important contribution to benthic deep-sea ecosystem functioning. Proc Natl Acad Sci 112:E2014–2019.
Diaz M. C., Rützler K., 2001 Sponges: an essential component of Caribbean coral reefs. Bulletin of Marine Science 69(2):535-546.
Dorigo U., Jacquet S., Humbert J. F., 2004 Cyanophage diversity, inferred from g20 gene analyses, in the largest natural lake in France, Lake Bourget. Appl Environ Microbiol. 70:1017–1022.
Doyle J. J., Doyle J. L., 1987 A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19:11–15.
Dunigan D. D., Fitzgerald L. A., Van Etten J. L., 2006 Phycodnaviruses: a peek at genetic diversity. Virus Res 117:119–132.
Edwards R. A., Rohwer F., 2005 Viral metagenomics. Nat Rev Microbiol 3(6):504-510.
Engelhard G., Righton D., Pinnegar J., 2013 Climate change and fishing: A century of shifting distribution in North Sea cod. Global Change Biology 20:2473–2483.
Evans O., Paul-Pont I., Whittington R. J., 2017 Detection of Ostreid herpesvirus 1 microvariant DNA in aquatic invertebrate species, sediment and other samples collected from the Georges River estuary, New South Wales, Australia. Aquat Org 122:247-255.
Fischer M. G., Allen M. J., Wilson W. H., Suttle C. A., 2010 Giant virus with a remarkable complement of genes infects marine zooplankton. Proceedings of the National Academy of Sciences 107(45):19508-19513.
Frederickson C. M., Short S. M., Suttle C. A., 2003 The physical environment affects cyanophage communities in British Columbia inlets. Microb Ecol 46:348–357.
Freymuth F., Eugene G., Vabret A., 1995 Detection of respiratory syncytial virus by reverse transcription-PCR and hybridization with a DNA enzyme immunoassay. J Clin Microbiol 33:3352–3355.
Fuhrman J. A., 1999 Marine viruses and their biogeochemical and ecological effects. Nature 399(6736):541-548.
Fuller N. J., Wilson W. H., Joint IR., Mann N. H., 1998 Occurrence of a sequence in marine cyanophages similar to that of T4 g20 and its application to PCR-based detection and quantification techniques. Appl Environ Microbiol 64:2051–2060.
Gibson U. E., Heid C. A., Williams P. M., 1996 A novel method for real time quantitative RT-PCR. Genome Research 6(10):995-1001.
Gili J. M., Coma R., 1998 Benthic suspension feeders: their paramount role in littoral marine food webs. Trends in Ecology & Evolution 13(8):316-321.

Gelderblom H. R., 1996 Structure and Classification of Viruses. In: Baron S. (ed.), Medical Microbiology. 4th edn, University of Texas Medical Branch at Galveston, Galveston (TX). Chapter 41.
Gimenes M., Zanotto P., Suttle C., 2012 Phylodynamics and movement of Phycodnaviruses among aquatic environments. ISME J 6:237–247.
Gorokhova E., 2005 Effects of preservation and storage of microcrustaceans in RNAlater on RNA and DNA degradation. Limnol Oceanogr Methods 3:143-148.
Guatelli J. C., Gingeras T. R., Richman D. D., 1989 Nucleic acid amplification in vitro: detection of sequences with low copy numbers and application to diagnosis of human immunodeficiency virus type 1 infection. Clinical Microbiology Reviews: 2(2):217-226.
Gregory A. C., Zayed A. A., Conceição-Neto N., et al., 2019 Marine DNA viral macro-and microdiversity from pole to pole. Cell 177(5):1109-1123.
Griffiths R. I., Whiteley A.S., O'Donnell A.G., Bailey M. J., 2000 Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA- and rRNA-based microbial community composition. Appl Environ Microbiol 66:5488-5491.
Hadas E., Marie D., Shpigel M., Ilan, M., 2006 Virus predation by sponges is a new nutrient-flow pathway in coral reef food webs. Limnology and Oceanography 51(3):1548-1550.
Harrington C., Del Casale A., Kennedy J., Neve H., Picton B. E., Mooij M. J., O'Gara F., Kulakov L. A., Larkin M. J., Dobson A. D. W., 2012 Evidence of bacteriophage-mediated horizontal transfer of bacterial 16S rRNA genes in the viral metagenome of the marine sponge *Hymeniacidon perlevis*. Microbiology 158(11):2789–2795.
Hentschel U., Hopke J., Horn M., Friedrich A. B., Wagner M., Hacker J., Moore B. S., 2002 Molecular evidence for a uniform microbial community in sponges from different oceans. Appl Environ Microbiol 68:4431–4440.
Hentschel U., Usher K. M., Taylor M. W., 2006 Marine sponges as microbial fermenters. FEMS Microb Ecol 55:167–177.
Hewson I., Button J. B., Gudenkauf B. M., Miner B., Newton A. L., Gaydos J. K., Wynne J., Groves C. L., Hendler G., Murray M., Fradkin S., Breitbart M., Fahsbender E., Lafferty K. D., Kilpatrick A. M., Miner C. M., Raimondi P., Lahner L., Friedman C. S., Daniels S., 2014 Densovirus associated with sea-star wasting disease and mass mortality. Proceedings of the National Academy of Sciences 111(48):17278–17283.
