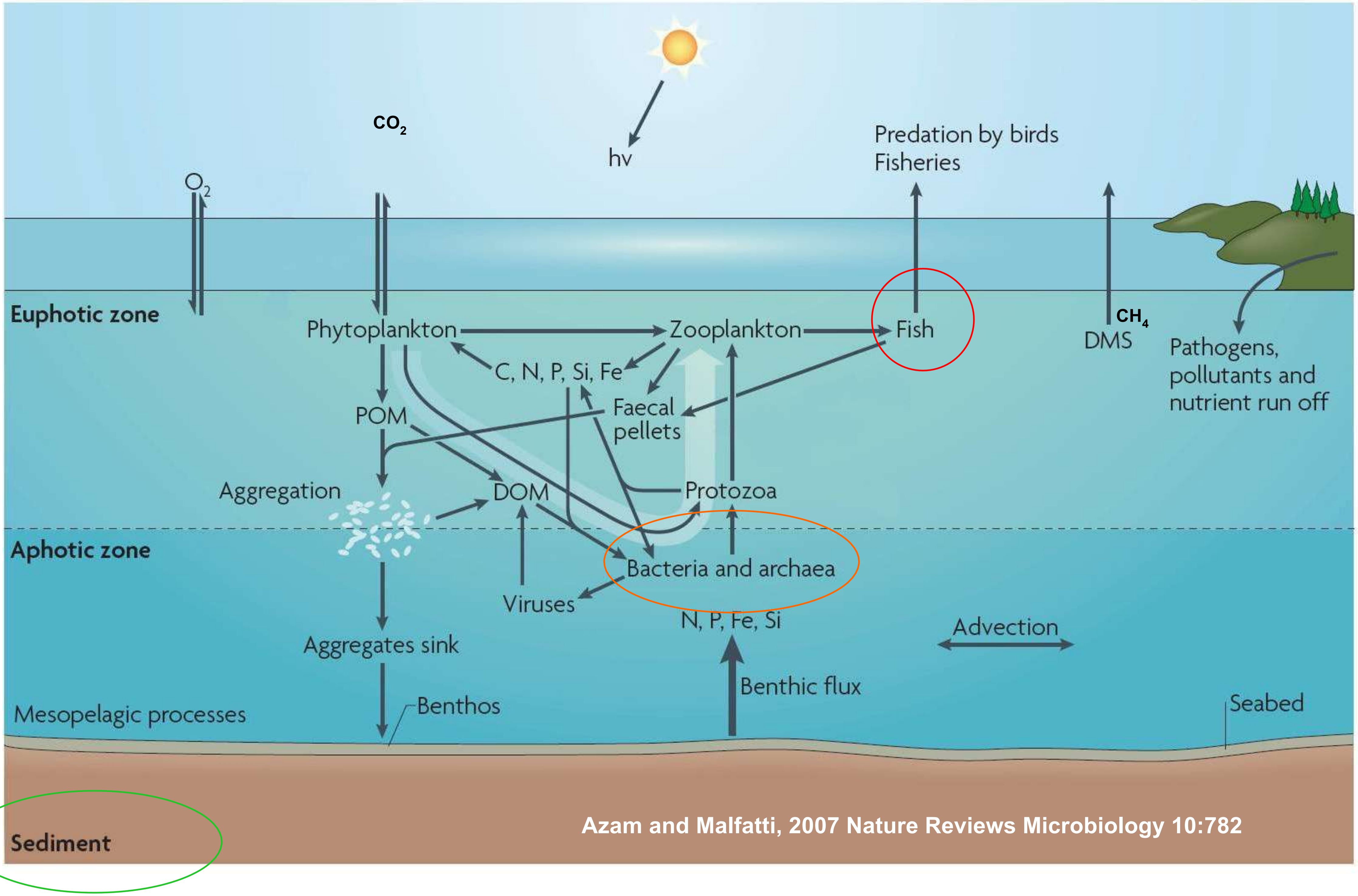
L03c: Diversity of Marine Bacteria, Archaea, Eukarya and Viruses

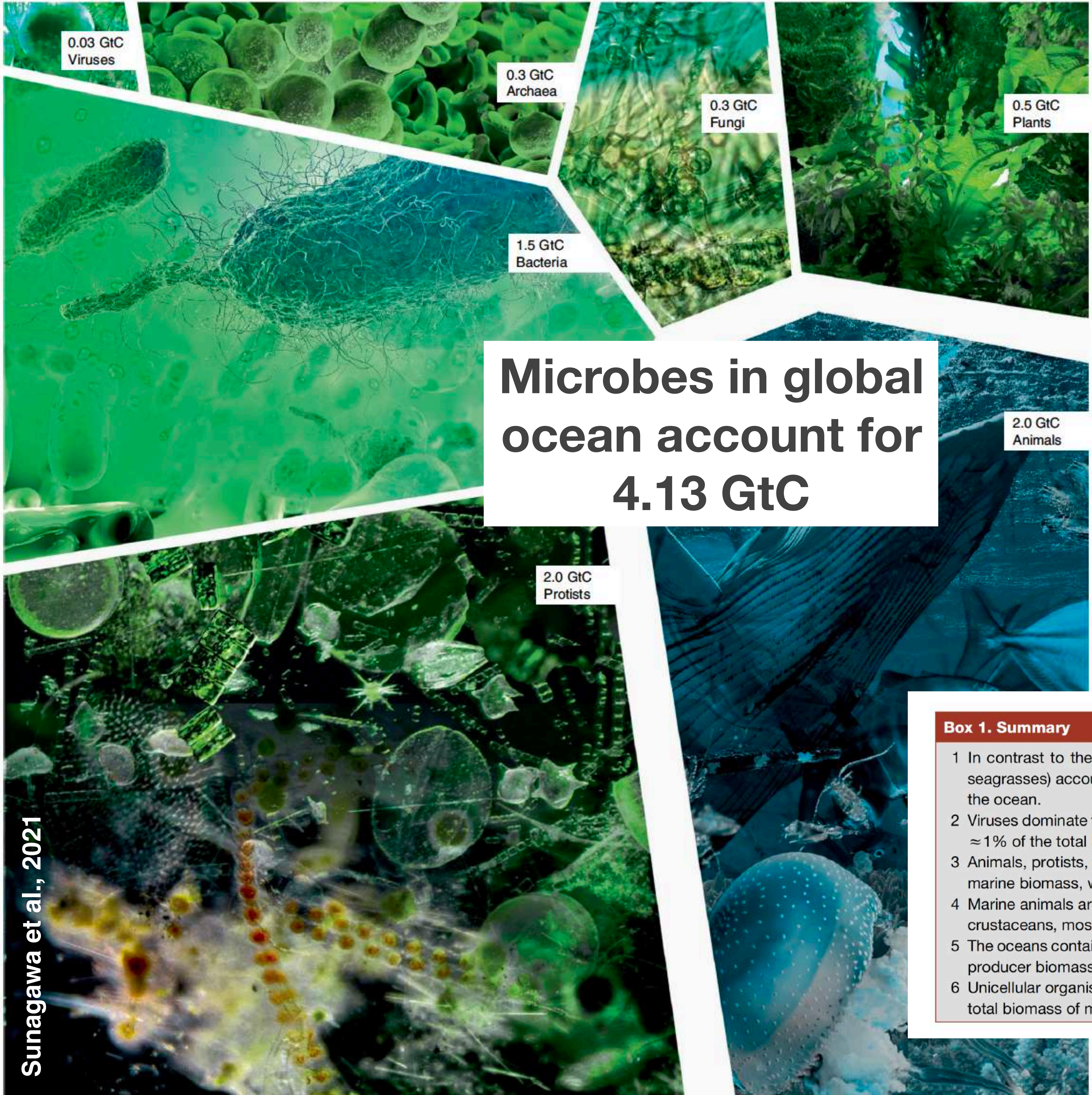
Marine microbes are highly diverse



Azam and Malfatti, 2007 Nature Reviews Microbiology 10:782

Microbes in global ocean account for 4.13 GtC

Gigatons of carbon



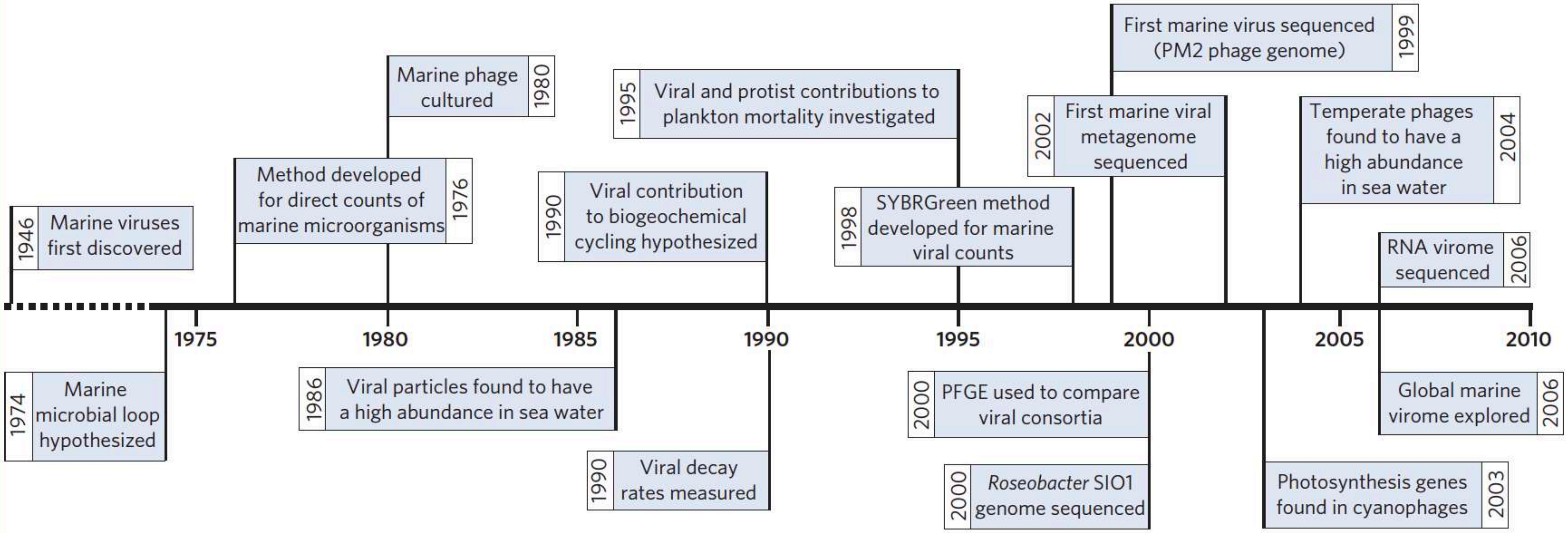
Sunagawa et al., 2021

Bar-On & Milo, 2019

Box 1. Summary

1 In contrast to their domination on land, plants (green algae and seagrasses) account for less than 10% of the total biomass in the ocean.
2 Viruses dominate the ocean in terms of number but constitute only ≈1% of the total biomass.
3 Animals, protists, and bacteria together account for ≈80% of the marine biomass, whereas on land they comprise only ≈2%.
4 Marine animals are dominated by small mesopelagic fish and crustaceans, mostly copepods, shrimp, and krill.
5 The oceans contain much more consumer biomass (≈5 Gt C) than producer biomass (≈1 Gt C).
6 Unicellular organisms contribute approximately two-thirds of the total biomass of marine organisms.

Box 1 | Highlights in marine virology research



1946 Marine viruses first discovered
1974 Marine microbial loop hypothesized
1976 Method developed for direct counts of marine microorganisms
1980 Marine phage cultured
1986 Viral particles found to have a high abundance in sea water
1990 Viral contribution to biogeochemical cycling hypothesized
1990 Viral decay rates measured
1995 Viral and protist contributions to plankton mortality investigated
1998 SYBRGreen method developed for marine viral counts
1999 First marine virus sequenced (PM2 phage genome)
2000 PFGE used to compare viral consortia
2000 *Roseobacter* SIO1 genome sequenced
2002 First marine viral metagenome sequenced
2003 Photosynthesis genes found in cyanophages
2004 Temperate phages found to have a high abundance in sea water
2006 RNA virome sequenced
2006 Global marine virome explored

