Marine viral *macro-* and *micro-*diversity from pole to pole

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Summary: Microbes drive most ecosystems and are modulated by viruses that impact their lifespan, gene flow and metabolic outputs. However, the influence of viral community diversity at the ecosystem level remains difficult to assess due to classification issues and few reference genomes. Here we establish a ~12-fold expanded global ocean virome dataset of 195,728 viral populations, now including the Arctic Ocean, and validate that these populations form discrete genotypic clusters. Meta-community analyses revealed just five ecological zones throughout the global ocean, and established local and global patterns and drivers in viral community diversity at levels of both *macro*diversity (*inter*-population diversity) and *micro*diversity (*intra*-population genetic variation). These patterns sometimes, but not always, paralleled those from macro-organisms and revealed temperate and tropical surface waters and the Arctic as biodiversity hotspots and mechanistic hypotheses to explain them. With this further understanding of viral populations and ecology in the ocean, viruses can be more broadly included in ecosystem models.

Introduction:

Biodiversity is essential for maintaining ecosystem functions and services (reviewed by Tilman *et al.*, 2014). Marine ecosystems represent 90% of the Earth's habitable volume and play an integral role in supporting human wellbeing, including food resources for more than 3 billion people (Hazen *et al.*, 2018). Meta-analyses looking at changes in marine biodiversity show that biodiversity loss increasingly impairs the ocean's capacity to produce food, maintain water quality, and recover from perturbations (Worm *et al.*, 2006). To date, marine conservation efforts have focused on specific organismal communities, such as fisheries or coral reefs, rather than conserving whole ecosystem biodiversity. However, emerging studies across diverse environments show that the stability and diversity of higher trophic level organisms rely upon diversity throughout the food web (e.g. Soliveres *et al.*, 2016). In the oceans, microbes represent

~70% of marine organismal biomass and are the foundation of the food web (Bar-On *et al.*, 2018). For ocean microbes and their viruses, global surveys that parallel century-old global terrestrial and decades-old marine macro-organismal global biodiversity surveys (Reiners *et al.*, 2017) are now emerging (e.g. de Vargas *et al.*, 2015; Sungawa *et al.*, 2015; Brum *et al.*, 2015; Roux *et al.*, 2016; **Table S1**). Key to assessing biodiversity changes across marine ecosystems is improving our understanding of current microbial biodiversity levels, distribution patterns, and their ecological drivers.

Despite their tiny size, viruses play a large role in marine ecosystems and food webs. For example, viral mortality is credited with lysing approximately 20-40% of bacteria per day and releasing carbon and other nutrients that impact the food web (reviewed by Suttle, 2007). Beyond mortality, viruses can alter evolutionary trajectories of microbial communities by transferring ~10^{29} genes per day globally (Paul, 1999) and biogeochemical cycling by metabolically reprogramming host photosynthesis, as well as central carbon metabolism and nitrogen and sulfur cycling (reviewed in Hurwitz & U'Ren, 2016). Finally, as the oceans are estimated to capture half of human-caused carbon emissions (Le Quéré *et al.*, 2018), it is notable that genes-to-ecosystems modeling has placed viruses as central players of the ocean 'biological pump' (Guidi *et al.*, 2016). Many of these discoveries are very recent as ocean viral genome sequence space is explored at the level of viral *macro*diversity, i.e., *inter*-population diversity, throughout the global oceans -- at least for the most abundant double-stranded DNA viruses sampled (**Table S2**).

In spite of this progress in studying marine viral *macro*diversity, virtually nothing is known about *micro*diversity, i.e., *intra*-population genetic variation. Such *micro*diversity, at least in eukaryotic organisms, is thought to drive adaptation and speciation to promote and maintain stability in ecosystems (Hughes *et al.*, 2008; Larkin & Martiny, 2017). This is likely also true in viruses since even a few mutations can alter host interactions and alter ecological and evolutionary dynamics for the genotype (e.g. Marston *et al.*, 2012; Petrie *et al.*, 2018). In nature, viral *micro*diversity measurements have been limited to marker genes (e.g. genes encoding major capsid proteins), which do not capture community-wide variability and genome-wide evidence of selection (e.g. Achtman & Wagner 2008; Sullivan, 2015). Recently, deeper metagenomic sequencing and population genetic theory-grounded species delimitations (Shapiro *et al.*, 2012; Cadillo-Quiroz *et al.*, 2012) have begun to reveal such *micro*diversity in microbes, and this has elucidated unknown features of speciation, adaptation, pathogenicity and transmission (e.g. Snitkin *et al.*, 2011; Schloissnig *et al.*, 2013; Rosen *et al.*, 2015; Lee *et al.*, 2017; Smillie *et al.*, 2018). Although parallel species delimitations are now available for viruses (Gregory *et al.*, 2016; Bobay *et al.*, 2018), no datasets are yet available to explore genome-wide *micro*diversity in viruses, particularly at the global scale.

Here we leverage the *Tara* Oceans global oceanographic research expedition sampling to establish a deeply-sequenced, global-scale ocean virome dataset and use it to assess the validity of the current viral population definition and to establish and explore baseline *macro*- and *micro*-diversity patterns with their associated drivers across local to global scales. These data have been collected and analyzed in the context of the larger *Tara* Oceans Consortium systematically-sampled, global-scale, viruses-to-fish-larvae datasets (de Vargas *et al.*, 2015; Sungawa *et al.*, 2015; Brum *et al.*, 2015; Lima-Mendez *et al.*, 2015; Pesant *et al.* 2015; Roux *et al.*, 2016), and help establish foundational ecological hypotheses for the field and a roadmap for the broader life sciences community to better study viruses in complex communities.

Results & Discussion:

The dataset. The Global Ocean Viromes 2.0 (GOV 2.0) dataset is derived from 3.95 Tb of sequencing across 145 samples distributed throughout the world's oceans (**Fig. 1A** and **Table S3;** see **Methods**). These data build on the prior GOV dataset (Roux *et al.*, 2016) by increased sequencing for mesopelagic (empirically defined as deep waters below 150m to 1,000m in our dataset) and Southern Ocean samples and upgrading assemblies for all 104 original GOV samples -- both of which drastically improved sampling of the ocean viruses in these samples (results below). Additionally, we added 41 new samples derived from the *Tara* Oceans Polar Circle (TOPC) expedition, which traveled 25,000 km around the Arctic Ocean in 2013. These 41 Arctic Ocean viromes were generated to represent the most significantly climate-impacted region of the ocean, and an extreme environment. No such metagenome-based viral data exist for the Arctic region (Deming & Collins 2017), and more generally, for many planktonic organisms, systematic sampling is uneven throughout the Arctic Ocean (CAFF State of the Arctic Marine Biodiversity Report) due to geopolitical and physical challenges of sampling these regions.

The first step to studying viral biodiversity from the assembled GOV 2.0 dataset (see **Methods**) was to identify contigs that likely derive from viruses using tools that collectively utilize homology to viral reference databases, probabilistic models on viral genomic features, and viral k-mer signatures (see **Methods**). These putative viral contigs were then assigned to 'populations', which are currently defined as viral contigs ≥10 kb where ≥70% of the shared genes have ≥95% average nucleotide identity (ANI) across its members (Brum *et al.*, 2015; Roux *et al.*, 2016; Roux *et al.*, 2018 *in press*; population definition also discussed below). This process identified 195,728 viral populations in the GOV 2.0 dataset, which is a ~12-fold increase over the 15,280 identified in the original GOV dataset and assemblies (Roux *et al.*, 2016) and augments prior marine viromic work (**Tables S2**). Of these original GOV viral populations, 12,708 were represented by single contigs and most (92%) were recovered in GOV 2.0 (**Fig. 1B-inset**), though with average lengths increased 2.4-fold from 18 kbp to 44 kbp (**Fig. 1B**). Outside these GOV-known and now improved viral populations, an additional 180,448 new GOV 2.0 viral populations were identified -- derived mostly (58%) from improved assemblies and deeper sequencing of the original GOV samples, and the rest (42%) from the 41 new Arctic Ocean viromes. Finally, new methods to identify shorter viral contigs (see **Methods**) were applied and these identified another 292,402 contigs as viral (5-10 kb length and/or circular), which, when added to the earlier data and clustered at ≥95% ANI, resulted in a total of 488,130 viral populations. While the annotatable fraction consisted of dsDNA viral families (**Fig. 1C**), most (90.2%) did not classify into any known viral family. Separately, known biases of the methods available at the time select against large dsDNA or any ssDNA and RNA viruses (see **Methods**), so these groups remain unexplored in the GOV 2.0 dataset.

Validating viral 'population' boundaries. Defining species is controversial for eukaryotes and prokaryotes (Kunz 2013; Cohan 2002; Fraser *et al.*, 2009) and arguably even more controversial for viruses (Bobay *et al.*, 2018), probably because of the paradigm of rampant mosaicism stemming from the rapid evolutionary rates of ssDNA and RNA viruses [reviewed by (Duffy *et al.*, 2008)]. The biological species concept, often referred to as the gold standard for defining species, defines species as interbreeding individuals that remain reproductively isolated from other such groups. To adapt this to prokaryotes and viruses, studies have explored patterns of gene flow to determine whether they might maintain discrete lineages as reproductive isolation does in eukaryotes. Indeed, gene flow and selection define clear boundaries between groups of bacteria, archaea and viruses (Shapiro *et al.*, 2012; Cadillo-Quiroz *et al.*, 2012; Gregory *et al.*, 2016; Bobay *et al.*, 2018). Because gene flow between groups is impossible to

measure for many groups, the term 'species' is rarely used for prokaryotes or viruses described in this way, and instead discrete lineages are described as 'populations.'

Separate from these population genetic theory grounded observations, evidence for gene flow constrained lineage cohesiveness in prokaryotes has emerged from evaluating whether metagenomic read-mapping reveals sequence-discrete populations or not. Indeed, this has been observed for over a decade in prokaryotes (Konstantinidis & Tiedje 2005) and more recently for some dsDNA viruses (viral-tagged metagenomes and 142 isolate genomes for marine cyanophages; Deng *et al*. 2014, Gregory *et al*. 2016; **Table S4**). Buoyed by these signatures of dsDNA viruses obeying the biological species concept (Bobay *et al.*, 2018), viral ecologists have established the definition of viral populations described above (Brum *et al.*, 2015; Roux *et al.*, 2016; Roux *et al.*, 2018 *in press*). Because this empirically-derived ≥95% ANI cut-off requires deeply sequenced groups, so only cyano- and myco-phages have been evaluated (Gregory *et al.*, 2016; Bobay *et al.*, 2018). Further, an emergent hypothesis suggests that phages evolve with very different modes and tempos driven by differing temperate or obligately lytic lifestyles (Mavrich & Hatfull, 2017). Thus there is need to evaluate how generalizable this viral population definition is in nature.