Hewson I., Meyers M. E. J., Fuhrman J. A., 2007 Diversity and biogeography of bacterial assemblages in surface sediments across the San Pedro Basin, Southern California Borderlands. Environ. Microbiol 9(4):923–933.
Hobbs Z., Abedon S. T., 2016 Diversity of phage infection types and associated terminology: the problem with 'Lytic or lysogenic'. FEMS Microbiology Letters 363(7): fnw047.
Holland P. M., Abramson R. D., Watson R., Gelfand D. H., 1991 Detection of specific polymerase chain reaction product by utilizing the 5'----3'exonuclease activity of *Thermus aquaticus* DNA polymerase. Proc Natl Acad Sci 88(16):7276-7280.
Hoffmann F., Radax R., Woebken D., Holtappels M., Lavik G., Rapp H. T., 2009 Complex nitrogen cycling inthe sponge *Geodia barretti*. Environ Microbiol 11: 2228–2243.
Howard-Varona C., Hargreaves K. R., Abedon S. T., Sullivan M. B., 2017 Lysogeny in nature: mechanisms, impact and ecology of temperate phages. The ISME Journal 11(7):1511-1520.
Humar A., Kumar D., Boivin G., Caliendo A. M., 2002 Cytomegalovirus (CMV) virus load kinetics topredict recurrent disease in solid-organ transplant patients with CMV disease. J Infect Dis 186(6):829–833.
Hurwitz B. L., Sullivan M. B., 2013 The pacific oceans virome (pov): a marine viral metagenomics dataset and associated protein clusters for quantitative viral ecology. PLoS One 8(2):e57355.
Hurwitz B. L., Brum J. R., Sullivan M. B., 2015 Depth-stratified functional and taxonomic niche specialization in the "core" and "flexible" Pacific Ocean Virome. The ISME Journal 9:472–484. Doi:10.1038/ismej.2014.143.

Hurwitz B. L., Westveld A. H., Brum J. R., Sullivan M. B., 2014 Modeling ecological drivers in marine viral communities using comparative metagenomics and network analyses. Proc Natl Acad Sci 111:10714–10719.
Jahn M. T., Arkhipova K., Markert S. M., Stigloher C., Lachnit T., Pita L., Kupczok A., Ribes M., Stengel S. T., Rosenstiel P., Dutilh B. E., Hentschel U., 2019 A phage protein aids bacterialsymbionts in eukaryote immune evasion. Cell Host & Microbe 26(4):542–550.
Jiang S. C., Paul J. H., 1998 Gene transfer by transduction in the marine environment. Applied and Environmental Microbiology 64:2780-2787.
Jover L. F., Effler T. C., Buchan A., Wilhelm S. W., Weitz J. S., 2014 The elemental composition of virus particles: implications for marine biogeochemical cycles. Nat Rev Microbiol 12:519–528.
Kamke J., Taylor M. W., Schmitt S., 2010 Activity profiles for marine sponge-associated bacteria obtained by 16S rRNAvs 16S rRNA gene comparisons. ISME J 4:498–508.
Kang M., Dunigan D. D., Van Etten J. L., 2005 Chlorovirus: a genus of Phycodnaviridae that infects certain chlorella-like green algae. Mol Plant Pathol 6:213–224.
Katayama H., Shimasaki A., Ohgaki S., 2002 Development of a virus concentration method and its application to detection of enterovirus and Norwalk virus from coastal seawater. Applied and Environmental Microbiology 68(3):1033-1039.
Kim M. S., Whon T. W., Bae J. W., 2013 Comparative viral metagenomics of environmental samples from Korea. Genomics & Informatics 11:121-128.
Kirkland P. A., Busby J., Stevens S. Jr., Maupin-Furlow J. A., 2006 Trizol-based method for sample preparation and isoelectric focusing of halophilic proteins. Anal Biochem 351:254–259.
Kitamura S. I., Tomaru Y., Kawabata Z., Suzuki S., 2002 Detection of marine birnavirus in the Japanese pearl oyster *Pinctada fucata* and seawater from different depths. Diseases of Aquatic Organisms 50(3):211-217.
Kriss A. E., Rukina E. A., 1947 Bacteriophages in the sea. Dokl Akad Nauk SSSR 57:833-836.
Kristensen D. M., Mushegian A. R., Dolja V. V., Koonin E. V., 2010 New dimensions of the virus world discovered through metagenomics. Trends in Microbiology 18(1):11-19.
Laffy P. W., Wood-Charlson E. M., Turaev D., et al, 2016 HoloVir: a workflow for investigating the diversity and function of viruses in invertebrate holobionts. Frontiers in Microbiology 7:822.
Laffy P. W., Wood-Charlson E. M., Turaev D., Jutz S., Pascelli C., Botté E. S., Bell S. C., Peirce T. E., Weynberg K. D., van Oppen M. J., Rattei T., 2018 Reef invertebrate viromics: diversity, host specificity and functional capacity. Environmental Microbiology 20(6):2125-2141.
Laffy P. W., Botté E. S., Wood-Charlson E. M., Weynberg K. D., Rattei T., Webster N. S., 2019 Thermal stress modifies the marine sponge virome. Environmental Microbiology Reports 11(5):690-698.
