Viruses of the eukaryotic plankton are predicted to increase carbon export efficiency in the global sunlit ocean

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Abstract

The biological carbon pump (BCP) is the process by which ocean organisms transfer carbon from surface waters to the ocean interior and seafloor sediments for sequestration. Viruses are thought to increase the efficiency of the BCP by fostering primary production and facilitating the export of carbon-enriched materials in the deep sea (the viral "shunt and pump"). A prior study using an oligotrophic ocean-dominated dataset from the *Tara* Oceans expedition revealed that bacterial dsDNA viruses are better associated with variation in carbon export than either prokaryotes or eukaryotes, but eukaryotic viruses were not examined. Because eukaryotes contribute significantly to ocean biomass and net production (> 40%), their viruses might also play a role in the BCP. Here, we leveraged deep-sequencing molecular data generated in the framework of *Tara* Oceans to identify and quantify diverse lineages of large dsDNA and smaller RNA viruses of eukaryotes. We found that the abundance of these viruses explained 49% of the variation in carbon export (compared with 89% by bacterial dsDNA viruses) and also substantially explained the variation in net primary production (76%) and carbon export efficiency (50%). Prasinoviruses infecting Mamiellales as well as *Mimivirus* relatives putatively infecting haptophytes are among the eukaryotic virus lineages predicted to be the best contributors to BCP efficiency. These findings collectively provide a first-level window into how eukaryotic viruses impact the BCP and suggest that the virus-mediated shunt and pump indeed plays a role.

Introduction

The oceanic biological carbon pump (BCP) is an organism-driven process by which atmospheric carbon (*i.e.* CO_2) is transferred to the ocean interior and seafloor for sequestration over periods ranging from centuries to those of geological time-scale durations. Between 15% and 20% of net primary production (NPP) is exported out of the euphotic zone, with 1% to 3% (ca. 0.2 gigatons) of fixed carbon reaching the seafloor annually (De La Rocha and Passow 2007; Herndl and Reinthaler 2013; Quéré et al. 2018; Zhang et al. 2018).

Three components of the BCP, namely, carbon fixation, export and remineralization, are governed by complex interactions between numerous members of planktonic communities (Falkowski et al. 1998). Among these organisms, diatoms (Tréguer et al. 2018) and zooplankton (Turner 2015) have been identified as important contributors to the BCP in nutrient-replete oceanic regions. In the oligotrophic ocean, Cyanobacteria and Collodaria (Lomas and Moran 2011), diatoms (Agusti et al. 2015; Leblanc et al. 2018) and other small (pico- to nano-) plankton (Richardson and Jackson 2007; Lomas and Moran 2011) have been implicated in the BCP. Overall, the composition of the planktonic community in surface waters, rather than a single species, is better associated with the intensity of the BCP (Boyd and Newton 1995; Guidi et al. 2009; Guidi et al. 2016).

A recent gene correlation network analysis based on *Tara* Oceans genomic data, ranging from viruses to zooplankton, outlined predicted species interactions associated with carbon export and revealed a remarkably strong association with bacterial dsDNA viruses relative to either prokaryotes or eukaryotes (Guidi et al. 2016). Cell lysis caused

by viruses promotes the production of dissolved organic matter and accelerates the recycling of potentially growth-limiting nutrient elements (*i.e.* nitrogen and phosphorus) in the photic zone (the "viral shunt") (Weinbauer and Peduzzi 1995; Gobler et al. 1997; Wilhelm and Suttle 1999; Weinbauer 2004; Motegi et al. 2009). This recycling in turn may increase primary production and carbon export (Brussaard et al. 2008; Weitz et al. 2015). Viruses have also been proposed to drive particle aggregation and transfer into the deep sea *via* the release of sticky, carbon-rich viral lysate (the "viral shuttle") (Proctor and Fuhrman 1991; Peduzzi and Weinbauer 1993; Shibata et al. 1997; Weinbauer 2004). The combined effect of these two viral properties, coined "shunt and pump", is proposed to enhance the magnitude and efficiency of the BCP (Suttle 2007). A study by Guidi et al. (2016) revealed that populations of bacterial dsDNA viruses in the sunlit oligotrophic ocean are strongly associated with variation in the magnitude of the flux of particulate organic carbon. Although viruses of eukaryotes were not included in the above-mentioned study, the significant contribution of their hosts to ocean biomass and net production (Li 1995; Nelson et al. 1995; Worden et al. 2004; Liu et al. 2009) and their observed predominance over prokaryotes in sinking materials of Sargasso Sea oligotrophic surface waters (Fawcett et al. 2011; Lomas and Moran 2011) suggest that eukaryotic viruses are responsible for a substantial part of the variation in exported carbon. Furthermore, the mechanisms causing this association remain to be dissected (Sullivan et al. 2017), as such an association might emerge through indirect correlation with other parameters, such as NPP.

In this study, we explored the association between eukaryotic viruses and the BCP as well as the viral mechanisms enhancing this process. We exploited comprehensive

98 organismal data from the *Tara* Oceans expedition (Sunagawa et al. 2015; Carradec et al.
99 2018) as well as related measurements of carbon export estimated from particle
100 concentration and size distributions observed *in situ* (Guidi et al. 2016). The investigation
101 of eukaryotic viruses based on environmental genomics has long been difficult because of
102 their lower concentration in the water column compared with prokaryotic dsDNA viruses
103 (Hingamp et al. 2013). Deep sequencing of planktonic community DNA and RNA, as
104 carried out in *Tara* Oceans, has enabled the identification of marker genes of major viral
105 groups infecting eukaryotes and begun to reveal that these groups represent a sizeable
106 fraction of the marine virosphere (Hingamp et al. 2013; Allen et al. 2017; Moniruzzaman
107 et al. 2017; Carradec et al. 2018; Mihara et al. 2018). In the present study, we identified
108 several hundred marker-gene sequences of nucleocytoplasmic large DNA viruses
109 (NCLDVs; so-called "giant viruses") in the prokaryotic size fraction. We also identified
110 RNA viruses in meta-transcriptomes of four eukaryotic size fractions. The resulting
111 profile of viral distributions was compared with the magnitude of carbon export (CE) and
112 its efficiency (CEE) to identify lineages of viruses predicted to contribute to the BCP.

