Diatom Viruses

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Abstract

The discovery, isolation, and cultivation of the first diatom-infecting virus less than two decades ago revealed an enigmatic, ecological interaction that altered our understanding of diatom ecosystem functioning. Since that discovery, characterization of additional diatom host-virus systems has brought important insight into unique aspects of these viruses and the biogeochemical consequences of virus-mediated mortality. Emerging approaches for identifying these pathogens in natural populations are revealing widespread prevalence and geographic distribution of diatom viruses and the environmental factors that influence host-virus interactions. In this chapter, we summarize the existing literature and highlight the latest research on diatom viruses and the potential of these viruses to impact one of the most significant groups of phytoplankton on the planet. We conclude with thoughts for the future generation of diatom viral ecologists.

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Abbreviations

dsDNA	Double-stranded DNA
dsRNA	Double-stranded RNA
FLDS	Fragmented and primer ligated dsRNA sequencing
ICTV	International Committee on Taxonomy of Viruses
MPN	Most probable number
ORF	Open-reading frame
RdRp	RNA-dependent RNA polymerase
ssDNA	Single-stranded DNA
ssRNA	Single-stranded RNA
TEM	Transmission electron microscopy
TEP	Transparent exopolymers

1 Introduction

The discovery that viruses are the most abundant biological entities in a wide range of marine and freshwater ecosystems (averaging 10^7 particles per milliliter of water; Bergh et al. 1989; Breitbart 2012) has considerably changed our view of the aquatic microbial food-web (Fuhrman 1999; Wilhelm and Suttle 1999). This seminal discovery has promoted research on these infectious agents and the role they play in marine environments. As obligatory pathogens, viruses depend on a living host to replicate. Virions, individual virus particles, consist of nucleic acids surrounded by a protective protein coat called the capsid. A lipid membrane can be found inside or outside of the capsid, the latter describing enveloped viruses. Viruses are traditionally classified by genome type (e.g., DNA, RNA, single or double-stranded, circular or linear, segmented or not), structural features (e.g., the symmetry and size of the virion, the capsid protein composition, the presence of an envelope), replication strategy, and host organism. Viral infection involves host recognition, adsorption, entry, and co-opting host machinery for viral genome replication and virion production. Viruses are thus specialized pathogens that act as important drivers of host population dynamics and evolution, and of ecosystem function globally (Suttle 2007; Breitbart 2012).

The ecological and evolutionary consequences of viral infection depend, in part, on the virus replication strategy. Through the lytic cycle, viral progeny is released into the environment via lysis of the host cell. For unicellular organisms, lytic infection leads to host mortality, altering community structure, and stimulating the microbial loop through the release of nutrients and organic matter (Suttle 2007; Brussaard et al. 2008)—a process referred to as the "viral shunt" (Wilhelm and Suttle 1999). In contrast, temperate viruses do not cause immediate host lysis, but rather are maintained in a latent state called lysogeny (Lwoff 1953; Paul 2008), and can alter host physiology and metabolism by introducing novel functions such as virulence

factor production (Waldor and Mekalanos 1996; Sumby and Waldor 2003; Vidgen et al. 2006) or immunity to infection by related viruses (super-infection; Lwoff 1953, Zinder 1958, Paul 2008, Blasdel and Abedon 2017). Continuous release or intermittent budding of viral progeny without host lysis can also occur, but the prevalence and environmental consequence of this mode of chronic infection is not well documented in aquatic viruses (Thomas et al. 2011; Demory et al. 2017).

The first viruses discovered in the ocean were largely phages—viruses that infect bacteria—with genomes comprising double-stranded (ds) DNA (reviewed in Breitbart 2012). Among the first eukaryotic algal viruses discovered were the *Phycodnaviridae*—large, dsDNA-containing viruses that infect a wide range of phytoplankton including chlorophytes, prasinophytes, dinoflagellates, and haptophytes (reviewed in Brussaard 2004). Advances in high-throughput sequencing later revealed a novel community of picorna-like viruses—small, single-stranded (ss) RNA-containing viruses (Culley et al. 2003, 2006) that have since been shown to include viruses similar to those that infect diatoms and dinoflagellates (Tai et al. 2003; Nagasaki et al. 2004).

Arguably one of the most globally distributed and ecologically successful protist groups in the ocean, diatoms are major players in silicon (Si) and carbon biogeochemistry, processing over 240 Tmol Si annually (Treguer and De La Rocha 2013) and contributing ~40% of marine primary production (Nelson et al. 1995) and carbon export (Falkowski et al. 1998; Smetacek 1999). The relatively recent discovery of diatom-infecting viruses revealed a unique group of marine viruses distinct in genome structure (ssRNA and ssDNA) and a virion size among the smallest on the planet (~20 to 40 nm in diameter; Nagasaki et al. 2004, Tomaru et al. 2015b). Although still in its infancy, our understanding of diatom viruses and the impact of host-virus interactions on biogeochemical cycling and ecosystem function is improving with the growing number of observations and experimental studies.

In this chapter, we summarize current knowledge about diatom-infecting viruses, starting with the discovery, diversity, and phylogeny of these unique viruses. We then describe the ecology of diatom viruses, including host-virus dynamics, environmental factors that influence infection, and the role diatom viruses play in natural communities. Finally, we discuss future outlooks of this developing frontier in diatom research, implications of emerging technologies and strategies toward better integration of diatom viruses in modeling ecosystem function.

2 Discovery, Isolation, and Characterization of Diatom Host-Virus Systems

2.1 Discovery and Isolation

The first diatom virus was isolated from Ariake Sound (Japan) in 2004 by filtering surface water through a 0.2 μm pore-size filter and challenging 22 exponentially growing diatom strains with the resulting filtrate. Following inhibition of algal growth and multiple rounds of dilution to extinction, a clonal pathogen of the centric

diatom, *Rhizosolenia setigera*, was isolated (Nagasaki et al. 2004). Since then, a number of diatom viruses have been isolated (Tables 1 and 2) from resuspended sediments or through a range of approaches such as dilution to extinction of filtered surface seawater, enrichment cultures, or tangential flow filtration (Wilhelm et al. 2010).

2.2 Morphological and Genomic Features

The *R. setigera* virus was identified as a positive-sense (+) ssRNA-containing virus and designated RsRNAV. Viral replication occurs within the host cytoplasm where small (~32 nm in diameter), naked (i.e., non-enveloped) and non-tailed hexagonal particles, suggestive of icosahedral symmetry, are formed. The linear genome (~9 kb) of RsRNAV encodes two open reading frames (ORFs; Shirai et al. 2006). ORF1 is a polyprotein gene encoding for replication proteins, including a helicase and an RNA-dependent RNA Polymerase (RdRp), a highly conserved sequence among the *Picornavirales* (Koonin et al. 1993). ORF2 encodes structural proteins of the viral capsid (Shirai et al. 2006). Subsequent discovery of other diatom-infecting +ssRNA viruses revealed similar features with genomes ranging between 8 and 10 kb encoding 2 ORFs, virion replication and assembly in the cytoplasm, and virion diameters ranging from 22 to 50 nm (Fig. 1, Table 1).