Larsen A., Castberg T., Sandaa R. A., Brussaard C. P. D. Egge, J., Heldal M., Paulino A., Thyrhaug R., van Hannen E. J., Bratbak G., 2001 Population dynamics and diversity of phytoplankton, bacteria and viruses in a seawater enclosure. Marine Ecology Progress Series 221:47-57.
Larsen J. B., Larsen A., Bratbak G., Sandaa R. A., 2008 Phylogenetic analysis of members of the Phycodnaviridae virus family, using amplified fragments of the major capsid protein gene. Appl Environ Microbiol 74:3048–3057.
Lee L. G., Connell C. R., Bloch W., 1993 Allelic discrimination by nick-translation PCR with fluorogenic probes. Nucleic Acids Res 21:3761–3766.
Lehahn Y., Koren I., Schatz D., Frada M., Sheyn U., Boss E., Efrati S., Rudich Y., Trainic M., Sharoni S., 2014 Decoupling physical from biological processes to assess the impact of viruses on a mesoscale algal bloom. Curr Biol 24:2041–2046.
Levin R. A., Voolstra C. R., Weynberg K. D., Van Oppen M. J. H., 2017 Evidence for a role of virusesin the thermal sensitivity of coral photosymbionts. ISME Journal 11:808-812.

Lindell D., Jaffe J., Coleman M., Futschik M., Axmann I., Rector T., Kettler G., Sullivan M., Steen R., Hess W., Church G., Chisholm S., 2007 Genome-wide expression dynamics of a marine virus and host reveal features of co-evolution. Nature 449: 83-86.
Lindell D., Sullivan M. B., Johnson Z. I., Tolonen A. C., Rohwer F., Chisholm S. W., 2004 Transfer of photosynthesis genes to and from *Prochlorococcus* viruses. Proc Natl Acad Sci 101:11013–11018.
Lohr J. E., Chen F., Hill R. T., 2005 Genomic analysis of bacteriophage ΦJL001: insights into its interaction with a sponge-associated alpha proteobacterium. Applied and Environmental Microbiology 71(3):1598-1609.
Lohr J., Munn C. B., Wilson W. H., 2007 Characterization of a latent virus-like infection of symbiotic zooxanthellae. Appl Environ Microbiol 73:2976–2981.
Lomeli H., Tyagi S., Pritchard C. G., Lizardi P. M., Kramer F. R., 1989 Quantitative assays based on the use of replicatable hybridization probes. Clinical Chemistry 35(9):1826-1831.
Luter H. M., Whalan S., Webster N. S., 2010 Exploring the role of microorganisms in the disease-like syndrome affecting the sponge *Ianthella basta*. Applied and Environmental Microbiology 76(17):5736-5744.
Lurgi M., Thomas T., Wemheuer B., Webster N. S., Montoya J. M., 2019 Modularity and predicted functions of the global sponge-microbiome network. Nature Communications 10(1):1-12.
MacIntyre D. A., Smith R., Chan E.C., 2006 Differential enrichment of high- and low-molecular weight proteins and concurrent RNA extraction. Anal Biochem 359:274–276.
Mackay I. M., Arden K. E., Nitsche A. 2002 Real-time PCR in virology. Nucleic Acids Research 30(6):1292-1305.
Maldonado M., Carmona M. C., Velásquez Z., Puig A., Cruzado A., López A., Young C. M., 2005 Siliceous sponges as a silicon sink: an overlooked aspect of benthopelagic coupling in the marine silicon cycle. Limnology and Oceanography 50(3):799-809.
Man T. K., Li Y., Dang T. A., Shen J., Perlaky L., Lau C. C., 2006 Optimizing the use of TRIzol-extracted proteins in surface enhanced laser desorption/ionization (SELDI) analysis. Proteome Sci 4(1):1-7.
Mann N. H., 2003 Phages of the marine cyanobacterial picophytoplankton. FEMS Microbiol Rev:17-34.
Margulis L., 1981 Symbiosis in cell evolution: Life and its environment on the early earth. J Basic Microbiol 22(6):427.
Martínez J. M., Swan B. K., Wilson W. H., 2014 Marine viruses, a genetic reservoir revealed by targeted viromics. The ISME Journal 8(5):1079-1088.
Marston M. F., Sallee J. L., 2003 Genetic diversity and temporal variation in the cyanophage community infecting marine *Synechococcus* species in Rhode Island's coastal waters. Appl Environ Microbiol 69:4639–4647.
Middelboe M., Lyck P. G., 2002 Regeneration of dissolved organic matter by viral lysis in marine microbial communities. Aquat Microb Ecol 27(2):187−194.
Middelboe M., Glud R. N., 2006 Viral activity along a trophicgradient in continental margin sediments off central Chile. Mar Biol Res 2:41–51.
Middelboe M., Jacquet S., Weinbauer M., 2008 Viruses in freshwater ecosystems: an introduction to the exploration of viruses in new aquatic habitats. Freshwater Biology 53(6):1069-1075.
Milling A., Gomes N., Oros-Sichler M., Goetz M., Smalla K., 2005 Nucleic acid extraction from environmental samples. In: Molecular microbial ecology. Osborn A. M., Smith C. J. (eds), pp 1–24. Taylor & Francis New York.
Ming H. X., Zhu L., Feng J. F., Yang G., Fan J. F., 2014 Risk assessment of rotavirus infection in surface seawater from Bohai Bay, China. Human and Ecological Risk Assessment: An International Journal 20(4):929-940.