113 # Results and Discussion

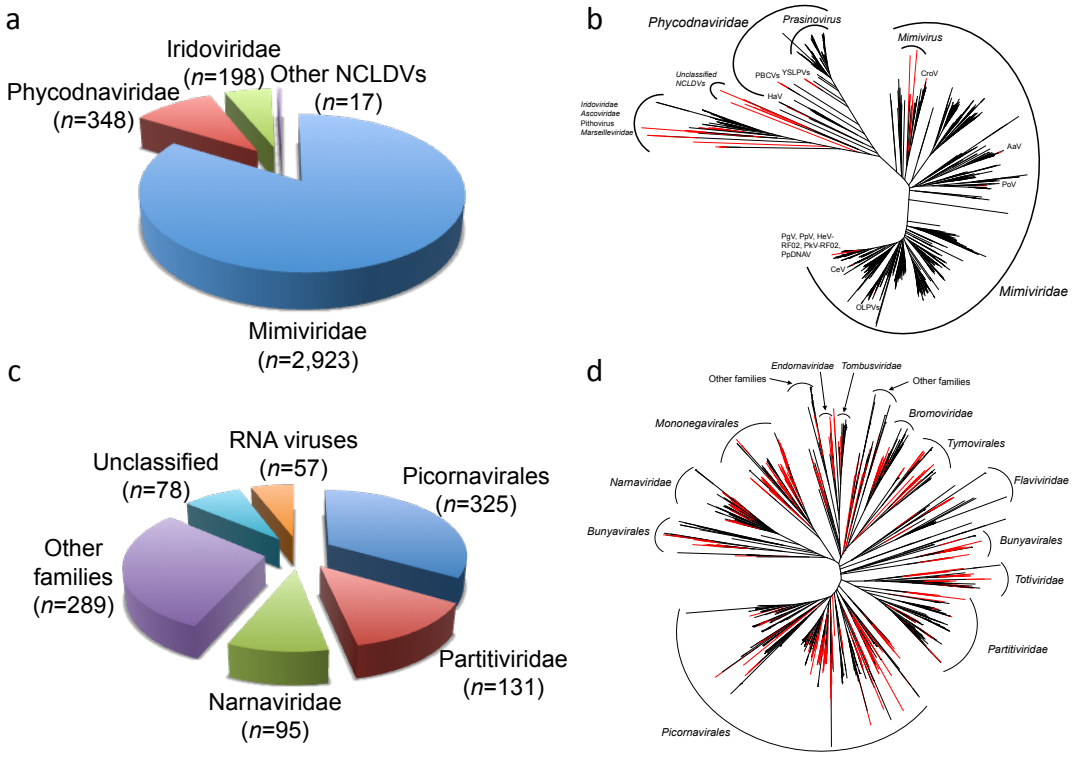
114 ## The discovery of diverse NCLDVs and RNA viruses in *Tara* Oceans gene
115 ## catalogs

116 We used profile hidden Markov model-based homology searching to identify marker-
117 gene sequences of viruses of eukaryotes in two ocean gene catalogs. These catalogs were
118 previously constructed from environmental shotgun sequence data of samples collected
119 during the *Tara* Oceans expedition. The first catalog, the Ocean Microbial Reference

Gene Catalog (OM-RGC), contains 40 million non-redundant genes predicted from the assemblies of *Tara* Oceans viral and microbial metagenomes (Sunagawa et al. 2015). We searched this catalog for NCLDV DNA polymerase family B (PolB) genes, as dsDNA viruses may be present in microbial metagenomes because large virions (> 0.2 μm) have been retained on the filter or viral genomes replicating within picoeukaryotic cells have been captured. The second gene catalog, the Marine Atlas of *Tara* Oceans Unigenes (MATOU), contains 116 million non-redundant genes predicted from meta-transcriptomes of single-cell microeukaryotes and small multicellular zooplankton (Carradec et al. 2018). We searched this catalog for RNA-dependent RNA polymerase (RdRP) genes of RNA viruses because transcripts of viruses actively infecting their host or genomes of RNA viruses have been captured in it.

We identified 3,486 NCLDV PolB sequences and 975 RNA virus RdRP sequences. All except 17 of the NCLDV PolBs were assigned to the families *Mimiviridae* (n = 2,923), *Phycodnaviridae* (n = 348) and *Iridoviridae* (n = 198) (Figure 1a). The large number of PolB sequences assigned to *Mimiviridae* and *Phycodnaviridae* compared with other NCLDV families is consistent with a previous observation based on a smaller dataset (Hingamp et al. 2013). The divergence between these environmental sequences and reference sequences from known viral genomes was greater in *Mimiviridae* than *Phycodnaviridae* (Figure 1b, Supplementary Figure 1a). Within *Mimiviridae,* 83% of the sequences were most similar to those from algae-infecting *Mimivirus* relatives. Among the sequences classified in *Phycodnaviridae,* 92% were most similar to those in *Prasinovirus*, while 5% were closest to *Yellowstone lake phycodnavirus*, which is closely related to *Prasinovirus*. RdRP sequences were assigned mostly to the order

143 *Picornavirales* (*n* = 325) followed by the families *Partitiviridae* (*n* = 131), *Narnaviridae*
144 (*n* = 95), *Tombusviridae* (*n* = 45) and *Virgaviridae* (*n* = 33) (Figure 1c), with most
145 sequences being distant (30% to 40% amino acid identity) from reference viruses (Figure
146 1d, Supplementary Figure 1b). This result is consistent with previous studies on the
147 diversity of marine RNA viruses, in which RNA virus sequences were found to
148 correspond to diverse positive-polarity ssRNA and dsRNA viruses distantly related to
149 well-characterized viruses (reviewed in Culley [2018]).



150

151 **Figure 1: Numerous lineages of nucleocytoplasmic large DNA viruses (NCLDVs)**
152 **and RNA viruses were identified in environmental samples collected during the**
153 ***Tara* Oceans expedition (2009-2013). a** and **c** Taxonomic breakdown of environmental
154 sequences of NCLDV DNA polymerase family B and RNA virus RNA-dependent RNA
155 polymerase. **b** and **d** Unrooted maximum likelihood phylogenetic trees of environmental
156 (black) and reference (red) viral sequences.

Eukaryotic viruses linked to carbon export efficiency in the sunlit ocean

Among the PolB and RdRP sequences identified in the *Tara* Oceans gene catalogs, 37% and 17% were respectively present in at least five samples and had accompanying carbon export measurement data (Supplementary Table 1). Using abundance profiles of these 1,454 marker-gene sequences at 61 sampling sites in the photic zone of 40 *Tara* Oceans stations (Figure 2a–c), we tested for associations with estimates of carbon export flux at 150 meters (CE_{150}) and measurements of carbon export efficiency (CEE). PLS regression model explained 49% ($R^2 = 49\%$) of the variation in CE_{150}, with a Pearson correlation coefficient between observed and predicted values of 0.67 ($P < 1 \times 10^{-4}$) (Figure 2d); this result demonstrates that viruses of eukaryotes are associated with the magnitude of carbon export. A comparison of viral abundances with CEE revealed that viruses are also strongly associated with CEE ($R^2 = 50\%$, $r = 0.72$, $P < 1 \times 10^{-4}$) (Figure 2e and Supplementary Figure 2a; see Supplementary Table 2 for details of PLS models and Supplementary Figure 2b for details of the permutation tests). In these PLS regression models, 62 and 26 viruses were considered to be important predictors (*i.e.* variable importance in the projection [VIP] score > 2 and regression coefficient > 0) of CE_{150} and CEE, respectively, and only two viruses were shared between them.

