南方科技大学
SOUTHERN UNIVERSITY OF SCIENCE AND TECHNOLOGY



Hourly-resolved marine microbial and viral dynamics in the subtropical Daya Bay, northern South China Sea

Shengwei Hou
Southern University of Science and Technology
2025/06/30

海洋科学与工程系
Department of Ocean Science and Engineering

Study & Work Experience

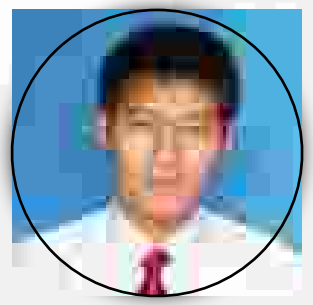
海洋科学与工程系
Department of Ocean Science and Engineering

Bachelor

2005-2009

Southwest University

Prof. Huamao Du



Master

2009-2013

Institute of Hydrobiology, CAS

Prof. Xudong Xu



Doctor

2013-2017

Freiburg University, Germany

Prof. **Wolfgang R. Hess**



Postdoc

2017-2018

Freiburg University, Germany

Prof. **Wolfgang R. Hess**

Postdoc

2018-2021

University of Southern California, USA

Prof. **Jed A. Fuhrman**



Research Interests

Harmful algal bloom

侯圣伟实验室





Computational Microbial Ecology and Evolution (CMEE)

明德求是 日新自强
VIRTUE | TRUTH | ADVANCE

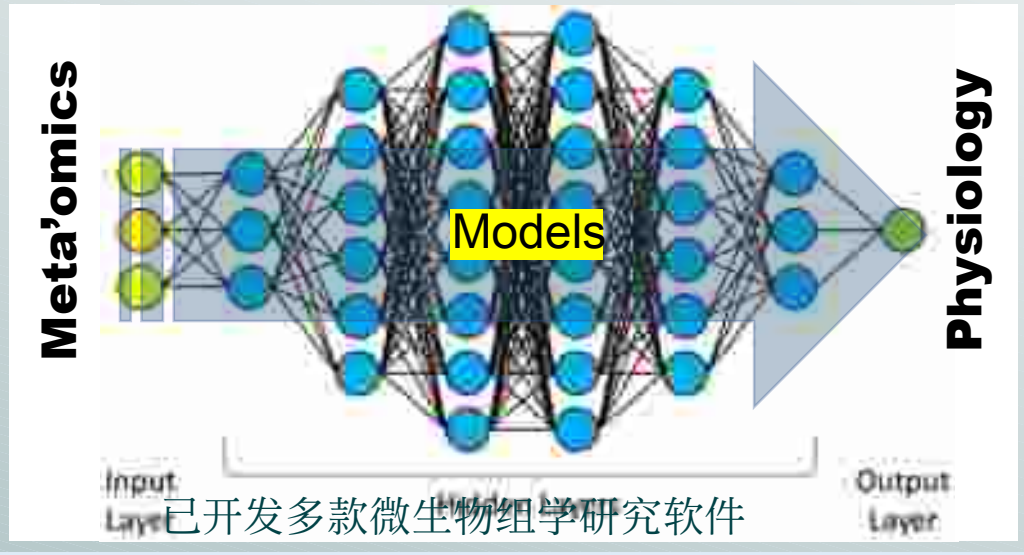
1. Computational Microbiology

- **Bioinformatics**

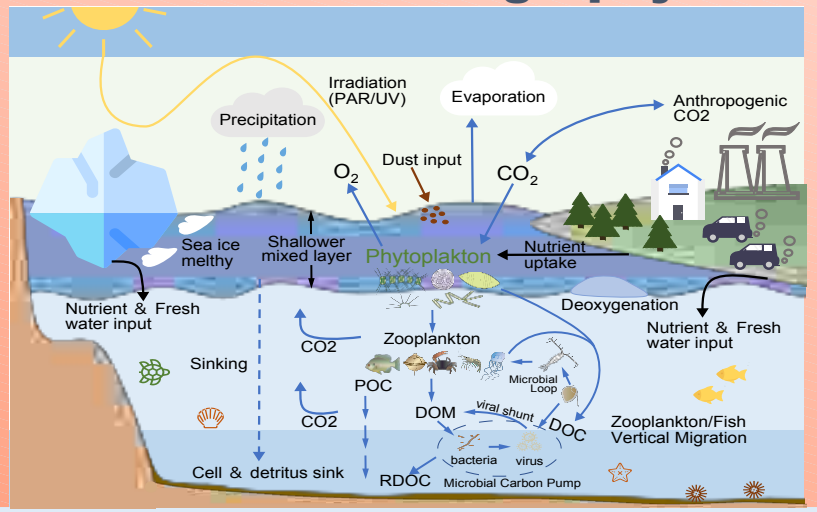
Tools developed: mdRNA-Seq, gRodon, contigNet, DeepMicroClass, DeepVirFinder, DeepVirFinder2, DeepMu, DeepBacOri, etc.

- **Microbial Ecology and Evolution**

Microbial adaptations to temperature, DO, pH, salinity, and other environmental stressors



2. Microbial Oceanography



- **Polar microbial oceanography**

Microbial and viral responses to polar warming

- **Microbial ecology in OMZs**

Microbial and viral responses to ocean deoxygenation

- **Microbial ecology in the dark ocean**

Microbial processes in hydrothermal vents, cold seeps, etc.



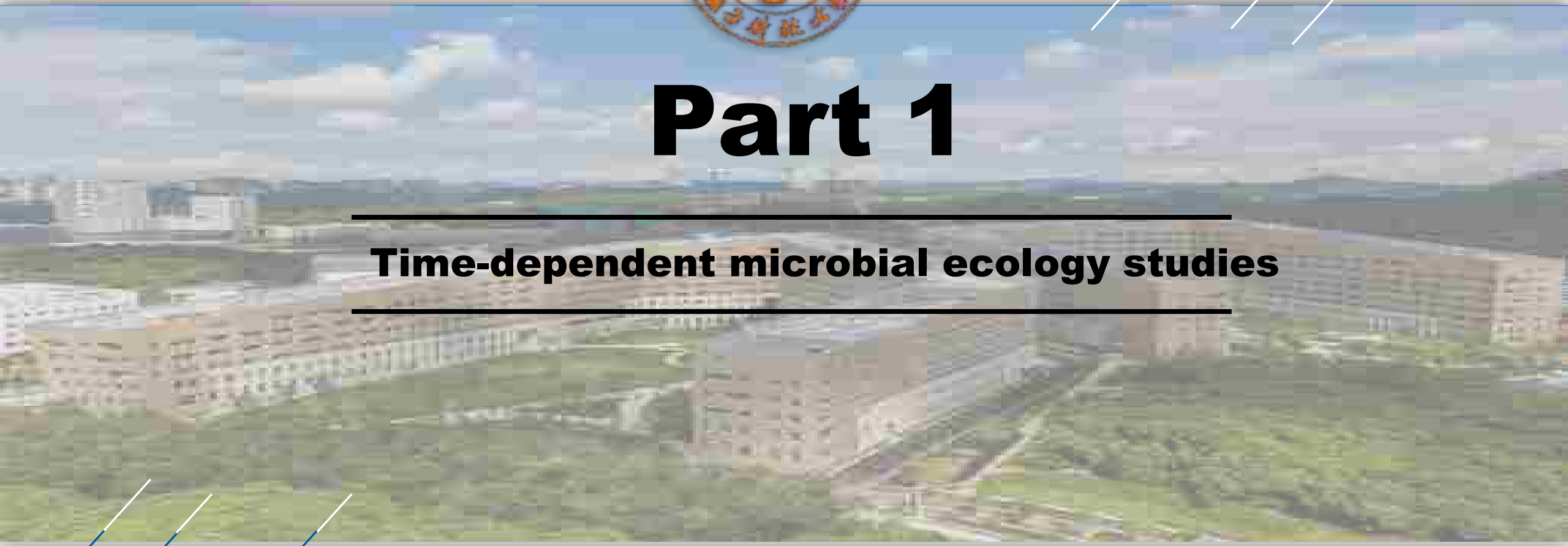
Content

01： Time-dependent microbial ecology studies

02： A UAV-based in situ community survey

03： Rhythmic changes of microbes in Daya Bay

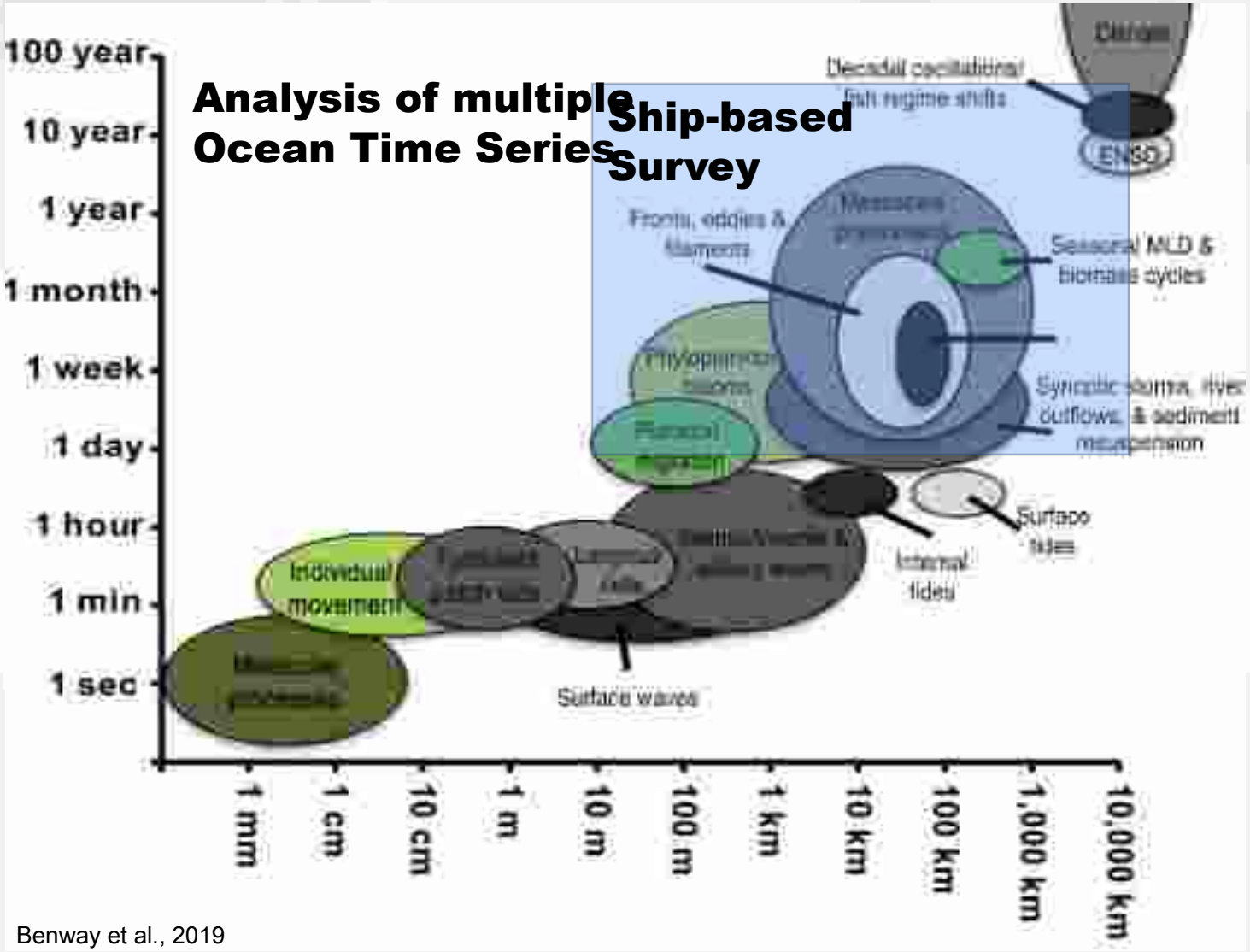
04： Rhythmic changes of viruses in Daya Bay