Moebus K., 1980 A method for the detection of bacteriophages from ocean water. Helgol Meeresunters 34:1–14.

Moebus K., Nattkemper H., 1981 Bacteriophage sensitivity patterns among bacteria isolated from marine waters. Helgol Meeresun 34:375–385.
Mohamed N. M., Enticknap J. J., Lohr J. E., McIntosh S. M., Hill R.T., 2008 Changes in bacterial communities ofthe marine sponge *Mycale laxissima* on transfer into aquaculture. Appl Environ Microbiol 74:1209–122.
Mojica K. D., Brussaard C. P., 2014 Factors affecting virus dynamics and microbial host–virus interactions in marine environments. FEMS Microbiology Ecology 89(3):495-515.
Mühling M., Fuller N. J., Millard A., Somerfield P. J., Marie D., Wilson W. H., Scanlan D. J., Post A. F., Joint I., Mann N. H., 2005 Genetic diversity of marine *Synechococcus* and co-occurring cyanophage communities: evidence for viral control of phytoplankton. Environ Microbiol 7:499–508.
Munn C. B., 2006 Viruses as pathogens of marine organisms-from bacteria to whales. Marine Biological Association of the United Kingdom. Journal of the Marine Biological Association of the United Kingdom 86(3):453.
Murray A. G., Jackson G. A., 1992 Viral dynamics: a model of the effects of size, shape, motion and abundance of single-celled planktonic organisms and other particles. Mar Ecol Prog Ser 89:103–116.
Nagasaki K., 2008 Dinoflagellates, diatoms, and their viruses. The Journal of Microbiology 46(3):235–243.
Niubò J., Pérez J. L., Carvajal A., Ardanuy C., Martín R., 1994 Effect of delayed processing of blood samples on performance of cytomegalovirus antigenemia assay. J Clin Microbiol 32:1119-1120.
Olson J. B., McCarthy P. J., 2005 Associated bacterial communities of two deep water sponges. Aquatic Microbial Ecology 39(1):47-55.
Ortmann A., Suttle C., 2005 High abundance of viruses in a deep-sea hydrothermal vent system indicates viral mediated microbial mortality. Deep Sea Research Part I: Oceanographic Research Papers 52:1515-1527.
Pallela R., Kim S. K., 2011 Ecobiotechnology of marine sponges and their symbionts-review and present status. Journal of Marine Bioscience and Biotechnology 5(4):15-25.
Park Y., Lee K., Lee Y. S., Kim S. W., Choi T. J., 2011 Detection of diverse marine algal viruses in the South Sea regions of Korea by PCR amplification of the DNA polymerase and major capsid protein genes. Virus Research 159(1):43-50.
Parvathi A., Zhong X., Jacquet S., 2012 Dynamics of various viral groups infecting autotrophic plankton in Lake Geneva. Adv Oceanogr Limnol 3:171–191.
Pascelli C., Laffy P. W., Kupresanin M., Ravasi T., Webster N. S., 2018 Morphological characterization of virus-like particles in coral reef sponges. PeerJ 6:e5625.
Patten N. L., Harrison P. L. Mitchell J. G., 2008 Prevalence of virus-like particles within the staghorn scleractinian coral (*Acropora muricata*) from the Great Barrier Reef Coral Reefs 27:569–580.
Patten N. L., Seymour J. R., Mitchell J. G., 2006 Flow cytometricanalysis of virus-like particles and heterotrophic bacteriawithin coral-associated reef water. J Mar Biolog Assoc UK 86:563– 566.
Patterson M. R., Chernykh V. I., Fialkov V. A., Savarese M., 1997 Trophic effects of sponge feeding within Lake Baikal's littoral zone. 1. Insitu pumping rates. Limnology and Oceanography 42(1):171-178.
Paul J. H., 2008 Prophages in marine bacteria: dangerous molecular time bombs or the key to survival in the seas? ISME J 2:579–589.
Paul J. H, Rose J. B., Jiang S. C., Kellogg C. A., Dickson L., 1993 Distribution of viral abundance in the reef environment of Key Largo, Florida. Appl Environ Microbiol 59:718.
Perdicaris S., Vlachogianni T., Valavanidis A., 2013 Bioactive natural substances from marine sponges: new developments and prospects for future pharmaceuticals. Nat Prod Chem Res 1(3):2329-6836.

Perez Sepulveda B., Redgwell T., Rihtman B., Pitt F., Scanlan D. J., Millard A., 2016 Marine phage genomics: The tip of the iceberg. FEMS Microbiology Letters 363 (15): fnw158.
Phillips K., McCallum N., Welch L., 2012 A comparison of methods for forensic DNA extraction: Chelex-100® and the QIAGEN DNA Investigator Kit (manual and automated). Forensic Science International: Genetics 6(2):282-285.
Pirtle E. C., Beran G. W., 1991 Virus survival in the environment. Rev Sci Tech 10(3):733-748.
Pollock F., Wood-Charlson E., van Oppen M., Bourne D., Willis B., Weynberg K., 2014 Abundance and morphology of virus-like particles associated with the coral *Acropora hyacinthus* differ between healthy and white syndrome-infected states. Marine Ecology Progress Series 510:39–43.