Recently, the capsid structure of an ssRNA virus, CtenRNAV-II, infecting *Chaetoceros tenuissimus* was resolved using cryo-electron microscopy (cryo-EM; Munke et al. 2020). Comparison to other *Picornavirales* viruses revealed conserved ancestral structural traits that provide insight into the evolutionary history of this order, but the presence of structures unique to CtenRNAV-II also leave open questions about the molecular details of viral infection and host-specificity. As this is the first diatom virus structure to be determined at near atomic-resolution, resolving the structure of additional members of this family will likely provide useful insight into the propagation and transmission of these viruses.

In addition to RNA viruses, a number of single-stranded DNA (ssDNA)-containing diatom viruses have been isolated and characterized (Table 2). Similar to ssRNA viruses, ssDNA viruses have small (25–38 nm in diameter), icosahedral capsids. In contrast, viral replication occurs in the nucleus where rod-shaped structures have been observed (Fig. 2a). However, these rod-shaped virus-like particles have never been observed extracellularly even following host lysis and have thus been hypothesized to represent precursors of mature virions (Eissler et al. 2009). The general genomic structure of diatom ssDNA viruses is a closed, circular, single-stranded molecule of DNA approximately 5–7 kb and composed of 3–4 ORFs (Fig. 2b). Two of these ORFs, denoted VP2 and VP3, encode a structural protein of the viral capsid and replication enzyme, respectively, with the function of the other ORF(s) unknown. With the exception of CdebDNAV and CsetDNAV (Tomaru et al. 2008; Tomaru et al. 2013b), the genome also contains a ~1 kb, double-stranded DNA region with unknown function. Intriguingly, diatoms are the only protists known to be infected by ssDNA viruses (Tomaru et al. 2015a) and thus far, no

Table 1 List and characteristics of ssRNA diatom host-virus systems

	Host	Host strain	Virus	Origin	Particle diameter (nm)	Genome size (nt)	Major proteins (kDa)	Latent period (h)	Burst size (infectious units cell^{-1})	NCBI Accession number	References
Centric	*Chaetoceros* sp.	SS08-C03	Csp03RNAV	Yatsushiro Sea, Japan	32	9417	42.0, 34.0, 28.0	<48	–	AB639040	Tomaru et al. (2013a)
	Chaetoceros socialis f. *radians*	L-4	CsfrRNAV	Hiroshima Bay, Japan	22	9467	32.0, 28.5, 25.0	<48	66	AB469874	Tomaru et al. (2009)
	Chaetoceros tenuissimus	2–10	CtenRNAV type-I	Ariake sound, Japan	31	9431	33.5, 31.5, 30.0	<24	1.0×10^4	AB37547	Shirai et al. (2008)
	Chaetoceros tenuissimus	2–10	CtenRNAV type-II	Hiroshima Bay, Japan	35	9562	32.2, 29.0, 26.1	24–28	136	AB971661	Kimura and Tomaru (2015)
	Guinardia delicatula	RCC3083	GdelRNAV	Western English Channel, France	35	9233	38.6, 33.9, 29.8, 27, 6.8	<12	9.34 x 10^4	MH706768	Arsenieff et al. (2019)
	Rhizosolenia setigera	S3	RsRNAV	Ariake sound, Japan	32	8847	41.5, 41.0, 29.5	48	3100	AB243297	Nagasaki et al. (2004)
	Skeletonema costatum	ME-SCM-1	ScosV[a]	Jaran Bay, Korea	45–50	–	–	<48	90–250	–	Kim et al. (2015a)
	Stephanopyxis palmeriana	NF-D-SPA-1	SpalV[a]	Jaran Bay, Korea	25–30	–	–	<80	92	–	Kim et al. (2015b)
	Thalassiosira gravida	IT Dia-1	TgraRNAV	Yatsushiro Sea, Japan	32	~9000[b]	–	–	–	LC013477	Tomaru et al. (2015b)

(continued)

Table 1 (continued)

	Host	Host strain	Virus	Origin	Particle diameter (nm)	Genome size (nt)	Major proteins (kDa)	Latent period (h)	Burst size (infectious units cell^{-1})	NCBI Accession number	References
	Thalassiosira sp.	–	ThalRNAV01	Kane'ohe bay, Hawaii	31–34	8951	–	–	–	–	Schvarcz (2019)
Pennate	*Amphiprora paludosa*	–	ApaIV	Kane'ohe bay, Hawaii	36–39	5172	–	–	–	–	Schvarcz (2019)
	Asterionellopsis glacialis	IT09-K25	AglaRNAV	Ago Bay, Japan	31	8842	–	–	–	AB973945	Tomaru et al. (2012)
	Cylindrotheca closterium	–	CCloRNAV03	Kane'ohe bay, Hawaii	29–32	8778	–	–	–	–	Schvarcz (2019)
	Nitzschia reversa	KT30	NitRevRNAV	Tokyo Bay, Japan	30	~9000[b]	36, 32, 30, 28	–	–	LC466844-LC466847	Toyoda et al. (2019)

Latent period (the time until the appearance of extracellular virus) and burst size are reported for batch culture in replete medium. Dashes indicate parameters that were not reported

[a]Not fully described, but have features similar to those of other diatom ssRNA viruses and are presumed to belong to this group

[b]Genome not fully sequenced

Table 2 List and characteristics of ssDNA diatom host-virus systems

	Host	Host strain	Virus	Origin	Particle diameter (nm)	Genome size (nt)	Major proteins (kDa)	Latent period (h)	Burst size (infectious units cell^{-1})	NCBI Accession number	References
Centric	*Chaetoceros debilis*	020810A04 Ch48	CdebDNAV	Ariake sound, Japan	32	~7000[b]	37.5, 41	12–24	55	AB504376	Tomaru et al. (2008)
	Chaetoceros cf. *gracilis*	–	CspNIV[a]	Chesapeake Bay, USA	25	–	–	<24	–	–	Bettarel et al. (2005)
	Chaetoceros lorenzianus	IT-Dia51	ClorDNAV	Hiroshoma Bay, Japan	34	5813	<225	48	2.2×10^4	AB553581	Tomaru et al. (2011c)
	Chaetoceros salsugineum	Ch42	CsalDNAV	Ariake sound, Japan	38	6000	43.5, 46	12–24	325	AB193315	Nagasaki et al. (2005)
	Chaetoceros setoensis	IT07-C11	CsetDNAV	Hiroshoma Bay, Japan	33	5836	31, 37	48	2.0×10^4	AB781089	Tomaru et al. (2013b)
	Chaetoceros sp.	TG07-C28	Csp05DNAV	Ago Bay, Japan	33	5785	40, 75	<24	–	AB647334	Toyoda et al. (2012)
	Chaetoceros sp.	SS628–11	Csp07DNAV	Hiroshoma Bay, Japan	34	5552	38.5	<12	29	AB844272	Kimura and Tomaru (2013)
	Chaetoceros sp.	–	CspDNAV-KB01	Kane'ohe bay, Hawaii	26–31	5903	–	–	–	–	Schvarcz (2019)
	Chaetoceros sp.	–	CspDNAV-KB02	Kane'ohe bay, Hawaii	32–36	5689	–	–	–	–	Schvarcz (2019)