Part 1

Time-dependent microbial ecology studies

Time scales in microbial ecology

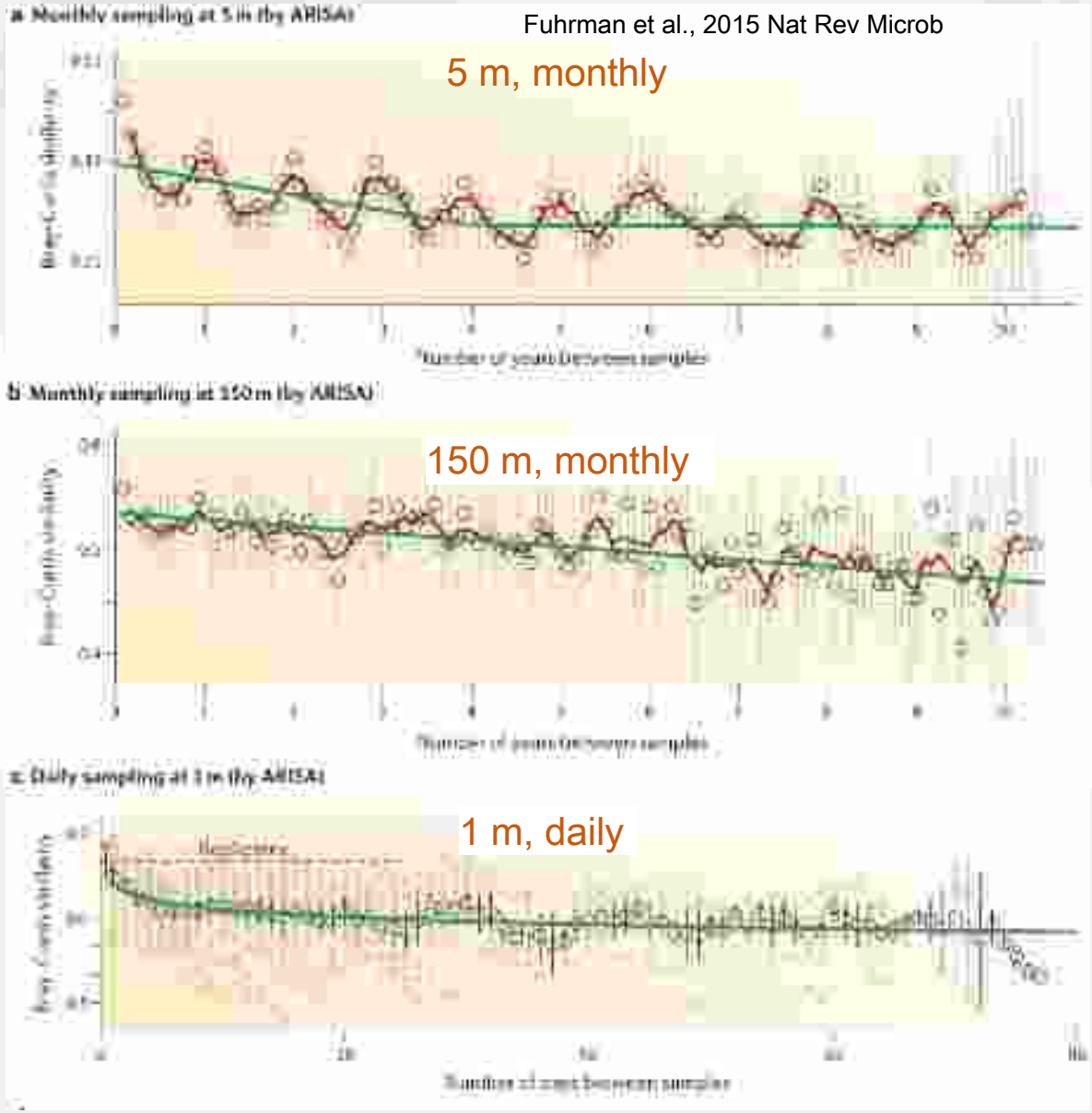


Benway et al., 2019

- **Temporal and spatial scales** are utilized in ecological studies to interpret patterns, processes, and environmental responses
- **Timescales** can range from minutes (e.g., wave height, temperature fluctuations) to decades (e.g., long-term climate trends, species distribution shifts)
- **Temporal resolution** (instantaneous, daily, seasonal, or climatological) should align with the ecological questions
- Ships, autonomous surface and underwater vehicles, and satellite-based platforms have taken on a greater importance in long-term, large-scale ocean observations

Time scales in microbial ecology

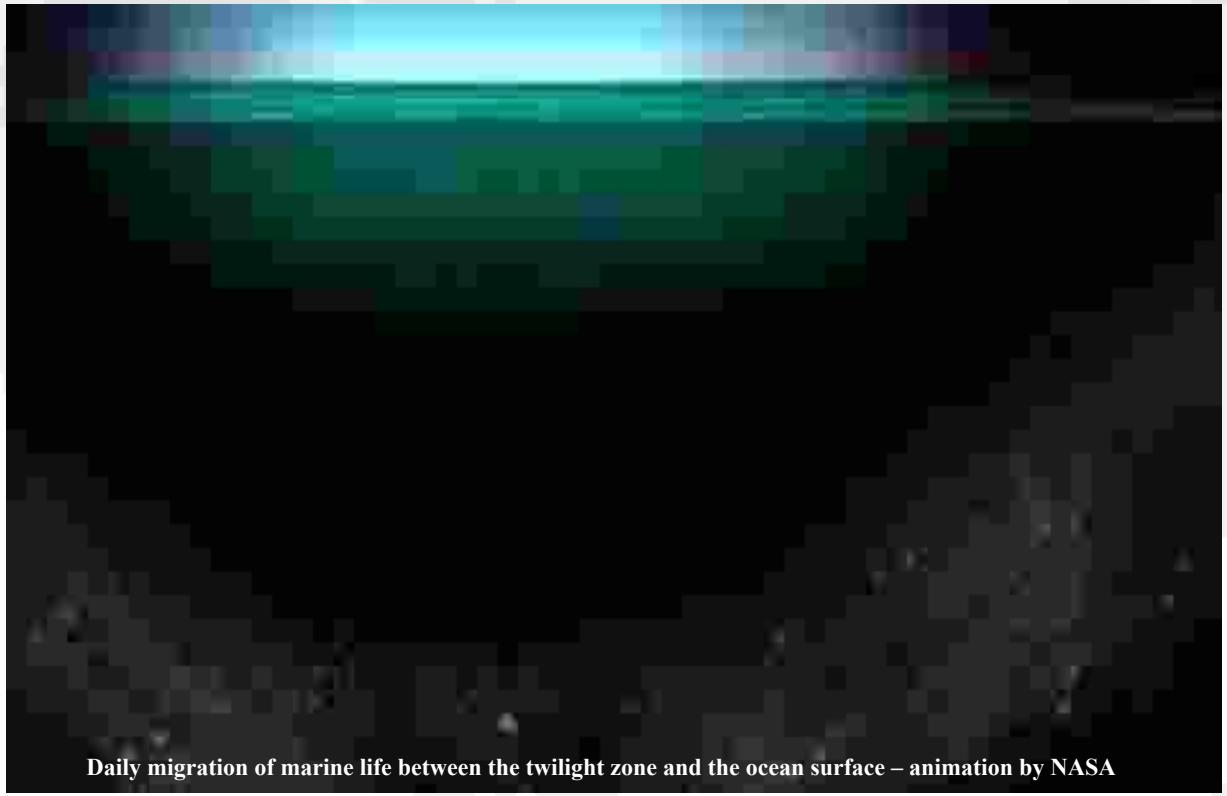
海洋科学与工程系
Department of Ocean Science and Engineering