Proctor L. M., Fuhrman J. A., 1990 Viral mortality of marine bactena and cyanobacteria. Nature 343:60.
Pruitt K. D., Tatusova T., Maglott D. R., 2007 NCBI reference sequences (RefSeq): a curated nonredundant sequence database of genomes, transcripts and proteins. Nucleic Acids Research 35:D61–D65.
Richelle-Maurer E., Degoudenne Y., Van de Vyver G., Dejonghe L., 1994 Some aspects of heavy metal tolerance in freshwater sponges. Van Soest, Van Kempen, Breakman (eds), Sponge in time and space. pp. 351-354. Balkema, Rotterdam.
Roberts D. E., Davis A. R., 1996 Patterns in sponge (Porifera) assemblages on temperate coastal reefs off Sydney, Australia. Marine and Freshwater Research 47(7):897-906.
Roberts P. C., Compans R. W., 1998 Host cell dependence of viral morphology. Proc Natl Acad Sci 95(10):5746-5751.
Rohwer F., Thurber R. V., 2009 Viruses manipulate the marine environment. Nature 459(7244):207-212.
Rohwer F., Edwards R., 2002 The Phage Proteomic Tree: a ge-nome-based taxonomy for phage. J Bacteriol 184:4529-4535.
Rosario K., Breitbart M., 2011 Exploring the viral world through metagenomics. Current Opinion in Virology 1:289297
Rosenberg E., Koren O., Reshef L., Efrony R., Zilber-Rosenberg I., 2007 The role of microorganisms in coral health, disease and evolution. Nature Reviews Microbiology 5(5):355-362.
Roux S., Enault F., Hurwitz B. L., Sullivan M. B., 2015 VirSorter: mining viral signal from microbial genomic data. PeerJ 3:e985.
Roux S., Brum J. R., Dutilh B. E., et al, 2016 Ecogenomics and potential biogeochemical impacts of globally abundant ocean viruses. Nature 537:689–693.
Roux S., 2019 A viral ecogenomics framework to uncover the secrets of nature's "Microbe Whisperers". mSystems 4:1–5.
Sandaa R. A., Heldal M., Castberg T., Thyrhaug R., Bratbak G., 2001 Isolation and characterization of two viruses with large genome size infecting *Chrysochromulina ericina* (Prymnesiophyceae) and *Pyramimonas orientalis* (Prasinophyceae). Virology 290(2):272-280.
Sandaa R. A., Clokie M., Mann N. H., 2008 Photosynthetic genes in viral populations with a large genomic size range from Norwegian coastal waters. FEMS Microbiology Ecology 63(1):2-11.
Sandaa R.-A., Larsen A., 2006 Seasonal variations in virus-host populations in Norwegian coastal waters: focusing on the cyanophage community infecting marine *Synechococcus* spp. Appl Environ Microbiol 72:4610–4618.
Sassoubre L. M., Love D. C., Silverman A. I., Nelson K. L., Boehm A. B., 2012 Comparison of enterovirus and adenovirus concentration and enumeration methods in seawater from Southern California, USA and Baja Malibu, Mexico. Journal of Water and Health 10(3):419-430.
Schmitt S., Weisz J. B., Lindquist N., Hentschel U., 2007 Vertical transmission of a phylogenetically complexmicrobial consortium in the viviparous sponge *Ircinia felix*. Appl Environ Microbiol 73:2067–2078.

Seymour J., Patten N., Bourne D., Mitchell J., 2005 Spatial dynamics of virus-like particles and heterotrophic bacteria within a shallow coral reef system. Marine Ecology Progress Series 288:1–8.
Sharon I., Battchikova N., Aro E.-M., Giglione C., Meinnel T., Glase, F., Pinter R. Y., Breitbart M., Rohwer F., Béjà O., 2011 Comparative metagenomics of microbial traits within oceanic viral communities. The ISME Journal 5(7):1178–1190.
Shelford E. J., Middelboe M., Møller E. F., Suttle C. A., 2012 Virus-driven nitrogen cycling enhances phytoplankton growth. Aquat Microb Ecol 66:41–46.
Shieh W. Y., Lin Y. M., 1994 Association of heterotrophic nitrogen-fixing bacteria with a marine sponge of *Halichondria* sp. Bulletin of Marinescience 54(2):557-564.
Short S. M., Suttle C. A., 2002 Sequence analysis of marine virus communities reveals that groups of related algal viruses are widely distributed in nature. Applied and Environmental Microbiology 68(3):1290-1296.
Short S. M., Chen F., Wilhelm S. W., 2010 The construction and analysis of marker gene libraries. Manual of Aquatic Viral Ecology: 82–91.
Short C. M., Suttle C. A., 2005 Nearly identical bacteriophage structural gene sequences are widely distributed in both marine and freshwater environments. Appl Environ Microbiol 71:480–486.
Short S. M., Short C. M., 2008 Diversity of algal viruses in various North American freshwater environments. Aquatic Microbial Ecology 51:13-21.
Short S. M., Suttle C. A., 2003 Temporal dynamics of natural communities of marine algal viruses and eukaryotes. Aquat Microb Ecol 32:107–119.
Short S. M., 2012 The ecology of viruses that infect eukaryotic algae. Environmental Microbiology 14(9):2253–2271.