(continued)

Table 2 (continued)

	Host	Host strain	Virus	Origin	Particle diameter (nm)	Genome size (nt)	Major proteins (kDa)	Latent period (h)	Burst size (infectious units $cell^{-1}$)	NCBI Accession number	References
	Chaetoceros tenuissimus	2–6	CtenDNAV type-I	Ariake sound, Japan	37	5639	38.5	96	320	AB597949	Tomaru et al. (2011b)
	Chaetoceros tenuissimus	2–10	CtenDNAV type-II	Hiroshoma Bay, Japan	37	5570	39	<24	1737	AB971658	Kimura and Tomaru (2015)
	Chaetoceros cf. *wighamii*	–	CwNIV[a]	Chesapeake Bay, USA	30	–	–	8	2.6×10^4	–	Eissler et al. (2009)
Pennate	*Thalassionema nitzschioides*	AR-TN01	TnitDNAV	Ariake sound, Japan	35	5573	–	–	–	AB781284	Tomaru et al. (2012)
	Halsea ostrearia	NCC148.78 NCC235.1	HOV-148 HOV-235	Bay of Bourgneuf, France	–	4567 4538	–	–	–	–	Gastineau et al. (2020)

Latent period (the time until the appearance of extracellular virus) and burst size are reported for batch cultures grown in replete medium. Dashes indicate parameters that were not reported

[a]Not fully described, but have features similar to those of other diatom ssDNA viruses and are presumed to belong to this group

[b]Genome not fully sequenced

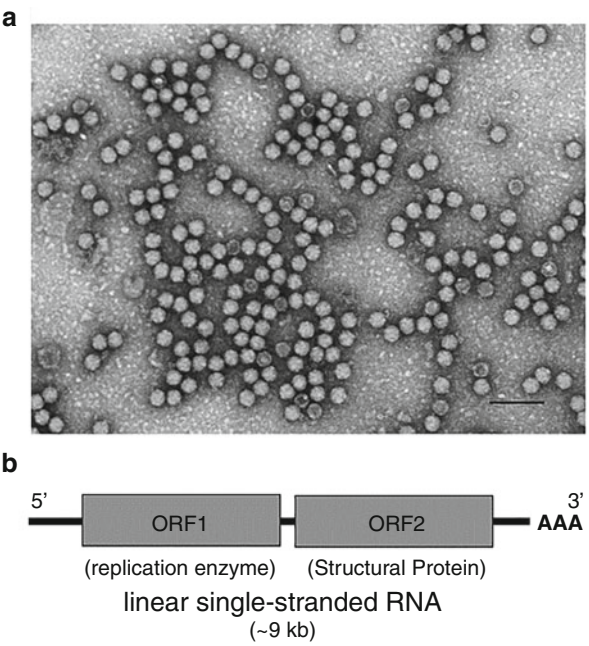

Fig. 1 General structure and genome organization of diatom ssRNA viruses. (**a**) Transmission electron micrograph of negatively stained CtenRNAV. Scale bar = 100 nm. (E. Yukabovskaya and K. Thamatrakoln, unpublished). (**b**) Genome structure of RsRNAV, representative of diatom ssRNA viruses. (Reproduced with permission from Tomaru et al. [2015a])

dsDNA viruses—the vast majority of known algal viruses that infect haptophytes, chlorophytes, and cyanobacteria (Coy et al. 2018)—have been reported to infect diatoms.

The majority of isolated diatom viruses infect centric diatom species, largely those within the genus *Chaetoceros*, perhaps not surprisingly, as this is one of the most globally distributed and diverse genera in the ocean, with approximately 400 species described (De Luca et al. 2019). Members of this genus are infected by either DNA or RNA viruses. However, in some species, both types of viruses can proliferate, as has been documented in *C. tenuissimus* (Kimura and Tomaru 2015). Viruses that infect centric diatoms in other genera such as *Guinardia, Minidiscus, Skeletonema, and Thalassiosira* have also been isolated (Tables 1 and 2; Arsenieff et al. 2020). Fewer viruses that infect pennate diatoms have been identified, with those infecting species in the genera *Amphiphora, Asterionellopsis, Cylindrotheca, Haslea, Nitzschia*, and *Thalassionema* (Tables 1 and 2). Given that the overwhelming majority of diatom viruses have been isolated from Japan, there is likely still considerable viral diversity that remains to be discovered.

2.3 Phylogeny

Over the past few years, the increasing number of diatom viral isolates have enabled more robust phylogenetic comparisons, clustering ssRNA and ssDNA viruses among two defined taxonomic groups. Based on the conserved phylogenetic marker,

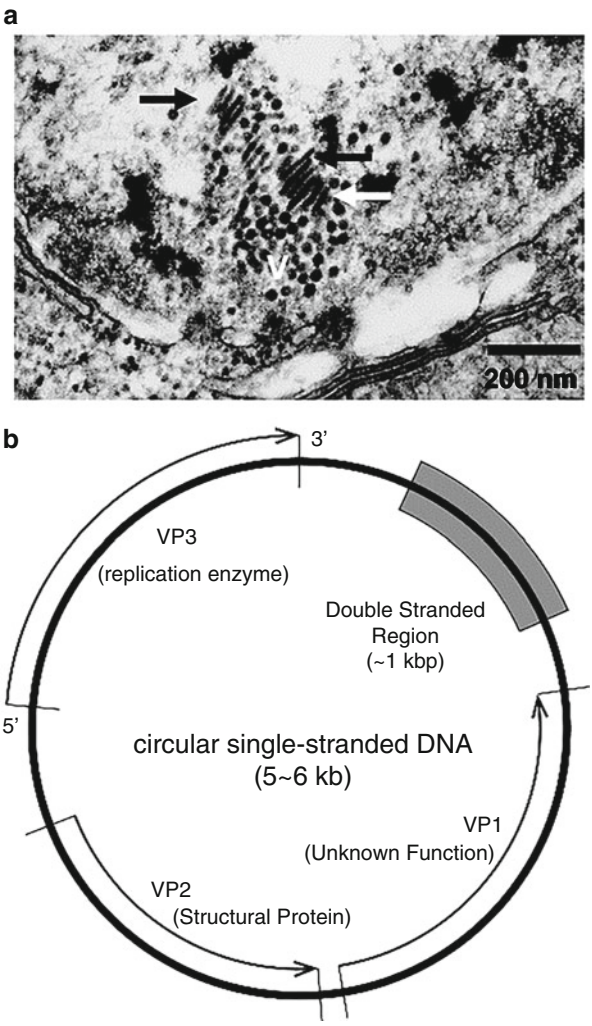

Fig. 2 General structure and genome organization of diatom ssDNA viruses. (**a**) Electron micrograph of intracellular Csp05DNAV. Reproduced with permission from Toyoda et al. (2012). *Arrows* indicate the rod-shaped form of the viral particle in the host nucleus. (b) A typical genome structure. (Reproduced with permission from Tomaru et al. [2015a])

RdRp, diatom ssRNA viruses belong to a monophyletic group that falls within the order *Picornavirales* and the family *Marnaviridae* (Fig. 3a; International Committee on Taxonomy of Viruses, ICTV, Lefkowitz et al. 2018), which includes a diverse range of cultured and uncultured marine ssRNA viruses. Seven genera comprise this family, three of which (*Bacillarnavirus*, *Kusarnavirus*, and *Sogarnavirus*) encompass known diatom viruses (Vlok et al. 2019). Species within these genera are further defined by amino acid similarity within the capsid protein.