CE_{150} was found to be correlated with NPP ($r = 0.76$, $P < 1 \times 10^{-11}$), which suggests that the association of viruses with CE_{150} is due to a viral shunt effect or to primary-production enhancement of viral production. In line with this interpretation, we found that viral abundances can predict NPP variations ($R^2 = 59\%$, $r = 0.78$, $P < 1 \times 10^{-4}$) (Figure 2f); in addition, a larger number of shared predictors were obtained under the PLS model for CE_{150} (44 viruses) than for CEE (3 viruses) ($P = 1.3 \times 10^{-7}$). In contrast,

CEE was not correlated with NPP ($r = 0.2$, $P = 0.1$) or CE_{150} ($r = 0.14$, $P = 0.3$). The association of some viruses with CEE is therefore not an indirect relationship caused by NPP; instead, viruses of eukaryotes may have a shuttling effect that enhances the efficiency of carbon export.

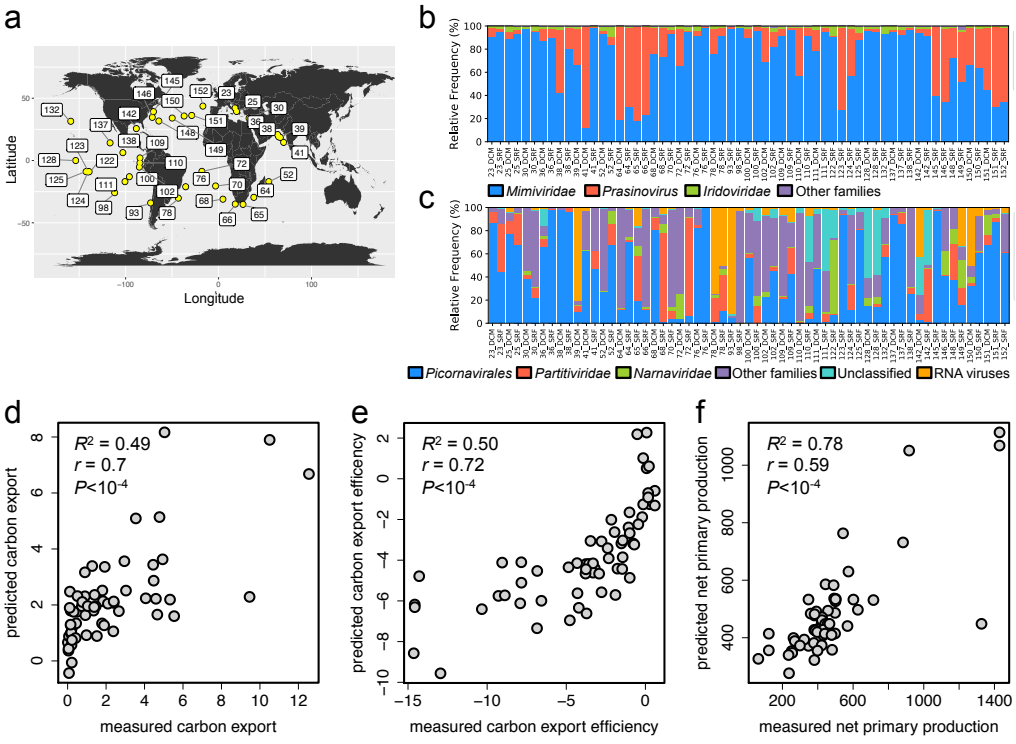


Figure 2: Abundance of nucleocytoplasmic large DNA viruses (NCLDVs) and RNA viruses is associated with carbon export efficiency and flux at 150m in the global ocean. a Location of 40 *Tara* Oceans stations that were the source of 61 prokaryote-enriched metagenomes and 244 eukaryotic meta-transcriptomes (61 sites x 4 organismal size fractions) from surface and DCM layers and measurements of carbon export. **b–c** Relative abundance of NLCVDs and RNA viruses in samples used for association analyses. **d–f** Plots showing the correlation between predicted and observed values in a leave-one-out cross-validation test for carbon export flux at 150 m (**d**), carbon export efficiency (**e**) and net primary production (**f**). Each PLS regression model was reconstructed using abundance profiles of 1,454 marker-gene sequences (1,282 PolBs and 172 RdRPs) derived from environmental samples. *r*, Pearson correlation coefficient; R^2, square of the correlation between measured response values and predicted response values. R^2, which was calculated as 1 – SSE / SST (sum of squares due to error and total), measures how successful the fit is in explaining the variation of the data. Model robustness was assessed using a permutation test ($n = 10{,}000$).

Giant viruses of small algae are predicted to enhance CEE

We considered 26 viruses positively associated with CEE with a VIP score > 2 (Supplementary File 1) to be important predictors of CEE in the PLS regression. These viruses are hereafter referred to as VIPs. Eleven VIPs (nine NCLDVs and two RNA viruses; red rectangle in Figure 3) were abundant in samples from Eastern India coast (EAFR) and Benguela current coast (BENG) provinces, with some (*e.g.* polb 00248170 and 002035391) also relatively abundant in samples from different oceanic provinces where CEE was also relatively high. This observation suggests that these viruses enhance the BCP in different regions of the global ocean.

Most VIPs (23 of 26) belonged to *Mimiviridae* (*n* = 11) and *Phycodnaviridae* (*n* = 12). All the phycodnavirus sequences were most closely related to those of prasinoviruses, with amino acid sequence identities to reference sequences ranging between 64% and 88%. The three remaining VIPs were RNA viruses. The closest homolog of one sequence (rdrp 36496887) was that of an octopus-associated member of *Picornavirales* (Beihai picorna-like virus 106), while the other two (90355641 and 58847823) were most similar to that of a crustacean-associated *Bunyavirales* virus (Wenling crustacean virus 9).