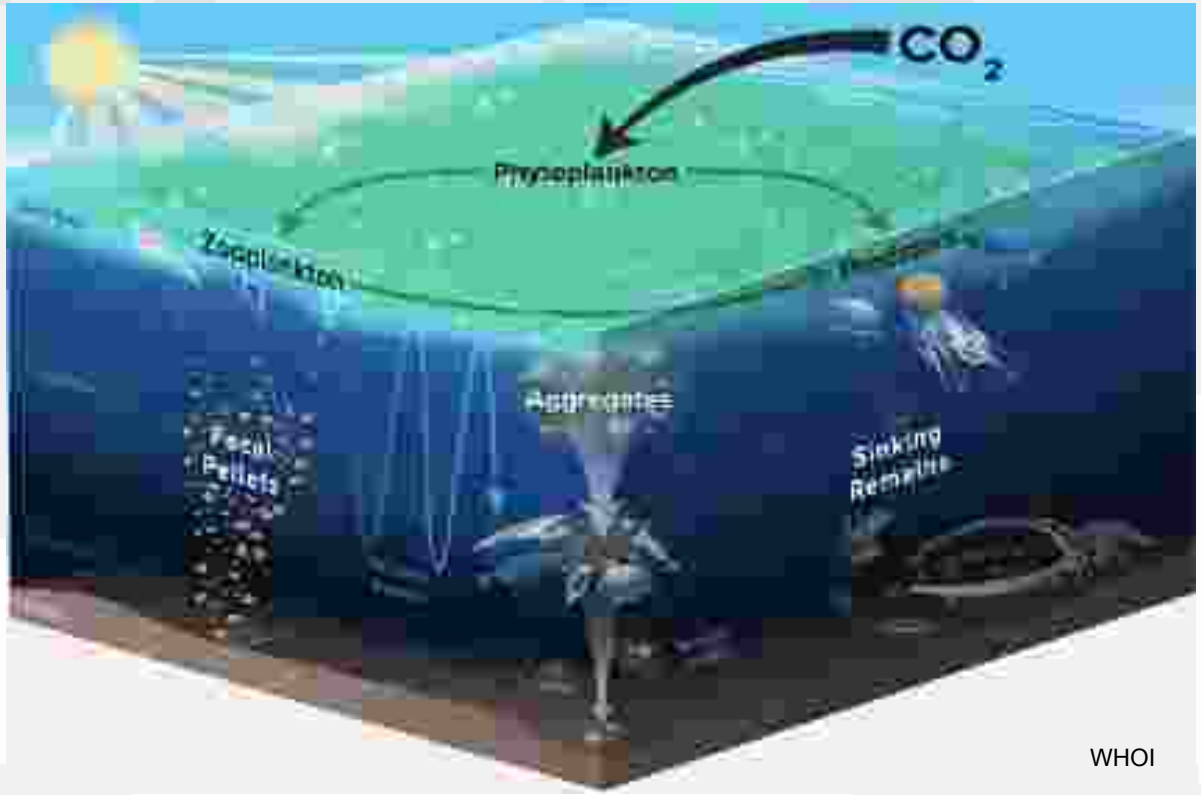
- Monthly dynamics of the surface microbial community at San Pedro Ocean Time-series (SPOT) have revealed patterns that indicate community-level resilience and, often, seasonality.
- The absence of a sinusoidal pattern at a depth of 150 m indicates that seasonality in community composition is not demonstrated at this depth, and the gentle downward trend (green line) indicates a degree of long-term variability.
- With a daily sampling resolution at a depth of 1 m, the microbial communities are more similar to each other.
- Dynamics also reveal that correlations vary among organisms depending on the timescale examined, indicating that different processes operate at different scales.
- More frequent daily observations are required.

Diel rhythms and marine ecology

海洋科学与工程系
Department of Ocean Science and Engineering



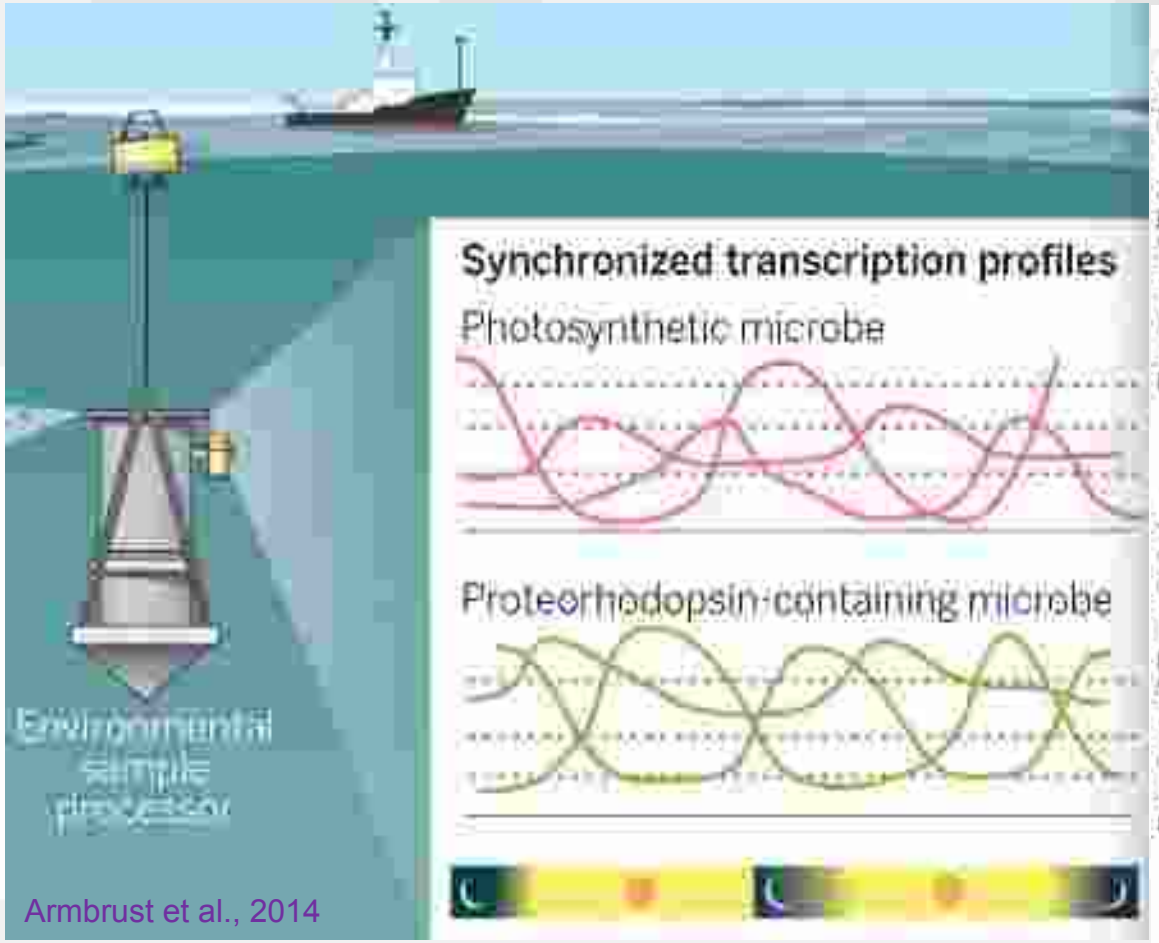
Daily migration of marine life between the twilight zone and the ocean surface – animation by NASA

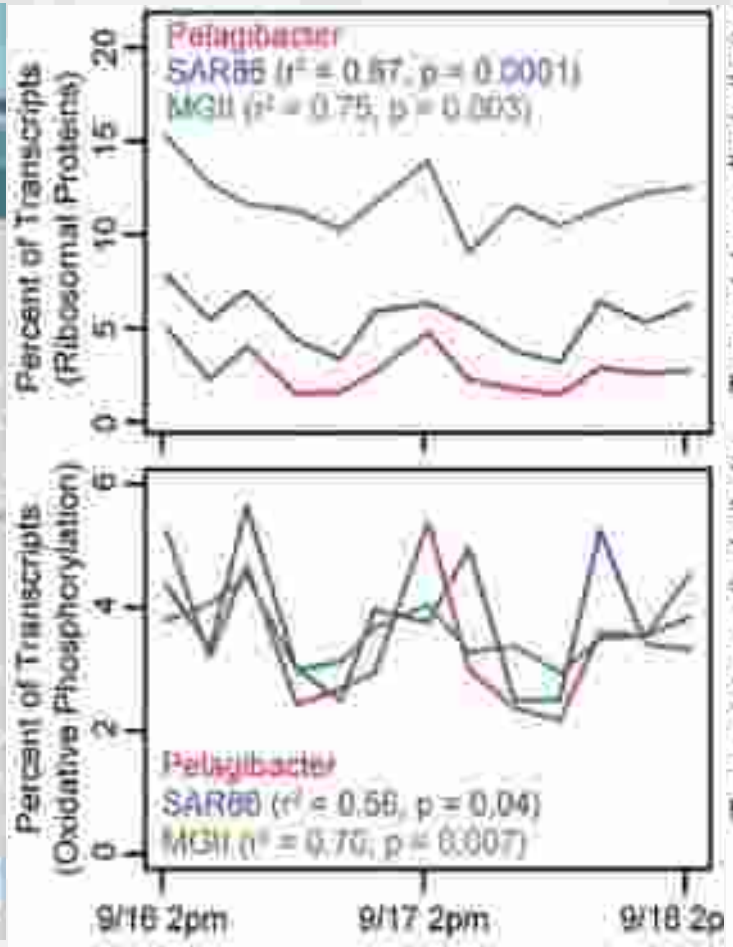


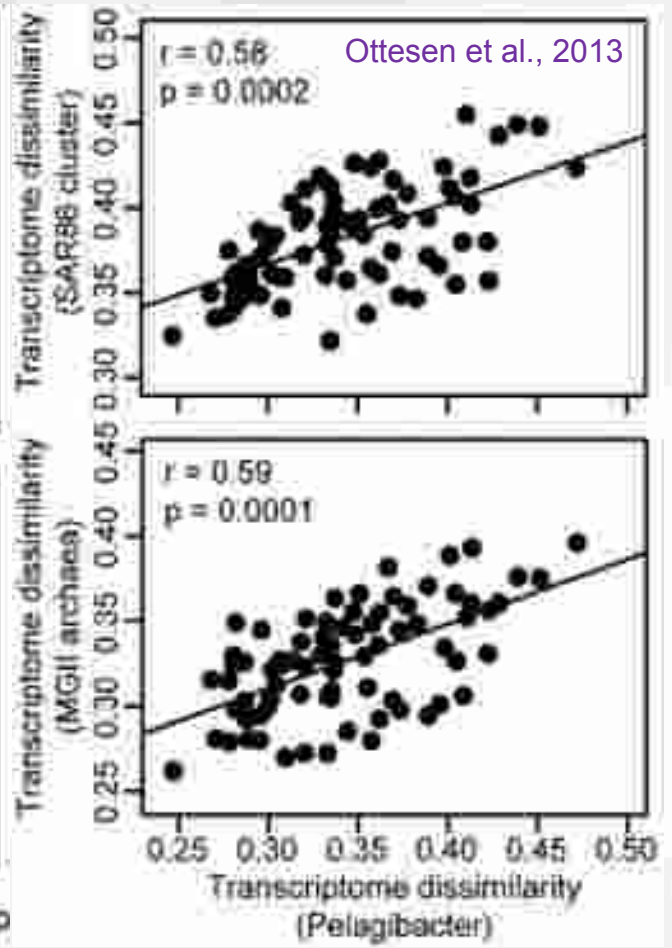
WHOI

- Diel rhythms—biological cycles repeating every 24 hours—are driven by the Earth's rotation and light-dark cycles.
- Diel rhythms influence the physiology, behavior, and interactions of marine organisms, from phytoplankton to zooplankton and fish.
- Light-driven rhythms are common in marine ecosystems.
- The marine carbon cycle is driven by the photosynthetic phytoplankton, which are controlled by light availability and intensity.
- The vertical migration of some zooplankton and predators is also regulated by the light cycle.
- Primary producers are consumed by higher trophic levels, bringing fixed carbon down to the aphotic zone—the biological carbon pump. Heterotrophic microbes are dependent on primary producers—the microbial carbon pump.