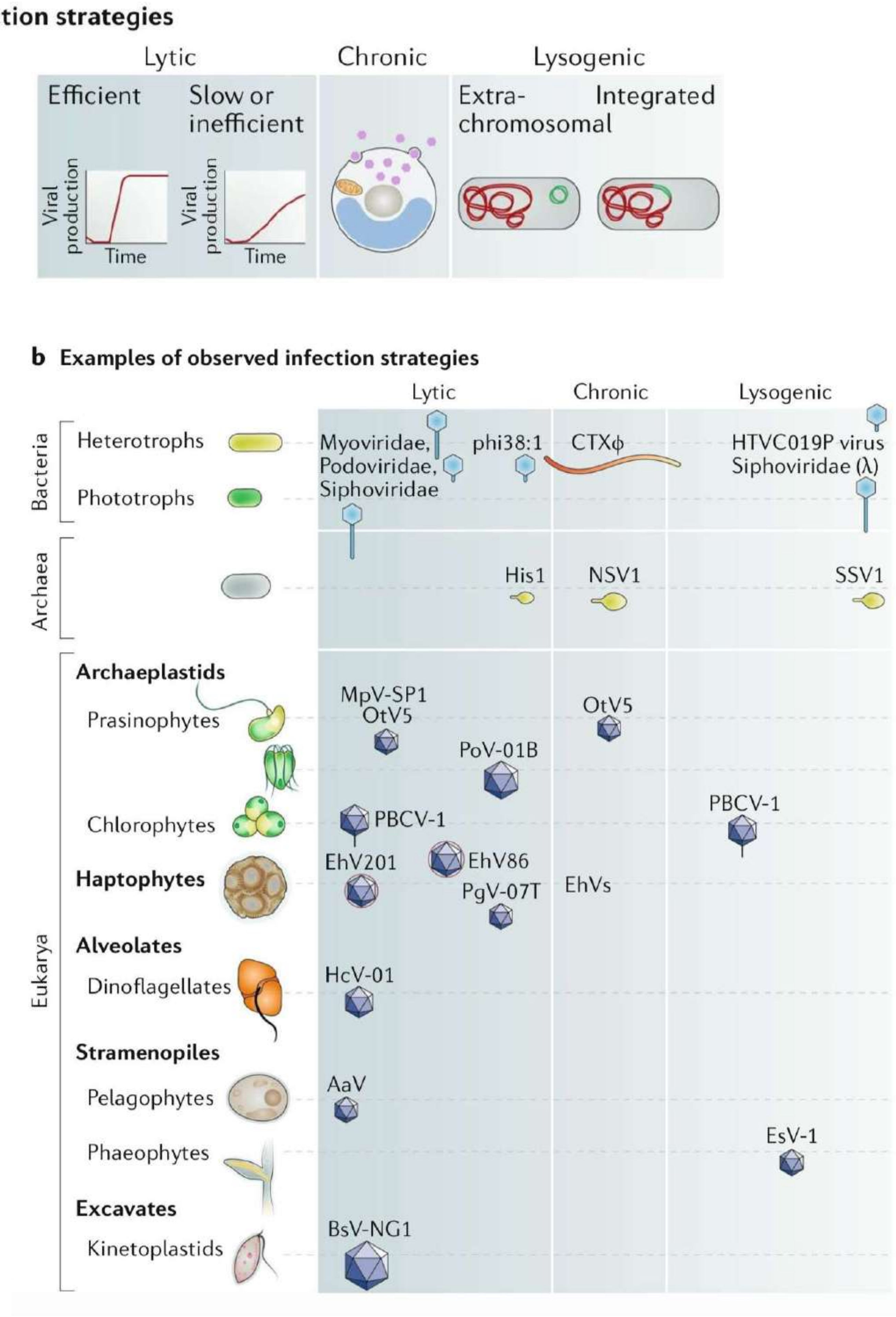
Bacterial viruses (phage) in sea water were first observed in the first half of the last century[59,60], although their presence remained unexplained until Lawrence Pomeroy hypothesized the 'marine microbial loop'[61] in 1974. In 1979, Francisco Torrella and Richard Morita discovered that marine viral particles were particularly abundant (10^4 per millilitre) and morphologically similar to phage[62], and phage from marine bacteria were soon cultured[63]. In the 1990s, much was learned about the genetic diversity of marine phage and eukaryotic viruses and their importance to the ecology of the marine plankton community. Numerous studies demonstrated the contribution of viruses and protists to global biogeochemical cycling arising from the lysis of plankton[4,39,64-66]. The first marine viral genomes were then sequenced[57,67], and genomics and metagenomics have since been used to characterize the diversity of both RNA viruses[68,69] and DNA viruses[7,70] in sea water, along with their effects on host physiology and ecology[71-73]. The timeline shown here (not to scale) lists the main events in marine virology research. PFGE, pulsed-field gel electrophoresis.

Rohwer & Thurber, 2009

Viruses

- Discovered in 1892, tobacco disease
- Moving genetic information
- Diverse life strategies
- Interaction with every domain of life
- Interaction with other viruses
- Cells can become resistant

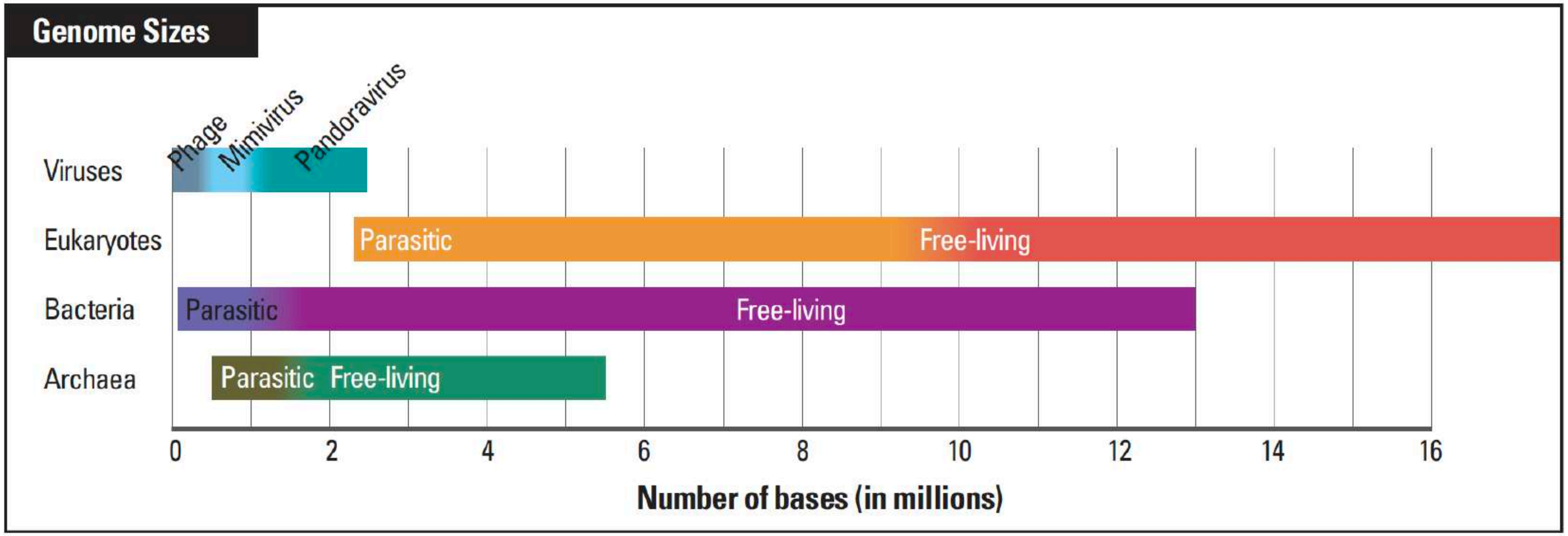
Zimmerman et al. 2020



Marine Viruses

- Total volume of the ocean ~$1.3x10^{21}$ litres —> ~$3x10^{9}$ viruses per litre = ~$4x10^{30}$ viruses
- 1% of biomass = 0.03 Gt = 75 millions blue whales
- 10^{30} viruses in the ocean, if stretched end to end, would span farther than the nearest 60 galaxies
- Predators of Bacteria, Archaea and Eukarya
- Top-down control —> viral shunt, futile cycle of C
- More productive habitat higher viral abundance in water and sediment
- Lytic, lysogenic, chronic cycle
- 1 in 10^{5} lysogenic cells will revert to lytic cycle due to host stress
- Lysogenic phage can survive periods when prey abundant is low or low metabolic activity
- Lysogeny very common ~50% in cultivated isolates of off-shore ocean
- Viral decay 1 or 3-10% per hour population due to UV, proteases, nucleases and adsorption on organic matter

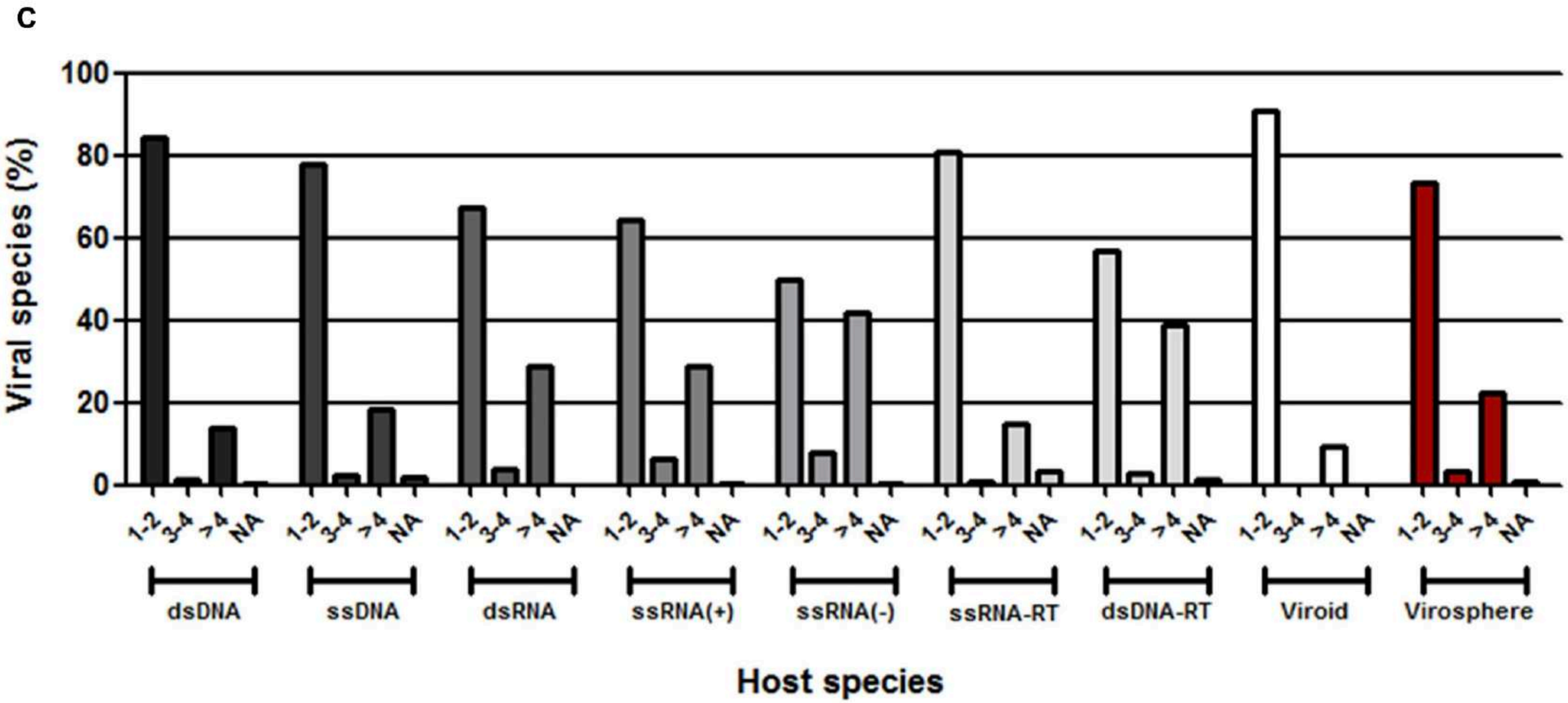
Marine Viruses, II



Pennisi, 2013

- At any given time, 1-4% of microbial cells have some virions inside
- ~40-50% of marine bacteria are killed every day
- 20-30% marine bacteria are infected
- 10^{23} viral infections occur every second
- Narrow host range
- Phytoplankton bloom can be terminated by viruses