For ssDNA viruses, the genus *Bacilladnavirus* was first proposed to encompass all of the diatom ssDNA viruses (ICTV; Tomaru et al. 2011b). However, this has since been revised and these viruses now reside within the family *Bacilladnaviridae* (Fig. 3b), which includes ssDNA viruses that infect marine mollusks (Kazlauskas

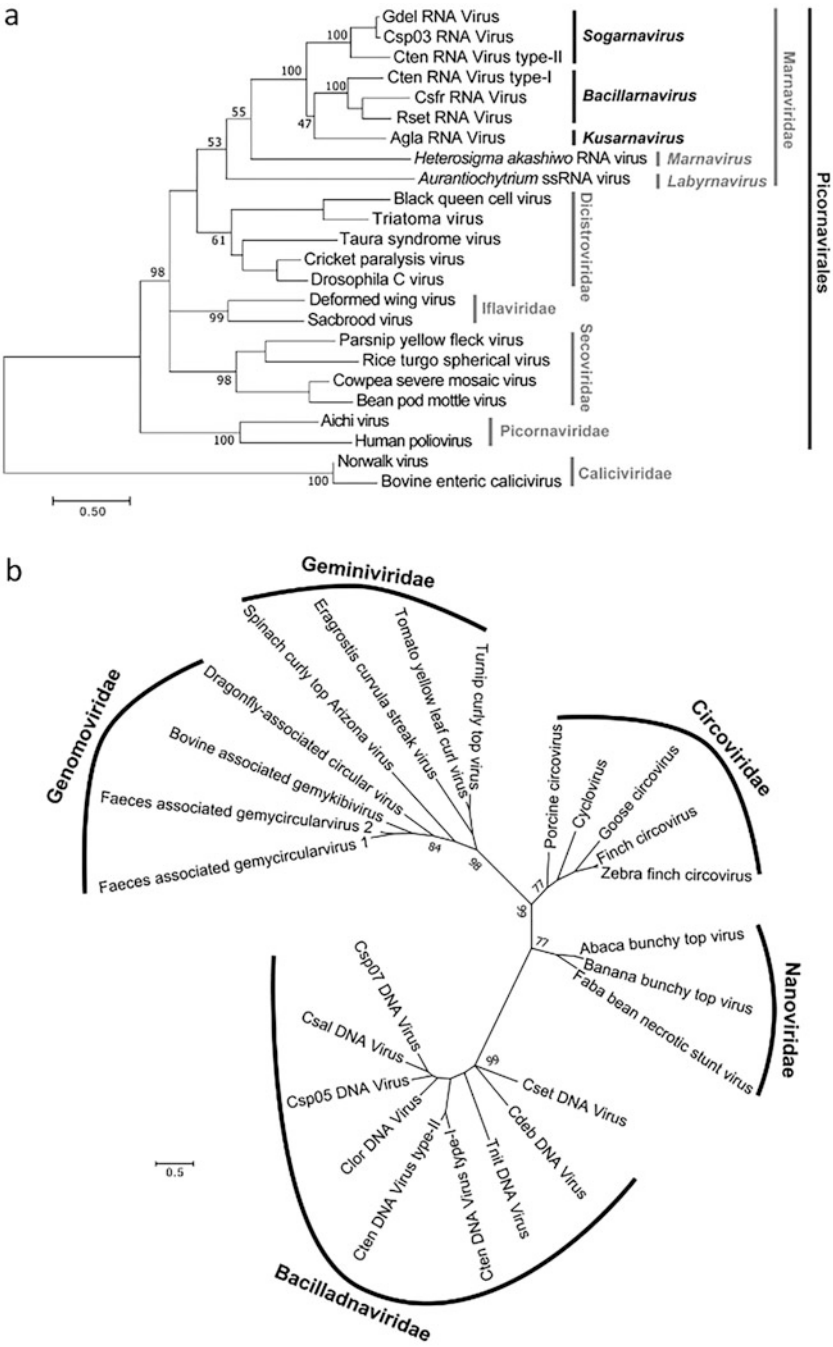

Fig. 3 Maximum likelihood phylogenetic trees of (**a**) ssRNA viruses constructed based on the amino acid sequence of RdRp and (**b**) ssDNA viruses constructed based on the amino acid sequences of replication-related proteins. Bootstrap values (%) from 1000 replications are shown. Scale bar indicate the number of substitutions per site. Full names of viruses are listed in Tables 1 and 2. (K. Kimura, unpublished)

et al. 2017). Within this family, *Chaetoceros*-infecting viruses were split into two newly proposed genera, *Diatodnavirus* and *Protobacilladnavirus,* based on conserved motifs in the replication protein (King et al. 2018). Interestingly, the capsid proteins of ssDNA viruses were found to be homologous to those of ssRNA viruses from the family *Nodaviridae* which infect insects and fish, suggesting a horizontal gene transfer between these two viral types (Kazlauskas et al. 2017). This hypothesis may be resolved with genome sequencing of additional diatom viruses that will allow comparisons with viruses of other marine organisms.

3 Diatom Host-Virus Interactions

3.1 Characteristics of Diatom Viral Infection

Infection Dynamics. All of the diatom viruses described thus far are lytic, causing host mortality within 2–10 days of inoculation. Generally, this coincides with the maximum release of infectious virions (Fig. 4), but in some systems, viral production can occur prior to host lysis (see Sect. 3.2). Impacts on host photophysiology, measured by a decrease in the maximum photochemical yield of photosystem II, have also been detected prior to host lysis, suggesting a potential impact of infection on photosynthesis and carbon fixation (Kranzler et al. 2019) that warrants further investigation.

Host Range. As commonly observed in other microalgal viruses, diatom viruses appear to be extremely limited in their host range, with most viruses capable of infecting only a single species, and in some cases, a single strain (Tomaru et al. 2011b). One exception found thus far is the ssRNA virus, CtenRNAV type-II, which can infect several different *Chaetoceros* species (Kimura and Tomaru 2015), raising intriguing questions about the mechanisms that determine host susceptibility and resistance.