Taxonomic analysis of genes in genome fragments containing VIP PolBs further confirmed their identity as *Mimiviridae* or *Phycodnaviridae* (Supplementary Figure 3). One contig (CNJ01018797) was predicted to encode a protein having a high amino-acid identity (94%) to a gene product from the transcriptome of the diatom *Triceratium dubium* (Supplementary Figure 3b). NCLDVs infecting diatoms have not been reported

so far, but a previous co-abundance analysis has linked diatoms and *Mimiviridae* (Moniruzzaman et al. 2017).

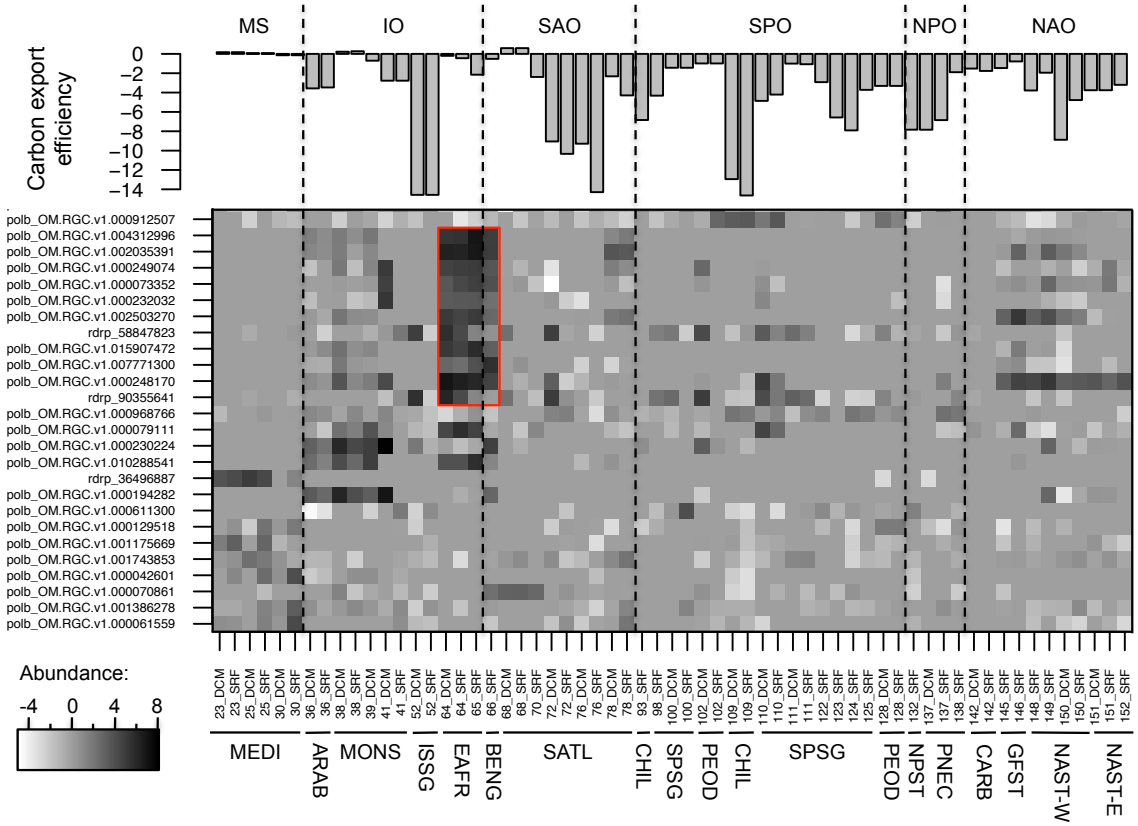


Figure 3: Biogeography of viral lineages associated with carbon export efficiency (CEE). The upper panel shows carbon export efficiency, ($CE_{deep} - CE_{surface}$) / CE_{deep}, for the 61 sampling sites. Sites with values above and below zero are those where carbon export flux was respectively higher and lower in deep than in surface waters; hence, they correspond to sites where the biological carbon pump was higher or lower compared with other sites. The bottom panel is a map reflecting abundances, expressed as center-log ratio transformed gene-length normalized read counts, of viruses positively associated with CEE and having a VIP score > 2. MS, Mediterranean Sea; IO, Indian Ocean; SAO, South Atlantic Ocean; SPO, South Pacific Ocean; NPO, North Pacific Ocean; NAO, North Atlantic Ocean. The bottom horizontal axis is labeled with *Tara* Oceans station numbers, sampling depth (SRF, surface; DCM, deep chlorophyll maximum) and abbreviations of biogeographic provinces.

Because most VIPs were only distantly related to isolated viruses, inferring characteristics such as host type and infection strategies was not straightforward. We therefore conducted a phylogeny-guided network-based host prediction analysis (Figure

241 4; see Methods). Within the *Prasinovirus* clade, which contained six VIPs, enrichment
242 analysis of predicted host groups along the phylogenetic tree uncovered 10 nodes
243 significantly enriched for five different eukaryotic orders. Mamiellales, the only known
244 host group of prasinoviruses, was detected at nine different nodes (seven of them had no
245 parent-to-children relationships), while the other four eukaryotic orders were found at
246 only one node (or two in the case of Eutreptiales) (Figure 4a). Within *Mimiviridae*,
247 significant enrichment was detected for 10 different orders; Collodaria was detected at 15
248 nodes (2 of them had no parent-to-children relationships) and Prymnesiales at 6 nodes (3
249 of them had no parent-to-children relationship), while all other orders were present at a
250 maximum of one node with no parent-to-children relationships. The nodes enriched in
251 Prymnesiales and Collodaria fell within or were sister to clades containing reference
252 viruses isolated from Prymnesiales and Phaeocystales (both are haptophytes orders)
253 species. This placement suggests that environmental PolB sequences in this clade belong
254 to *Mimiviridae* viruses that infect Prymnesiales. Interestingly four of these *Mimiviridae*
255 viruses were VIP viruses (Figure 4a). The detection of Collodaria may be the result of
256 indirect associations that reflect a symbiotic relationship with Prymnesiales, as some
257 acantharians, a lineage of Rhizaria related to Collodaria, are known to host Prymnesiales
258 species (Mars Brisbin et al. 2018). Nodes enriched in the remaining three other orders fell
259 within clades that were only distantly related to any reference *Mimiviridae*. The final
260 *Mimiviridae* VIP (polb 000079111) in the tree was a distant relative of *Aureococcus*
261 *anophagefferens virus* (AaV). No host groups were predicted for the clade containing the
262 Picornavirales VIP (Figure 4b). Overall, this host prediction analysis revealed that

lineages of prasinoviruses and putative prymnesioviruses are among the viral lineages best associated with CEE.