Siddappa N. B., Avinash A., Venkatramanan M., Ranga U., 2007 Regeneration of commercial nucleic acid extraction columns without the risk of carryover contamination. BioTechniques 42(2):186.
Simister R. L., Schmitt S., Taylor M. W., 2011 Evaluating methods for the preservation and extraction of DNA and RNA for analysis of microbial communities in marine sponges. Journal of Experimental Marine Biology and Ecology 397(1):38-43.
Snijders P. J., van den Brule A. J., Meijer C. J., 2003 The clinical relevance of human papilloma virus testing: relationship between analytical and clinical sensitivity. J Pathol 201(1):1–6.
Steger D., Ettinger-Epstein P., Whalan S., Hentschel U., de Nys R., Wagner M., and Taylor M. W., 2008 Diver-sity and mode of transmission of ammonia-oxidizing archaea in marine sponges. Environ Microbiol 10:1087–1094.
Steindler L., Beer S., Ilan M., 2002 Photosymbiosis in intertidal and subtidal tropical sponges. Symbiosis 33:253-273.
Steward G. F., Culley A. I., Mueller J. A., Wood-Charlson E. M., Belcaid M., Poisson G., 2013 Are we missing half of the viruses in the ocean? The ISME Journal 7(3):672-679.
Sullivan M. B., Coleman M., Weigele P., Rohwer F., Chisholm S. W., 2005 Three Prochlorococcus cyanophage genomes: signature features and ecological interpretations. PLoS Biol 3: e144.
Sun J. L., Zhang H. Y., Guo Z. Y., Ying W. T., Qian X. H., Wang J. L., 2009 Comparison of three methods of protein extraction from Dermatophagoides pteronyssinus for two-dimensional electrophoresis. Chin Med Sci J 24:64–68.
Suttle C. A., 2000 Ecological, evolutionary, and geochemical consequences of viral infection of cyanobacteria and eukaryotic algae. In: Hurst CJ (Ed) Viral Ecology (1st Edn, Vol 1), pp 247-296. Academic Press, San Diego, California.
Suttle C. A., 2007 Marine viruses—major players in the global ecosystem. Nature Reviews Microbiology 5(10):801-812.
Suttle C. A., Fuhrman J. A., 2010 Chapter 15: Enumeration of virus particles in aquatic or sediment samples by epifluorescence microscopy. In: Manual of Aquatic Viral Ecology. Wilhelm, S.W., Weinbauer, M.G., Suttle C.A. (eds.), American Society of Limnology and Oceanography, Waco, TX, pp.145-153.

Sweet M., Bythell J., 2017 The role of viruses in coral health and disease. Journal of Invertebrate Pathology 147:136-144.
Taylor M. W., Hill R. T., Piel J., Thacker R. W., Hentschel U., 2007 Soaking it up: the complex lives of marine sponges and their microbial associates. The ISME Journal 1(3):187-190.
Taylor M. W., Schupp P. J., de Nys R., Kjelleberg S., Steinberg P. D., 2005 Biogeography of bacteria associ-ated with the marine sponge *Cymbastela concentrica*. Environ Microbiol 7:419–433.
Taylor M. W., Schupp P. J., Dahllof I., Kjelleberg S., Steinberg P.D., 2004 Host specificity in marine sponge-associated bacteria, and potential implications for marine microbial diversity. Environ Microbiol 6:121–130.
Tesniere C., Torregrosa L., Pradal M., Souquet J.-M., Gilles C., Dos Santos K., Chatelet P., Gunata Z., 2006 Effects of genetic manipulation of alcohol dehydrogenase levels on the response to stress and the synthesis of secondary metabolites in grapevine leaves. Journal of Experimental Botany 57(1):91-99.
Thakuria D., Schmidt O., Liliensiek A. K., Egan D., Doohan F. M., 2009 Field preservation and DNA extraction methods for intestinal microbial diversity analysis in earthworms. Journal of Microbiological Methods 76(3):226-233.
Thomassen S., Riisgård H. U., 1995 Growth and energetics of the sponge *Halichondria panicea*. Marine Ecology Progress Series 128:239-246.
Thomas T., Rusch D., DeMaere M. Z., Yung P. Y., Lewis M., Halpern A., Heidelberg K. B., Egan S., Steinberg P. D., Kjelleberg S., 2010 Functional genomic signatures of sponge bacteria reveal unique and shared features of symbiosis. The ISME Journal 4(12):1557-1567.
Thomas T., Moitinho-Silva L., Lurgi M., Björk J. R., Easson C., Astudillo-García C., Olson J. B., Erwin P. M., López-Legentil S., Luter H., Chaves-Fonnegra A., 2016 Diversity, structure and convergent evolution of the global sponge microbiome. Nature Communications 7(1):1-12.
Thompson L. R., Zeng Q., Kelly L., Huang K. H., Singer A. U., Stubbe J., Chisholm S. W., 2011 Phage auxiliary metabolic genes and the redirection of cyanobacterial host carbon metabolism. Proc Natl Acad Sci 108(39):E757-E764.
Thurber R. V., Payet J. P., Thurber A. R., Correa A. M., 2017 Virus–host interactions and their roles in coral reef health and disease. Nature Reviews Microbiology 15(4):205-216.
Thurber R. L. V., Correa A. M. S., 2011 Viruses of reef-building scleractinia corals. J Exp Mar Bio Ecol 408:102–113.