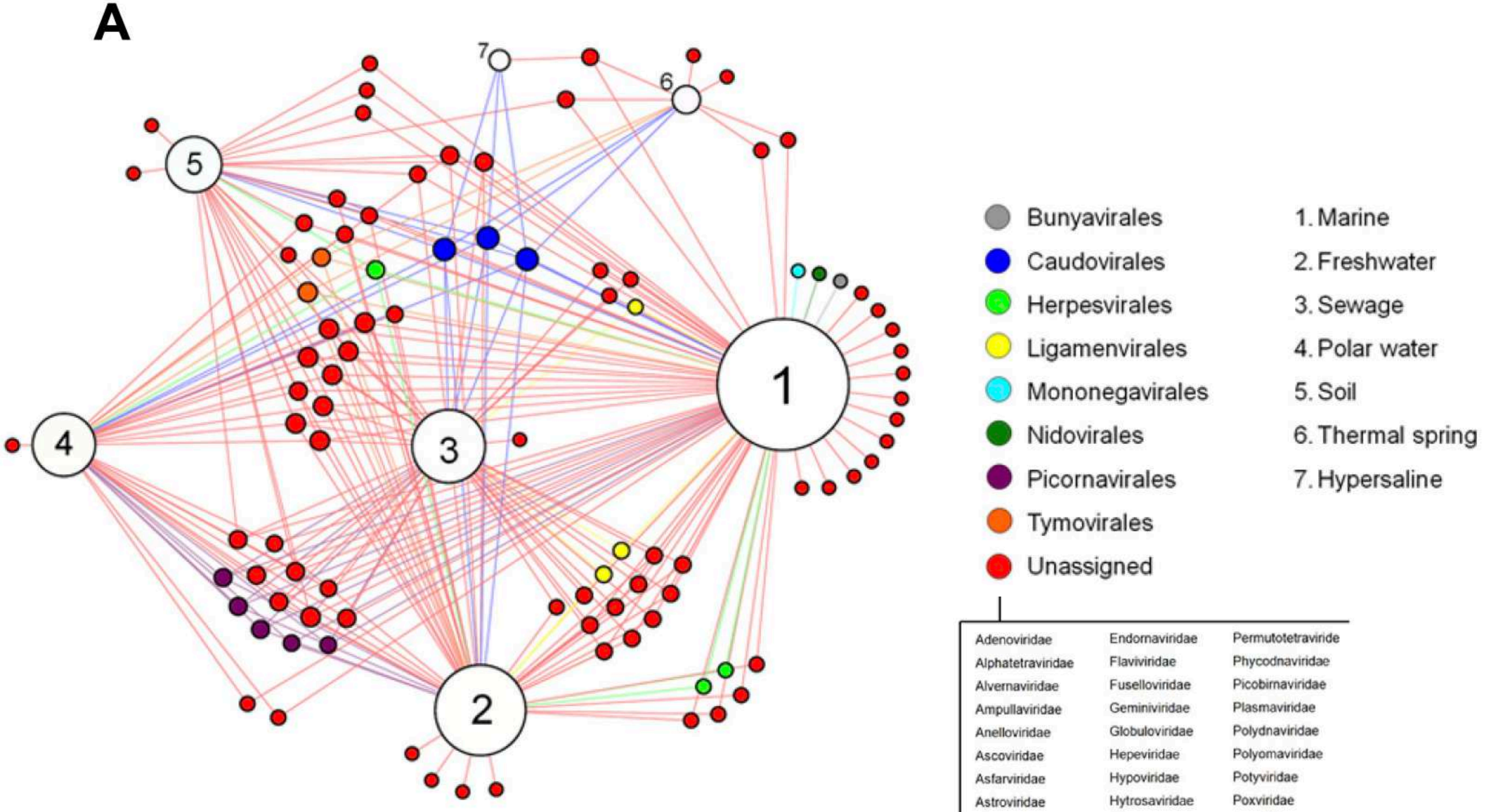
Host range of the known virosphere



Rodriguez et al., 2017

Viral diversity on Earth

Rodriguez et al., 2017



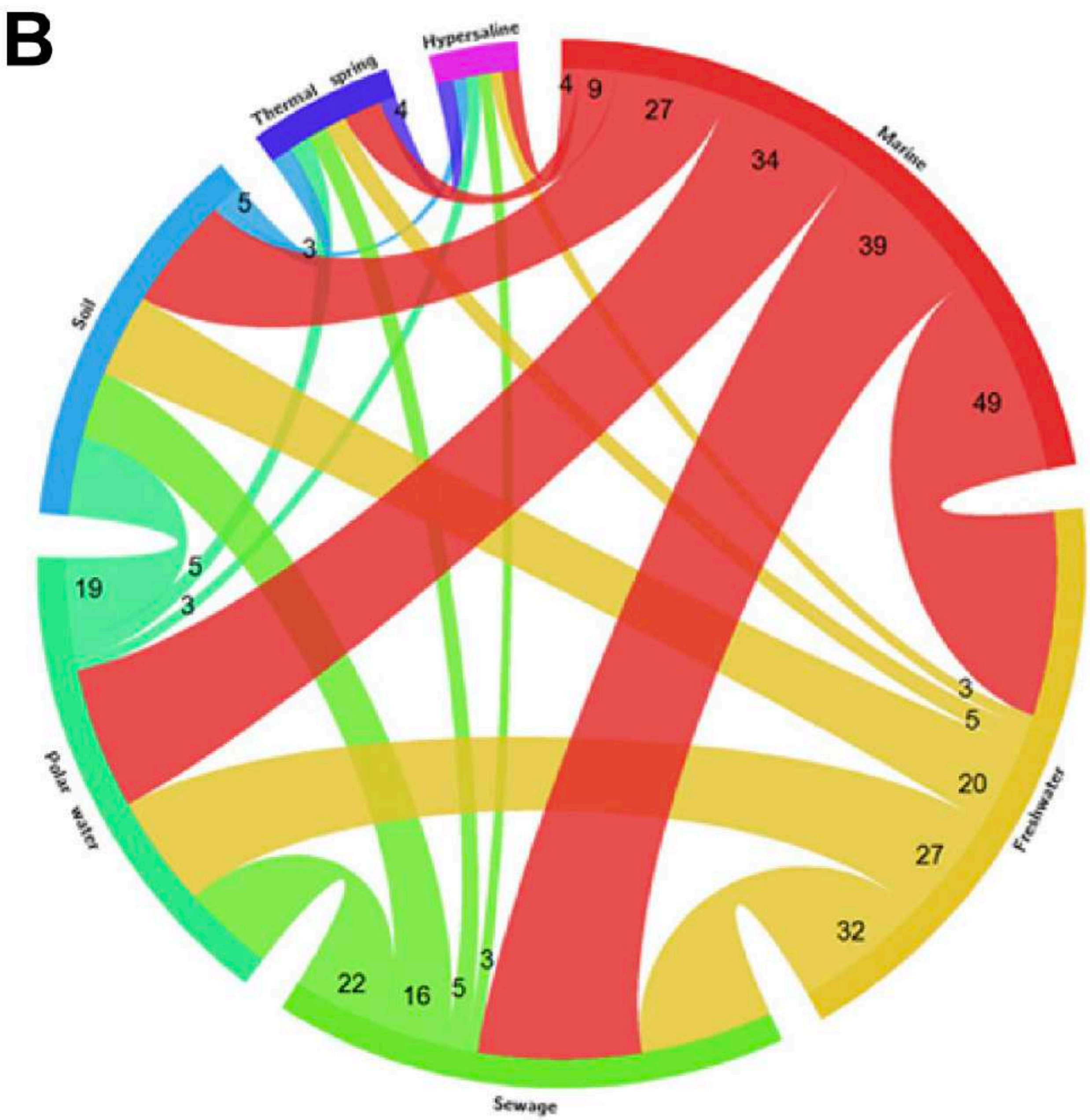
Adenoviridae
Alphatetraviridae
Alvernaviridae
Ampullaviridae
Anelloviridae
Ascoviridae
Asfarviridae
Astroviridae
Bacillariornaviridae
Bacillarnaviridae
Baculoviridae
Barnaviridae
Bicaudaviridae
Bidnaviridae
Birnaviridae
Bromoviridae
Caliciviridae
Carmotetraviridae
Caulimoviridae
Chrysoviridae
Circoviridae
Closteroviridae
Comoviridae
Corticoviridae
Cystoviridae
Endornaviridae
Flaviviridae
Fuselloviridae
Geminiviridae
Globuloviridae
Hepeviridae
Hypoviridae
Hytrosaviridae
Inoviridae
Iridoviridae
Lavidaviridae
Leviviridae
Luteoviridae
Marseilleviridae
Microviridae
Mimiviridae
Nanoviridae
Nimaviridae
Nodaviridae
Nudiviridae
Orthomyxoviridae
Papillomaviridae
Partitiviridae
Partiviridae
Parvoviridae
Permutotetraviride
Phycodnaviridae
Picobirnaviridae
Plasmaviridae
Polydnaviridae
Polyomaviridae
Potyviridae
Poxviridae
Reoviridae
Retroviridae
Sequiviridae
Spiraviridae
Tectiviridae
Tetraviridae
Tombusviridae
Totiviridae
Turriviridae
Virgaviridae
Sobemovirus
Bacilladnavirus
Cilevirus
Noravirus
Pandoravirus
Salterprovirus
Ourmiavirus

- Myoviridae, Podoviridae, and Siphoviridae (phages belonging to the order Caudovirales) were found in all of the searched environments —> “everything is everywhere and environment selects —> it is true for some but not for others

- Each node represents an environment (white) or viral groups (families or known viral genus – colored nodes) classified according to the orders formally recognized by the ICTV. The viruses not currently assigned in any order are listed. The node diameter is proportional to the edge degree. A total of 96 viral groups are represented

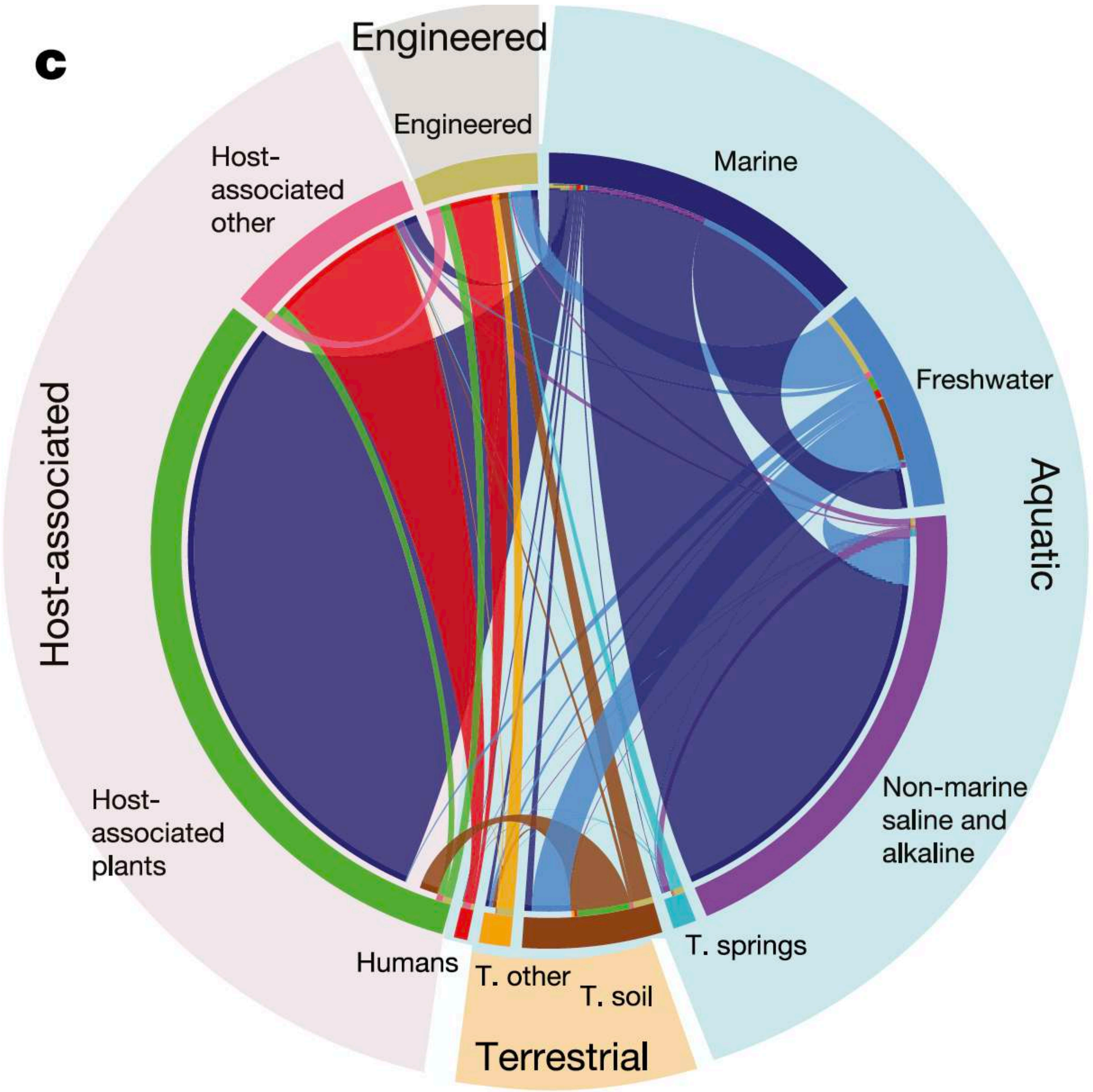
Viral connectivity among environments

Rodriguez et al., 2017



- Different amount of viral groups are shared among the environments
- Marine shared up to 49 viral groups with other environments
- Reinforcing the ubiquity of viruses on the planet
- Marine, Freshwater, Hypersaline, Sewage, Polar water, Soil, Thermal spring

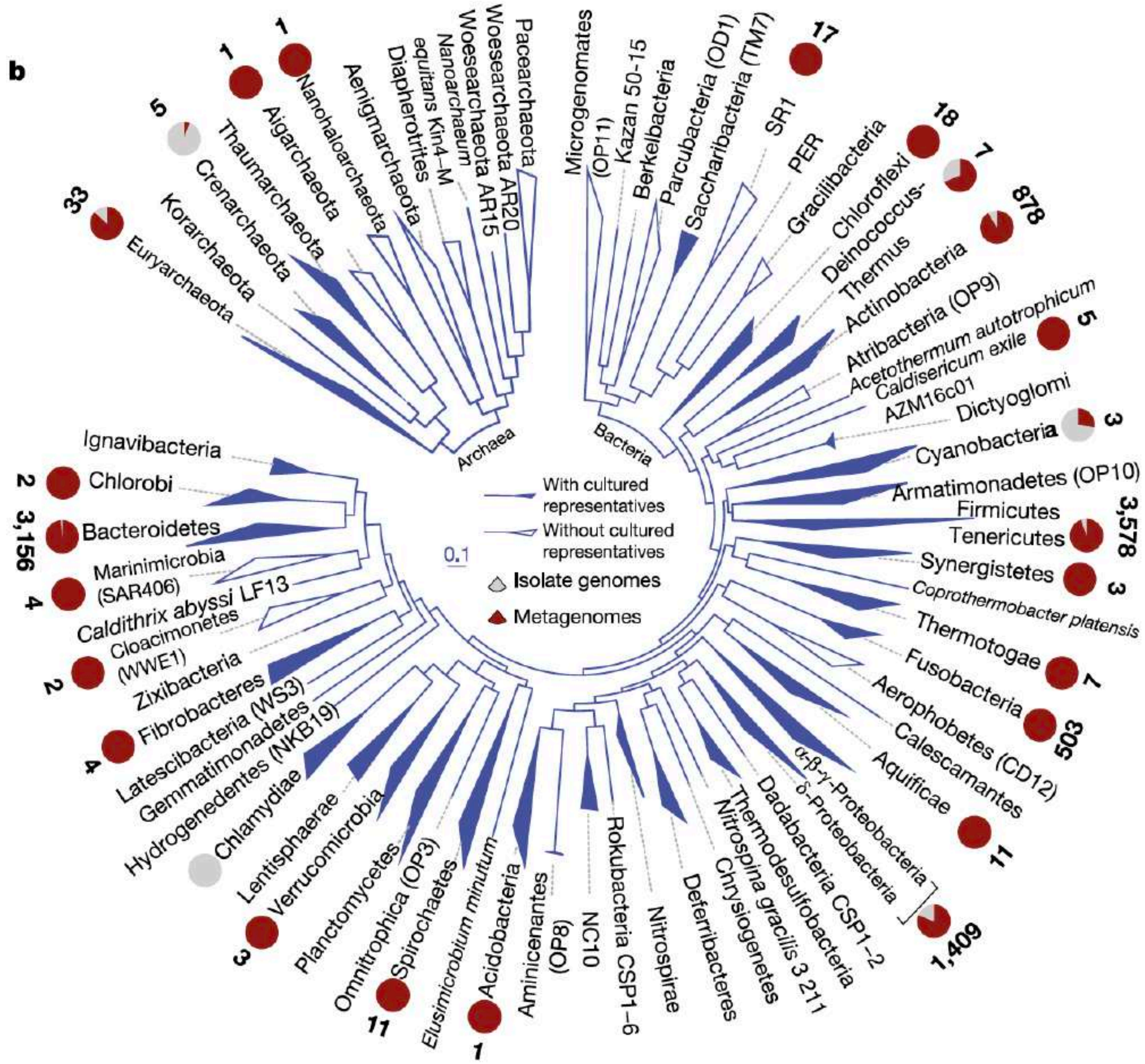
Viral connectivity in OneHealth



- Healthy Environment—> healty Plants and Animals —> Healthy Humans
- Pairwise connection between habitat types based on shared viral sequences
- Engineered: bioreactors and wastewater
- Humans are sharing viriome with host-associated and Engineered mostly