Aggregation and Spore Formation. In aquatic systems, viruses have been historically considered to act as "shunts", diverting energy away from higher trophic levels and back into the microbial loop (Wilhelm and Suttle 1999). However, recent evidence suggests virus may also "shuttle" carbon into the mesopelagic and deep ocean by stimulating processes that facilitate sinking (Lønborg et al. 2013; Guidi et al. 2016; Laber et al. 2018; Nissimov et al. 2018). These processes include the formation of large, ballasted particle aggregates—mediated by the production of polysaccharidic, transparent exopolymers (TEP)—and the induction of heavily silicified spore formation, both of which have been implicated in massive carbon export events in the ocean (Alldredge et al. 1995; Rynearson et al. 2013). Aggregation has been observed in infected *C. socialis* and *C. tenuissimus* cultures (Tomaru et al. 2009; Yamada et al. 2018); however, in the latter, this was mediated by proteinaceous, Coomassie-stainable particles, rather than TEP (Yamada et al. 2018). Viral infection of *C. socialis* also induces spore formation (Tomaru et al. 2009; Pelusi et al. 2020) that appears to serve as a defense mechanism, as spores produced during infection are unable to propagate the infection upon germination

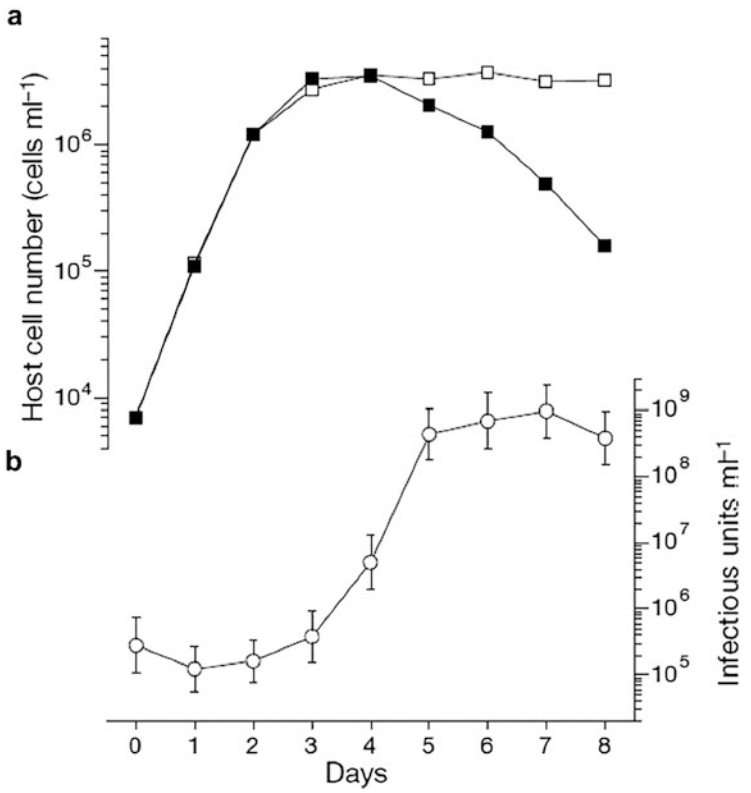

Fig. 4 Representative host-virus infection dynamics. (**a**) Host abundance in control, uninfected (open squares) and infected cultures (*black squares*) of *C. tenuissiumus* (**b**) viral abundance in infected cultures. (Reproduced with permission from Tomaru et al. [2011b])

(Pelusi et al. 2020). Taken together, these findings highlight the potential for viruses to influence both the life cycle of diatoms and the fate of diatom organic matter in the ocean.

Viral Production. The release of viruses from the host through budding or lysis is critical for viral propagation, and quantifying the abundance of viruses is necessary for understanding the ecological significance of viral infection. A major hindrance toward this goal has been our inability to rapidly and reliably enumerate these viruses in culture or natural populations. The genomic make-up (i.e., ssRNA and ssDNA) and small size of diatom viruses preclude quantifying viral abundance using high-throughput methods that employ dsDNA-specific fluorescent dyes combined with microscopy or flow cytometry (Tomaru and Nagasaki 2007). Thus, classical methods of viral enumeration, such as plaque assays or most probable number assays (MPN) are employed (Suttle 1993). Although both of these methods are rather easy to implement, they are time-consuming, dependent on host susceptibility, and prone to high variability and underestimation due to factors such as aggregation. They are,

however, advantageous because they provide the number of infectious particles, unlike fluorescent dyes and flow cytometry, which only give estimates of total viral abundance without accounting for infectivity. Using MPN assays in culture studies, diatom virus burst size (i.e., the number of viruses produced by a single host cell, calculated by dividing the number of infectious units by the number of dead host cells), ranges from 10^1 to 10^5 infectious units per host cell (Tables 1 and 2), the upper end of this range being among the highest reported burst sizes of any algal virus.

Prevalence of Infection. Determining the fraction of a population that is infected at any given time is a critical aspect of understanding not only how viruses are propagated and transmitted, but also the role viruses play in regulating bloom dynamics. One study using TEM image analysis of infected *C.* cf. *wighamii* found that only 20% of the culture was infected just prior to host lysis, suggesting that even within a clonal culture, there is unexplained variability in host susceptibility (Eissler et al. 2009). Adopting methods from other host-virus systems, such as fluorescence in situ hybridization (Robertson and Thach 2009; Castillo et al. 2020), iPolony, a solid-phase polony-based PCR approach (Mruwat et al. 2021), or single-cell viral sequencing (Zanini et al. 2018; Ku et al. 2020) will be instrumental in quantifying the proportion of infected cells within a diatom population and providing a more fundamental understanding of host-virus interactions.

3.2 Factors Impacting Host-Virus Interactions

With the availability of emerging model diatom host-virus systems, we are now starting to identify biotic and abiotic factors that influence infection dynamics and understand the ecological significance and biogeochemical consequence of diatom host-virus interactions in natural populations.

Host Physiology. Numerous studies suggest host growth phase and physiology influence infection dynamics and viral production, irrespective of viral genome type (i.e., ssDNA or ssRNA). In semi-continuous grown cultures, viral-induced host mortality by CtenDNAV-II or CtenRNAV-II was inversely correlated to *C. tenuissimus* growth rate (Tomaru et al. 2021). For some diatoms, host lysis and mortality appear to only occur once cultures reach late logarithmic or stationary phase (in some cases up to nine days post-infection), even when cultures are infected during early exponential growth. However, viral progeny can be detected extracellularly as early as 1–3 days post-infection and prior to host lysis (Fig. 4; Shirai et al. 2008, Tomaru et al. 2014, Kimura and Tomaru 2015). In contrast, when these same species are infected in stationary phase, host lysis is more rapid, occurring within 1–3 days. Intriguingly, the final viral titer between cultures infected during logarithmic or stationary phase does not significantly differ indicating the longer time to lysis does not result in increased viral production. The underlying cellular mechanism(s) driving this variability remains to be determined; however, there is a well-documented interplay between viruses and host cell cycle in other systems, whereby hosts in specific phases of the cycle are more susceptible to infection (Davy and

Doorbar 2007; Bagga and Bouchard 2014). Characterizing viral infection in synchronized cultures of diatoms would provide insight into this possibility.

Host lysis is not restricted to stationary phase in all diatom host-virus systems. For example, infection of *Skeletonema costatum* (Kim et al. 2015a) and *Stephanopyxis palmeriana* (Kim et al. 2015b) caused host lysis during exponential phase, concomitant with the appearance of high extracellular virus abundance. Infection of *Guinardia delicatula* by GdelRNAV also caused host lysis three days post-infection when cultures were still growing exponentially; however, an order of magnitude increase in extracellular viruses could be detected within 12 hours of infection (Arsenieff et al. 2019). These dynamics of host mortality and early viral production have also been observed in *C. debilis* (Tomaru et al. 2008), *C. setoenis* (Tomaru et al. 2013b) and *C.* cf. *wighamii* (Eissler et al. 2009).