Diel rhythms and microbial metabolism





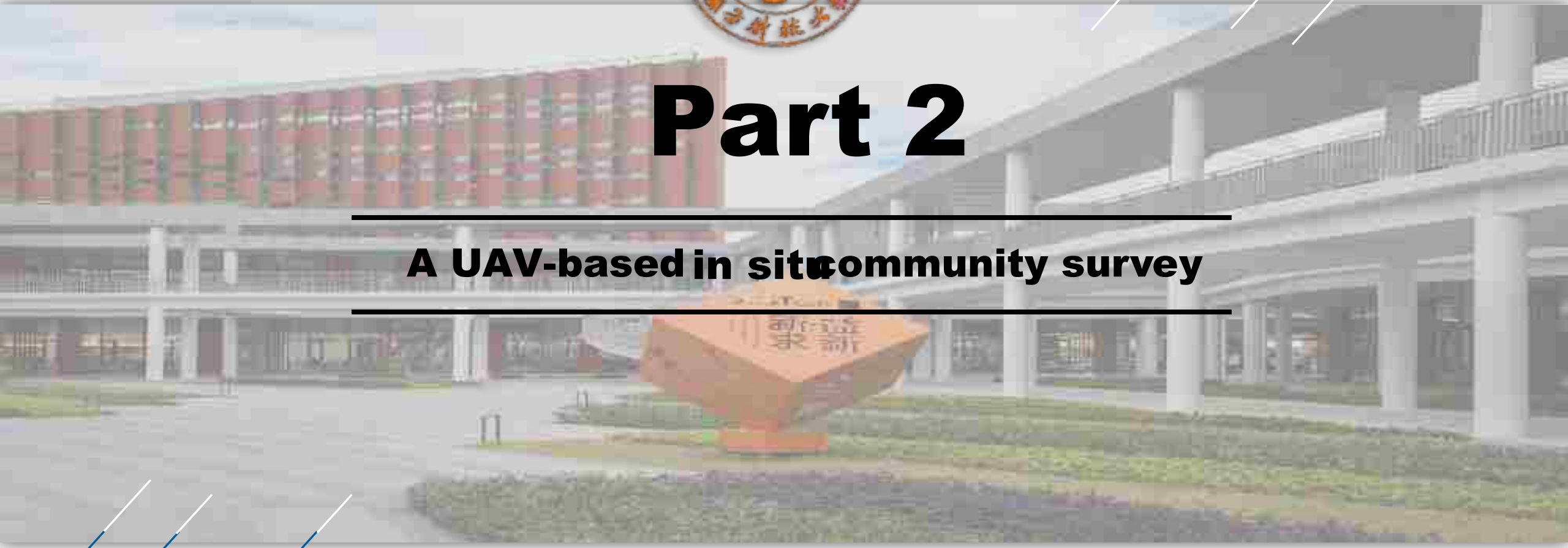


- Diel rhythms have been detected for photosynthetic or proteorhodopsin-containing microbes in the open ocean
- Synchronized diel expression of ribosomal and oxidative phosphorylation related proteins have been observed for SAR11, SAR86 and MGII
- Coastal waters are more dynamic than open ocean, diel patterns might be less obvious

Scientific questions

To answer :

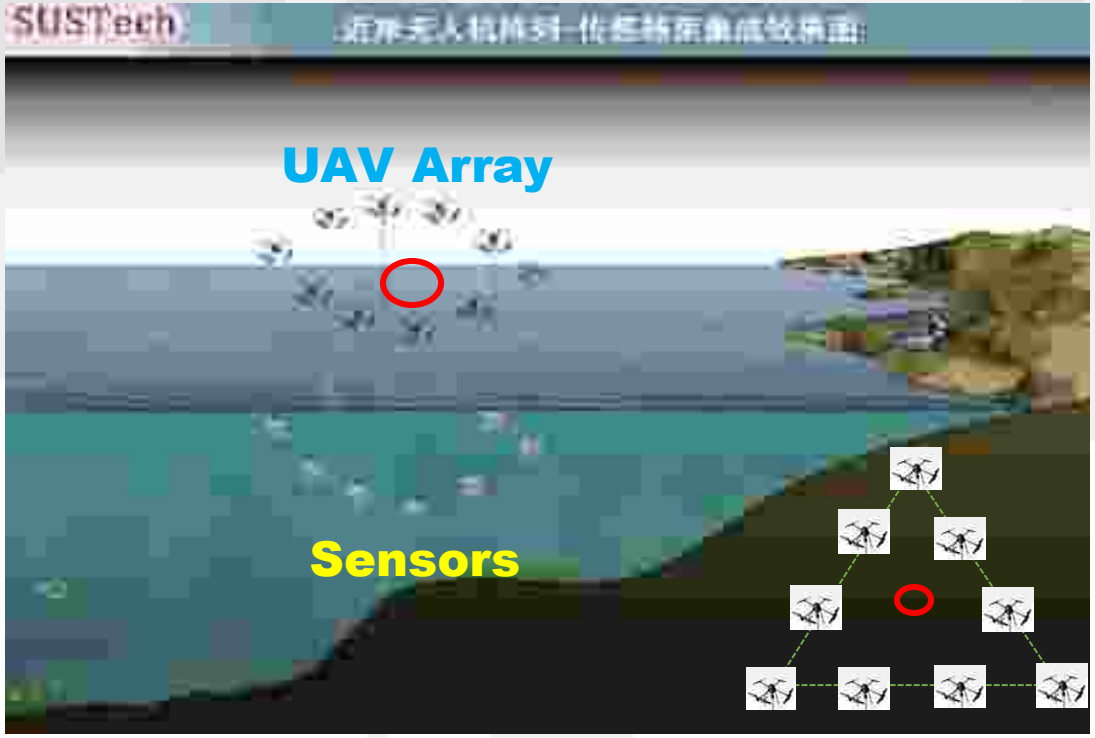
- Do coastal microbes and viruses have diel rhythms?
- If so, which ones show diel patterns, and why?
- If so, which ones have similar diel patterns, and why?