Paez-Espino et al., 2016

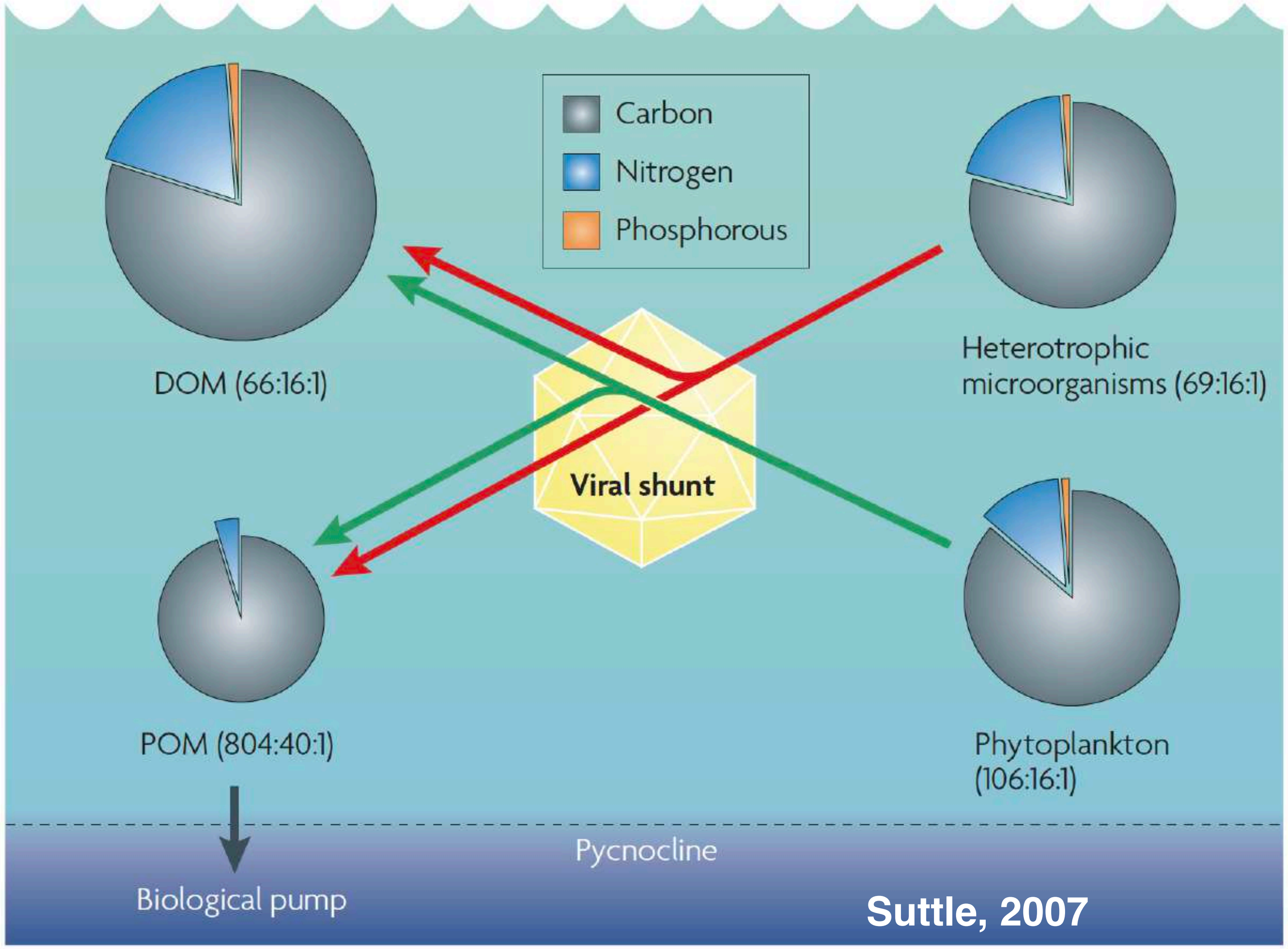
Virus-Host connectivity



Paez-Espino et al., 2016

- Phylogenetic distribution of bacterial and archaeal hosts
- For each phylum a pie chart indicates the fraction of sequences assigned to this phylum from metagenomic viral contigs (red), and isolate viruses (grey)
- The number of metagenomic viral contigs assigned to each phylum is indicated by the numbers next to pie charts

Viral shunt



Suttle, 2007

The numbers in parentheses are the estimated ratios of carbon:nitrogen:phosphorous (in atoms)

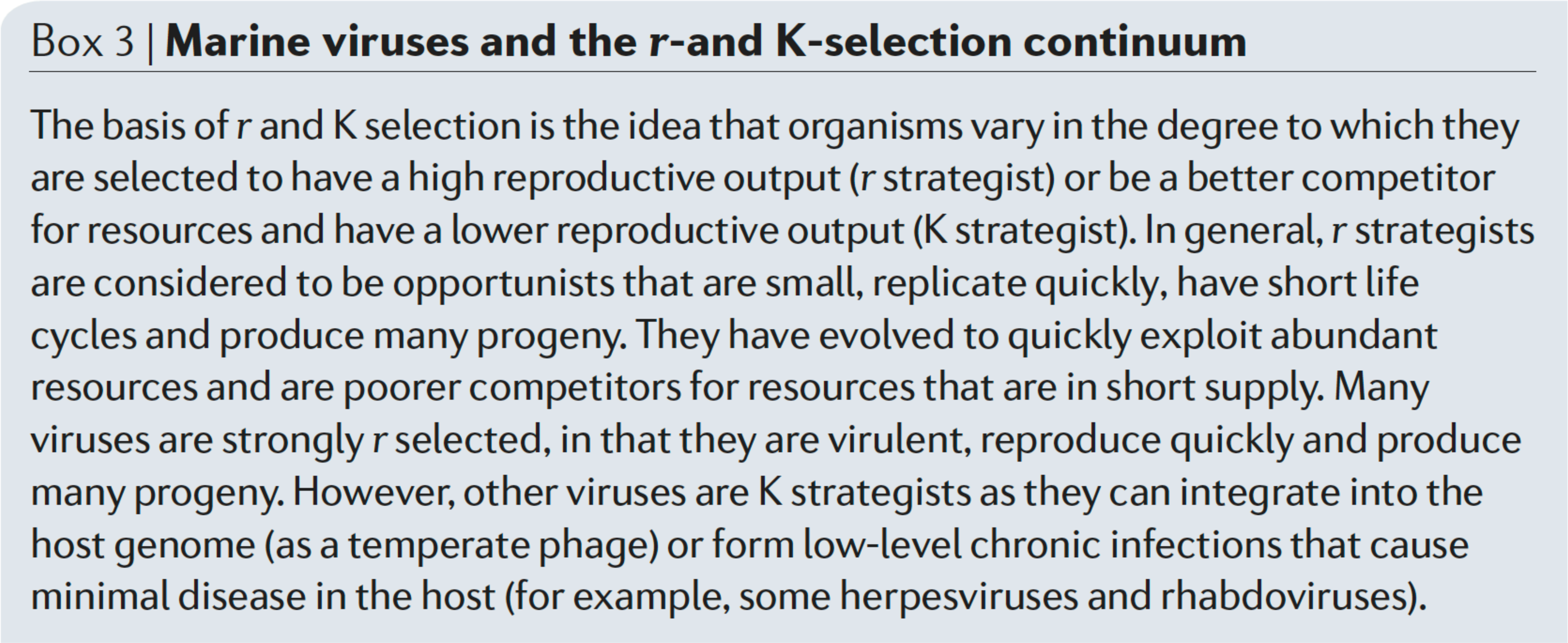
The viral shunt moves material from heterotrophs and photoautotrophs into POM and DOM

Stoichiometric effect, such that the POM and DOM pool chemical compositions are not the same as the composition of the organisms from which the material was derived

Increase CO_2 system respiration due to release of organic material

Highly labile materials (e.g. amino acids, nucleic acids), tend to be recycled in the photic zone, whereas more recalcitrant carbon-rich material, such as that found in cell walls, is probably exported to deeper waters

- Material that is exported to deeper waters by the viral shunt is probably more carbon rich than the material from which it was derived —> increase the efficiency of the biological pump: viral shuttle

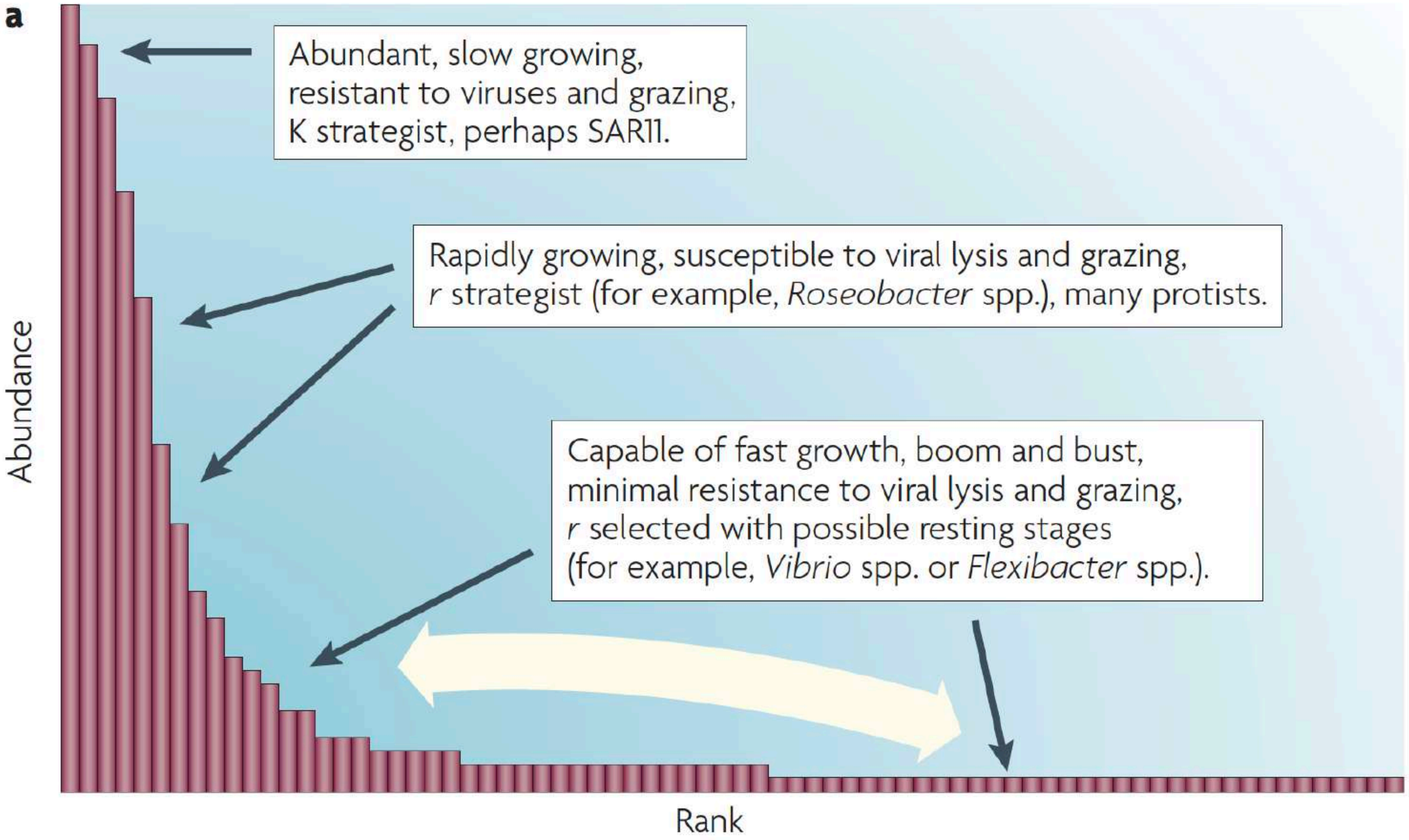


Box 3 | Marine viruses and the *r*-and K-selection continuum

The basis of *r* and K selection is the idea that organisms vary in the degree to which they are selected to have a high reproductive output (*r* strategist) or be a better competitor for resources and have a lower reproductive output (K strategist). In general, *r* strategists are considered to be opportunists that are small, replicate quickly, have short life cycles and produce many progeny. They have evolved to quickly exploit abundant resources and are poorer competitors for resources that are in short supply. Many viruses are strongly *r* selected, in that they are virulent, reproduce quickly and produce many progeny. However, other viruses are K strategists as they can integrate into the host genome (as a temperate phage) or form low-level chronic infections that cause minimal disease in the host (for example, some herpesviruses and rhabdoviruses).

Suttle, 2007

Distribution of marine microbes along the K- and r-selection continuum



Abundant, slow growing, resistant to viruses and grazing, K strategist, perhaps SAR11.