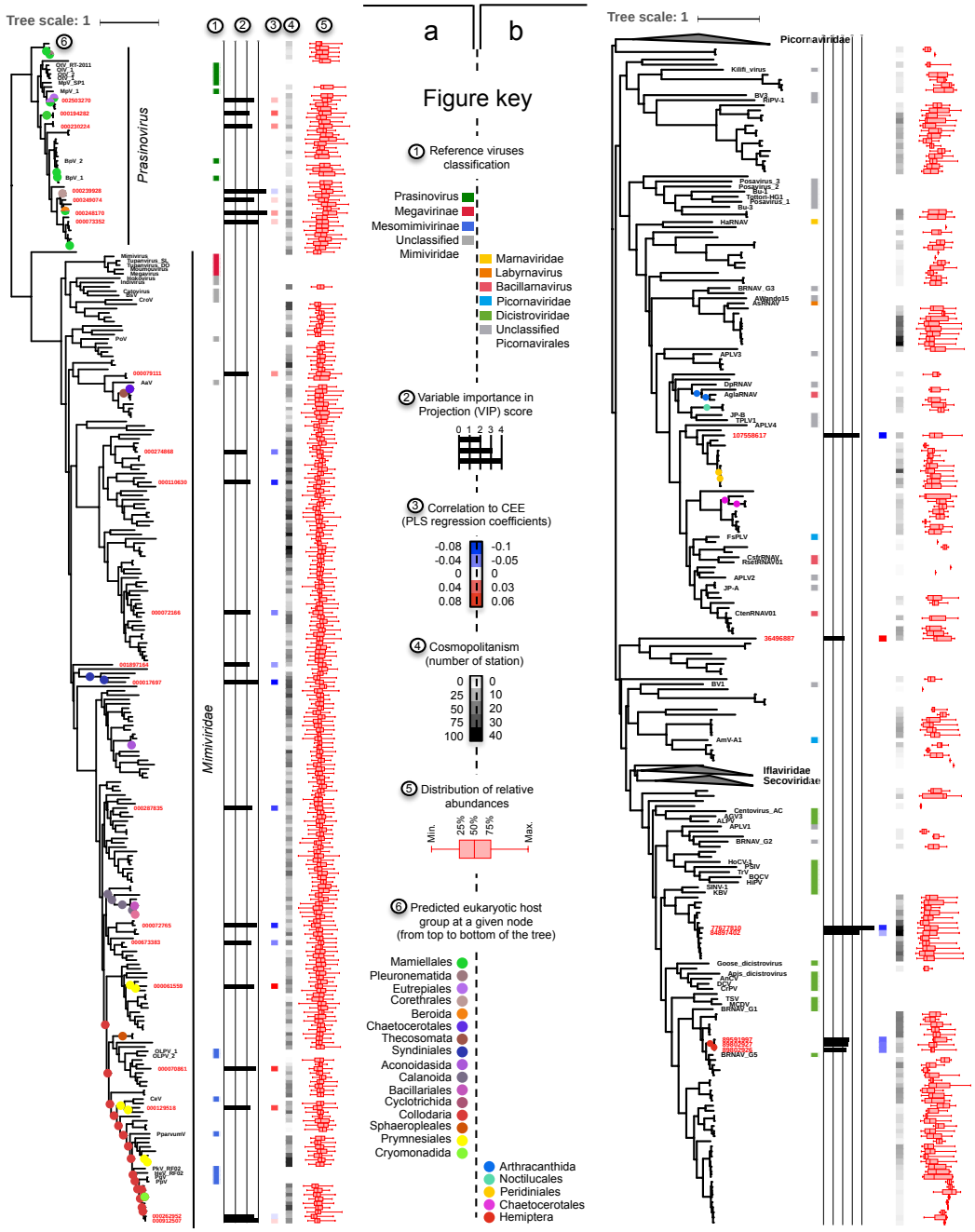


Figure 4: Phylogenetic position of viruses associated with the efficiency of carbon export. Details of the PLS model and viral global distribution, relative abundance and putative eukaryotic host group are superimposed on the trees. Phylogenetic trees contain environmental (labeled in red if VIP > 2) and reference (labeled in black) sequences of *Prasinovirus* and *Mimiviridae* DNA polymerase family B (**a**) and *Picornavirales* RdRP (**b**).

Functional traits of eukaryotic organisms interacting with CEE-associated viruses

The host assignment of Mamiellales, Prymnesiales (Figure 4a) and Chaetocerotales (Figure 4b) to viral clades containing reference viruses isolated from these organismal groups suggests that the network-based approach was able to predict virus–host relationships with a certain level of reliability using our large dataset despite the reported limitations of similar types of analyses (Coenen and Weitz 2018). This positive signal prompted us to use these species-association networks to investigate taxonomic and functional differences between the eukaryotic organisms predicted to interact with viruses that were either positively or negatively associated with CEE (Figure 5). At the functional level, viruses positively associated with CEE had a greater number of connections with chloroplast-bearing eukaryotes ($Q = 0.03$) and with silicified eukaryotes ($Q = 0.05$) compared with viruses negatively associated with CEE (Supplementary Table 4); this suggests that these virus–host systems contribute to CEE. This result also supports the proposed idea that viral lysis enhances the effect of the BCP; that is, lysis of autotrophs is expected to release growth-limiting nutrients (Gobler et al. 1997) that are recycled to grow new cells, while carbon-rich cell debris, potentially ballasted with silica, tends to aggregate and sink (Suttle 2007).