Environmental Factors. In addition to growth phase, host-virus dynamics can also be toggled by nutrient availability. In the centric, bloom-forming diatom *C. tenuissimus*, cultures grown under silicon (Si) limiting conditions experienced more rapid infection and mortality than cultures infected under replete conditions (Kranzler et al. 2019). However, similar to cultures infected during different growth phases, the burst size was not significantly different between replete and Si-limited cultures, suggesting diatom viral replication occurs almost immediately following entry and that the time to lysis is dictated by other factors, possibly host physiology. In contrast, in iron-limited *C. tenuissimus*, viral-induced mortality was delayed and significantly reduced and despite the longer latent period, viral burst size was lower when compared to cultures infected under replete conditions (Kranzler et al. 2021). These nutrient-driven host-virus dynamics were observed when cultures were infected with either CtenDNAV or CtenRNAV. Intriguingly, in this same system, temperature has also been found to impact infection dynamics, but in a viral-strain specific manner (Tomaru et al. 2014). While infection and mortality were accelerated at higher temperature when cultures of *C. tenuissimus* were infected with an ssDNA virus, there was no difference in the dynamics when the same species was infected with an ssRNA virus, alluding to possible niche differentiation between these two co-occurring viruses (Tomaru et al. 2014). In subsequent work using different combinations of *C. tenuissimus* host and virus strains, both temperature and salinity significantly impacted the timing of host lysis following infection, with the magnitude of the impact dependent on the host-virus combination (Kimura and Tomaru 2017).

Taken together, these findings highlight the importance of host physiology in viral infection dynamics and raise questions about the mechanism underlying the response to infection. However, the observed variability also demonstrates there is still much to learn about the nature of diatom host susceptibility to viral infection. In a wide range of host-virus systems, including algal hosts, oxidative stress is well-known to play a role in pathogenesis (Schwarz 1996; Sheyn et al. 2016; Moniruzzaman et al. 2018) and likely plays a role in diatoms as well (Kranzler et al. 2021). There may also be a role for the silicified cell wall in defense against infection. Although this has yet to be empirically established, it has been hypothesized that the intricate nano- and micro-scaled structures of the frustule

could serve as a semi-active filter providing a physical barrier to viral infection (Herringer et al. 2019). This is consistent with observations of increased susceptibility in both Si-limited diatoms (Kranzler et al. 2019) and stationary phase cultures (Shirai et al. 2008; Tomaru et al. 2014; Kimura and Tomaru 2015), as diatoms are well-documented to reduce silicification when Si is limiting (Paasche 1975; Brzezinski et al. 1990). Thinner frustules may lead to large pores providing easier access of diatom viruses to the cell membrane.

Biotic Interactions. Little is known about biotic interactions that influence infection dynamics. To date, only one study has explored bacteria-virus-diatom interactions and found that axenic cultures of *C. tenuissimus* were completely lysed during infection, but when xenic cultures were infected, a host sub-population survived and showed signs of regrowth (Kimura and Tomaru 2014). From this "resistant" sub-population, the bacterial community was characterized and clonal isolates of *Nautella* sp., *Polaribacter* sp., and. *Sulfitobacter* sp. were established. When these bacteria were added back to axenic infected, cultures of *C. tenuissimus,* a sub-population of cells were again observed to survive infection. The mechanism by which diatoms are able to escape viral infection in the presence of bacteria has not been elucidated, but presents interesting ecosystem interactions for further exploration.

A recent study reported the discovery of sub-viral agents in cultures of *C. debilis* infected with CdebDNAV, suggesting the presence of a co-occurring satellite virus (Tomaru et al. 2020). Satellite viruses are parasitic viruses of other viruses that hijack the replication machinery of the co-infecting virus for its own replication, thereby promoting the survival of the cellular host. This tripartite interaction between host, virus, and satellite virus, has been observed in other aquatic systems (La Scola et al. 2008), and the further characterization of this potentially similar system in diatoms will provide intriguing insight into the nature of the diatom host-virus relationship.

4 Diatom Viruses in Natural Populations

4.1 Diatom Viruses in Marine Systems

Even prior to the identification of diatom viruses, shotgun sequencing of amplified RdRp genes in coastal waters near British Columbia, Canada, revealed the presence of ssRNA viruses in the ocean (Culley et al. 2003). Phylogenetic analysis revealed these picorna-like viruses were similar, but distinct from the ssRNA virus known at the time to infect the bloom-forming dinoflagellate, *Heterosigma akashiwo* (Tai et al. 2003). As sequencing technologies improved, metagenomic studies revealed widespread presence and persistence of RNA viruses, leading to estimates that RNA viruses could rival, or even outnumber, the more well-characterized dsDNA-containing viruses in the ocean (Culley et al. 2006, 2014; Culley and Steward 2007; Steward et al. 2013; Gustavsen et al. 2014; Miranda et al. 2016; Vlok et al. 2019). Newly developed methods are now enabling the detection of previously unknown viruses. Fragmented and loop primer ligated dsRNA sequencing (FLDS)

is a novel method that efficiently captures RNA viruses by specifically purifying long dsRNA from living organisms, allowing the identification of both dsRNA viruses and replicative intermediates of ssRNA viruses. This method revealed the presence of multiple RNA viruses within diatom communities in a rocky marine environment (Urayama et al. 2016). Recently, FLDS led to the discovery of a novel member of non-segmented dsRNA viruses from the family *Totiviridae* and other unknown RNA viruses associated with the diatom holobiont, *Melosira* sp. that differ from all of the previously discovered diatom viruses (Chiba et al. 2020), suggesting the diversity of RNA viruses may be even more greatly underestimated than previously thought.

Few studies have explored the prevalence of ssDNA viruses in the environment, largely due to methodological limitations. Metagenomic analysis of ssDNA requires whole genome amplification, such as multiple displacement amplification (Kim and Bae 2011), which many studies do not employ, thereby largely excluding ssDNA and preferentially capturing dsDNA. Early studies targeting ssDNA viruses in metagenomic analyses highlight an unexplored diversity of ssDNA, but were conducted when few diatom-specific ssDNA virus genome sequences were available and thus could not be identified (Angly et al. 2006; Labonté and Suttle 2013; McDaniel et al. 2014). Similar to RNA viruses, ssDNA viruses may comprise a larger fraction of the DNA viral community than previously known (Labonté and Suttle 2013). A study on sediments collected from coastal Japan found that 96–100% of the total DNA viral assemblage comprised ssDNA viruses (Yoshida et al. 2018). Only one study directly reported the assembly of a full ssDNA virus genome, similar to the ssDNA virus that infects *C. lorenzianus,* from a metagenomic study of coastal waters near Florida (USA; McDaniel et al. 2014). However, as the number of sequenced diatom viral genomes has increased, so has our ability to detect these genetic signatures, and a reanalysis of existing datasets (both ssRNA and ssDNA) could reveal the presence of previously unidentified diatom viruses.