To test this, we permissively mapped metagenomic reads against our 488,130 GOV 2.0 viral populations by allowing 'local' matching as low as 18% nucleotide identity, and statistically identifying 'breaks' in the resulting read frequency histograms (see **Methods**). This revealed that, on average, the break occurred such that reads <92% nucleotide identity failed to map (**Fig. 2A; full results Table S5)**, which resulted in a genome-wide signature of ≥95% ANI for nearly all (99.9% or 487,875) of the GOV 2.0 viral populations, including the smaller 5-10 kb viral populations (**Fig. 2B**). This implies that the observed viral populations in the dataset are predominantly and detectably sequence-discrete. This result is consistent with data from viral-tagged metagenomes (Deng *et al.*, 2014) and gene-sharing networks of prokaryotic virus genomes (Iranzo *et al.*, 2016*,* Bolduc *et al.*, 2017), which also showed that sampled viral genome sequence space is clustered at each 'species' and 'genus' levels, respectively. Thus, while ssDNA and RNA viruses have variable and elevated genome evolutionary rates that can erode species boundaries [reviewed by (Duffy *et al.*, 2008)], it appears that metagenome-assembled dsDNA viral populations for the most part form discrete genotypic clusters and can be appropriately delineated via a ≥95% genome-wide ANI cut-off.

Meta-community analysis reveals 5 ecological zones. Having organized this global sequence space into discrete and biologically meaningful populations, we next sought to use metagenome-derived abundance estimates to establish patterns and drivers of viral population diversity across the global ocean across multiple levels of ecological organization (**Fig. 3**). This revealed that the 145 GOV 2.0 viral communities assorted into just five meta-communities, denoted ecological zones, whether assessed using Bray-Curtis dissimilarity distances in principle coordinate analysis (**Fig. 4A)**, non-metric multidimensional scaling (**Fig. S1A**), or hierarchical clustering (**Fig. S1B**) and after accounting for variable sample sizes (see **Methods**). We designated these 5 emergent ecological zones as the Arctic (ARC), Antarctic (ANT), bathypelagic (BATHY), temperate and tropical epipelagic (TT-EPI) and mesopelagic (TT-MES), and used these for further study. Depth ranges were defined as done previously (Reygondeau, *et al.* 2018), with epipelagic, mesopelagic, and bathypelagic being waters of depths 0 to 150 meters, 150 to 1,000 meters, and deeper than 2,000 meters, respectively.

Comparison of our virome-inferred ecological zones to those inferred for the oceans in other ways was telling. Our zones differed from traditional oceanographic biogeographical

biomes (e.g. Longhurst), where four biomes and ~50 provinces have been designated across surface ocean waters based on annual cycles of nutrient chlorophyll a (Longhurst *et al.* 1995, Longhurst 2007), and from mesopelagic ecoregions and biogeochemical provinces based on biogeography and environmental climatology, respectively (Sutton, *et al.* 2017; Reygondeau, *et al.* 2018). However, they were similar to those observed for marine bacterial communities, which clustered by mid-latitude surface, high-latitude, and deep waters (Ghiglione *et al.*, 2012). This implies that the physicochemical structuring of marine *microbial* communities is likely the most important factor in structuring marine viral communities, perhaps reflecting a relative stability in host range of viruses in the oceans (de Jonge *et al.* 2018). To evaluate this physicochemical structuring, we examined the universal predictors and drivers of viral ecological zones, across one (**Fig. 5A**) and multiple ordination dimensions (**Fig. 5B**; see **Methods**). This suggested that temperature was the major driver structuring these ecological zones, as previously shown from global microbial surveys (Sunagawa *et al.*, 2015) and our own smaller ocean virome surveys, where we posited previously that temperature likely directly impacts microbial community structure, and indirectly viral community structure (Brum *et al*., 2015). Moreover, temperature has been shown to play an important role in virus-host interactions, especially in the Arctic (Maat *et al.*, 2017).

Viral *macro-* and *micro-* diversity, and drivers, within and between ecological zones. To explore diversity patterns across ecological zones, we calculated per sample diversity using Shannon's *H*' for *macro*diversity and a newly established method for community-wide *micro*diversity. This new method, because it estimated average nucleotide diversity (or π) from the mean of 1,000 iterations of π averaged from 100 randomly subsampled well-sequenced populations (see **Methods**), is only able to assess well-sampled, abundant populations. These zone-normalized (see **Methods**) comparisons revealed that *macro*diversity was highest in TT-EPI ($p < 0.05$), closely followed by the ARC, and lowest in TT-MES and ANT (**Fig 4B – bottom**), whereas *micro*diversity was highest in TT-MES ($p < 0.05$) and lowest in ARC (**Fig. 4B –left**). At the zonal level, a negative trend between *macro-* and *micro-* diversity emerges (**Fig. 4B-right**), although we note that the small number of zonal points limits our statistical inferences, even in this global dataset.

Recent work suggests that higher *micro*-diversity can impede the maintenance of *macro*-diversity by promoting competitive exclusion (Hart *et al.*, 2016). Thus we posit that, if the zonal level negative *macro/micro* diversity trends are real, this may result from increased *intra*population niche variation that reduces *inter*population niche variation resulting in competitive exclusion by the superior competitors, which may occur slowly and may be why it only appears at this regional scale. Because estimates of *micro*diversity in our dataset and even currently available single virus genomics approaches (Martínez-Hernández *et al.* 2017) remain limited to only the most abundant populations, testing such a hypothesis awaits critically-needed advances and scalability in single-virus genomics technologies.

At the per-sample level, however, *macro-* and *micro-* diversity were not correlated, even within each zone (**Fig. 4B – right**). Although these are the first data available for viruses, for larger organisms, *macro-* and *micro*-diversity are often correlated across habitats sharing similar species pools, presumably due to habitat characteristics altering immigration, drift, and selection (Vallend & Gerber, 2005). These ecological correlations are generally positive and significantly stronger in discrete habitats (e.g. islands) in contrast to more connected communities like the ocean [reviewed in (Vallend *et al.*, 2014)]. Thus we posit that the lack of correlation between marine viral *macro-* and *micro-* diversity at this per-sample level is driven by differences in local

drivers (**Fig. 4C**). Consistent with this, local drivers differed as nutrients strongly (and negatively) correlated with viral *macro*diversity, whereas photosynthetically active radiation (PAR; an indicator of productivity) best (and positively) correlated with viral *micro*diversity in the epipelagic waters (**Fig. 4C**).

Mechanistically, these results suggest several possible hypotheses. At the viral *macro*diversity level, decreased host diversity in algal blooms, which themselves rely on nutrient pulses (Farooq & Malfatti, 2007), could skew viral rank abundance curves towards dominance by increasing abundance of bloom-associated viral populations. This is supported by the negative correlation between our viral *macro*diversity and particulate inorganic carbon (PIC; **Fig. 4C**), a hallmark of coccolithophore blooms (Groom & Holligan, 1987), and chlorophyll a (**Fig. 5C**). For viral *micro*diversity in epipelagic waters, PAR was the main driver. PAR is known to impact host diversity, particularly in nutrient-poor surface waters, by inhibiting photoautotrophs (Feng *et al.*, 2015) and the dominant heterotroph, SAR11 (Ruiz-González *et al.*, 2013), but stimulating other key microbes such as *Roseobacter*, *Gammaproteobacteria* and NOR5 (Ruiz-González *et al.*, 2013). We hypothesize that the shorter-term impacts of high PAR in the surface waters on host communities may create new niches for viruses, whereby *micro*diversity increases to enable differentiation of existing viral populations. As above, advances in single-virus genomics would be invaluable for testing this hypothesis.

Viral *macro-* and *micro-* diversity, and drivers, against classical ecological gradients. Ecologists have long explored the relationship between diversity and geographic range, which in eukaryotes and bacteria are highly correlated and thought to be due to the accumulation of niche-specific selective mutations across populations with large heterogeneous geographic ranges (i.e. the niche variation hypothesis; Van Valen 1965, Hedrick, 2006, Rosen *et al.*, 2015). No parallel studies have looked at viruses. To explore this for viruses, we determined the geographic range of viral populations based on their distribution within and between ecological zones (**Fig. 6A**) and then calculated their average π (see **Methods**) to assess patterns in *macro*- and *micro*-diversity, respectively. Viral populations were designated as 'multi-zonal' if they were observed in >1 ecological zone, 'zone-specific regional' if they were observed in only one zone, but $\geq$2 viral communities, or 'zone-specific local' if they were observed in only 1 viral community within a single zone.

These analyses first revealed differences in the dominant viral geographic ranges across the different ecological zones. For example, multi-zonal viral populations dominated ANT and BATHY (>60% of viral populations found within zone), both across the zone (**Fig. 6B**) and within each station (**Fig. S4**), whereas zone-specific regional viral populations dominated TT-EPI and ARC and the multi-zonal and zone specific viral populations were approximately equally represented in TT-MES (**Fig. 6B**). The high levels of zone-specific viral populations in TT-EPI and ARC, as well as the high levels of viral *macro*diversity (**Fig. 4B-bottom**), are indicative of high endemism and suggest these regions may be biodiversity hotspots for marine viruses. In contrast, the ANT and BATHY are composed mostly of multi-zonal viral populations suggesting that they may be sink habitats that are more dependent on migration (*sensu* Watkinson & Sutherland, 1995). However, across all ecological zones, viral population *micro*diversity decreased with virus geographic range (**Fig. 6C;** $p < 0.05$), presumably from varied ecologies providing differing selective niches for the single, widely-distributed population that then drive differentiation through isolation-by-environment processes (*sensu* Shapiro *et al.*, 2012). Such findings are new for viruses, but parallel the results for eukaryotes (Hedrick, 2006)

and bacteria (Rosen *et al.*, 2015) and suggest a universality to isolation-by-environment processes across organismal kingdoms and viruses.

Ecologists have also long observed, across most flora and fauna, that there are latitudinal patterns in diversity across both terrestrial and marine environments. Briefly, the latitude diversity gradient (LDG) suggests that both *macro*- and *micro*-diversity are highest at mid-latitudes and decrease poleward (Pianka 1966, Hillebrand 2004, Mannion *et al.*, 2013, Miraldo *et al.*, 2016). We found that both viral *macro*- and *micro*-diversity followed the LDG except in ARC, where both increased (**Fig. 7A**). This high equatorial *macro*- and *micro*-diversity was consistent across the Indian, Atlantic, and Pacific Oceans as expected (**Fig. 7B & C**). The Arctic Ocean, however, was not only unexpectedly elevated in diversity, but it also displayed a unique pattern. Specifically, two distinct zones – definable by climatology-derived water mass nutrient stoichiometry (N*; **Fig. 7D**; see *Comparing ARC-H and ARC-L* in **Methods**) – emerged as high (ARC-H) and low (ARC-L) diversity regions that were significantly differentiable at both *macro*- and *micro*-diversity levels (**Fig. 7E**). Further, ARC-H was characterized by low nutrient ratios (N*; >9X lower in ARC-H than ARC-L on average; $p < 5E-04$) and drove the divergence from the LDG (**Fig. S5)**.

Mechanistically, we interpret these observations as follows. Prior work in this region has shown (i) strong denitrification in the Bering Strait (Devol *et al.*, 1997), which explains the low N* in the west, and (ii) increasing oligotrophy in the Beaufort Gyre due to increasing vertical stratification, which selects against larger algae and for smaller algae and bacteria in the ARC-H (Li *et al.*, 2009). As above, we hypothesize that shorter-term increased host diversity results in increased viral *macro*- and *micro*-diversity in ARC-H. Though our GOV 2.0 dataset is confounded by seasonality of sampling, we posit that this elevated summer-time *macro*- and *micro*-diversity in ARC may fuel viral ecological differentiation and represent an unrecognized 'cradle' of viral biodiversity beyond the tropics. Though this elevated diversity in the Arctic was surprising, together with a similar deviation seen in mollusks (Valdovinos *et al.*, 2003) and recently reported in ray-finned fish (Rabosky *et al.*, 2018), these results call into question whether this decades-old paradigm needs revisiting and suggests that polar regions may be important biodiversity hotspots for viruses, as well as larger organisms.