Viral sequence ID	Reg. Coeff.	Taxa	Chloroplast	Silicification	Calcification
polb_OM.RGC.v1.001386278	0.064	Dinophyceae	■		
polb_OM.RGC.v1.000070861	0.063	Collodaria			
polb_OM.RGC.v1.000042601	0.062	Dinophyceae	■		
polb_OM.RGC.v1.001743853	0.057	Trachymedusae			
polb_OM.RGC.v1.001175669	0.056	Rotaliida			■
polb_OM.RGC.v1.000129518	0.055	Dinophyceae	■		
polb_OM.RGC.v1.000611300	0.047	Ulvophyceae	■		
polb_OM.RGC.v1.000194282	0.047	Gymnodiniales	■		
rdrp_36496887	0.038	Poecilostomatoida			
polb_OM.RGC.v1.010288541	0.038	Bacillariophyta	■	■	
polb_OM.RGC.v1.000230224	0.033	Arthra_Symphy_F1			
polb_OM.RGC.v1.000079111	0.032	Cephaloidophorida			
polb_OM.RGC.v1.000968766	0.032	Calanoida			
polb_OM.RGC.v1.000248170	0.028	Beroida			
polb_OM.RGC.v1.007771300	0.027	Dinophyceae			
polb_OM.RGC.v1.015907472	0.024	Coscinodiscophytina	■	■	
polb_OM.RGC.v1.000232032	0.013	Gregarinomorphea			
polb_OM.RGC.v1.000073352	0.012	Mamiellophyceae	■		
polb_OM.RGC.v1.000249074	0.010	Dinophysiales			
polb_OM.RGC.v1.002035391	0.010	Coscinodiscophytina	■	■	
polb_OM.RGC.v1.004312996	0.009	Coscinodiscophytina	■	■	
polb_OM.RGC.v1.000912507	0.009	Acanthoecida			
polb_OM.RGC.v1.000062429	-0.009	Peridiniales			
polb_OM.RGC.v1.000239928	-0.018	Dinophyceae	■		
polb_OM.RGC.v1.001445888	-0.026	Copepoda			
polb_OM.RGC.v1.001897164	-0.035	Copepoda			
polb_OM.RGC.v1.001883961	-0.043	Ostracoda			
polb_OM.RGC.v1.000844241	-0.043	Peridiniales			
polb_OM.RGC.v1.000673383	-0.043	Calanoida			
polb_OM.RGC.v1.000072166	-0.043	Labyrinthulida			
polb_OM.RGC.v1.000274868	-0.045	Chlorophyceae	■		
polb_OM.RGC.v1.001561949	-0.048	Ostracoda			
polb_OM.RGC.v1.000205020	-0.048	Collodaria			
polb_OM.RGC.v1.004472615	-0.049	Calanoida			
polb_OM.RGC.v1.000228894	-0.051	Variosea			
polb_OM.RGC.v1.009636901	-0.051	Collodaria			
polb_OM.RGC.v1.000495602	-0.056	MAST_3-12			
polb_OM.RGC.v1.000074398	-0.061	Ichthyosporea			
polb_OM.RGC.v1.015629864	-0.062	Peridiniales			
polb_OM.RGC.v1.000287835	-0.064	Collodaria			
polb_OM.RGC.v1.000471455	-0.065	Collodaria			
polb_OM.RGC.v1.000110630	-0.073	Acantharea			
polb_OM.RGC.v1.002652025	-0.075	Dinophyceae	■		
rdrp_89802927	-0.075	Calanoida			
rdrp_89802926	-0.075	Insecta			
polb_OM.RGC.v1.002767869	-0.078	Collodaria			
rdrp_103795290	-0.082	Collodaria			
polb_OM.RGC.v1.000017697	-0.083	Calanoida			
rdrp_77677810	-0.099	Leptothecata			

Figure 5: Functional traits and taxonomy of putative hosts of viruses associated with the efficiency of carbon export. "Putative hosts" refers to eukaryotic V9-OTUs with the highest absolute edge weight in FlashWeave interaction networks. Taxa correspond to higher rank classification of the V9-OTU. Black squares indicate the presence of a functional trait for the V9-OTU best connected to the virus.

Integrating previous observations to model a virus-driven BCP

Our PLS models revealed that viruses of eukaryotes are associated not only with the magnitude of carbon export, but also with the export efficiency of particles. This finding suggests that viruses contribute to BCP enhancement by promoting aggregate formation and subsequent sedimentation. Consistent with this view, prasinoviruses and putative prymnesioviruses were found to be among the viruses best associated with the efficiency of carbon export under our PLS model for CEE. Several prasinoviruses (fourteen with an available genome sequence) have been isolated for three genera of green microalgae, namely *Micromonas*, *Ostreococcus* and *Bathycoccus*. These genera belong to the order Mamiellales, which are bacterial-sized phytoplankton common in coastal and oceanic environments and considered to be influential actors in oceanic systems (Not et al. 2012; Weynberg et al. 2017). *Chrysochromulina ericina* virus, *Prymnesium parvum* DNA virus, *Prymnesium kappa* virus-RF02 and *Haptolina ericina* virus are *Mimiviridae* viruses isolated from Prymnesiales (Haptophyta) cultures (Figure 4). They are closely related to *Phaeocystis globosa* virus (PgV) and *Phaeocystis poutcheti* virus isolated from Phaeocystales (Haptophyta) cultures. Both Prymnesiales and Phaeocystales species have non-calcifying organic scales and some species can form blooms and colonies. The detection of several putative prymnesioviruses in samples containing small cells is consistent with the observation of diverse and abundant noncalcifying haptophytes in open oceans (Liu et al. 2009; Endo et al. 2018). According to the estimates of Liu et al (2009), these noncalcifying haptophytes contribute twice as much as diatoms or prokaryotes to photosynthetic production, thereby highlighting their importance in the BCP. Prymnesiales are an important mixotrophic group in oligotrophic ocean (Unrein et

319 al. 2014) and mixotrophy is proposed to increase vertical carbon flux by enabling the
320 uptake organic forms of nutrients (Ward and Follows 2016). Viral infection of such
321 organisms may thus help increase BCP efficiency.

322 As most other cultured algal viruses, prasinoviruses and prymnesioviruses are
323 lytic. Viral lysis not only generates the cellular debris used to build aggregates; it also
324 facilitates the process of aggregation (Weinbauer 2004) *via* viral-induced production of
325 agglomerative compounds, such as transparent exopolymer particles (TEPs). Lønborg et
326 al. (2013) proposed that the increased DOC and TEP production observed in cultures of
327 *Micromonas pusilla* infected with prasinoviruses (compared with non-infected cultures)
328 could stimulate particle aggregation and thus carbon export out of the photic zone. Some
329 prasinoviruses encode glycosyltransferases of the GT2 family. Similar to the a098r gene
330 (GT2) in *Paramecium bursaria Chlorella virus 1*, the expression of GT2 family members
331 during infection possibly leads to the production of a dense fibrous hyaluronan network at
332 the surface of infected cells. Such a network may trigger the aggregation of host cells,
333 facilitate viral propagation (Van Etten et al. 2017) and increase the cell wall C:N ratio.
334 PgV, closely related to prymnesioviruses (Figure 4), has been linked with increased TEP
335 production and aggregate formation during the termination of *Phaeocystis* bloom
336 (Brussaard et al. 2007).

337 Other previous experimental and *in situ* studies have linked viruses and viral
338 activity to vertical carbon flux, cell sinking, and aggregate formation. Several laboratory
339 experiments have revealed associations between viruses and sinking cells or between
340 viruses and aggregate formation. For example, sinking rates of the toxic bloom former
341 *Heterosigma akashiwo* are elevated following viral infection (Lawrence and Suttle 2004).