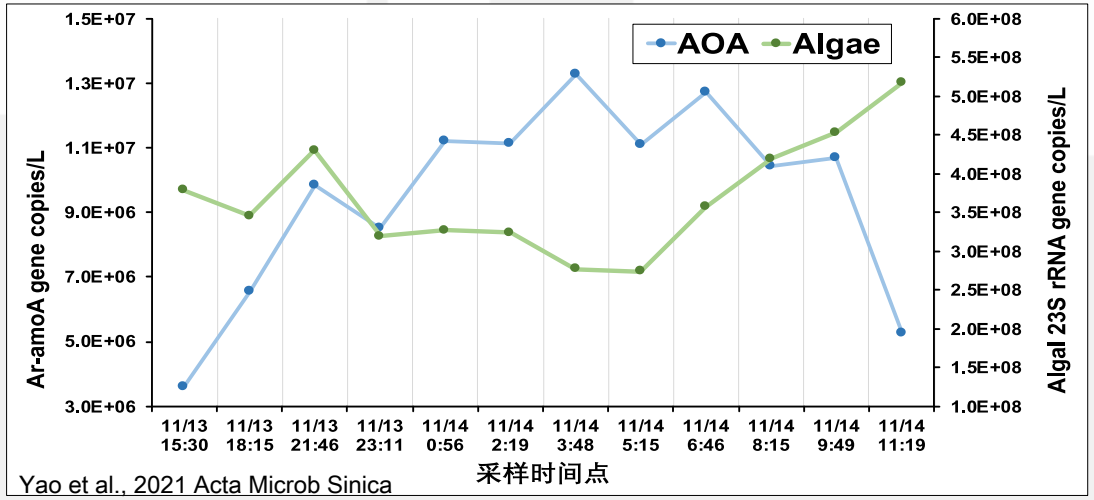


Part 2

A UAV-based in situ community survey

A UAV-based in situ community survey





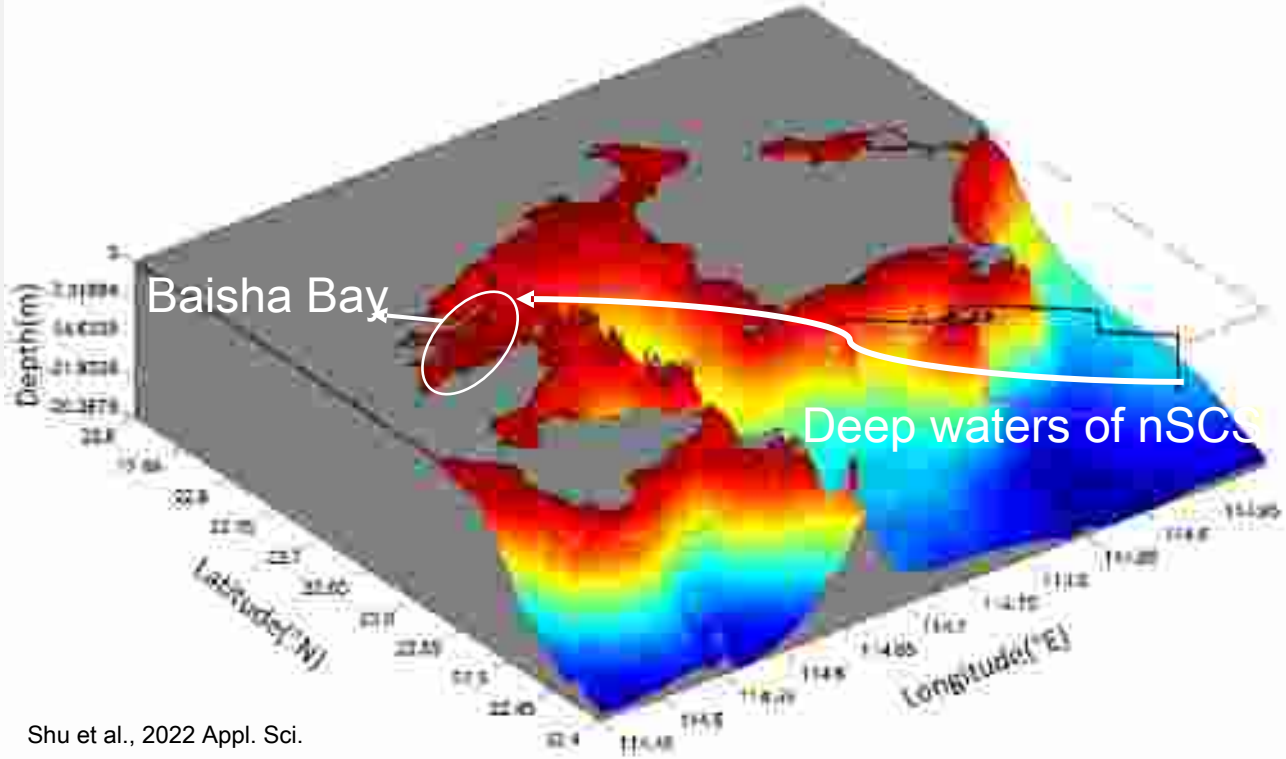
Yao et al., 2021 Acta Microb Sinica



- With UAV-attached multi-channel sensors, time-intensive field investigation is practical
- Within one day, strong variations could be observed for both env factors and microbes
- Negative correlation between AOA and algae

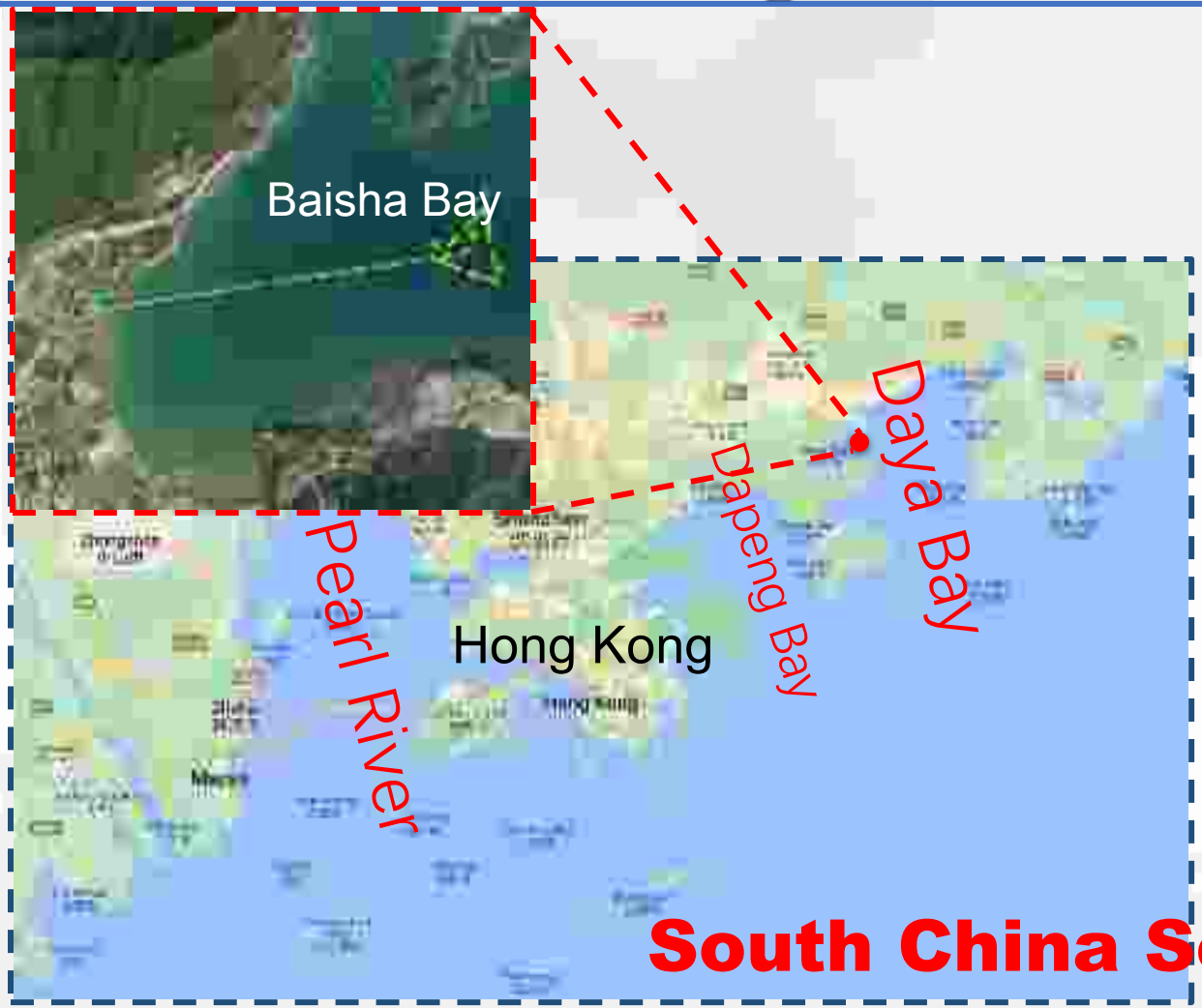
Study area

海洋科学与工程系
Department of Ocean Science and Engineering



Shu et al., 2022 Appl. Sci.

- Baisha Bay (<5 m), in the corner of Daya Bay
- Physical models predicted waters of Baisha Bay circulated from ~15 m in the northern SCS
- Oct 28-31, 2021, UAV array sampling (1 L) with a 2h time interval for 72 h