Finally, as ocean exploration accelerates, patterns in diversity through the vertical layers of the ocean have become a focus. An emergent depth diversity gradient (DDG) hypothesis suggests that *macro*diversity decreases with depth (Costello & Chaudhary, 2017), which has been explored across the World Register of Marine Species that includes some microbes and viruses (http://www.marinespecies.org/), but *micro*diversity has not yet been explored for any organism. Overall, our virome-inferred diversity patterns were less obviously consistent with the DDG, although deep water ocean data were limited (**Fig. 7F**). Briefly, viral *macro*diversity largely followed the DDG with high diversity in the surface waters and decreased diversity with depth, whereas viral *micro*diversity did not as it decreased until 200 m depth, but then sharply increased (**Fig. 7F**). This deep water increase coincided with an increase in bacterial *macro*diversity in the mesopelagic region (**Fig. S6A & B**), and in TT-MES, this bacterial *macro*diversity correlated with viral *micro*diversity (**Fig. S6C**).

If more extensive deep water sampling confirms these patterns, we see several scenarios that could explain these data. First, we hypothesize that viral *micro*diversity may, in part, be driven by an increase in *macro*diversity of zone-specific bacterial populations in TT-MES, which we interpret as an expansion of host 'niches' available for infection that could drive diversification in viruses (Elena *et al*., 2009). Second, we hypothesize that the decrease in viral

*macro*diversity may be driven by increased viral *micro*diversity in the mesopelagic region that can promote competitive exclusion (*sensu* Hart *et al.*, 2016) as discussed above. Alternatively, lower cell density in the mesopelagic layer (Sunagawa *et al.* 2015) may result in less encounters between the "predators" and their "preys", reducing viral speciation (as a function of reduced number of viral generations), but selecting for viruses with broader host range. Again, testing these hypotheses will require technological advances to measure *in situ* host ranges and sensitivities of viruses and cells, respectively, at scales relevant to the diversity in nature.

Conclusions:

This study provides a systematic and global-scale view of patterns and drivers of marine viral *macro*- and *micro*- diversity that reveals three overarching advances. First, five ecological zones emerge for the global ocean, which contrasts known Longhurst biogeographic patterning in other organisms, but is consistent with observations from the largely co-sampled ocean microbiome (Sunagawa *et al.* 2015). Second, patterns and drivers of viral *macro*- and *micro*-diversity differ per-sample and correlate to geographic range. These findings offer hints at underlying mechanisms that impact these two levels of diversity that will guide researchers from discovery to hypothesis-testing as technologies, such as scalable single virus genomics and *in situ* host range assays, advance towards sampling scales relevant to those in nature. Third, epipelagic waters and the Arctic Ocean emerge from our work as biodiversity hotspots for viruses. While this is surprising given the LDG paradigm that the tropics rather than the poles are the cradles of diversity, it is in line with other observations in larger organisms (Valdovinos *et al.*, 2003, Rabosky *et al.*, 2018) and emphasizes the importance of these drastically climate-impacted Arctic regions for global biodiversity. Together, these advances, along with the parallel global-scale ecosystem-wide measurements of *Tara* Oceans (e.g. de Vargas *et al.*, 2015; Sungawa *et al.*, 2015; Brum *et al.*, 2015; Lima-Mendez *et al.*, 2015; Roux *et al.*, 2016) provide the foundation for incorporating viruses into emerging genes-to-ecosystems models (e.g. Guidi *et al.* 2016, Garza *et al.*, 2018) that guide ocean ecosystem management decisions that are likely needed if humans and the Earth System are to survive the current epoch of the planet-altering Anthropocene.

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Main Text Figure Legends:

Fig. 1. The Global Ocean Viromes 2.0. A. Arctic projection of the global ocean highlighting the new sampling stations of viromes in the GOV 2.0 dataset. Datasets from non-arctic samples were previously published in (Brum *et al.*, 2015; Roux *et al.*, 2016). **B.** Histograms of the average assembled contig lengths for viral populations >10 kb shared between GOV and GOV 2.0. **B-inset.** More than 92% of the unbinned GOV viral populations were reassembled and identified in GOV 2.0 >10 kb populations. **C.** Pie charts showing how many of the 488,130 total viral populations comprising GOV 2.0 can be annotated and, of those, their viral family level taxonomy.

Fig. 2. GOV 2.0 viral population have discrete population boundaries. (A) Histogram showing the read distribution frequency break between spuriously mapped reads and legitimate reads mapping to the genome. **(B)** Histograms showing the average percent identity of reads mapped to each genome after removing spuriously mapped reads.

Fig. 3. Ecological levels of organization. Schematic showing the different ecological levels of organization studied in this paper.

Fig. 4. Viral communities partition into five ecological zones with different *macro-* and *micro-* diversity levels. (A) Principal coordinate analysis (PCoA) of a Bray-Curtis dissimilarity matrix calculated from GOV 2.0. Analyses show that viromes significantly (Permanova p = 0.001) structure into five distinct global ecological zones: ARC, ANT, BATHY, TT-EPI, and TT-MES zones. Ellipses in the PCoA plot are drawn around the centroids of each group at 95% (inner) and 97.5% (outer) confidence intervals. Four outlier viromes that did not cluster with their ecological zones were removed (**Fig. S2A**) and all the sequencing reads were used (see **Fig. S2B** and **Methods**). **(B – right)** Scatterplots showing correlations between *macro-* (Shannon's *H'*) and *micro-* (average π for viral populations with $\geq$ 10x median read depth coverage; see **Methods**) diversity values for each sample across GOV 2.0. The larger circles represent the average per zone. **(B – left)** Boxplots showing median and quartiles of average *micro*diversity per ecological zone. (**B – bottom**) Boxplots showing median and quartiles of *macrodiversity* for each ecological zone. Zonal samples were randomly downsampled to n = 5 to account for zone sampling difference. All pairwise comparisons shown were statistically significant (p<0.01) using two-tailed Mann-Whitney U-tests. **(C)** Pearson's correlation results comparing *macro-* and *micro-*diversity with different biogeographical and biogeochemical parameters at the global scale (see **Fig. S3**, **Table S3** for all abbreviations, and **Methods**).

Fig. 5. Ecological drivers of global viral *macro*diversity. (A) Regression analysis between the first coordinate of a PCoA (**Fig. 4A**) and temperature showed that samples were separated by their local temperatures with an r^2 of 0.822. **(B)** Potential ecological drivers & predictors of beta-diversity across GOV 2.0 for the first two dimensions (Goodness of fit r^2 using a generalized additive model) and across all dimensions (Mantel test based on Spearman's correlation). Temperature was uniformly reported as the best predictor of viral beta-diversity globally. **(C)** Regression analysis between viral *macro*diversity at the deep chlorophyll maximum (DCM) layer and areal chlorophyll a concentration (after cube transformation) showed that viral *macro*diversity correlation with nutrients (**Fig. 4C**) is mediated (at least partially) by primary productivity. The untransformed values are provided on the lower axis for reference. The

Shannon's *H* outlier 32_DCM (**Fig. S3**) and a chlorophyll a concentration outlier (173_DCM; **Fig. 5D**) have been excluded from the regression analysis. **(D)** Boxplot analysis of areal chlorophyll a concentrations showing a single outlier concentration that fell above the fourth quantile of the data points (function geom_boxplot of ggplot).

Fig. 6. Size of geographic range positively correlates with *micro*diversity. (A) Venn diagram showing the number of viral populations found only in one zone (zone-specific) and those that are shared between and among the five ecological zones (multi-zonal). **(B)** Stacked barplots showing the number of multi-zonal, regional, and local viral populations found within the species pool of each ecological zone. **(C)** Boxplots showing median and quartiles of *micro*diversity (average π for viral populations with $\geq$ 10x median read depth coverage) per populations found within each zone defined as multi-zonal, regional, or local. Statistics were the same as in Fig. 2.

Fig. 7. Viral *macro-* and *micro-* diversity global biodiversity trends. (A) Loess smooth plots showing the latitudinal distributions of *macro-* and *micro-*diversity. **(B & C)** Equirectangular projections of the globe showing *macro-* and *micro-*diversity levels within each sample, respectively, across the global ocean. Samples collected at different depths from the same latitude and longitude are overlaid and the colors representing their *macro-* and *micro-* diversity values are merged. **(D)** Arctic projection of the global ocean showing the geographical division between ARC-H and ARC-L stations. The patterns are largely concordant with the Arctic division by climatology-derived N*. While we did sample across different seasons, the calculated N* values are not dependent on the season (see *impact of the coast, depth, and seasons* in **Methods**). **(E)** Boxplots showing median and quartiles of *macro-* (left) and *micro-* (right) diversity of the ARC-H and ARC-L regions. Statistics were the same as in Fig. 2. **(F)** Loess smooth plots showing the depth distributions of *macro-* and *micro-* population diversity. On all the smooth plots, the line represents the Loess best fit, while the lighter band corresponds to the 95% confidence window of the fit. Abbreviations: N*, the departure from dissolved N:P stoichiometry in the Redfield ratio and a geochemical tracer of Pacific and Atlantic water mass (see **Methods**).

Main Text Figures:

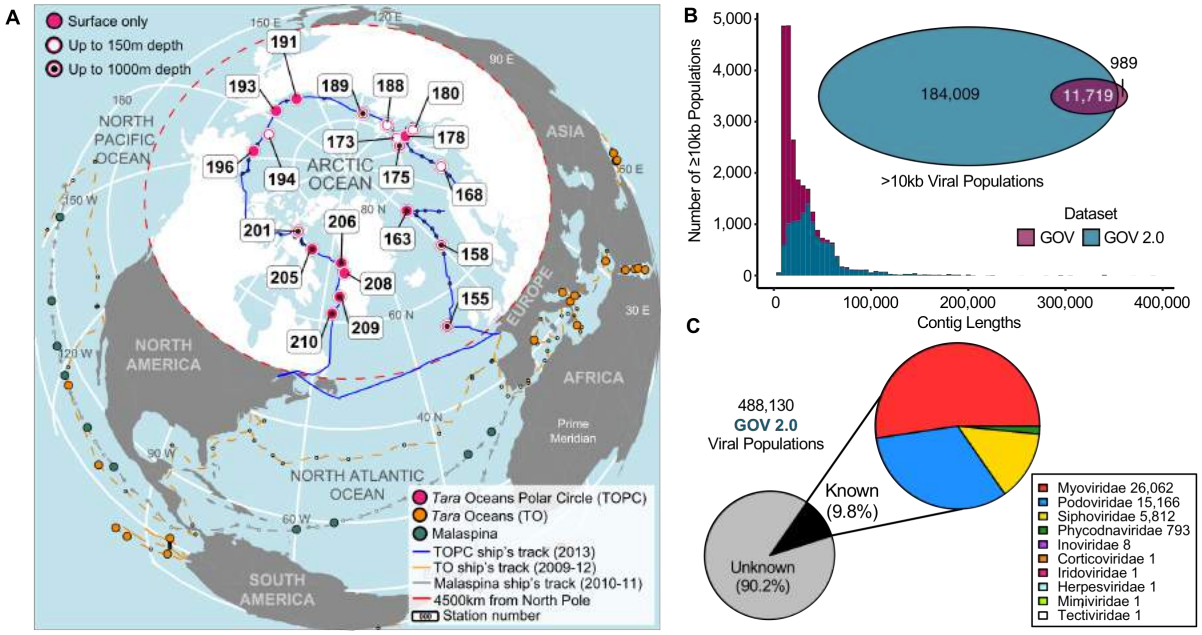

Fig. 1. The Global Ocean Viromes 2.0.