As early as 1993, an experimental study revealed increased particle aggregation and primary productivity upon addition of virally enriched material to seawater samples (Peduzzi and Weinbauer 1993). More recently, cultures of the diatom *Chaetoceros tenuissimus* infected with a DNA virus (CtenDNAV type II) have been shown to produce higher levels of large-sized particles (50 to 400 μm) compared with non-infected cultures (Yamada et al. 2018). *In situ* studies have uncovered vertical transport of viruses between photic and aphotic zones in the Pacific Ocean (Hurwitz et al. 2015) and in *Tara* Oceans samples (Brum et al. 2015), which suggests their association with sinking material. In addition, the level of active *Emiliania huxleyi* virus (EhV) infection in a coccolithophore bloom has been found to be correlated with water column depth, which suggests that infected cells are exported from the surface to deeper waters in aggregate form (Sheyn et al. 2018). EhVs infecting *E. huxleyi* have also been found to facilitate particle aggregation and downward vertical flux of both particulate organic and particulate inorganic carbon in the North Atlantic (Laber et al. 2018). No *Phycodnaviridae* PolB had their best hits to EhVs in our study, which is probably because of sampling regions and periods. These previous observations come as potential mechanistic explanations that provide support for our results suggesting eukaryotic viruses have a "shuttle effect" that enhance carbon export.

Viruses *versus* hosts as key markers of CE and CEE variations

Our analysis used similar data and analytical methods as those of Guidi et al. (2016), who explored the link between planktonic networks and the BCP. Those authors reported that bacterial viruses are better associated with CE_{150} than are cellular organisms. In their study, host lineages for prasinoviruses (Mamiellales) and prymnesioviruses

(Prymnesiales) were not found to be associated with CE_{150}. The fact that we detected these viruses as predictors of CEE may mean that viruses are better predictors than their hosts, most likely because viruses may better reflect the overall process and mechanisms by which carbon is exported. Viral infection causes lysis and aggregation of cells, enhances sinking rates and increases the export of organic carbon out of the sunlit ocean. Among the eukaryotic organisms studied by Guidi et al. (2016), collodarians, dinoflagellates and copepods were reported to be important contributors to CE_{150}. Some of the viral taxa we found to be associated with CEE were connected with collodarians and dinoflagellates in our host prediction analysis, although not as clearly as with Mamiellales and Prymnesiales. Known copepod viruses have a ssDNA genome and hence were not captured in our data. Only one genome sequence of a ssRNA virus and one PolB sequence of a NCLDV are available for dinoflagellates viruses, and none have been reported for collodarians. Viruses infecting these organisms may be among those we found to be associated with CEE, but the lack of reference viruses hampered their identification from the environmental samples.

Conclusions

In this study, we identified associations between CEE and diverse groups of planktonic eukaryotic viruses collected in the photic ocean from 61 sampling sites during the *Tara* Oceans expedition. Two lineages were predicted to be important groups facilitating carbon export in the global ocean: prasinoviruses infecting tiny green algae of the order Mamiellales, and *Mimivirus* relatives putatively infecting haptophytes of the order Prymnesiales. This finding suggests that these eukaryotic viruses, like cyanophages

previously reported to be strongly associated with carbon export, are important factors in the BCP. This idea is in agreement with observations that their hosts, despite being one to two orders of magnitude less abundant than cyanobacteria, likely dominate carbon biomass and net production in the ocean. The global-scale association between eukaryotic viruses and CEE is empirical evidence supporting the shunt and pump hypothesis, namely, that viruses enhance carbon pump efficiency by increasing the relative amount of organic carbon exported from the surface to the deeper sea.

Methods

Data context

We used publicly available data generated in the framework of the *Tara* Oceans expedition (Karsenti et al. 2011). Single-copy marker-gene sequences for NCLDVs and RNA viruses were identified from two gene catalogs: the Ocean Microbial Reference Gene Catalog (OM-RGC) and the Marine Atlas of Tara Oceans Unigenes (MATOU). The viral marker-gene abundance profiles used in our study for prokaryotic-sized metagenomes and eukaryotic-sized meta-transcriptomes were from Sunagawa et al. (2015) and Carradec et al. (2018), respectively. For eukaryotic 18S rDNA V9 OTUs, we used an updated version of the data of de Vargas et al. (2015) that included functional trait annotations (chloroplast-bearing, silicified and calcified organisms) of V9-OTUs. Abundance profiles are compositional matrices in which gene abundances are expressed as unnormalized (V9 barcode data) or gene-length normalized (shotgun data) read counts. Eukaryotic plankton samples were filtered for categorization into the following size classes: piconano (0.8–5 μm), nano (5–20 μm), micro (20–180 μm) and meso (180–2000

μm) (Pesant et al. 2015). Indirect measurements of carbon export (mg^{-1} m^{-2} d^{-1}) in 5-m increments from the surface to a 1000-m depth were taken from Guidi et al. (2016). The original measurements were derived from the distribution of particle sizes and abundances collected using an underwater vision profiler (Picheral et al. 2010). These raw data are available from PANGEA (Picheral et al. 2014). Net primary production (NPP) data were extracted from 8-day composites of the vertically generalized production model (VGPM) (Behrenfeld and Falkowski 1997) at the week of sampling.

Carbon export and carbon export efficiency

Carbon flux profiles (mg^{-1} m^{-2} d^{-1}) based on particle size distribution and abundances were estimated according to Guidi et al. (2008). Carbon flux values from depths of 30 to 970 m were divided into 20-m bins, each obtained by averaging the carbon flux values from the designated 20 m from profiles gathered during biological sampling within a 25-km radius during 24 h and having less than 50% missing data (Supplementary Figure 4). For compatibility with Guidi et al. (2016), carbon export was defined as the carbon flux at 150 m. Carbon export efficiency was calculated as follows: CEE = (CE_{deep} – $CE_{surface}$)/CE_{deep} (Sarmiento 2013) and CEE′ = CE_{deep}/$CE_{surface}$ (Francois et al. 2002). In these formulas, $CE_{surface}$ is the maximum average carbon flux within the first 150 m (the maximum layer depth varied between 20 to 130 m in this dataset), and CE_{deep} is the average carbon flux 200 m below this maximum.