Baisha Bay（Daya Bay）UAV Sampling

3 stations, 500 m apart, forming a triangle

Study area



Sampling and data analysis

海洋科学与工程系
Department of Ocean Science and Engineering

Env. Sampling



UAV-attached Sensors

Temp、pH、Salinity、DO、Chla

Bio. Sampling

1 L seawater at each station / 2h

Cellular fraction（>0.2 μm）

Viral fraction（0.02 μm）

DNA、RNA extraction

Library Prep

Metagenomics

Metatranscriptomics

Amplicon

Viromics

Data Analysis

Microbial Diversity

Microbial Rhythms

Micro. Metabolism

Micro. Activities

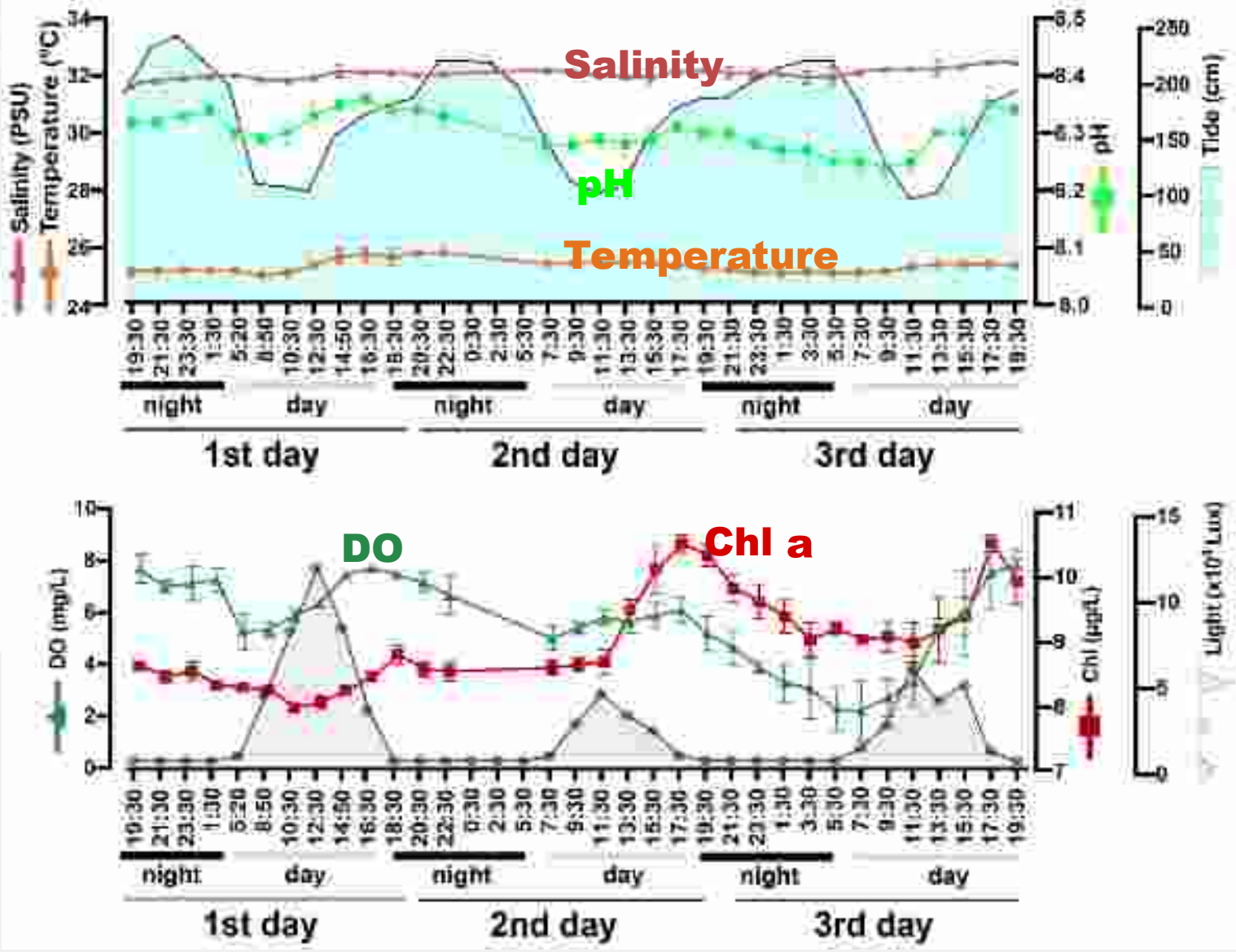
Viral Diversity

Viral AMGs

Viral Rhythms

Viral Activities

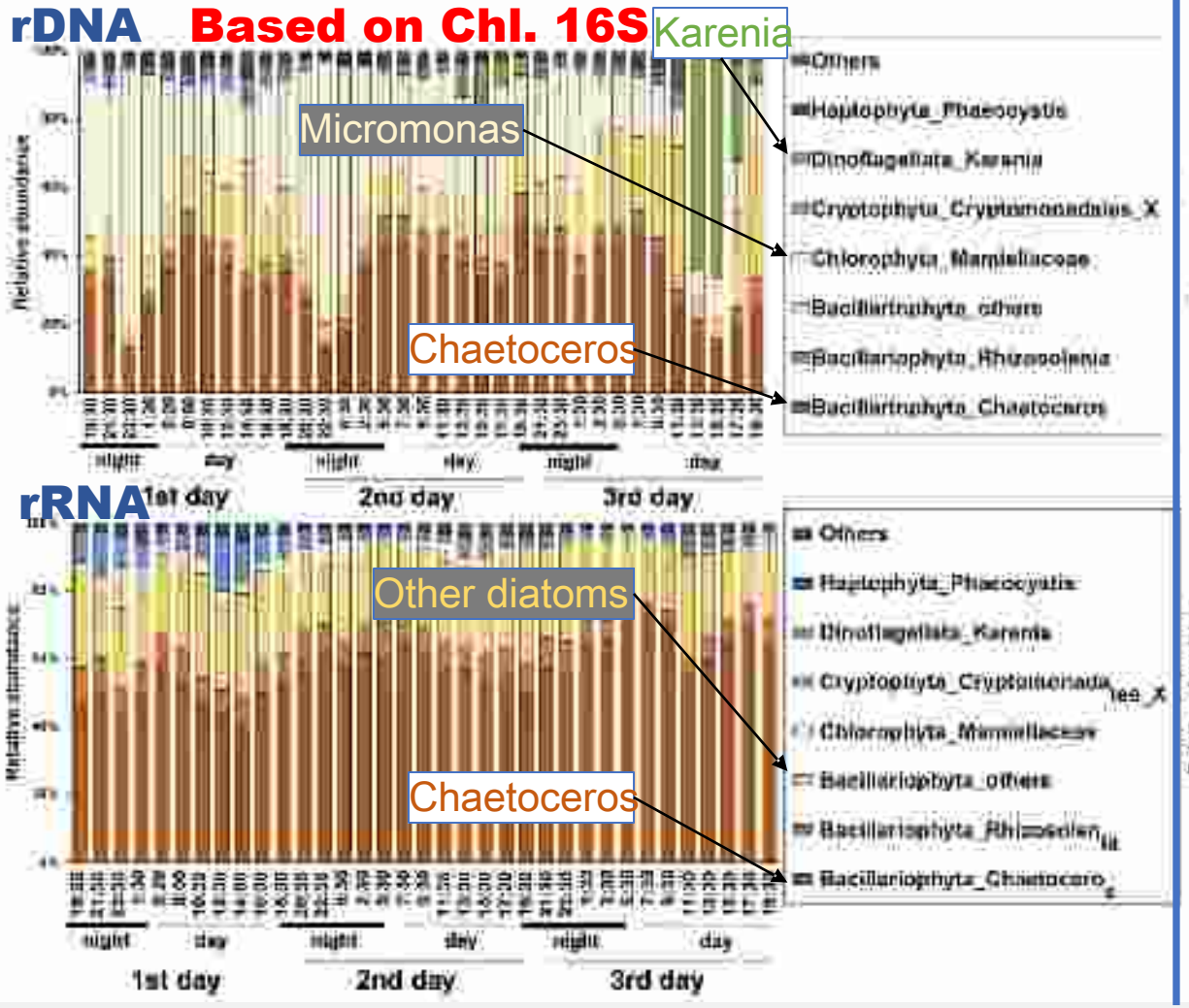
Daily changes of env. factors

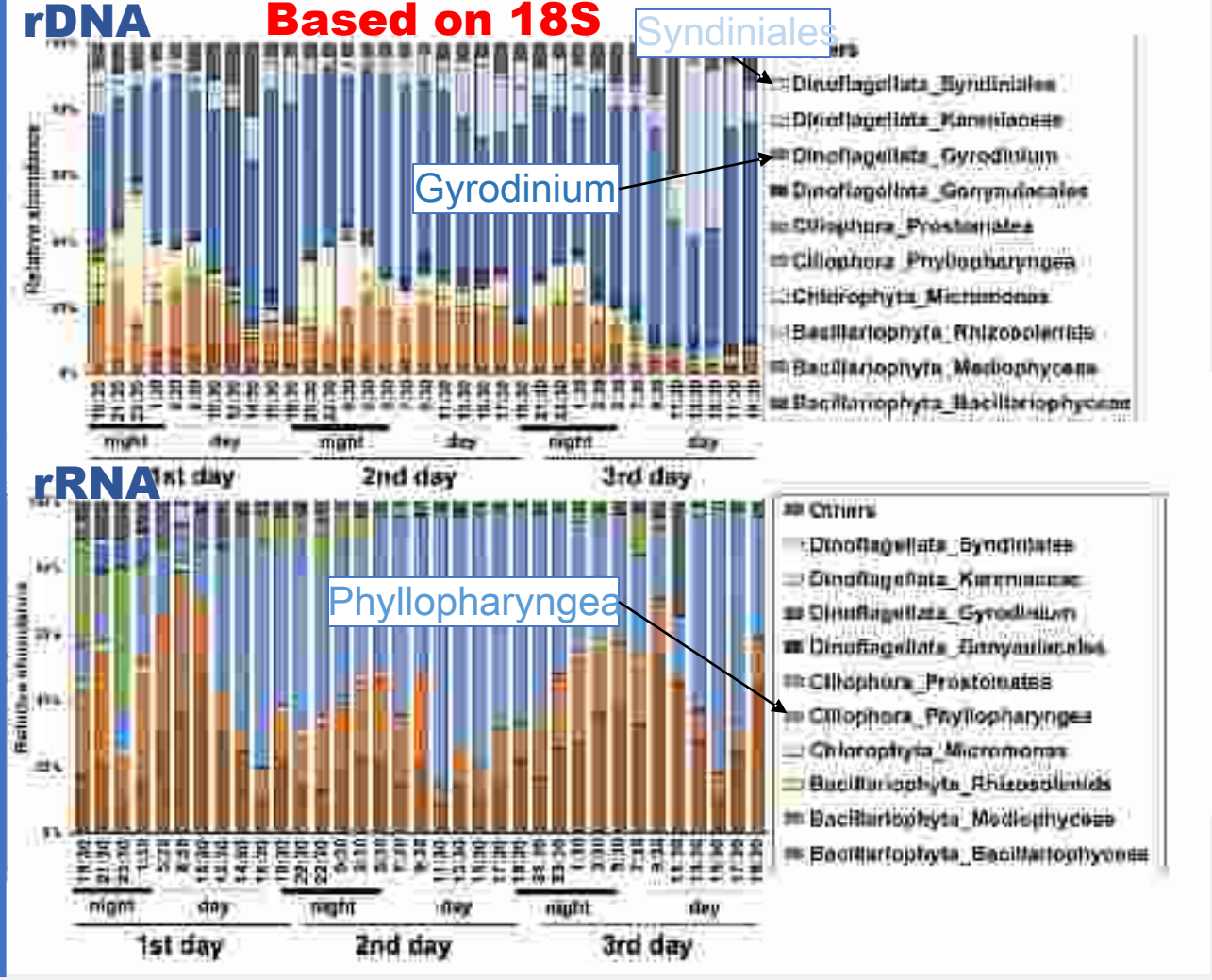


- Both salinity and temperature remained stable during sampling, while light intensity and tidal height showed clear diurnal variation
- Chl a exhibited diurnal changes, with a decrease from dusk to midday, followed by an increase until sunset
- Light intensity decreased from the 2nd day, while Chl a increased
- DO correlated well when Chl a was low (<5μg/L), and decreased dramatically when Chl a was high (still correlated)

Community structures of phytoplankton

海洋科学与工程系
Department of Ocean Science and Engineering



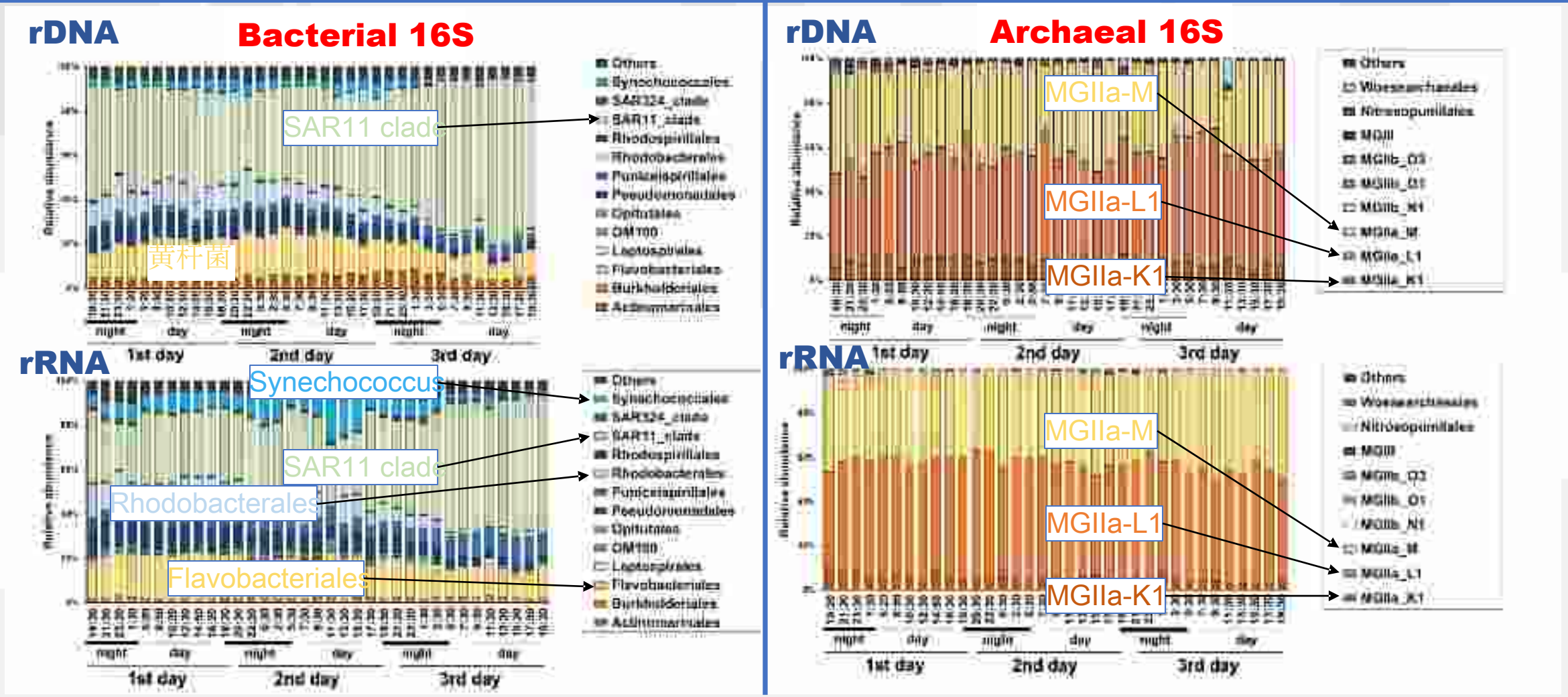


- Chlorophyta and Bacillariophyta dominated the microeukaryotic phytoplankton based on Chl. 16S
- Chaetocerosshowed the highest activity