The first study to specifically explore diatom virus dynamics in natural communities was in 2005 in Chesapeake Bay, USA (Bettarel et al. 2005). To explore the spatiotemporal dynamics of lytic viral infection, viral concentrates from distinct regions in the bay were generated by tangential flow filtration throughout the year and used to inoculate laboratory cultures of *C.* cf. *gracilis*. The highest incidence of lytic infection occurred throughout the bay during late winter-early summer, with no infection detected late summer through fall, except in a few isolated sites. Using a similar approach, temporal dynamics of diatom host and virus abundance was explored in coastal waters near Japan (Tomaru et al. 2011a), where seasonal variability of lytic viral abundance was also observed. Strain specificity of infection has also been observed in natural populations. By challenging isolates of *Pseudo-nitzschia* from waters collected in Puget Sound, Washington (USA) with viral concentrates throughout the year, it was found that only 8–16% of isolates could be infected (Carlson et al. 2016), again highlighting the heterogeneity of permissive cells within a population.

Advances in sequencing technology and bioinformatic analysis are expanding our ability to detect infection by these once enigmatic entities. A seminal study using

metatranscriptomic analysis of eukaryotic communities identified putative host-virus relationships independent of cultured isolates and demonstrated the applicability of using cell-associated viruses as an indicator of active viral infection (Fig. 5a; Moniruzzaman et al. 2017). This approach has proven powerful for characterizing and diagnosing infection in natural diatom assemblages. In the Northeast Pacific Ocean, metatranscriptomic analysis of diatom communities revealed reduced virus diversity and production in iron-limited diatoms, similar to findings in laboratory cultures (Kranzler et al. 2021). Coupling metatranscriptomic analysis of cell-associated viruses with targeted quantification of free, extracellular viruses enabled the diagnosis of different stages of infection in natural diatom assemblages (Fig. 5b) and revealed enhanced infection of Si limited diatom communities (Kranzler et al. 2019). Taken together, these studies demonstrate that nutrient availability could drive diatom viral infection and mortality in natural populations.

4.2 Diatom Viruses in Freshwater Systems

Given the ubiquity of diatoms in aquatic environments, we might expect an equally widespread presence of diverse diatom viruses in rivers and lakes. However, there has been no reported isolation and cultivation of a freshwater diatom host-virus system, so our ability to identify freshwater diatom viruses is limited by our ability to detect them through sequence similarity to marine diatom viruses. Identification of ssRNA viruses has been reported in a few freshwater systems by sequence analysis of RdRp genes. In an antarctic lake, viruses with reported similarity to *Bacillarnavirus* displayed strong seasonality in abundance, being only present in summer and not in spring (López-Bueno et al. 2015). Temporal seasonality was also reported in a temperate lake in the eastern United States where a larger proportion of potential diatom infecting viruses were found in winter compared to summer (Djikeng et al. 2009). In the St. Lawrence Estuary (Canada), spatio-variability was found with distinct diatom viruses occupying discrete salinity regimes (i.e., freshwater, brackish, marine) within the estuarine system (Labbé et al. 2018). Identification of ssDNA diatom viruses in freshwater systems has been more elusive. Although few in number, there are studies that have targeted ssDNA viruses in polar and temperate environments (e.g., López-Bueno et al. 2009; Roux et al. 2012; Zawar-Reza et al. 2014; Aguirre de Cárcer et al. 2015), but none have directly identified those with similarity to known diatom ssDNA viruses. However, those studies suggest freshwater viral communities may be quite unique, with as little as 3% similarity to viral sequences from marine environments (López-Bueno et al. 2009; Roux et al. 2012), highlighting that the absence of freshwater diatom ssDNA viral genomes may be hindering our ability to identify them in natural populations.

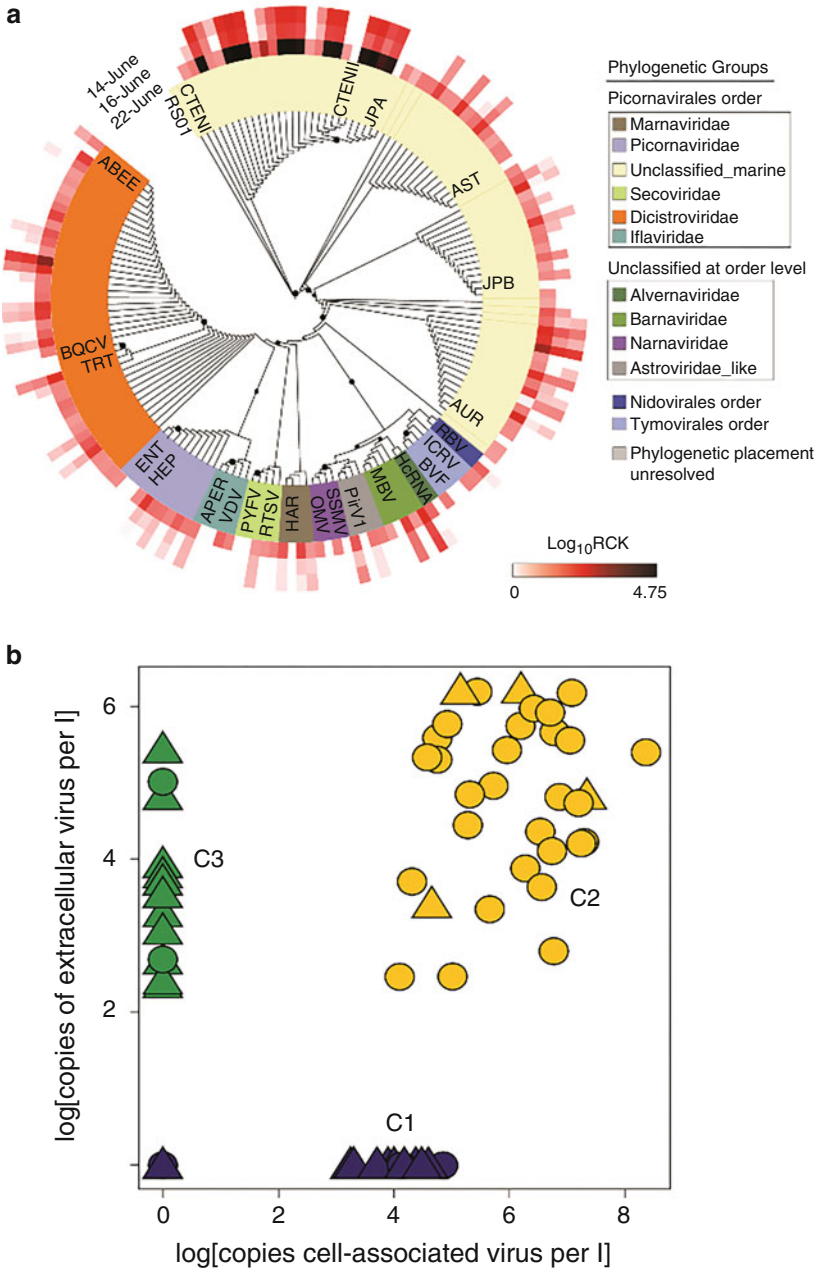

Fig. 5 Detecting and quantifying diatom viruses in natural populations. (**a**) Phylogenetic placement of RdRp motifs of ssRNA viruses from Quantuck Bay, NY. Temporal expression level of each is shown on the outer ring as rarefied read counts per kilobase ($\log_{10}$RCK). See original publication for further detail. Reproduced from Moniruzzaman et al. 2017 with minor formatting modifications under the license http://creativecommons.org/licenses/by/4.0/. (**b**) *k*-means cluster analysis of metatranscriptomic data of cell-associated diatom viruses and targeted, quantitative, reverse transcription-PCR data of extracellular diatom viruses reveals three distinct groups characterized