Acquisition of viral marker genes from ocean gene catalogs

Viral genes were collected from two gene catalogs: OM-RGC version 1 (Sunagawa et al. 2015) and MATOU (Carradec et al. 2018). The OM-RGC data were taxonomically re-

annotated as in Carradec et al. (2018). Importantly, the NCBI reference tree used to determine the last common ancestor was modified to reflect the current NCLDVs classification (see Carradec et al. [2018] for details). We classified viral gene sequences as eukaryotic or prokaryotic according to their best BLAST score against viral sequences in the Virus-Host DB (Mihara et al. 2016). DNA polymerase B (PolB) and RNA-dependent RNA polymerase (RdRP) genes were used as markers for NCLDVs and RNA viruses, respectively. For PolB, reference proteins from the NCLDV orthologous gene cluster NCVOG0038 (Yutin et al. 2009) were aligned using *linsi* (Katoh and Standley 2013). A HMM profile was constructed from the resulting alignment using *hmmbuild* (Eddy 1998). This PolB HMM profile was searched against OM-RGC amino acid sequences annotated as NCLDVs, and sequences longer than 200 amino acids and having hits with *E*-values $< 1 \times 10^{-5}$ were selected as putative PolBs. RdRP sequences were chosen from the MATOU catalog as follows: sequences assigned to Pfam profiles PF00680, PF00946, PF00972, PF00978, PF00998, PF02123, PF04196, PF04197 or PF05919 and annotated as RNA viruses were retained as RdRPs. The resulting 3,486 PolB sequences and 975 RdRP sequences were used along with their abundance matrices for PLS regression analyses, co-abundance network reconstructions and to build phylogenies.

Testing for associations of viral abundance with CE_{150} and CEE

To test for an association between the abundance of viral marker genes and CEE, we proceeded as follows. Only marker genes represented by at least two reads in five or more samples were retained. To cope with the sparsity and composition of the data, gene-length normalized read-count matrices were center-log transformed separately for RNA

viruses and NCLDVs. We next selected genes with a Spearman correlation coefficient to CE_{150} or CEE greater than 0.2 or smaller than -0.2 (zero values were removed). To assess the association between these marker genes and CEE, we used partial least square (PLS) regression analysis as described in Guidi et al. (2016). The number of components selected for the PLS model was chosen to minimize the root mean square error of prediction (Supplementary Table 2). We assessed the strength of the association between carbon export (the response variable) and viral abundance (the explanatory variable) by correlating leave-one-out cross-validation predicted values with the measured carbon export values. We tested the significance of the correlation by comparing the original Pearson coefficient between explanatory and response variables with the distribution of Pearson coefficients obtained from PLS models reconstructed on permutated data (10,000 iterations). We estimated the contribution of each gene (predictor) according to its variable importance in the projection (VIP) (Chong and Jun 2005). The VIP measure of a predictor estimates its contribution in the PLS regression. Predictors having high VIP values (> 2) are assumed to be important for the PLS prediction of the response variable.

Phylogenetic analysis

Environmental PolB sequences annotated as *Phycodnaviridae* or *Mimiviridae* were searched against reference PolB sequences using BLAST. Environmental sequences with hits to a reference sequence having >40% identity and an alignment length greater than 400 amino acids were kept and aligned with reference sequences using *linsi* (Katoh and Standley 2013). We further removed sequences that occurred in fewer than 10 samples to reduce the size of the tree. Environmental RdRP sequences annotated as *Picornavirales* were translated into six frames of amino acid sequences, and reference *Picornavirales*

RdRP sequences collected from the Virus-Host DB (Mihara et al. 2016) were searched against the CDD database (Marchler-Bauer et al. 2015) using rpsBLAST. The resulting alignment was used to trim reference and environmental RdRP sequences to the conserved part corresponding to the domain, CDD: 279070, before alignment with *linsi*. PolB and RdRP multiple sequence alignments were manually curated to discard poorly aligned sequences. Trees were constructed using the *build* function of ETE3 (Huerta-Cepas et al. 2016) as implemented at GenomeNet (https://www.genome.jp/tools-bin/ete). Columns were automatically trimmed using *trimAl* (Capella-Gutiérrez et al. 2009), and trees were constructed using FastTree with default settings (Price et al. 2009).

Virus–eukaryote co-occurrence analysis

We used FlashWeave (Tackmann et al. 2018) with Julia 1.0 to detect virus–host associations on the basis of their co-abundance patterns. Read count matrices for the 3,486 PolB, 975 RdRP and 18S V9 DNA barcodes obtained from samples collected at the same location were fed into FlashWeave. 18S-V9 data were filtered to retain OTUs with an informative taxonomic annotation. 18S-V9 OTUs and viral marker sequences were further filtered to conserve only those present in at least five samples. FlashWeave analyses were run separately for each of the four eukaryotic size fractions. The number of samples per size fraction ranged between 51 and 99 for NCLDVs and between 36 and 62 for RNA viruses. The number of retained OTUs per size fraction varied between 1,775 and 2,269 for NCLDVs and between 48 and 125 for RNA viruses (Supplementary Table 3). FlashWeave was run under the settings heterogenous = false and sensitive = true, and PolB/RdRP-V9-OTU edges receiving a weight > 0.2 and a Q-value < 0.01(the default) were retained.

Mapping of putative hosts onto viral phylogenies

In our association networks, individual viral sequences were often associated with multiple 18S-V9 OTUs belonging to drastically different eukaryotic groups, a situation that can reflect interactions among multiple organisms but also noise associated with this type of analysis (Coenen and Weitz 2018). To extract meaningful information from these networks we reasoned as follow: We assumed that evolutionarily related viruses infect evolutionary related organisms, similar to the case of phycodnaviruses (Clasen and Suttle 2009). In the interaction networks, the number of connections between viruses in a given clade and its eukaryotic host group should accordingly be enriched compared with the number of connections with non-host organisms arising by chance. Following this reasoning, we assigned the most likely eukaryotic host group as follows. The tree constructed from viral marker-gene sequences (PolB or RdRP) was traversed from root to tips to visit every node. We counted how many connections existed between leaves of each node and the V9-OTUs of a given eukaryotic group (order level). We then tested whether the node was enriched compared with the rest of the tree using Fischer's exact test and applied the Benjamini-Hochberg procedure to control the FDR among comparisons of each eukaryotic taxon (order level). To avoid the appearance of significant associations driven by a few highly connected leaves, we required half of the leaves within a node to be connected to a given eukaryotic group. Significant enrichment of connections between a virus clade and a eukaryotic order was considered to be indicative of a possible virus–host relationship. We refer to the above approach, in which taxon interactions are mapped onto a phylogenetic tree of a target group using the organism's associations predicted from a species co-abundance-based network, as TIM,

for Taxon Interaction Mapper. The script is available upon request. This approach can be extended to interactions other than virus–host relationships.

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Supplementary material

- Supplement.docx: supplementary figures and tables.
- Supplementary_file_1: Information on the predictors (viruses) in the PLS model predicting carbon export efficiency. Predictors are sorted by VIP score.
- Supplementary data available at GenomeNet FTP: ftp://ftp.genome.jp/pub/db/community/tara/Cpump/