- Gyrodinium (diatom) were abundant but not active
- Predatory Phyllopharyngea (ciliate) were rare but highly active

Community structures of bacterioplankton

海洋科学与工程系
Department of Ocean Science and Engineering



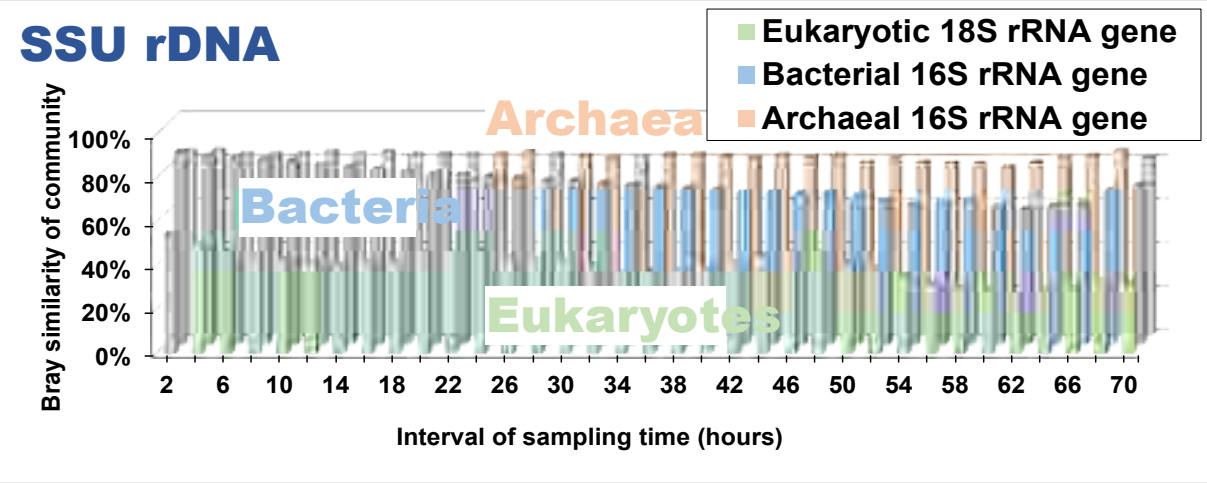
- SAR11 clade, Synechococcus, Rhodobacterales, and Flavobacteriales were the dominating microbes
- MGII dominated the archaeal DNA and RNA, mainly from the MGIIa clade (M, L1, K1 genera)

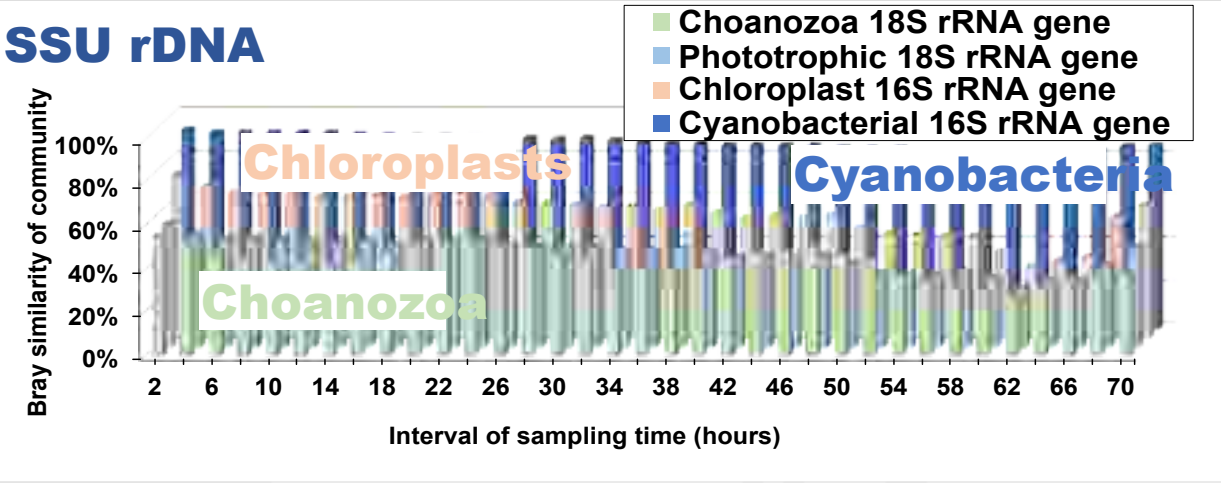
Part 3

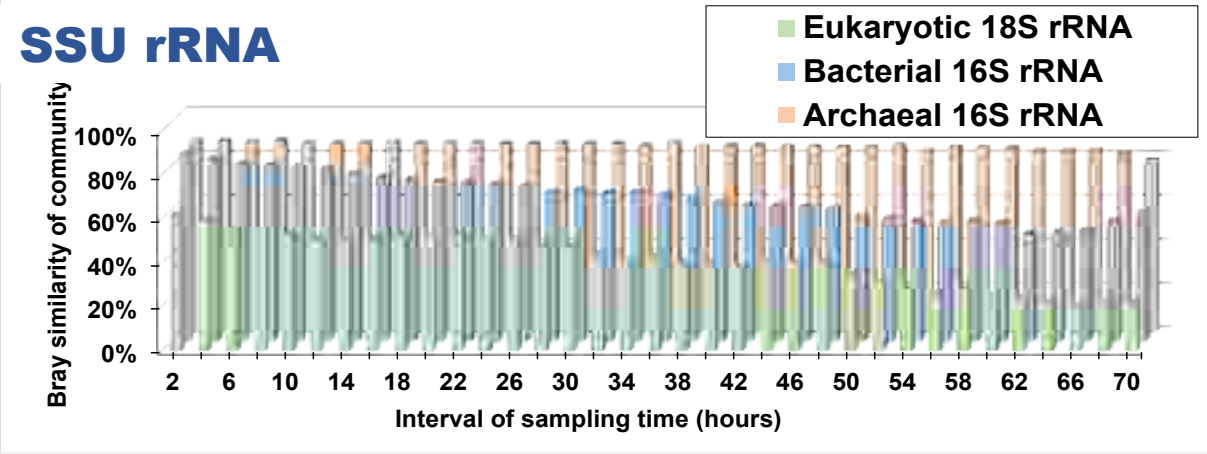
Rhythmic changes of microbes in Daya Bay