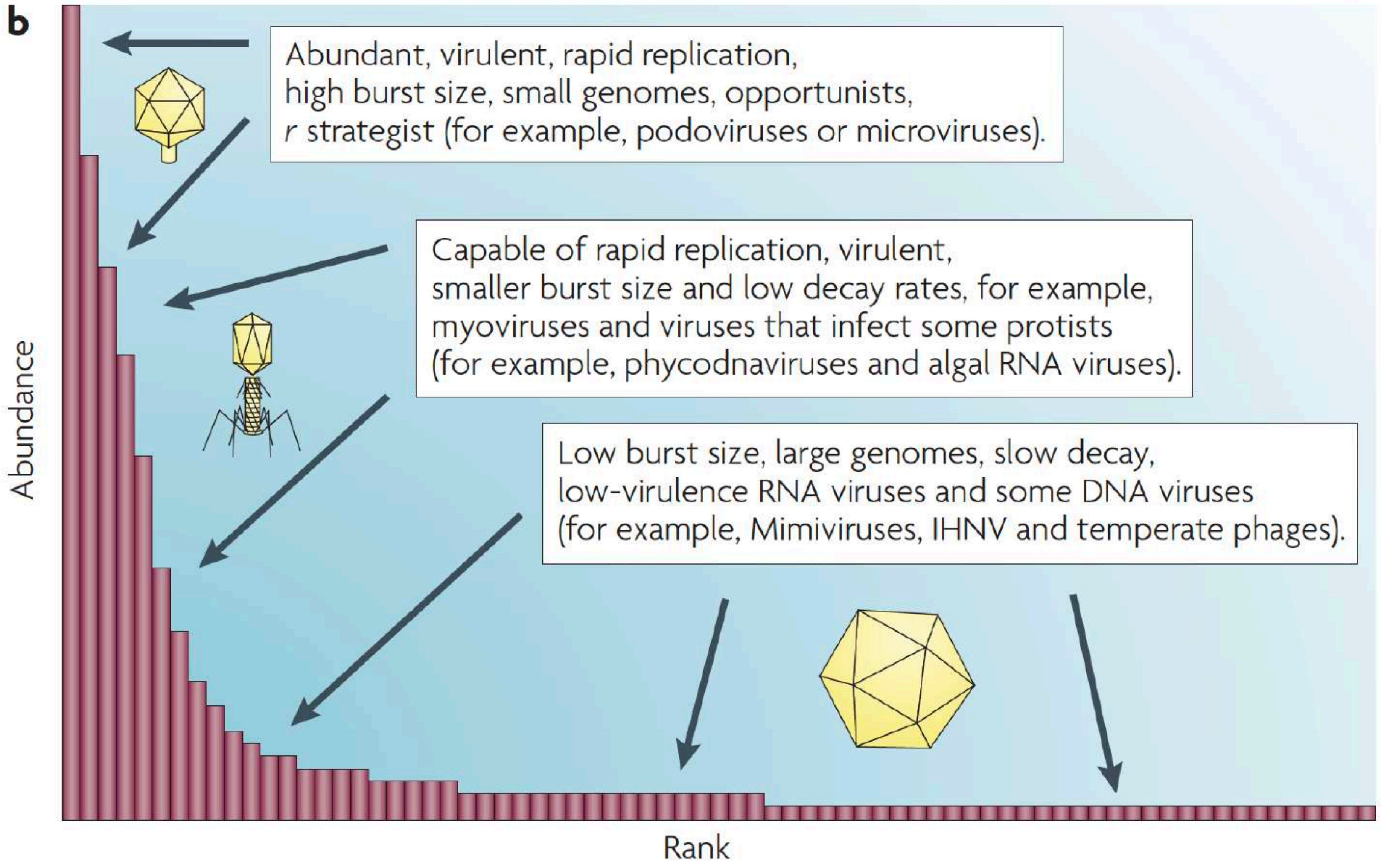
Rapidly growing, susceptible to viral lysis and grazing, *r* strategist (for example, *Roseobacter* spp.), many protists.

Capable of fast growth, boom and bust, minimal resistance to viral lysis and grazing, *r* selected with possible resting stages (for example, *Vibrio* spp. or *Flexibacter* spp.).

Suttle, 2007

- **A rank–abundance curve**
- **Most abundant organism in the ocean, SAR11, probably K-selected organism: slow maximum growth rates but are resistant to viral lysis and grazing**
- **Less abundant organisms, such as *Roseobacter* spp. and *Vibrio* spp., are capable of rapid growth but are highly susceptible to viral infection and grazing**
- **Rarer microorganisms are more r selected, whereas the microorganisms that dominate the biomass are the most K selected**
- **Yellow arrow represents low abundance taxa that periodically encounter conditions that are conducive to rapid growth, but as their abundance increases the rates of viral infection also increase, resulting in host cell lysis and a return to low abundance**

Marine viruses along the K- and r-selection continuum



Abundant, virulent, rapid replication, high burst size, small genomes, opportunists, *r* strategist (for example, podoviruses or microviruses).

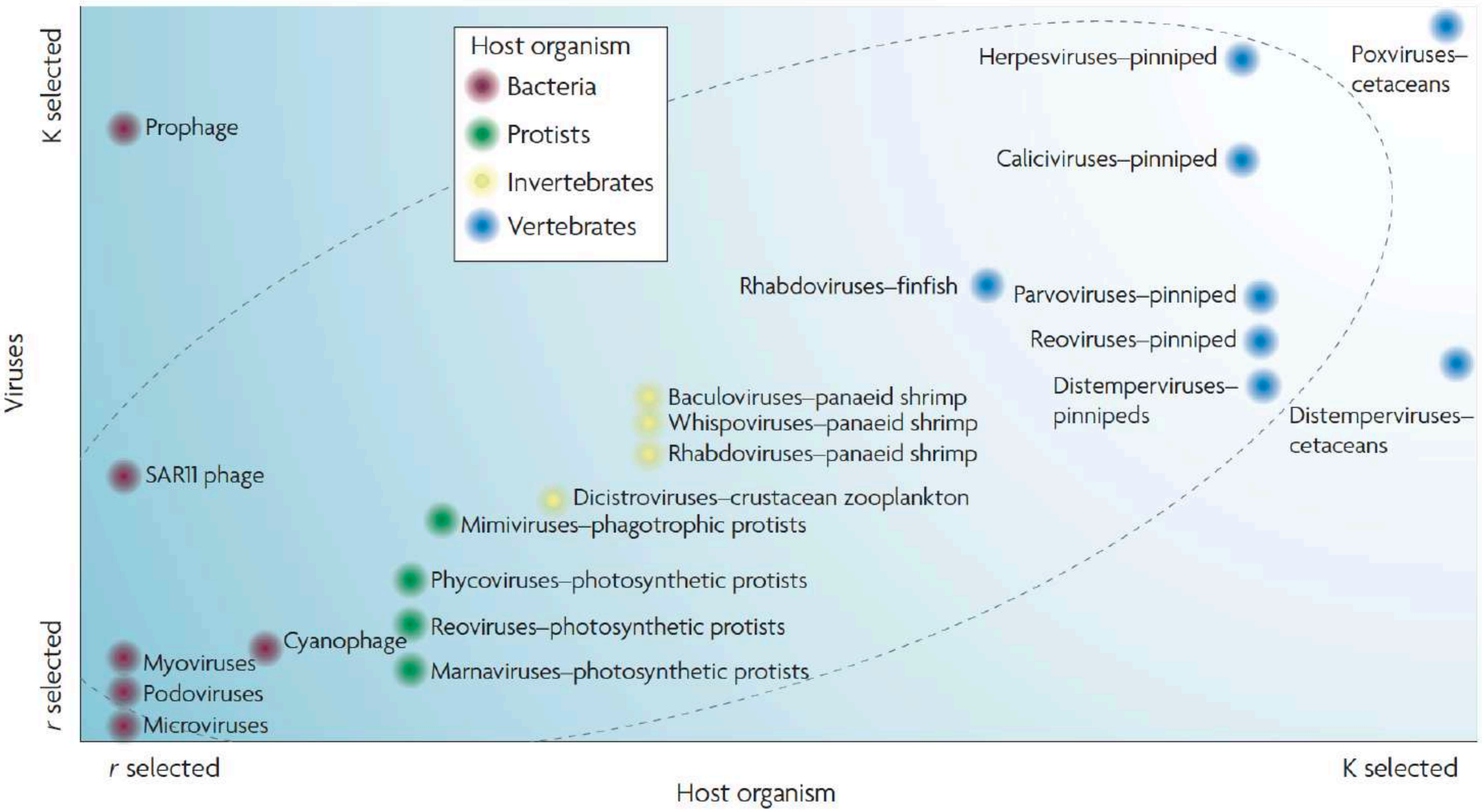
Capable of rapid replication, virulent, smaller burst size and low decay rates, for example, myoviruses and viruses that infect some protists (for example, phycodnaviruses and algal RNA viruses).

Low burst size, large genomes, slow decay, low-virulence RNA viruses and some DNA viruses (for example, Mimiviruses, IHNV and temperate phages).

Suttle, 2007

- **A rank–abundance curve showing marine viruses**
- **In contrast to SAR11, the most abundant viruses are r selected, virulent, have small genome sizes and are short-lived, population structure is probably uneven, with many of the viruses at any given time being progeny from a limited number of lytic events**
- **The rarer, more K-selected viruses have larger genomes, decay slowly and can form stable associations with their hosts, some RNA and DNA viruses that are long-lived and have low virulence. IHNV, infectious haematopoietic necrosis virus.**

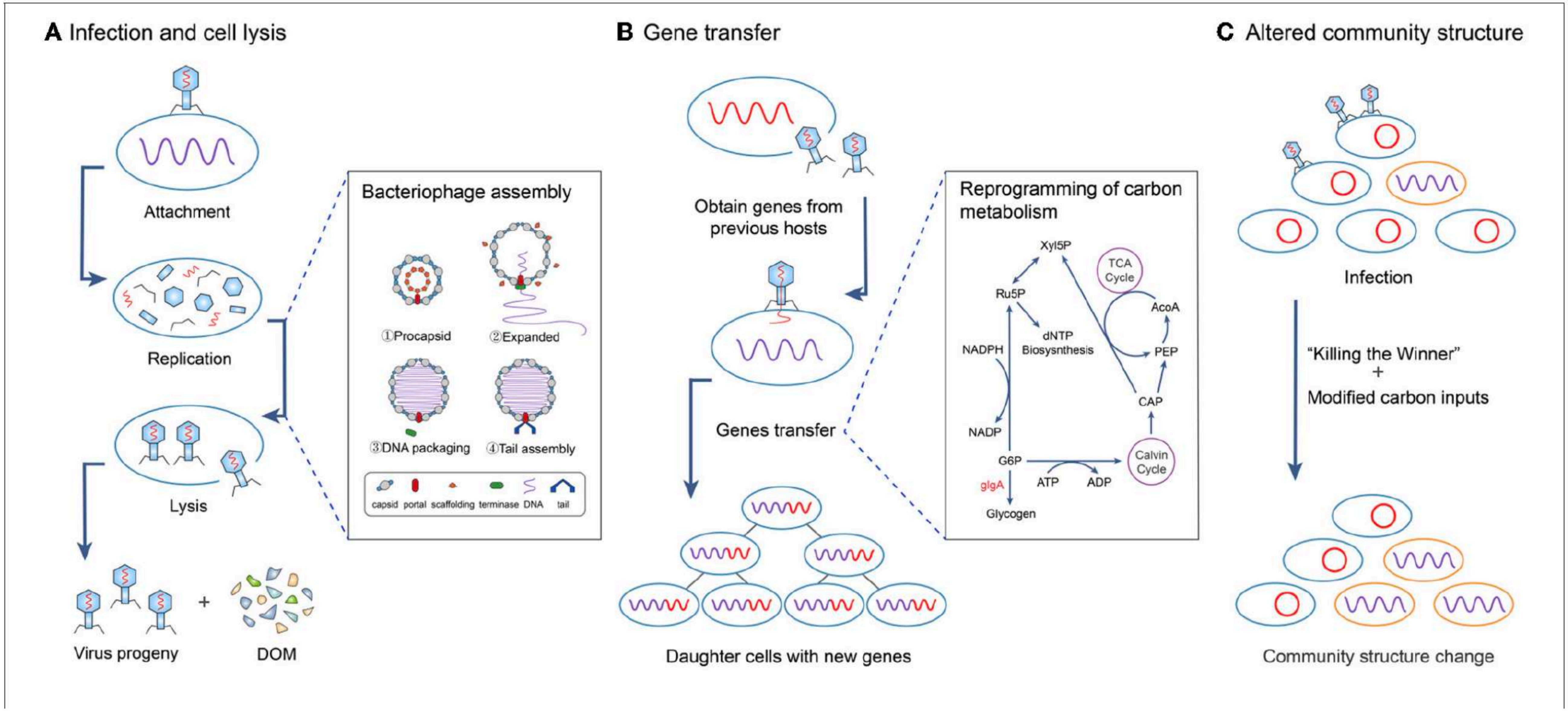
Marine viruses-hosts distribution along an r- and K-selection continuum



Suttle, 2007

- Prokaryotes and the viruses that infect them are more r selected
- Temperate phages that form stable associations with the hosts they infect are more K selected than lytic phages
- Viruses that infect larger, longer-lived organisms are more K selected, tend to have lower virulence and, in some cases, form stable associations with the organisms they infect
- The individual host–virus combinations should be considered as a ‘cloud’ rather than discrete points

Viruses affect microbial community structures and regulate C cycling



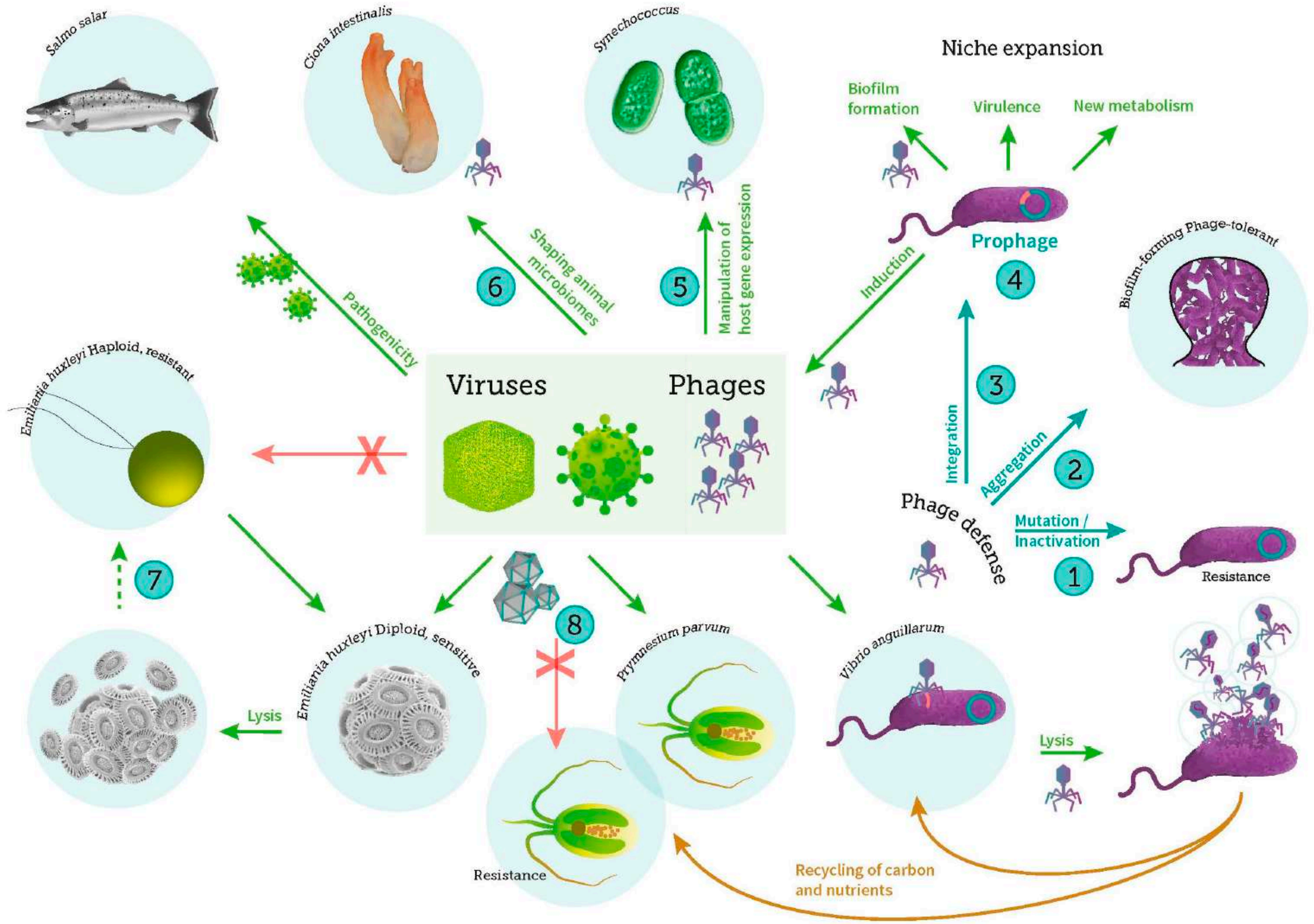
Gao et al., 2022

(A) Viruses infect microbial hosts and invade and destroy microbial cells (lysis) leading to C direct release in dissolved organic matter (DOM)