5 Implications for Ecosystem Function and Biogeochemical Cycling

Diatom viruses are emerging as widespread and prevalent pathogens with the potential to significantly impact diatom-mediated primary productivity and biological pump efficiency (Culley et al. 2003, 2014; Shirai et al. 2006; Culley and Steward 2007; Steward et al. 2013; Gustavsen et al. 2014; Miranda et al. 2016; Kranzler et al. 2019; Vlok et al. 2019; Kranzler et al. 2021). By facilitating host lysis, diatom viruses are a mechanism for the turnover and remineralization of diatom organic matter and associated elements in the surface ocean. With a global mean estimate that 58% of diatom silica production (Nelson et al. 1995) is supported by recycled silicic acid, and in some regions of the ocean up to 100% (Treguer and De La Rocha 2013), turnover of diatoms by viral infection may represent a heretofore unappreciated component of diatom-mediated silicon cycling. At the same time, infection-induced aggregation and spore formation may counter the "viral shunt" by facilitating sinking and stimulating export through a "viral shuttle", a biogeochemical consequence that may be even more accentuated under conditions that delay host lysis (i.e., exponential growth, temperature, or iron limitation). With the biogenic silica-based cell wall serving as ballast, diatoms are estimated to contribute ~40% of carbon export (Jin et al. 2006). However, we still cannot explain the high spatiotemporal variability in diatom-mediated export (Tréguer et al. 2018). Viral infection mediated processes that serve to shunt or shuttle diatom organic matter and associated elements may be a critical, overlooked component of marine biogeochemical cycling and the diatom-mediated biological pump.

6 Future Outlooks

Given the key role diatoms play in aquatic ecosystems, elucidating how these populations are regulated by viral pathogens is essential to fully understand the biogeochemical impact of diatoms and the fate of diatom organic matter. Laboratory studies on model host-virus systems have revealed a role for biotic and abiotic interactions in the dynamics of infection, including viral replication and host mortality. Although both are critical for transmission and propagation of viral infection, the role that diatom viruses play in bloom formation and termination is still being elucidated. Moreover, the environmental drivers influencing host-virus interactions are understudied and the degree to which natural diatoms populations are infected remains largely unknown.

The discovery, isolation, and cultivation of more than 20 distinct diatom host-virus systems has been a critical step in understanding infection dynamics and the

Fig. 5 (continued) as early infected (C1, *blue symbols*), actively infected (C2, *yellow symbols*), and post-lytic (C3, *green symbols*) populations in the California Current Ecosystem. (Reproduced with permission from Kranzler et al. [2019])

potential ecological and biogeochemical impacts of diatom host-virus interactions. However, properties of infection such as the latent period, rate of viral production, viral infectivity, host specificity, and impacts on host physiology and metabolism are needed to elucidate the role of viruses in bloom formation and termination and subsequent impacts on diatom-mediated biogeochemical cycling. Characterizing the genetic variability of diatom virus populations (e.g., rate of mutation per infection event, genome recombination) may also shed light on the role and regulation of diatom virus diversity. Critically lacking is a comparative transcriptomic, proteomic, and metabolomic analysis during infection that would provide insight into the molecular and biochemical mechanisms underlying the host response to infection, as well as potentially identify diagnostic markers specific for infection. Given the availability and widespread use of genetic transformation systems in both centric and pennate model diatoms (Falciatore et al. 2020), adaptation of these techniques to established diatom host-virus systems would further facilitate a mechanistic and molecular understanding of diatom host-virus interactions.

Over the past two decades, ssDNA and ssRNA viruses that infect diatoms have emerged as significant and diverse members of the viral community. However, the large majority of diatom host-virus systems isolated to date are from Japan and all are from the marine environment. Having additional systems from distinct geographical regions, as well as from different host genera, will be instrumental in determining how conserved host-virus interactions are within this globally dominant and diverse group of phytoplankton (Malviya et al. 2016). Additional viral genome sequences would also enrich the current reference dataset and improve our ability to identify diatom viral signatures in metagenomic surveys, the latter of which would be facilitated by focused sampling of the smaller size fraction (0.02–0.2 μm) on oceanographic campaigns. High-throughput sequencing of targeted "viromes" is already revealing the immense diversity of RNA viruses (Wolf et al. 2020) and, when used quantitatively and in combination with metatranscriptomic analysis of cell-associated viruses, can diagnose stages of infection in natural populations (Kranzler et al. 2019, 2021). Additional methods for high-throughput, absolute quantification of diatom viruses, taking advantage, for example, of commercially available fluorescent dyes specific for ssDNA and ssRNA, and more powerful flow cytometers and microscopes capable of detecting particles down to 20 nm would provide an even broader view of the prevalence, pervasiveness, and distribution of these viruses in the global ocean.

When metrics of infection are taken in context with biogeochemical and physiological metrics, driving factors of infection itself can be elucidated (Kranzler et al. 2019). Combining this with network analyses of community sequence data to identify specific host-virus interactions within a mixed community (Moniruzzaman et al. 2017) sets the stage for further exploration of host specificity and mechanisms of resistance across both space and time. Rapidly advancing single-cell technologies applied in other algal systems have enabled the characterization of host-virus interactions at an unprecedented scale (Rosenwasser et al. 2019; Ku et al. 2020). These approaches will be useful in cultured diatom systems, as well as in natural communities where the heterogeneity of host-virus interactions can be assessed

across spatial and temporal gradients (Yoon et al. 2011; Martínez-García et al. 2014). Ultimately, parameterization of diatom host-virus interactions will be needed to model the impact of viral-mediated mortality on diatom productivity. This has been done in other host-virus systems (Record et al. 2016; Talmy et al. 2019; Middelboe 2000; Thingstad 2000; Thamatrakoln et al. 2019; Demory et al. 2021), but incorporation of viral-mediated losses into broader global ecosystem and biogeochemical models must account for the impact of diatom viruses given their role in regulating one of the most globally dominant and ecologically relevant phytoplankton groups in the modern ocean.

We have come a long way in the 16 years since the first diatom virus was reported with the cultivation of numerous diatom host-virus systems, the sequencing of diatom viral genomes, the characterization of lytic infection dynamics, and the identification of environmental parameters that influence host-virus dynamics. We have developed methods to detect and quantify diatom viruses in natural populations and can diagnose different stages of infection. However, there is still much to learn. How globally distributed are diatom viruses in the ocean? To what degree are diatom populations infected? What are the consequences of viral infection on diatom-mediated biogeochemical cycling and biological pump efficiency? Answers to these questions and more are essential to our understanding of the impact viral infection has on the flow of diatom organic carbon and associated matter in both the modern and future oceans.

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