Triant D. A., Whitehead A., 2009 Simultaneous extraction of high-quality RNA and DNA from small tissue samples. Journal of Heredity 100:246–250.
Vacelet J., Gallissian M. F., 1978 Virus-like particles in cells of the sponge *Verongia cavernicola* (Demospongiae, Dictyoceratida) and accompanying tissues changes. Journal of Invertebrate Pathology 31(2):246-254.
Van Etten J. L., Graves M. V., Müller D. G., Boland W., Delaroque N., 2002 Phycodnaviridae-large DNA algal viruses. Arch Virol 147:1479–1516.
Van Etten J. L., Meints R. H., 1999 Giant viruses infecting algae. Annual Review of Microbiology 53:447-94. doi: 10.1146/annurev.micro.53.1.447.
van Oppen M. J. H., Baker A. C., Coffroth M. A., Willis B. L., 2009 Bleaching resistance and the role of algal endosymbionts. Ecological Studies, pp. 83-102. Springer-Verlag, Berlin.
Van Soest R. W., Boury-Esnault N., Vacelet J., Dohrmann M., Erpenbeck D., De Voogd N. J., Santodomingo N., Vanhoorne B., Kelly M., Hooper J. N., 2012 Global diversity of sponges (Porifera). PLoS One 7(4):e35105.
Villarreal L. P., 2005 Viruses and the evolution of life. American Society for Microbiology Press, Washington.
Vlok M., Lang A. S., Suttle C. A., 2019 Application of a sequence-based taxonomic classification method to uncultured and unclassified marine single stranded RNA viruses in the order Picornavirales. Virus Evolution 5(2):vez056.

von Wintzingerode F., Gobel U. B., Stackebrandt E., 1997 Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. FEMS Microbiol Rev 21:213–229
Wagner H. J., Cheng Y. C., Huls M. H., Gee A. P., Kuehnle I., Krance R. A., Brenner M. K., Rooney C. M., Heslop H. E., 2004 Prompt versus preemptive intervention for EBV lymphoproliferative disease. Blood 103(10):3979–3981.
Walters K. D., Pawlik J. R., 2005 Is there a trade-off between wound-healing and chemical defenses among Caribbean reef sponges? Integrative and Comparative Biology 45(2):352-358.
Wang K., Chen F., 2008 Prevalence of highly host-specific cyanophages in the estuarine environment. Environ Microbiol 10:300–312.
Wang G., Murase J., Asakawa S., Kimura M., 2010 Unique viral capsid assembly protein gene (g20) of cyanophages in the floodwater of a Japanese paddy field. Biol Fertil Soils 46:93–102.
Warwick-Dugdale J., Solonenko N., Moore K., Chittick L., Gregory A. C., Allen M. J., Sullivan M. B., Temperton B., 2019 Long-read viral metagenomics captures abundant and microdiverse viral populations and their niche-defining genomic islands. PeerJ 7: e6800.
Watzinger F., Hörth E., Lion T., 2001 Quantification of mRNA expression by competitive PCR using non-homologous competitors containing a shifted restriction site. Nucleic Acids Research 29(11):e52-e52.
Watzinger F., Suda M., Preuner S., Baumgartinger R., Ebner K., Baskova L., Niesters H. G. M., Lawitschka A., Lion T., 2004 Real-time quantitative PCR assays for detection and monitoring of pathogenic human viruses in immunosuppressed pediatric patients. Journal of Clinical Microbiology 42(11): 5189-5198.
Webster N., Cobb R., Soo R., Anthony S., Battershill C., Whalan S., Evans-Illidge E., 2011 Bacterial community dynamics in the marine sponge *Rhopaloeides odorabile* under in situ and ex situ cultivation. Marine Biotechnology 13:296-304.
Webster N. S., 2007 Sponge disease: A global threat? Environmental Microbiology 9:1363–1375.
Webster N. S., Cobb R. E., Negri A. P., 2008 Tempera-ture thresholds for bacterial symbiosis with a sponge. ISMEJ 2:830–842.
Webster N. S., Taylor M. W., 2012 Marine sponges and their microbial symbionts: love and other relationships. Environmental Microbiology 14(2):335-346.
Webster N. S., Thomas T., 2016 The sponge hologenome. MBio 7(2): e00135-16.
Weinbauer M. G., 2004 Ecology of prokaryotic viruses. FEMS Microbiology Reviews 28(2):127-181.
Weinbauer M. G., Fritz I., Wenderoth D. F., Höfle M. G., 2002 Simultaneous extraction from bacterioplankton of total RNA and DNA suitable for quantitative structure and function analyses. Appl Environ Microbiol 68:1082-1087.
Weinbauer M. G., Suttle C. A., 1997 Comparison of epifluorescence and transmission electron microscopy for counting viruses in natural marine waters. Aquatic Microbial Ecology 13:225–232.
Weynberg K. D., 2018 Viruses in Marine Ecosystems: From Open Waters to Coral Reefs. Adv Virus Res 101:1-38.
Weynberg K. D., Laffy P. W., Wood-Charlson E. M., Turaev D., Rattei T., Webster N. S., van Oppen M. J. H., 2017a Coral-associated viral communities show high levels of diversity and host auxiliary functions. Peer J 5:e4054.