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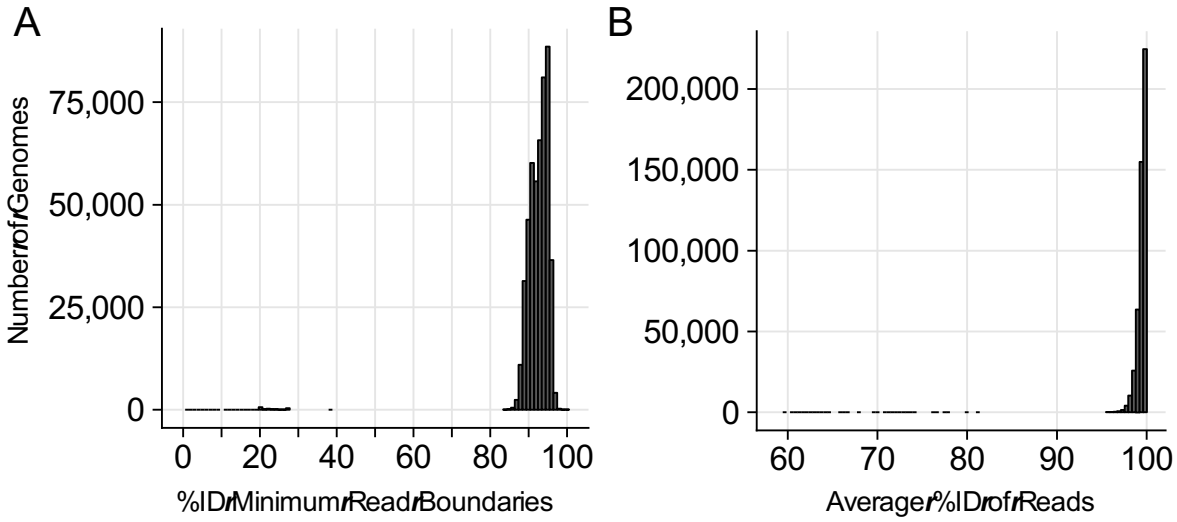
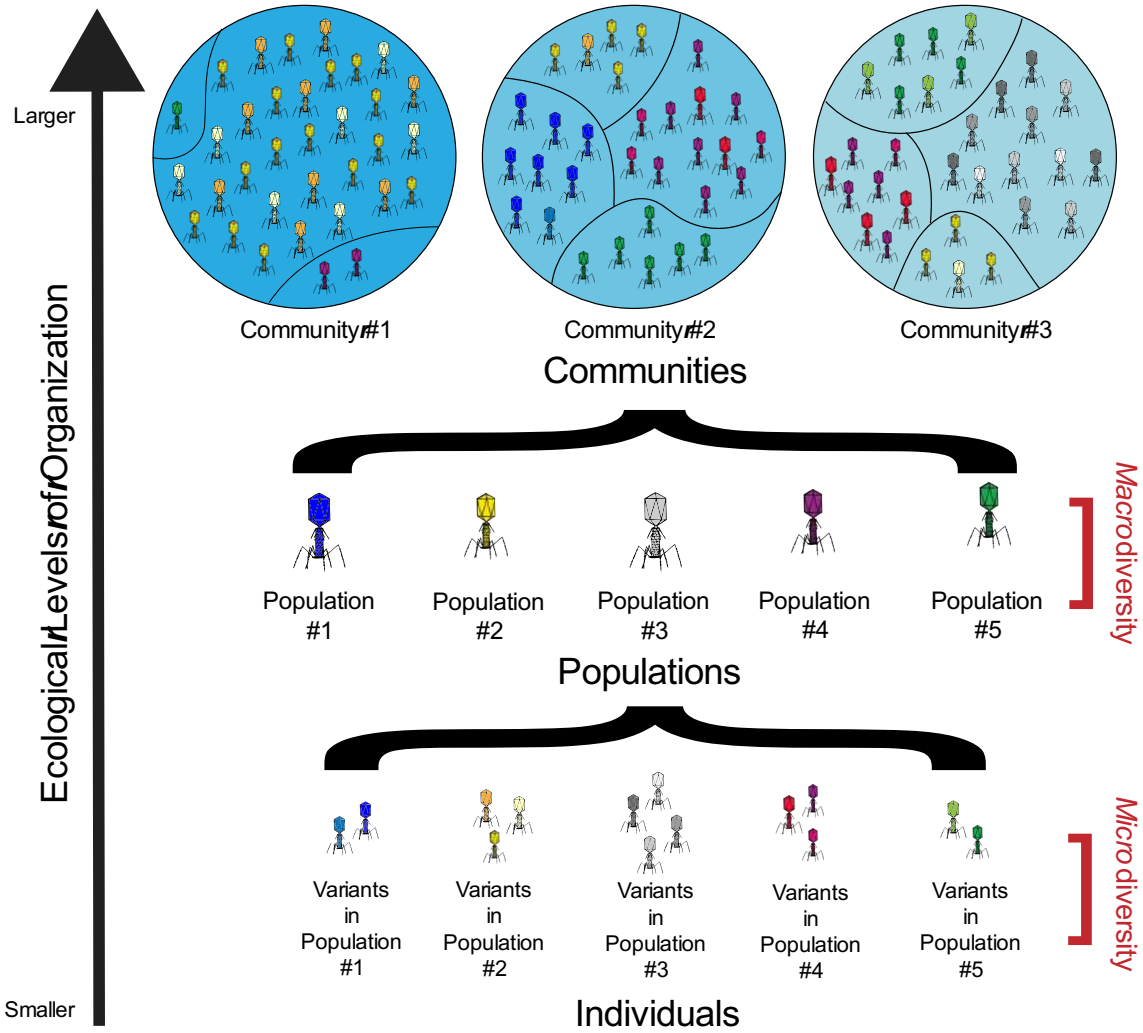


Fig. 2. GOV 2.0 viral population have discrete population boundaries.



665 **Fig. 3. Ecological levels of organization.**

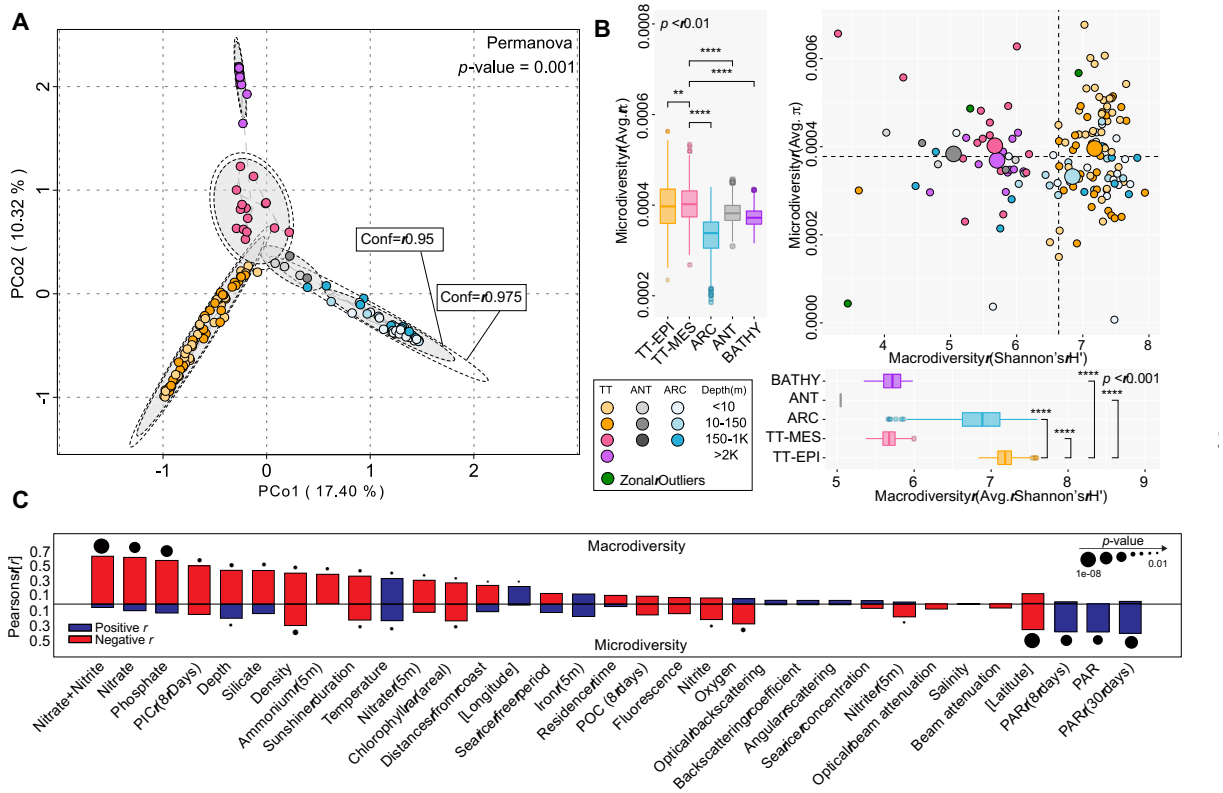


Fig. 4. Viral communities partition into five ecological zones with different *macro-* and *micro-* diversity levels.

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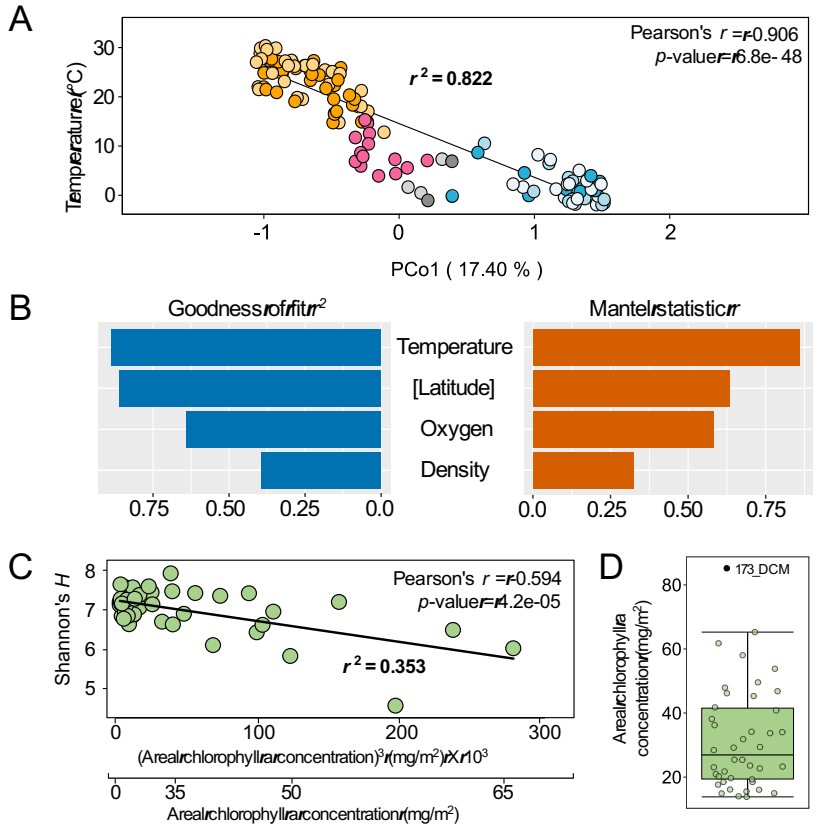

Fig. 5. Ecological drivers of global viral *macro*diversity.