(B) After virus infection, gene transfer from virus (and/or previous host) previous host reprograms C metabolism

(C) Virus infection changes magnitude of C inputs and changes microbial community structure

Virus-host interactions in marine ecosystem



Middelboe & Brussard, 2017

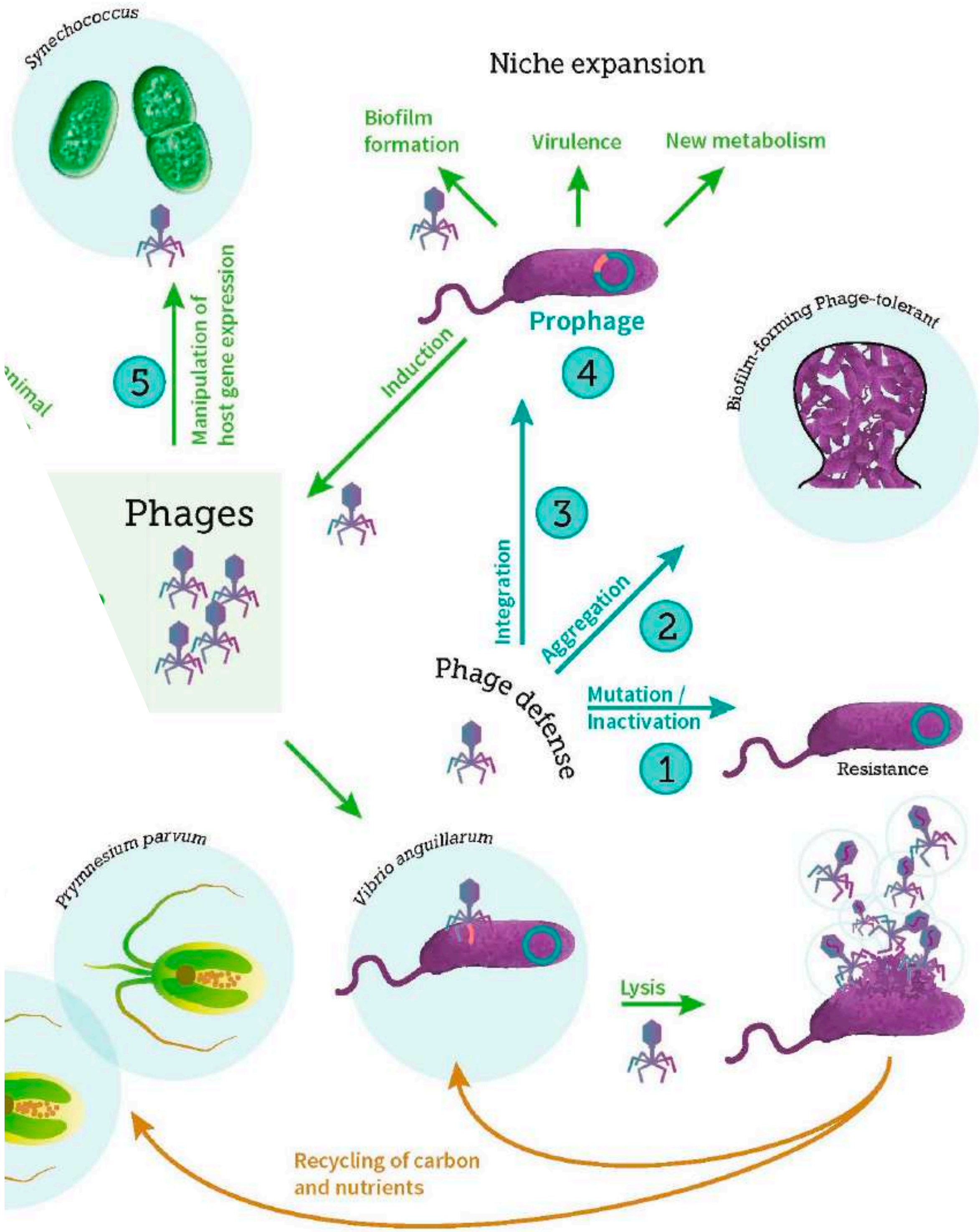
(1) Bacteria can prevent phage infection by mutational modification of surface receptors or by enzymatic degradation of the incoming phage DNA

(2) Protection of cells in aggregates or biofilms can be a defense strategy against phage infection

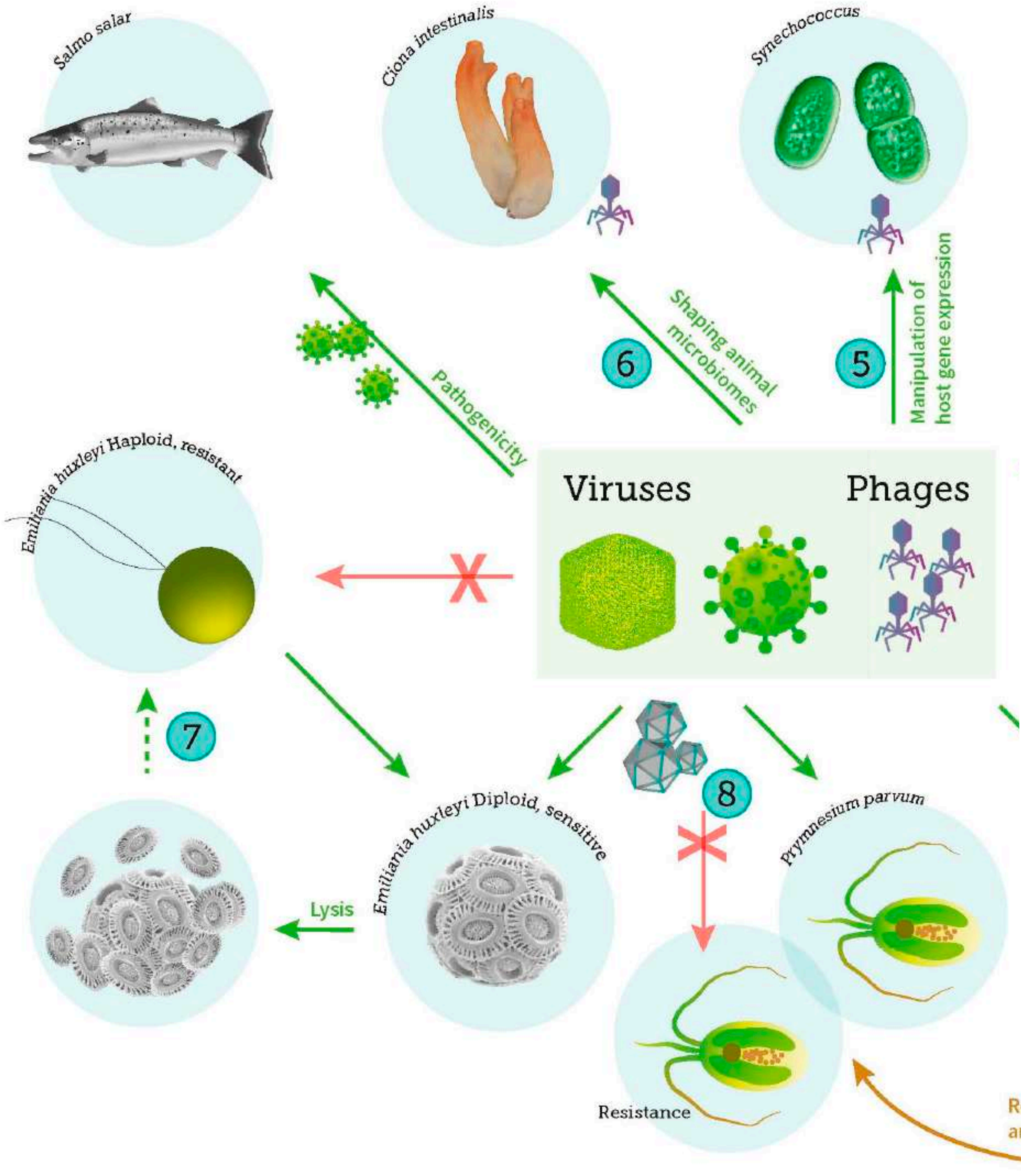
(3) Infection by temperate phages can result in the integration of the phage DNA in the host genome as prophage —>The integrated prophage can prevent infection by similar phages (Superinfection exclusion mechanism)

(4) Integrated phage can contribute with important genetic information to the host that may expand its metabolic or virulence properties —> Prophage induction leads to the release of new phages and may also stimulate biofilm formation

(5) Phages can manipulate host gene expression in cyanobacteria for improved infection efficiency, either by exploiting the host genes or by encoding host photosynthesis genes which are then expressed during infection



Middelboe & Brussard, 2017



(6) Phage interaction with bacterial hosts contributes to shaping the gut microbiome of invertebrates (e.g., tunicates), thus affecting the symbiotic relationship between gut microbes and their hosts

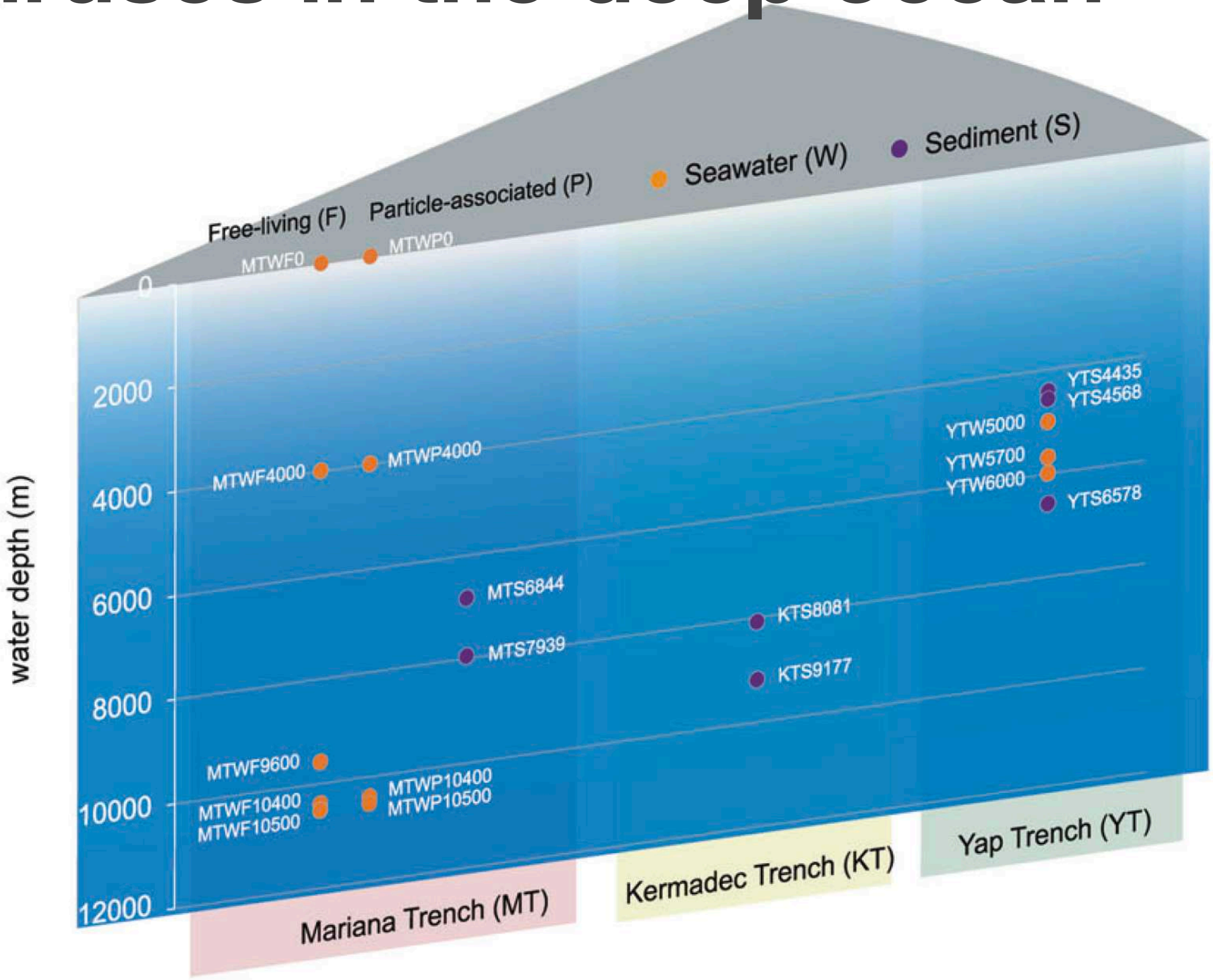
(7) In the coccolithophorid phytoplankton Emiliania huxleyi the diploid virally infected cells may undergo viral induced lysis or re-emerge (dotted arrow) as haploid cells containing viral RNA and lipids. These haploid cells are thought to resist virus infection (as indicated with the X) and develop into the diploid cells by karyogamy

(8) The large and diverse group of nucleocytoplasmic large DNA viruses (NCLDV) infects a range of photosynthetic protists (e.g. prasinophytes Micromonas pusilla and Ostreococcus tauri, the toxin-producing haptophyte Prymnesium parvum) thus affecting mortality, diversity and production of phytoplankton