Weynberg K. D., Neave M., Clode P. L., Voolstra C. R., Brownlee C., Laffy P., Webster N. S., Levin R. A., Wood-Charlson E. M., van Oppen M. J. H., 2017b Prevalent and persistent viral infection in cultures of the coral algal endosymbiont *Symbiodinium*. Coral Reefs 36:773–784.
Weynberg K. D., Wood-Charlson E. M., Suttle C. A., van Oppen M. J. H., 2014 Generating viral metagenomes from the coral holobiont. Frontiers in Microbiology 5:1–11.
Whalley S. A., Murray J. M., Brown D., Webster G. J., Emery V. C., Dusheiko G. M., Perelson A. S., 2001 Kinetics of acute hepatitis B virus infection in humans. J Exp Med 193(7):847–854.

Wilhelm S. W., Carberry M. J., Eldridge M. L., Poorvin L., Saxton M. A., Doblin M. A., 2006 Marine and freshwater cyanophages in a Laurentian Great Lake: evidence from infectivity assays and molecular analyses of g20 genes. Appl Environ Microbiol 72:4957–4963.
Wilhelm S., Suttle C., 1999 Viruses and nutrient cycles in the sea: Viruses play critical roles in the structure and function of aquatic food webs. BioScience 49(10):781-788.
Williamson S. J., McLaughlin M. R., Paul J. H., 2001 Interaction of the ΦHSIC Virus with its host: Lysogeny or Pseudolysogeny? Applied and Environmental Microbiology 67(4):1682–1688.
Wilkinson C. R., Fay P., 1979 Nitrogen fixation in coral reef sponges with symbiotic cyanobacteria. Nature 279(5713):527-529.
Wilkinson C. R., 1980 Nutrient translocation from green algal symbionts to the freshwater sponge Ephydatia fluviatilis. Hydrobiologia 75:241-250.
Willner D., Hugenholtz P., 2013 From deep sequencing to viral tagging: Recent advances in viral metagenomics. BioEssays 35:436–442.
Wilson W. H., Francis I., Ryan K., Davy S. K., 2011 Temperature induction of viruses in symbiotic dinoflagellates. Aquat Microb Ecol 25:99–102.
Wilson W. H., Dale A. L., Davy J. E., Davy S. K., 2005 An enemy within? Observations of virus-like particles in reef corals. Coral Reefs 24:145–148.
Wilson W. H., Chapman D. M., 2001 Observation of virus-like particles in thin sections of the plumose anemone, *Metridium senile*. Journal of the Marine Biological Association of the United Kingdom 81(5):879.
Wilson W. H., Fuller N. J., Joint I. R., Mann N. H., 1999 Analysis of cyanophage diversity and population structure in a south-north transect of the Atlantic Ocean. Bull Inst Oceanogr 1999:209–216.
Wilson W. H., Van Etten J. L., Allen M. J., 2009 The Phycodnaviridae: the story of how tiny giants rule the world. Current Topics in Microbiology and Immunology 328:1–42.
Wommack K. E., Colwell R. R., 2000 Virioplankton: viruses in aquatic ecosystems. Microbiology and Molecular Biology Reviews 64(1):69-114.
Wommack K. E., Bhavsar J., Ravel J., 2008 Metagenomics: Read length matters. Applied and Environmental Microbiology 74:1453-1463.
Wood-Charlson E. M., Weynberg K. D., Suttle C. A., Roux S., van Oppen M. J. H., 2015 Metagenomic characterization of viral communities in corals: mining biological signal from methodological noise. Environ Microbiol 17:3440–3449.
Yamada T., Onimatsu H., Van Etten J., 2006 *Chlorella* viruses. Advances in Virus Research 66:293–336.
Zhang X., Liu Y., Wang M., et al, 2020 Characterization and genome analysis of a novel marine *Alteromonas* phage P24. Current Microbiology 77(10):2813-2820.
Zhong X., Jacquet S., 2013 Prevalence of viral photosynthetic and capsid protein genes from cyanophages in two large and deep perialpine lakes. Applied and Environmental Microbiology 79(23):7169–7178.
Zhong Y., Chen F., Wilhelm S. W., Poorvin L., Hodson R. E., 2002 Phylogenetic diversity of marine cyanophage isolates and natural virus communities as revealed by sequences of viral capsid assembly protein gene g20. Appl Environ Microbiol 68:1576–1584.

Received: 07 June 2021. Accepted: 30 June 2021. Published: 30 June 2021.
Authors:
Effa Syaqilah Binti Jamal, Faculty of Fisheries and Food Sciences, University of Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia, e-mail: effajamal2017@gmail.com
Logajothiswaran Ambalavanan, Faculty of Fisheries and Food Sciences, University of Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia, e-mail: loga_5427@yahoo.com
Shumpei Iehata, Faculty of Fisheries and Food Sciences, University of Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia, e-mail: shumpei@umt.edu.my
Sandra C. Zainathan, Faculty of Fisheries and Food Sciences, University of Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia; Institute of Marine Biotechnology, University of Malaysia Terengganu, 21030 Kuala Nerus, Kuala Terengganu, Malaysia, e-mail: sandra@umt.edu.my

How to cite this article:
Jamal E. S., Ambalavanan L., Iehata S., Zainathan S. C., 2021 A review on marine viruses in sponges and seawater. AACL Bioflux 14(3):1828-1854.