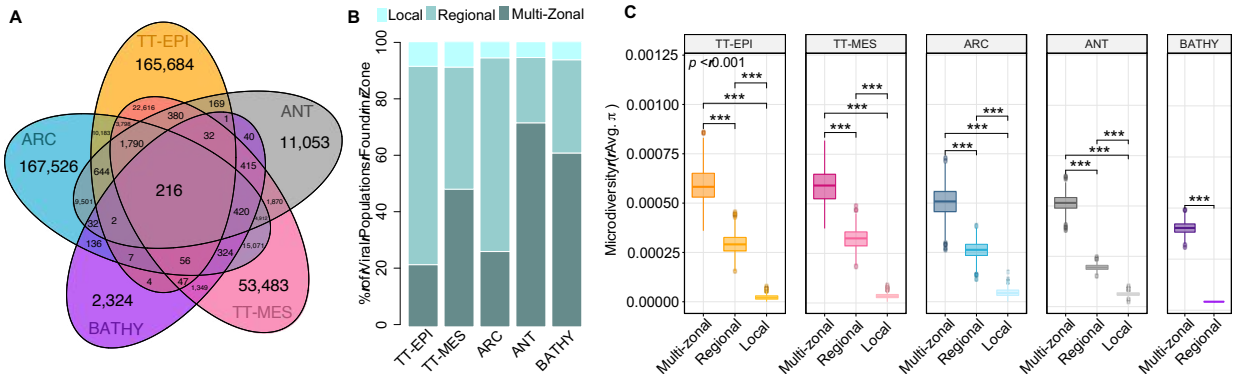

Fig. 6. Size of geographic range positively correlates with *micro*diversity.

675

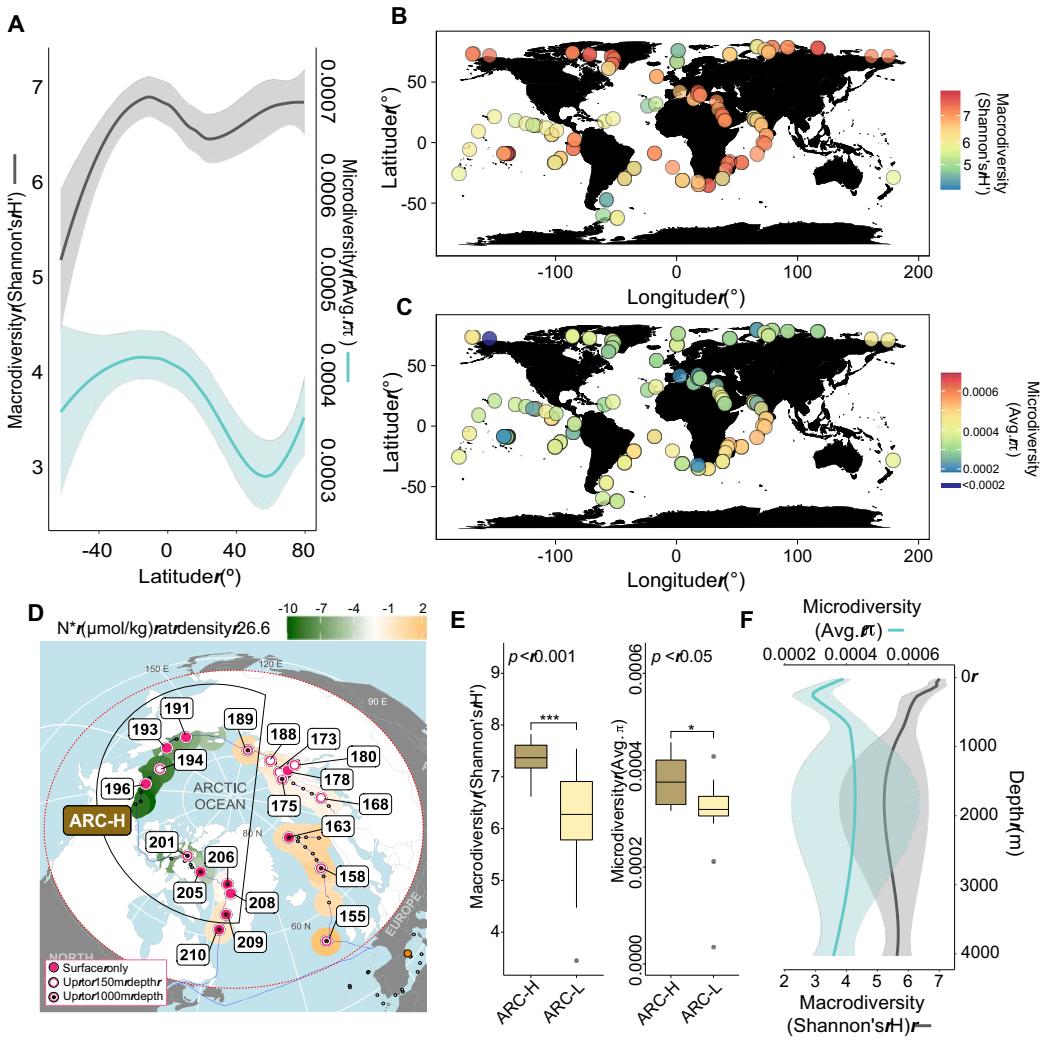

Fig. 7. Viral *macro-* and *micro-* diversity global biodiversity trends.

STAR Methods Text

Key Resources Table

Reagent or Resource	Source	Identifier(s)
Sequencing Reagents and Kits		
NEBNext DNA Sample Prep Master Mix	New England Biolabs, Ipswich, MA	Cat n° E6040S
NEXTflex PCR free barcodes	Bioo Scientific, Austin, TX	Cat n° NOVA-514110
Kapa Hifi Hot Start Library Amplification kit	KAPA Biosystems, Wilmington, MA	Cat n° KK2611
DNA SMART ChIPSeq Kit	Takara Bio USA, Mountain View, CA	Cat N° 634865
Deposited Data		
Tara Oceans Viromes Raw Reads	Brum *et al.*, 2015; Roux *et al.*, 2016	European Nucleotide Archive (ENA) - see **Table S3** for details
Tara Oceans Polar Circle Raw Reads	This paper	European Nucleotide Archive (ENA) - see **Table S3** for details
Malaspania Viromes Raw Reads	Roux *et al.*, 2016	Integrated Microbial Genomes (IMG) with Joint Genome Institute - see **Table S3** for details
16S rRNA gene *Tara* Oceans data	Logares *et al.*, 2014	Supplementary materials in Logares *et al.*, 2014
Biogeographical and Physicochemical data	Pesant *et al.*, 2015	PANGAEA (Data Publisher for Earth & Environmental Science) - see **Table S3** for details
N* Arctic Data	This paper	**Table S3**

Software and Algorithms		
nucmer (MUMmer3.23)	Kurtz *et al.*, 2004	https://sourceforge.net/projects/mummer/
bbmap 37.57	https://jgi.doe.gov/data-and-tools/bbtools/	https://jgi.doe.gov/data-and-tools/bbtools/
metaSPAdes 3.11	Nurk *et al.*, 2017	https://github.com/ablab/spades/releases
prodigal 2.6.1	Hyatt *et al.*, 2010	https://github.com/hyattpd/Prodigal
diamond	Buchfink *et al.*, 2014	https://github.com/bbuchfink/diamond
VirSorter v2	Roux *et al.*, 2015	https://github.com/simroux/VirSorter
VirFinder	Ren *et al.*, 2017	https://github.com/jessieren/VirFinder
CAT	Cambuy *et al.*, 2016	https://github.com/dutilh/CAT
blast 2.4.0+	ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/	ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/
vConTACT2	Jang *et al.*, *in press* 2018	https://bitbucket.org/MAVERICLab/vcontact2
bowtie2	Langmead & Salzberg, 2012	https://github.com/BenLangmead/bowtie2
BamM	https://github.com/Ecogenomics/BamM	https://github.com/Ecogenomics/BamM
Bedtools	Quinlan & Hall, 2010	https://github.com/arq5x/bedtools2/blob/master/docs/content/overview.rst
Vegan (R package)	Dixon, 2003	https://cran.r-project.org/web/packages/vegan/index.html
heatmap3 (R package)	https://cran.r-project.org/web/packages/heatmap3/in	https://cran.r-project.org/web/packages/he

	dex.html	atmap3/index.html
ggplot2 (R package)	https://cran.r-project.org/web/packages/ggplot2/index.html	https://cran.r-project.org/web/packages/ggplot2/index.html
ggpubr (R package)	https://cran.r-project.org/web/packages/ggpubr/index.html	https://cran.r-project.org/web/packages/ggpubr/index.html
Analyses scripts (per Figure)	This paper	https://bitbucket.org/MAVERICLab/GOV2

Contact for Reagent and Resource Sharing

Further information and requests for resources and reagents should be directed to and will be fulfilled by the corresponding contact, Matthew Sullivan (mbsulli@gmail.com).

Experimental Model and Subject Details

Not applicable.

Methods Details

Tara Oceans Polar Circle (TOPC) expedition sample collection, processing, and sequencing

Between June 2013 and December 2013, 41 samples were collected at different depths from 20 different sites near or within the Arctic Ocean (see full list of samples in **Table S3**). Physicochemical measurements, sample collection, and DNA extractions were performed using the methods described in (Roux *et al.*, 2016). Extracted DNA was prepared for sequencing using library preparation method described in (Alberti *et al.*, 2017) for viral samples collected during the *TOPC* campaign (section 4.2) and sequenced using the HiSeq 2000 system (101 bp, paired end reads). Importantly, our sample collection and library preparation methods have known bias towards <0.2um dsDNA viruses (Roux *et al.*, 2017). The *TOPC* samples were combined with the previously published viromes in (Brum *et al.*, 2015; Roux *et al.*, 2016). Of the previously published dataset, the mesopelagic samples at (*Tara* stations 37, 39, 56, 64, 68, 70, 76, 78, 111, 122, 137, 138) and the Southern Ocean samples (*Tara* stations 82, 84, 85) were sequenced deeper. These combined samples comprise the GOV 2.0 dataset. The number of reads found in each sample can be found in **Table S3**.

Due to different library preparation for the *TOPC* samples than the original GOV samples, the previously sequenced mesopelagic samples (*Tara* stations 68, 78, 111, 137) were prepped using the *TOPC* library preparation to determine if it impacted our ability to assemble viral populations. We found no significant difference between library preparations (**Fig. S7**). For two surface samples (*Tara* Stations 100 and 102), we also re-prepped the DNA using the DNA SMART ChIP-Seq kit which allows to catch ssDNA in the library preparation (Takara) and further sequenced these two samples using the HiSeq 2000 system.