These interactions are strongly controlled by environmental factors such as temperature, nutrient availability and light

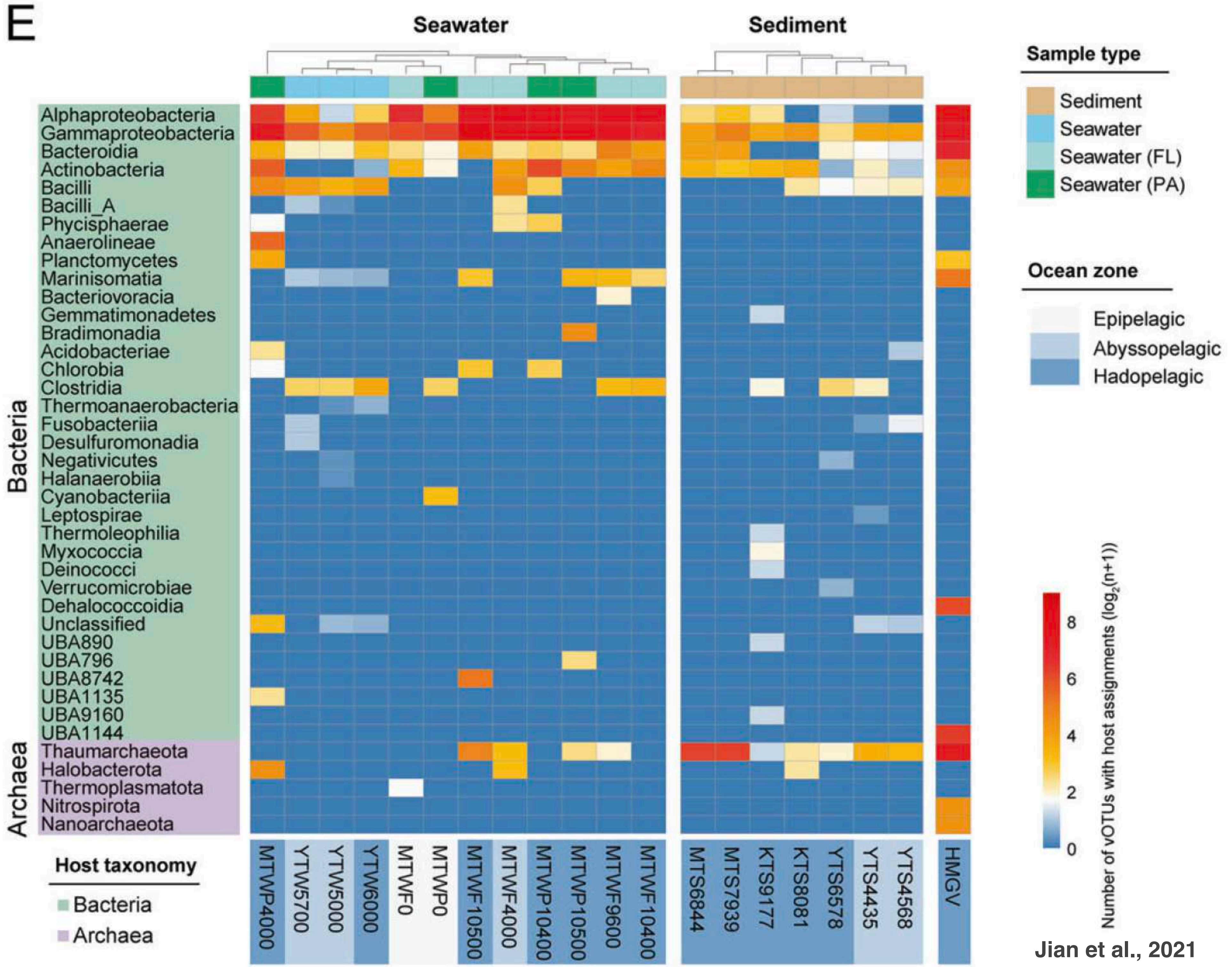
Middelboe & Brussard, 2017

Viruses in the deep ocean



Jian et al., 2021

Distribution of predicted bacterial and archaeal hosts of trench viruses



Jian et al., 2021

- Heatmaps show number of vOTUs with host assignments, which are hierarchically clustered by samples
- Bacterial and archaeal host taxonomy shown are in the class and phylum level
- Bars on the top and bottom of the figure indicate the type and ocean zone of each sample
- FL free living, PA particle associated

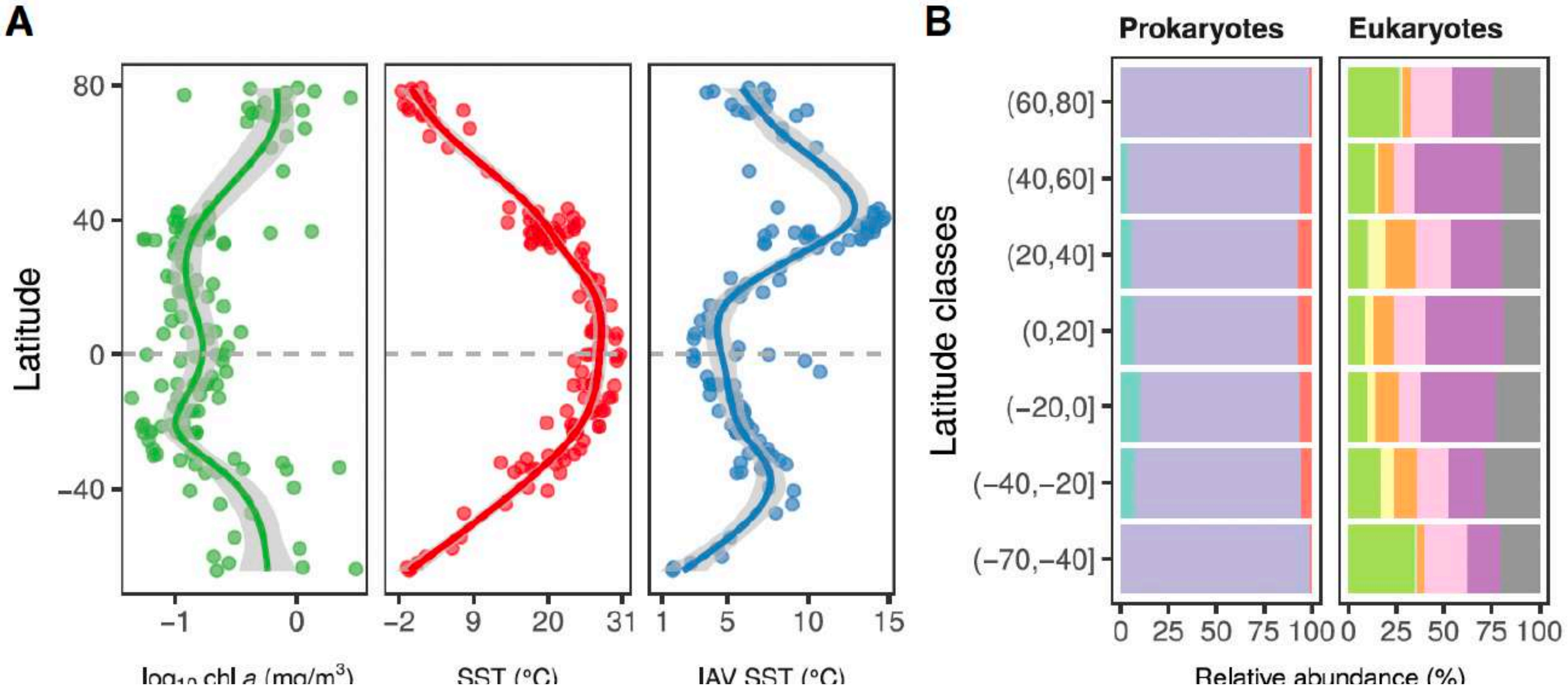
- Niche-specific viral community

- Hadopelagic seawater preferably adopting lysogenic lifestyles

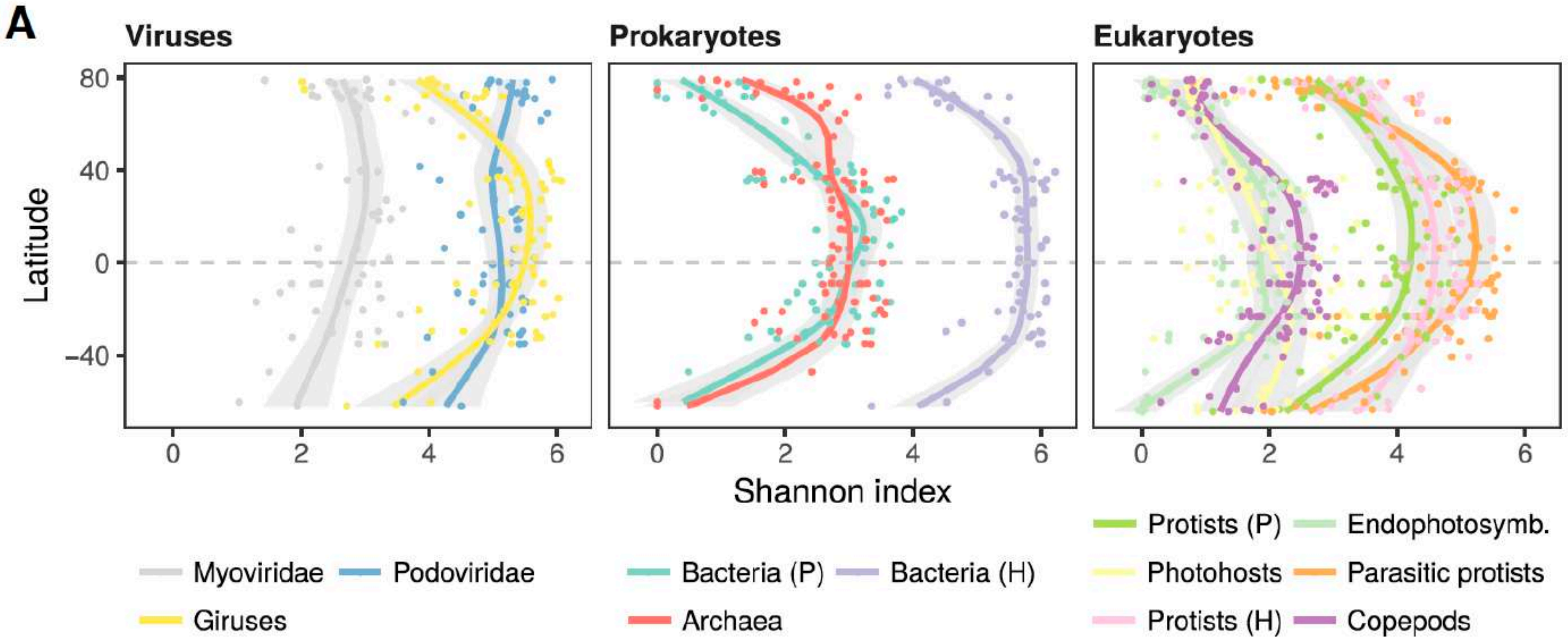
- In hadopelagic, auxiliary genes for utilization of refractory organic matter