All the remaining STAR Methods we used are quantifications and statistical analyses. All the details related to these STAR Methods are therefore provided in the following section, ***Quantification and Statistical Analyses***

Quantification and Statistical Analyses

Viral contig assembly, identification, and dereplication

All samples in the GOV 2.0 dataset (Roux *et al.*, 2016) as well as the previously sequenced *TOPC* library-prepped mesopelagic samples and the DNA SMART ChIP-Seq kit surface samples were individually assembled using metaSPAdes 3.11.1 (Nurk *et al.*, 2017). Prior to assembly, Malaspina samples from GOV 2.0 were further quality controlled. Briefly, adaptors and Phix174 reads were removed and reads were trimmed using bbduk.sh (https://jgi.doe.gov/data-and-tools/bbtools/; minlength=30 qtrim=rl maq=20 maxns=0 trimq=14 qtrim=rl). Following assembly, contigs ≥1.5kb were piped through VirSorter (Roux *et al.*, 2015) and VirFinder (Ren *et al.*, 2017) and those that mapped to the human, cat or dog genomes were removed. Contigs ≥5kb or ≥1.5kb and circular that were sorted as VirSorter categories 1-6 and/or VirFinder score ≥0.7 and p <0.05 were pulled for further investigation. Of these contigs, those sorted as VirSorter categories 1 and 2, VirFinder score ≥0.9 and p <0.05 or were identified as viral by both VirSorter (categories 1-6) and VirFinder (score ≥0.7 and p <0.05) were classified as viral. The remaining contigs were run through CAT (Cambuy *et al.*, 2016) and those with <40% (based on an average gene size of 1000) of the genome classified as bacterial, archaeal, or eukaryotic were considered viral. In total, 848,507 viral contigs were identified. Viral contigs were grouped into populations if they shared ≥95% nucleotide identity across ≥80% of the genome (*sensu* Brum *et al.*, 2015) using nucmer (Kurtz *et al.*, 2004). This resulted in 488,130 total viral populations found in GOV 2.0 (see **Table S5** for VirSorter, VirFinder, and CAT results), of which 195,728 were ≥10kb.

Viral taxonomy

For each viral population, ORFs were called using Prodigal (Hyatt *et al.*, 2010) and the resulting protein sequences were used as input for vConTACT2 (Jang *et al.*, *in press* 2018) and for blastp. Viral populations represented by contigs >10kb were clustered with Viral RefSeq release 85 viral genomes using vConTACT2. Those that clustered with a virus from RefSeq based on amino acid homology based on diamond (Buchfink *et al.*, 2014) alignments were able to be assigned to a known viral taxonomic genus and family. For GOV 2.0 viral populations that could not be assigned taxonomy or were <10kb, family level taxonomy was assigned using a majority-rules approach, where if >50% of a genome's proteins were assigned to the same viral family using a blastp bitscore ≥50 with a Viral RefSeq virus, it was considered part of that viral family.

Viral population boundaries

To determine if our viral populations had discrete sequence boundaries, all reads across the GOV 2.0 dataset (excluding the *Tara* stations 68, 78, 111, 137 prepped using the *TOPC* library preparation methods and the DNA SMART ChIP-Seq kit prepped libraries) were pooled and mapped non-deterministically to our viral populations using the 'very-sensitive-local' setting in bowtie2 (Langmead & Salzberg, 2012). The percent nucleotide identity (% ID) of each mapped read and the positions in the genome where the read mapped were determined. The frequency of reads mapping at a specific % IDs were weighted based on the length of each read mapped across the genomes. Frequencies of reads mapping at specific % IDs were smoothed using Loess smooth functions (span = 1 to be more permissive of lower % ID reads) to create read frequency histograms (% ID vs. frequency). To determine break in the distribution of read

frequencies between the different % IDs, Euclidean distances calculated were calculated between % ID frequencies and then hierarchically clustered in R.

Calculating viral population relative abundances, and average read depths

To calculate the relative abundances of the different viral populations in each sample, reads from each GOV 2.0 virome were first non-deterministically mapped to the GOV 2.0 viral population genomes using bowtie2. BamM (https://github.com/ecogenomics/BamM) was used to remove reads that mapped at <95% nucleotide identity to the contigs, bedtools genomecov (Quinlan & Hall, 2010) was used to determine how many positions across each genome were covered by reads, and custom Perl scripts were used to further filter out contigs without enough coverage across the length of the contig. For downstream *macro*diversity calculations, contigs ≥5kb in length that had <5kb coverage or less than the total length of the contig covered for contigs <5kb were removed. For downstream *micro*diversity calculations, all contigs with <70% of the contig covered were removed. BamM was used to calculate the average read depth ('tpmean' -minus the top and bottom 10% depths) across each contig. For the *macro*diversity calculations, the average read depth was used as a proxy for abundance and normalized by total read number per metagenome to allow for sample-to-sample comparison.

Subsampling reads

Unequal sequencing depth can have large impacts on diversity measurements, specifically α-diversity measurements (Lemos *et al.*, 2011). Due to 5x more sequencing depth in *TOPC* samples and the deeply sequenced mesopelagic and Southern Ocean samples (**Table S3**), all viromes in the GOV 2.0 dataset were randomly subsampled without replacement to 10,000 paired reads or 10,000 single-end reads using reformat.sh from bbtools suite (https://sourceforge.net/projects/bbmap/). The subsampled read libraries were assembled using metaSPAdes 3.11.1. Contigs ≥1.5kb that shared ≥95% nucleotide identity across ≥80% of the genome with the 488,130 viral populations in GOV 2.0 were pulled out and grouped into populations to be used as the subsampled GOV 2.0 viral populations. In total, there were 46,699 viral populations. Relative abundances were calculated per sample as aforementioned for *macro*diversity calculations, but using the subsampled GOV 2.0 viral populations and the subsampled reads.

Macrodiversity calculations

The *macro*diversity α- (Shannon's *H*) and β- (Bray-Curtis dissimilarity) diversity statistics were performed using vegan in R (Dixon, 2003). The α-diversity calculations were based on the relative abundances produced from the subsampled reads. Loess smooth plots with 95% confidence windows in ggplot2 in R were used to look at changes in Shannon's *H* across latitude (**Fig. 7A)** and depth (**Fig. 7F)**. For the β-diversity, both the subsampled and the total reads abundances were used to look at community structure (**Fig. S2**). Principle Coordinate analysis (function capscale of vegan package with no constraints applied) and NMDS analysis (function metaMDS; K=2 and trymax=100) were used as the ordination methods on the Bray-Curtis dissimilarity matrices from both the subsampled and total reads calculated from GOV 2.0 (function vegdist; method "bray") after a cube root transformation (function n^{th} root; n=3). The ecological zones that emerged were verified using a permanova test (function "adonis") and the confidence intervals were plotted using function "ordiellipse" at the specified confidence limits (95% and 97.5%) using the standard deviation method. There were no significant differences in

clustering between the subsampled and all reads Bray-Curtis dissimilarity PCoA plots (**Fig. S2**). Hierarchical clustering (function pvclust; method.dist="cor" and method.hclust="average") was conducted on the same Bray-Curtis dissimilarity matrices using 1000 bootstrap iterations and only the approximately unbiased (AU) bootstrap values were reported. The heatmaps were generated using the heatmap3 package with appropriate rotations of the branches in the dendrograms. Samples that did not cluster with their ecological zone (*Tara* mesopelagic stations 72, 85, and 102 and *Tara* surface station 155) were considered outliers and removed from further analyses (**Fig. S2A & C**).

Microdiversity calculations

Viral populations with an average read depth of ≥10x across 70% of their representative contig in at least one sample in the GOV 2.0 dataset were flagged for *micro*diversity analyses. We used 10x as the minimum coverage because population genetic statistics were found to be relatively consistent down to 10x based on previous downsampling coverage analyses (Schloissnig *et al.*, 2013). BAM files containing reads mapping at ≥95% nucleotide identity were filtered for just the flagged viral populations. Samtools mpileup and bcftools were used to call single nucleotide variants (SNVs) across these populations. SNV calls with a quality call > 30 threshold were kept. Coverage for each allele for each SNV locus was summed across all the metagenomes. For each SNV locus, the consensus allele was re-verified and those with alternative alleles that had a frequency >1% (1000 Genomes Project Consortium, 2012), the classical definition of a polymorphism, and supported by at least 4 reads were considered SNP loci (Schloissnig *et al.,* 2013). Nucleotide diversity (π) per genome were calculated using equation from (Schloissnig *et al.*, 2013). Due to the variable coverage across the genome, coverage was randomly downsampled to 10x coverage per locus in the genome. For the downsampling, if there was not the target 10x coverage for the locus, all of the alleles were sampled. Nucleotide diversity (π) was calculated for each genome with an average read depth ≥10x across 70% of their contig in each sample. For each sample, π values of 100 viral populations were randomly selected and averaged. This was repeated 1000x and the average of the all 1000 subsamplings was used as the final microdiversity value for each sample. Loess smooth plots with 95% confidence windows in ggplot2 in R were used to look at changes in average π across latitude (**Fig. 7A)** and depth (**Fig. 7F)**.

Drivers of Macro- and Micro-diversity

Regression analysis between the first coordinate of the PCoA (**Fig. 5A)** and available temperature measurements was conducted using the lm function in R. The environmental variables were fitted to the first two dimensions of the PCoA using a generalized additive model (function envfit; permutations=9999 and na.rm = TRUE). Then, they were correlated with all the PCoA dimensions using a mantel test (function mantel; permutations=9999 and method="spear") after scaling (function scale) and calculating their distance matrices (function vegdist; method "euclid" and na.rm = TRUE). Finally, they were correlated with Shannon's *H* and π using Pearson's correlation (function cor; use="pairwise.complete.obs") after removing Shannon's *H* outliers based on a boxplot analysis (**Fig. S3**).

Subsampling macro- and micro- diversity

Due to unequal sampling across each ecological zone, we chose to normalize the number of samples between each ecological zone by subsampling the down to lowest zone sample size

(ANT; n = 5). Shannon's *H* outliers were not included in the subsampling. Five samples within each zone were randomly subsampled without replacement and their *macro*- and *micro*- diversity values averaged, respectively. We subsampled 1000x and plotted the averages and assessed for significant differences using Mann-Whitney U-tests in ggboxplot from the R package ggpubr (**Fig. 4B**).

Classifying multi-zonal, regional, and local viral populations

To determine geographic range, viral populations were evaluated for their distributions across the five ecological zones and plotted using the VennDiagram package in R (**Fig. 6A**). If present in ≥1 sample in more than one ecological zone, it was considered multi-zonal (58% GOV 2.0 viral populations). If present only in samples found within a single zone, it was considered zone-specific (48% GOV 2.0 viral populations). Zone-specific viral populations were further divided into regional (≥2 samples within a zone) and local (only 1 sample within a zone). The proportion of multi-zonal, regional, and local viral populations found across each zone (**Fig. 6B**) and across each station (**Fig. S4**) were calculated by dividing the number of each type by the total number of viral populations found across a zone or station, respectively. To assess the impact of geographic range on *micro*diversity per zone, stations were randomly subsampled without replacement as described above. Within each sample, π values of 50, 100, and 20 viral populations of each geographic distribution (multi-zonal, regional, and local, respectively) were randomly selected and averaged. All the viral populations with a geographic range were sampled and averaged in samples that lacked enough deeply-sequenced viral populations with particular geographic range. This was repeated 1000x and the averages plotted and assessed for significant differences using Mann-Whitney U-tests in ggboxplot from the R package ggpubr (**Fig. 6C**).

Comparing ARC-H and ARC-L

The ARC-H and ARC-L regions were defined based on their biogeography; the ARC-H stations were located in the Pacific Arctic region, the Arctic Archipelago, and the Davis-Baffin Bay, in addition to one station (Station 189) in the Kara-Laptev sea, which was separated by a land mass from the rest of the stations in the same area (**Fig. 7D**). The ARC-L stations were located in the Kara-Laptev Sea (except Station 189), the Barents Sea, and subpolar areas (stations 155 and 210). The departure from the dissolved N:P stoichiometry in the Redfield ratio (N*) was calculated as in (Tremblay *et al.*, 2015) to represent the deficit in dissolved inorganic nitrogen (DIN) in the ratio and as a geochemical tracer of pacific and atlantic water masses. *Macro*- and *micro*- diversity values for each station in ARC-H and ARC-L were plotted and assessed for significant differences using Mann-Whitney U-tests in ggboxplot from the R package ggpubr (**Fig. 7E**).

Comparing GOV to GOV 2.0

Viral populations assembled in the GOV (Roux *et al.*, 2016) were compared to the GOV 2.0 viral populations (**Fig. 1B**) using blastn. Unbinned GOV viral populations with a nucleotide alignment to a GOV 2.0 viral populations with ≥95% nucleotide identity and an alignment length ≥50% the length were considered present in the GOV 2.0. These results were plotted in a venn diagram using the VennDiagram package in R. The frequency of contig lengths of viral populations that were shared across both samples were plotted using ggplot2 (function "geom_histogram"; binwidth =5000).

Calculating 16S OTU Macrodiversity

Previously published 16S OTU data were taken from (Logares *et al.*, 2014). The *macro*diversity α- (Shannon's *H*) statistics were performed using vegan in R (Dixon, 2003). Loess smooth plots with 95% confidence windows in ggplot2 in R were used to look at changes in bacterial Shannon's H down the depth gradient. Differences between surface, deep chlorophyll maximum, and mesopelagic bacterial samples were compared using Mann-Whitney U-tests and plotted in ggboxplot from the R package ggpubr. Finally, viral *micro*diversity was correlated with bacterial Shannon's *H* using Pearson's correlation (function cor; use="pairwise.complete.obs") and a linear regression (**Fig. S7C**).

Impact of the coast, depth, and seasons

GOV 2.0 samples are largely open ocean samples. Even though the arctic samples were more coastal, we didn't observe any significant coastal impact on the global *macro*diversity (Pearson's r = -0.25; Bonferroni-corrected p-value = 0.18) and *micro*diversity (Pearson's r = 0.1; p-value = 0.16) levels (**Fig. 4C**). Although nitrate and phosphate levels generally increase with depth, we observed higher correlations and significantly lower p-values for these nutrients with *macro*diversity levels than between depth and *macro*diversity (**Fig. 4C**) which suggests an impact of nutrients on viral diversity via primary production (**Fig. 5C**). Additionally, since the sampling was largely at discrete depth layers with different densities in the TT region (epipelagic, mesopelagic, and bathypelagic), rather than sampling gradients, we discerned a clearer signal for the separation between these ecological zones (**Fig. 4A**). On the other hand, all the arctic epipelagic and mesopelagic samples fell within the same ecological zone due to the absence of a pycnocline in this area (**Fig. 4A**). Finally, the circumnavigation of the Arctic Ocean spanned multiple seasons (spring, summer, and fall). Based on our previous observation from a time-series data in a sub-arctic system (Hurwitz & Sullivan, 2013), our viral *macro*diversity is expected to be lowest during the spring and summer and increase towards the winter season. However, our calculated N* values are not dependant on the season and represent the largest magnitude of change among all of the environmental variables that correlated with *macro*diversity between the ARC-H and ARC-L regions.

Data and Software Availability

Code availability

Scripts used in this manuscript are available on the Sullivan laboratory bitbucket under GOV 2.0.

Data availability

All raw reads are available through ENA (*Tara* Oceans and *TOPC*) or IMG (Malapsina) using the identifiers listed in **Table S3**. Processed data are available through iVirus, including all assembled contigs, viral populations and genes.

Author contributions:

MC, CD, JF, SK-L, CM, SPe, MP, SPi, JP, and *Tara* Oceans coordinators conceptualized and organized sampling efforts for the *Tara* Oceans Polar Circle expedition. SPe annotated, curated, and managed all biogeochemical data. AA, CC, and PW coordinated all sequencing efforts. ACG, AAZ, NC-N, BT, BB, KA, YL, DV, J-ET, MB, CB, CdV, BED, DI, LK-B, SR, SS, PW, and MBS created the study design, analyzed the data, and wrote the manuscript. All authors approved the final manuscript. **Competing interests:** The authors declare no competing interests.

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List of Supplementary Materials:

Tara Oceans Coordinators and Affiliations

Figures S1-S7

Tables S1-S5

Supplementary Materials:

Tara Oceans Coordinators and Affiliations

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Supplementary Figures

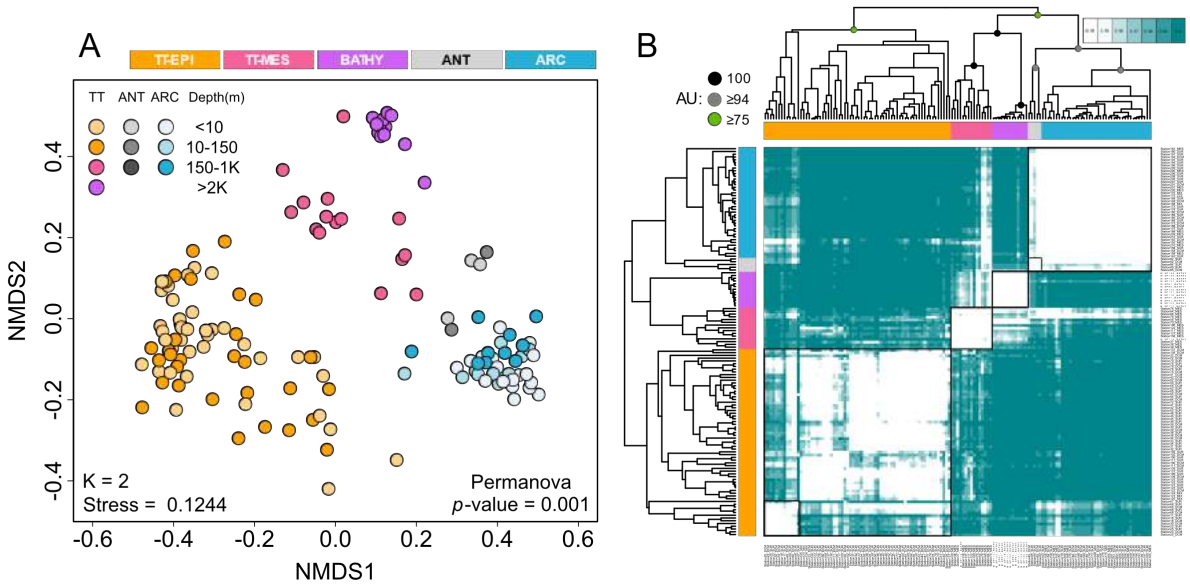

Fig. S1. Non-metric multidimensional scaling (NMDS) and hierarchical clustering of GOV 2.0. As observed with the Principle Coordinate analysis **(Fig. 2A)**, NMDS analysis **(A)** and correlation-based hierarchical clustering **(B)** of a Bray-Curtis dissimilarity matrix calculated from GOV 2.0 structured the viromes into five distinct global ecological zones with an approximately unbiased (AU) bootstrap value ≥ 77 in the hierarchical clustering. Four outlier viromes were removed and all the sequencing reads were used, with justification provided in (**Fig. S5, C and D**), respectively. Abbreviations: ARC, Arctic; ANT, Antarctic; BATHY, bathypelagic; TT-EPI, temperate and tropical epipelagic; TT-MES, temperate and tropical mesopelagic.