Tara surface ocean Viruses across a latitudinal gradient 0-1000 m

Ibarbalz et al., 2019

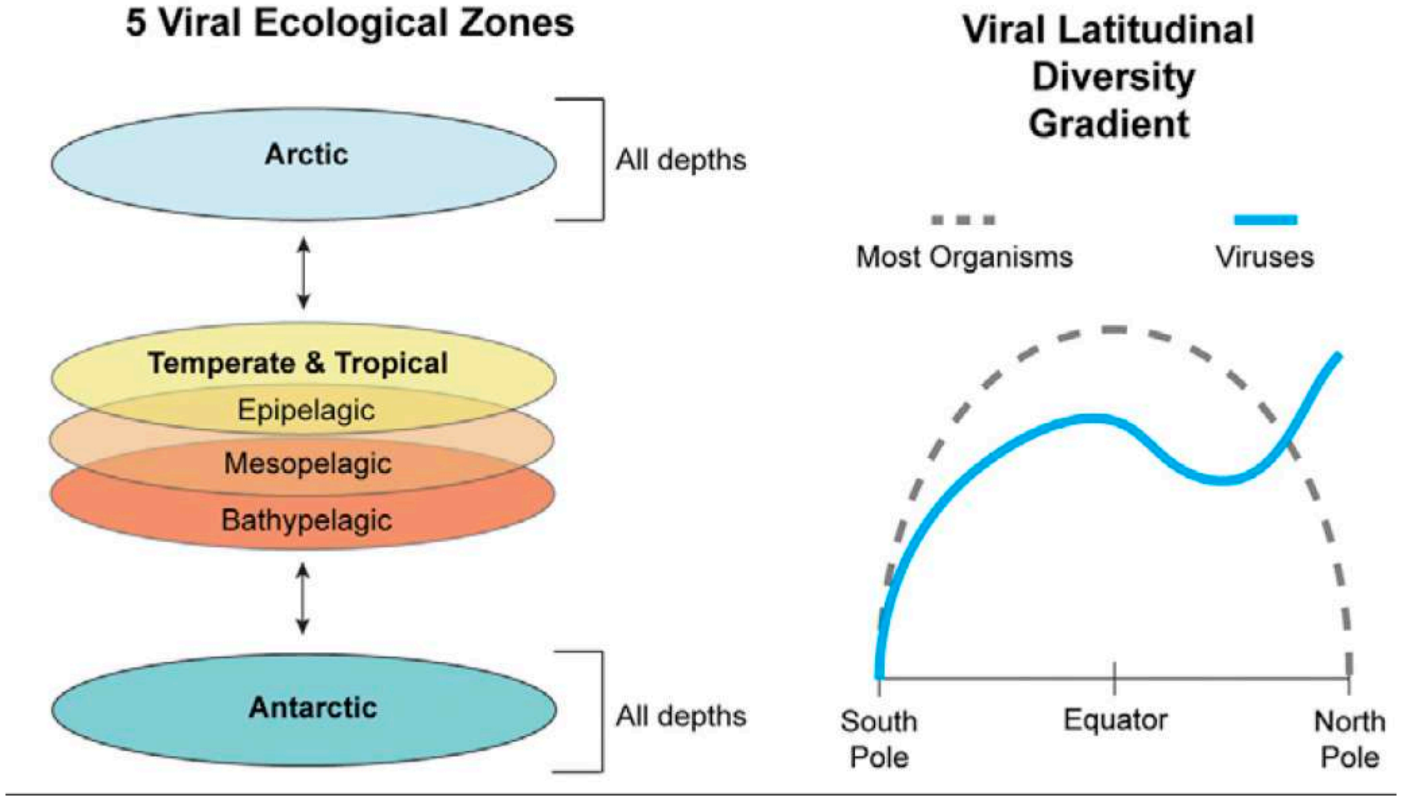


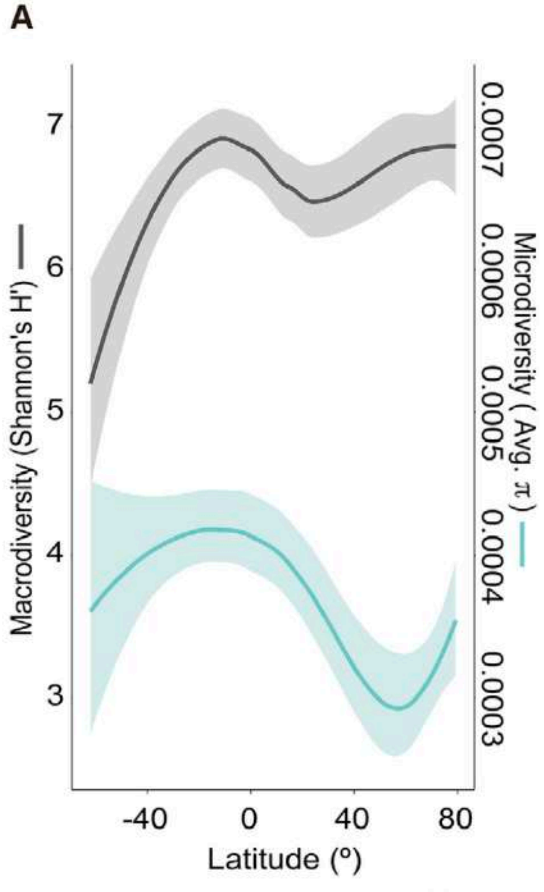
- 🟩 Bacteria (P)
- 🟪 Bacteria (H)
- 🟥 Archaea
- 🟩 Protists (P)
- 🟩 Endophotosymb.
- 🟨 Photohosts
- 🟧 Parasitic protists
- 🟪 Protists (H)
- 🟪 Copepods
- ⬜ Others

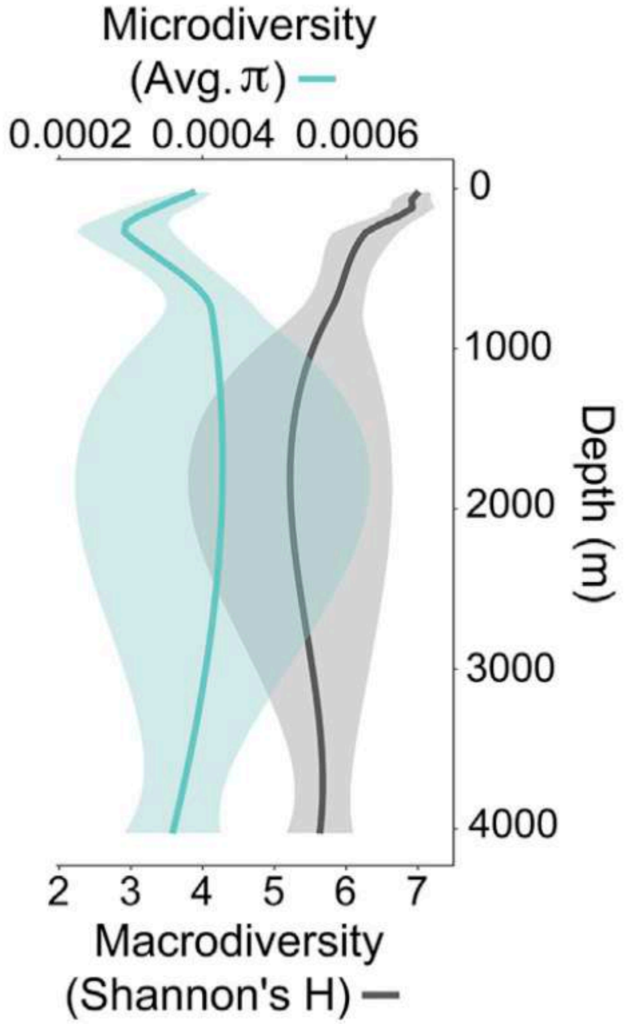


- **Temperature is an important driver in shaping microbial community**
- **Drop in diversity at the Tropics-Poles**

Ocean Viruses from 0 to 4000 m



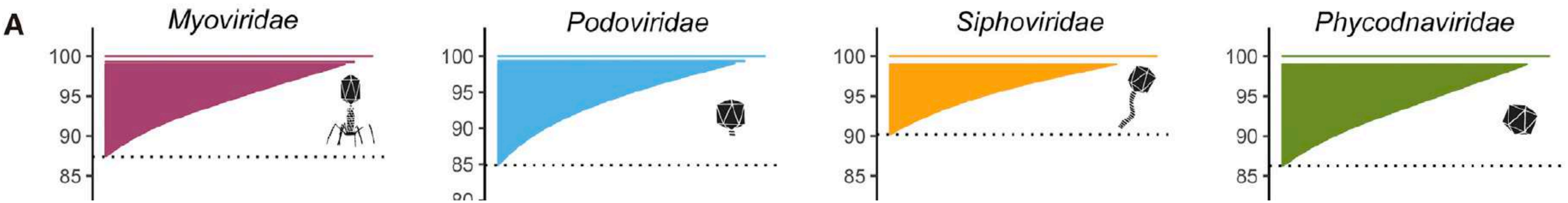




Gregory et al., 2019

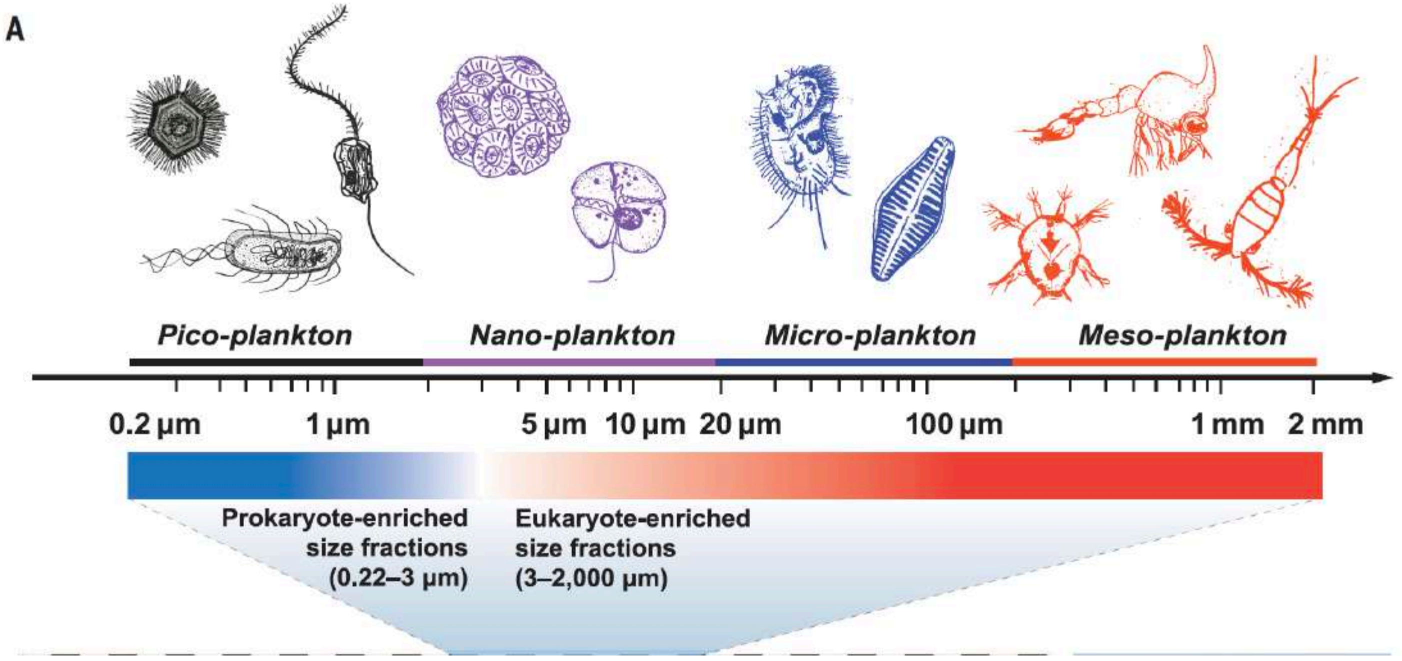
- Macrodiversity: inter-population diversity
- Microdiversity: intra-population genetic variation
- Five ecological zones (not biogeography) as for Bacteria

Ocean Viruses from 0 to 4000 m



- Most abundant viral populations
- Myoviridae: contractile tail : Synechococcus, Prochlorococcus and Cyanobacteria
- Podoviridae: short tail: Vibrio, Roseobacter
- Siphoviridae: long flexible tail, Roseobacter
- Phycodnaviridae: no tail —>dsDNA: Emiliania huxleyi, Ostreococcus, Micromonas, Phaeocystis
- RNA viruses: protists

Dominguez-Huerta et al., 2022



Viral species level diversity

- Cyan: temperature
- White: nutrients
- Violet: primary productivity
- Light green: particulate inorganic carbon

- ✷ ***Virus community composition (which encompasses identity and abundance of vOTUs)***
- ✷ ***Species diversity (which encompasses***
- ✷ ***vOTUs' richness and distribution evenness)***

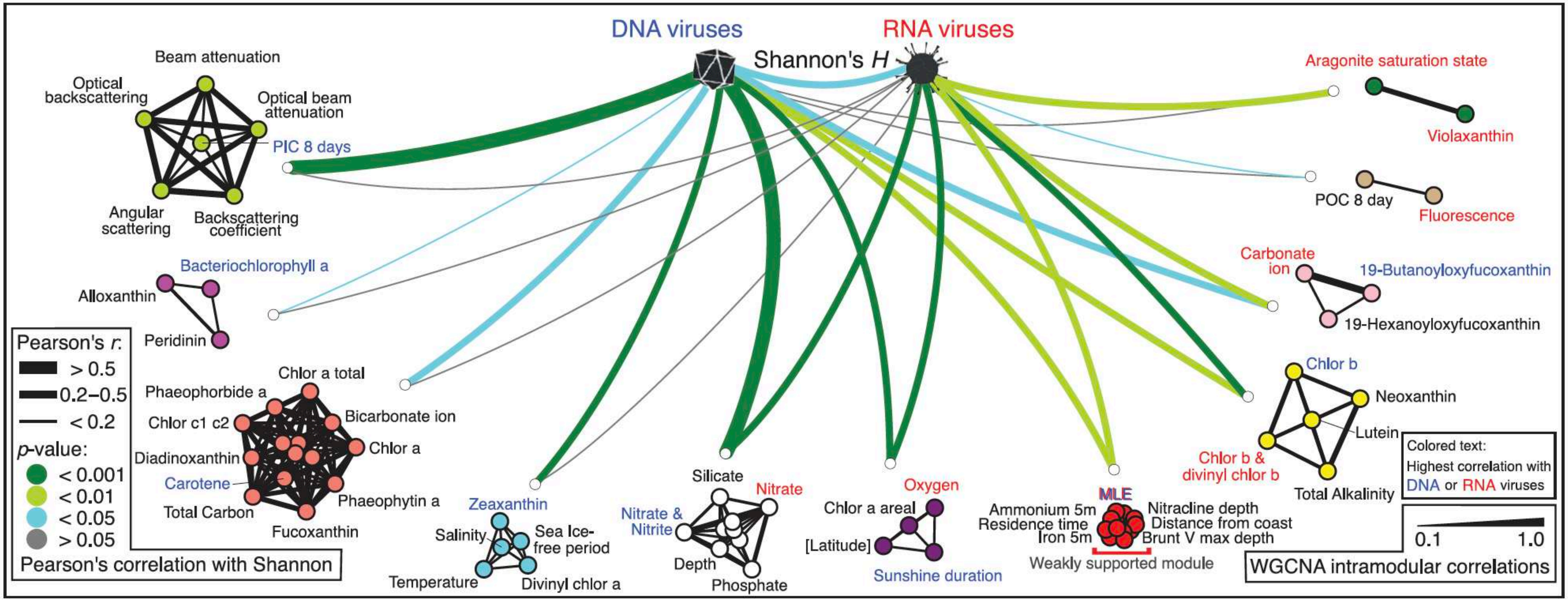


Fig. 3. "Species"-level diversity correlates of marine RNA viruses. Weighted gene correlation network analysis (WGCNA)–supported modules (to account for collinearity) of environmental variables (materials and methods) showing the cofactors of RNA and DNA virus diversity. Modules are Pearson correlated to the Shannon's *H* values of each virus group. Shown are only those relationships that were statistically supported by both Pearson's and Spearman's tests. Only RNA viruses from the prokaryotic fraction were used (see Fig. 2 for explanation). Notably, aragonite and carbonates could be indicative of coccolithophores, whereas violaxanthin and the latitude-chlor a signal could be related to diatoms. MLE, maximum Lyapunov exponent; POC, particulate organic carbon.

Dominguez-Huerta et al., 2022

Co-evolution: predator-prey

- **RED QUEEN: continuous army race via mutation and counter-mutation resulting in continual selection pressure for the host to evolve resistance and for the phage to evolve to overcome such resistance**
    - **Not infinite, some mutations may impair host fitness, fluctuating selection**
    - **Cyclic changes in abundance through negative frequency-dependent selection**

- **KILL THE WINNER: where “winner” refers not necessarily to the most abundant but to the most active prokaryotic population. Grazers and viruses are controlling bacteria population**
    - **Fluctuation in abundance due to new niches due to lysis**
    - **Reset of the winner since phage pressure diminishes**

- **PIGGYBACK THE WINNER: lysogen as a strategy when host in a favourable environment, protection from other viral attack, reduced control of phage on bacterial abundance**