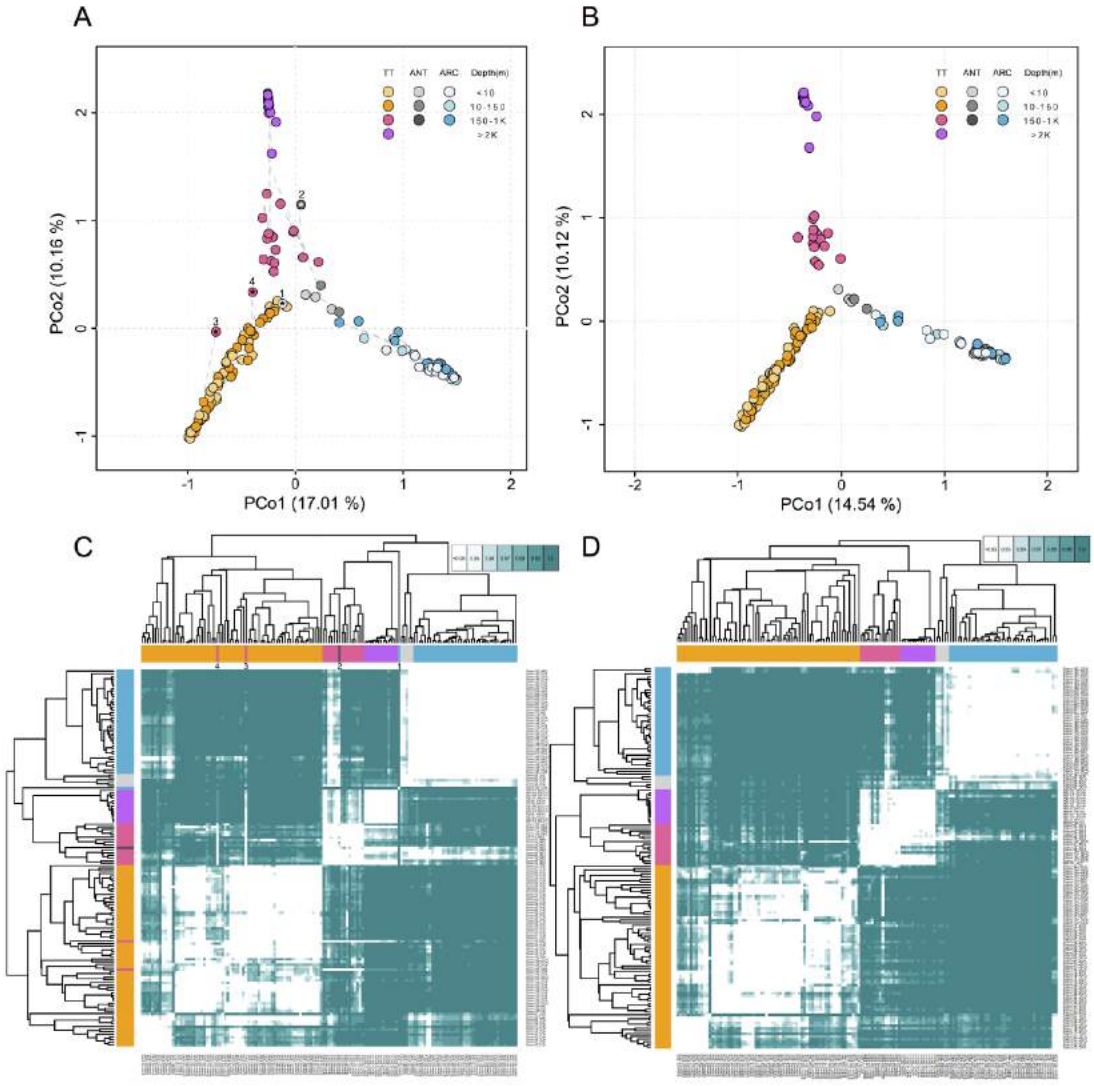

Fig. S2. Beta-diversity of the total reads and subsampled reads GOV 2.0 dataset. PCoA of a Bray-Curtis dissimilarity matrix calculated from GOV 2.0 using all the sequencing reads **(A)** and after randomly subsampling the reads to the same sequencing depth **(B)**. The dissimilarity matrices from **(A)** and **(B)** were used to conduct hierarchical clustering on the samples as shown in **(C)** and **(D)**, respectively. The four viromes which were removed from (**Fig. 4)** and **(Fig. S1)** are highlighted with asterisks; sample 1 (station 155_SUR) is the only surface sample in the North Atlantic Drift Province and could have been influenced by the warm surface currents going northward due to the Atlantic Meridional Overturning Circulation; sample 2 (station 85_MES) is the only mesopelagic sample from the Southern Ocean and could have been influenced by the upwelling of ancient deep ocean water (which is also congruent with the similarity observed between deep water bacterial communities of polar and lower latitude (Ghiglione *et al.*, 2012)); sample 3 (station72_MES) fell outside the 97.5% confidence intervals of all the ecological zones; sample 4 (station102_MES) was located in El Niño-Southern

Oscillation region and could have been influenced by the upwellings and downwellings in this area. Additionally, samples 1, 3, and 4 were among the Shannon's *H* outliers (**Fig. S3**). Viral communities still partitioned into five ecological zones after subsampling the reads as shown by the PCoA **(B)** and hierarchical clustering **(D)** plots.

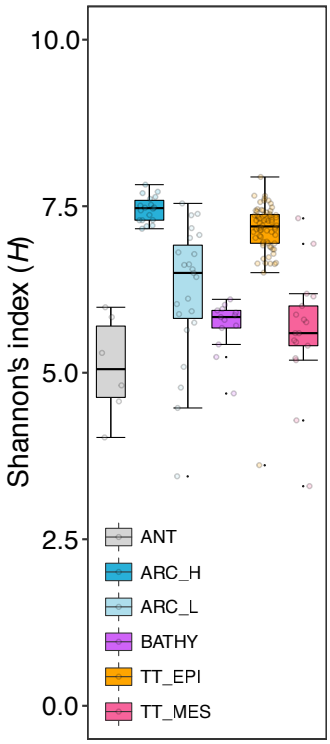

Fig. S3. Boxplot analysis of viral *macro*diversity across GOV 2.0 ecological zones. Outliers that fell below the first quantile or above the fourth quantile (function geom_boxplot of ggplot) of each ecological zone were removed before examining the predictors of viral *macro*diversity (**Fig. 4C**). Outliers: 32_SUR, 155_SUR, 56_MES, 70_MES, 72_MES, 102_MES, MSP131, and MSP144.

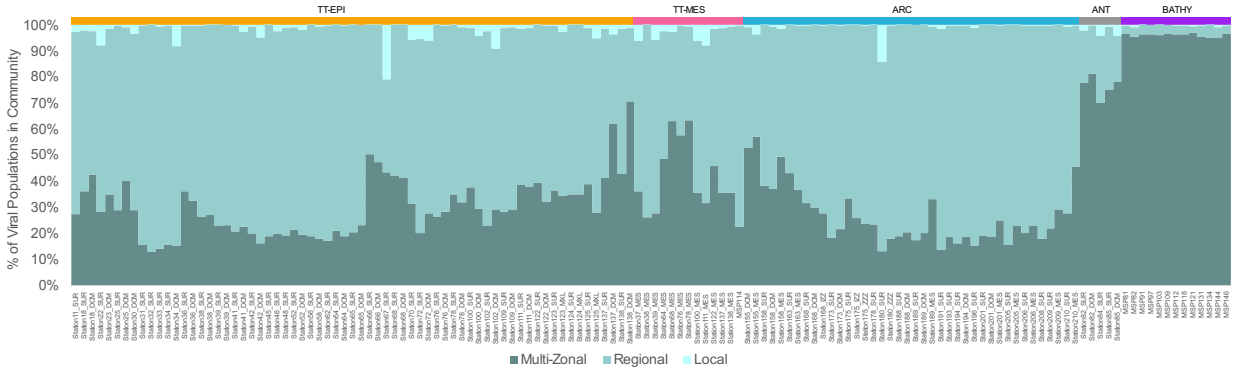

Fig. S4. Stacked barplots showing the number of multi-zonal, regional, and local viral populations found within the species pool of each station. Ecological zone outliers (see **Fig. S5**) are excluded.

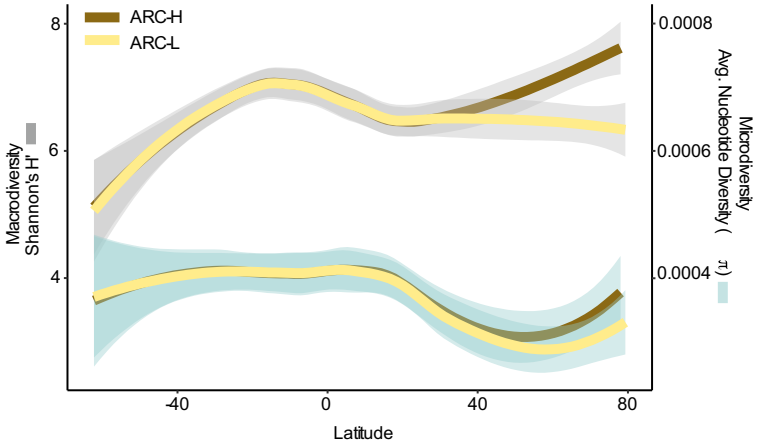

Fig. S5. ARC-H drives the divergence from the LBG. Loess smooth plots showing the latitudinal distributions of *macro*- and *micro*- population diversity with ARC-H and ARC-L regions. The line represents the loess best fit, while the lighter band corresponds to the 95% confidence window of the fit.

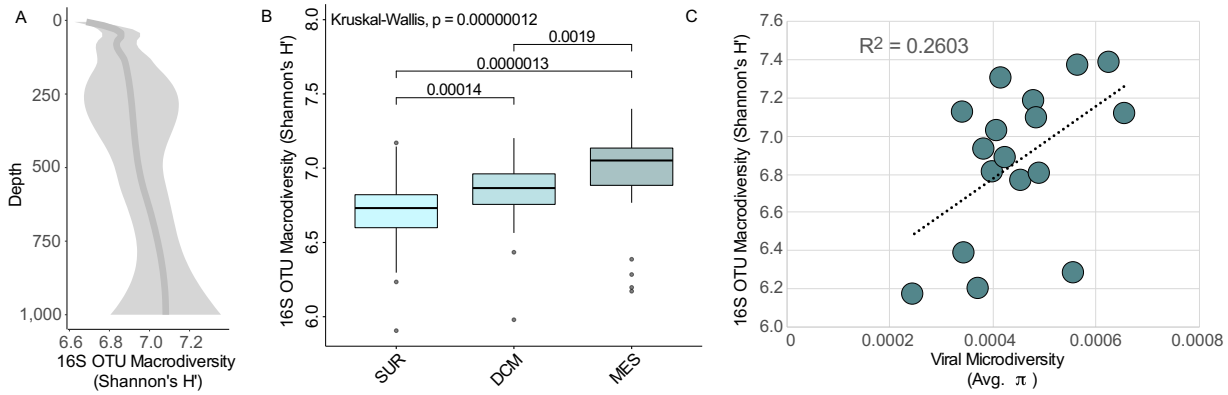

Fig. S6. Microbial 16S OTUs biodiversity deviate from the DBG and correlates with viral microdiversity in the mesopelagic. (A) Loess smooth plots showing 16S OTUs (Logares *et al.*, 2014) *macro*diversity distributions down the depth gradient. The line represents the loess best fit, while the lighter band corresponds to the 95% confidence window of the fit. **(B)** Boxplots showing median and quartiles of surface, deep chlorophyll maximum (DCM), and mesopelagic 16S OTU data taken from (Logares *et al.*, 2014). All pairwise comparisons shown were statistically significant (p<0.05) using two-tailed Mann-Whitney U-tests. **(C)** Scatterplot showing the correlation (Pearson's correlation r = 0.51; p-value = 0.036) and linear regression (r^2 = 0.26) between *Tara* Oceans mesopelagic samples shared between the 16S OTU samples in (Logares *et al.*, 2014) and our viral samples in GOV 2.0.

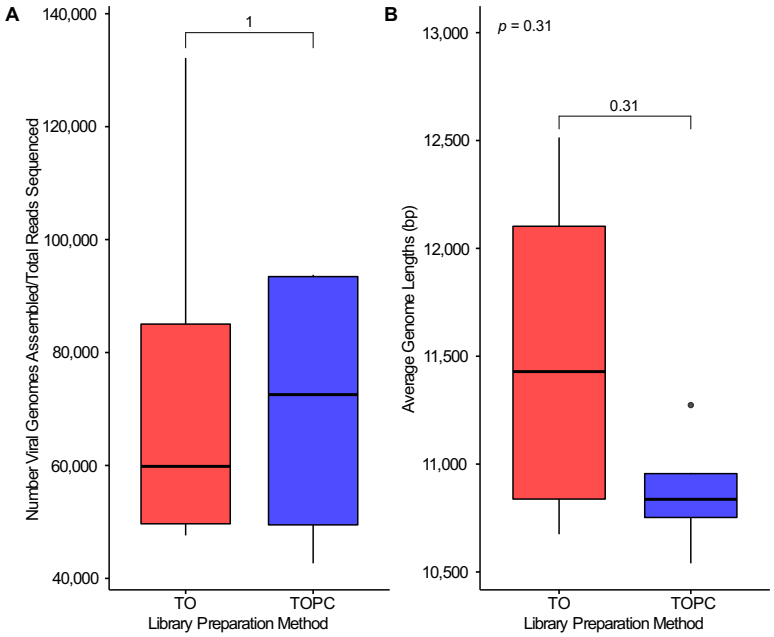

Fig. S7. TO and TOPC library preparation comparisons. (A & B) Boxplots showing median and quartiles of the number of assembled viral genomes per total reads sequenced and the average genome lengths in TO and TOPC preparations of *Tara* mesopelagic stations 68, 78, 111, and 137, respectively. All pairwise comparisons shown were not statistically significant using two-tailed Mann-Whitney U-tests.

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