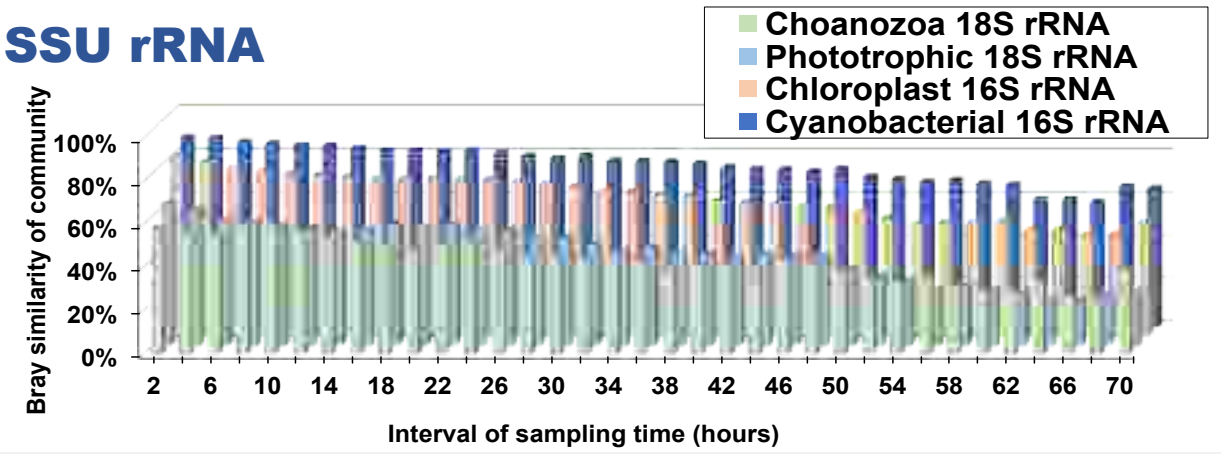
Community-level similarity patterns

海洋科学与工程系
Department of Ocean Science and Engineering





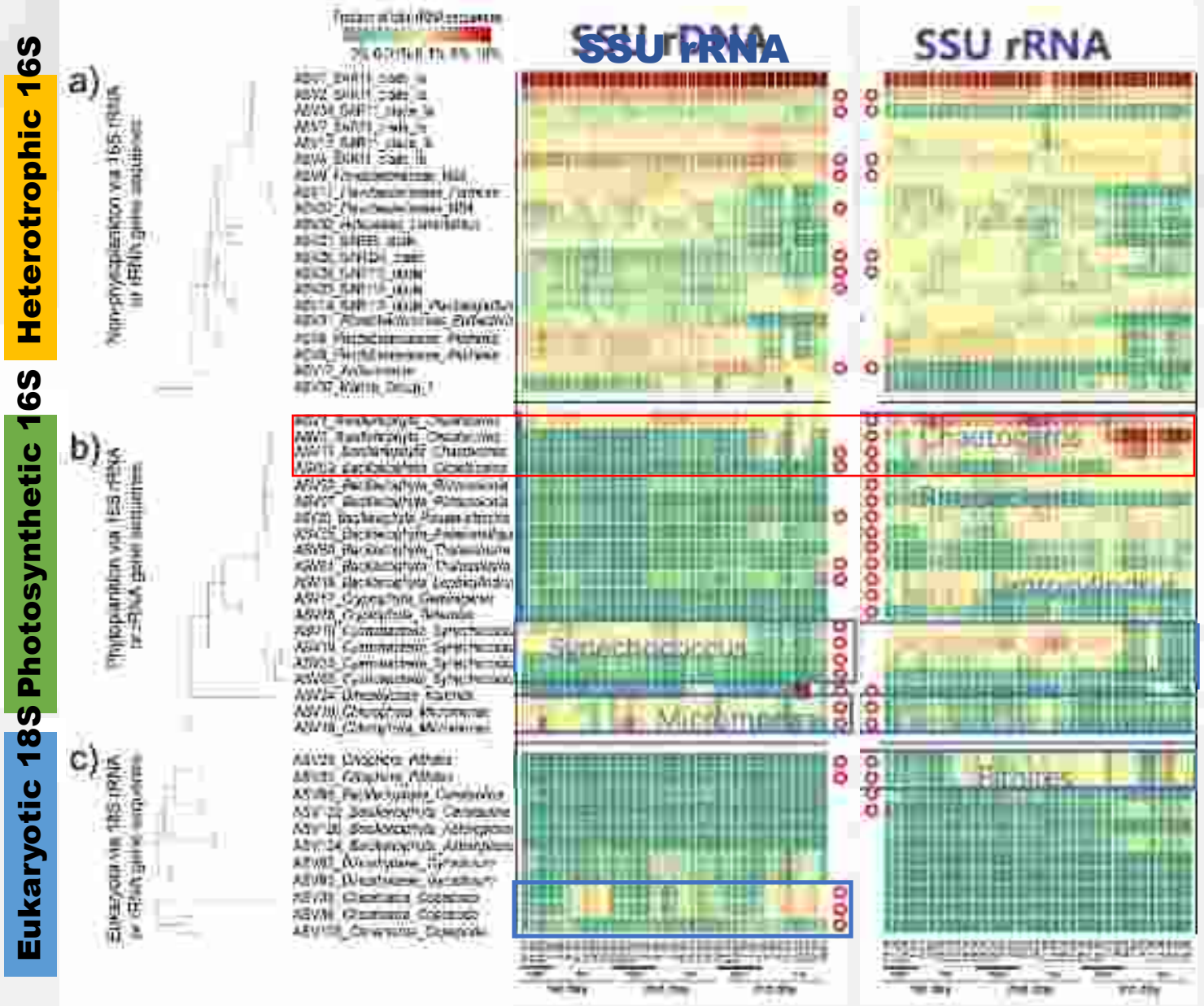




- Archaeal communities remained stable
- Bacterial communities drifted with time
- Eukaryotic communities showed a 24h rhythm

- Cyano were more stable than microalgal comm.
- **Choanozoa rDNA showed strong diel rhythm**
- Similarity decayed faster at the rRNA level

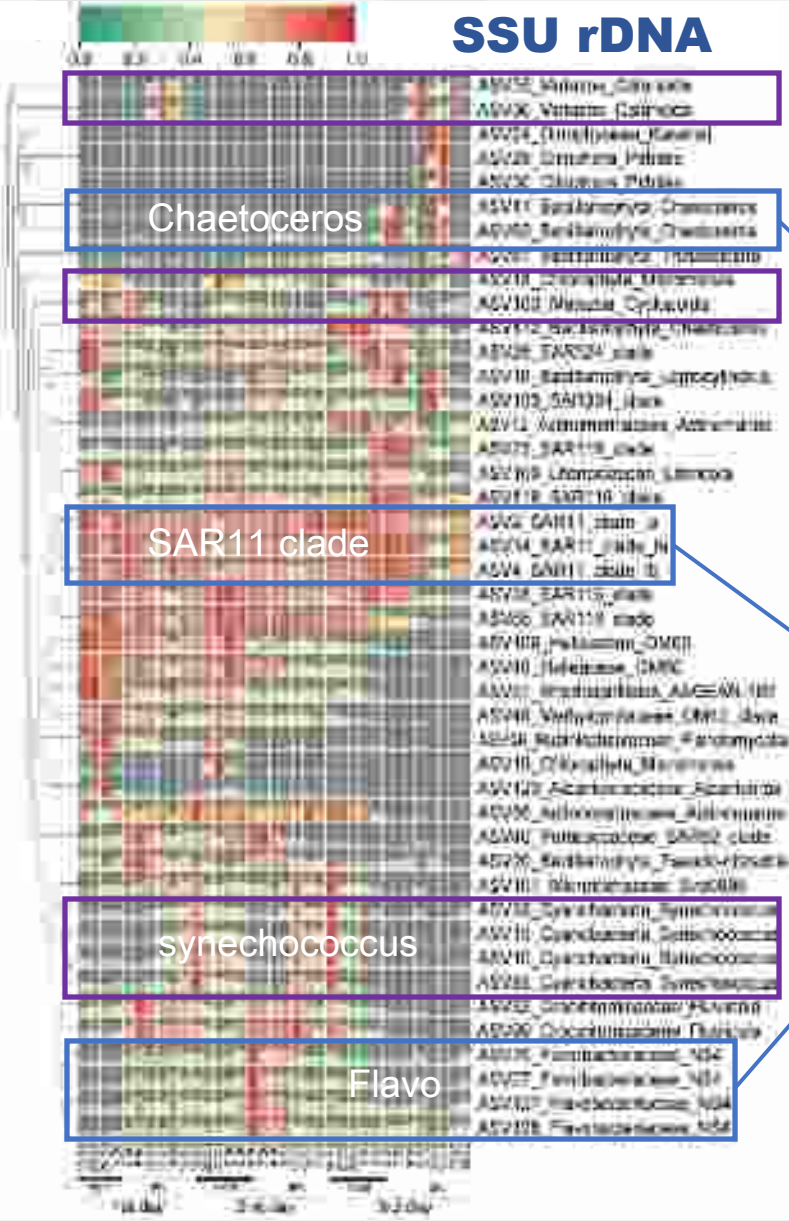
Microbes of diel rhythms

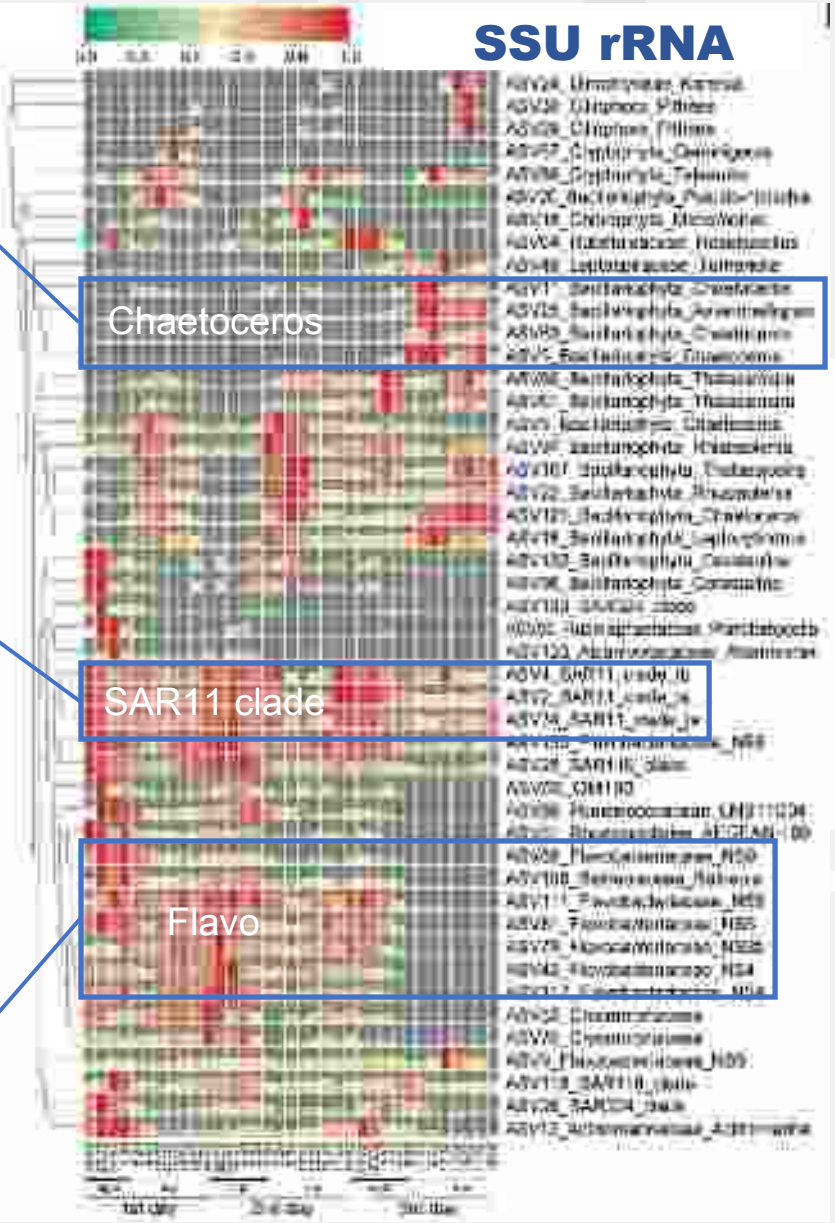


- Photosynthetic microbes, including Cyano. and microalgae were mostly diel at either rDNA or rRNA levels
- Picoeukaryotes (Micromonas) showed lower rRNA activity than rDNA, while diatoms and Cryptophyta showed higher rRNA than rDNA proportions
- Ciliates showed stronger diel patterns at rRNA level, while copepods showed stronger diel rhythms at rDNA level
- Heterotrophic bacteria (SAR11 、 Flavo 、 SAR116) showed diel patterns at both rDNA and rRNA levels
- Diel patterns for MGII were weak

Microbes of diel rhythms

海洋科学与工程系
Department of Ocean Science and Engineering

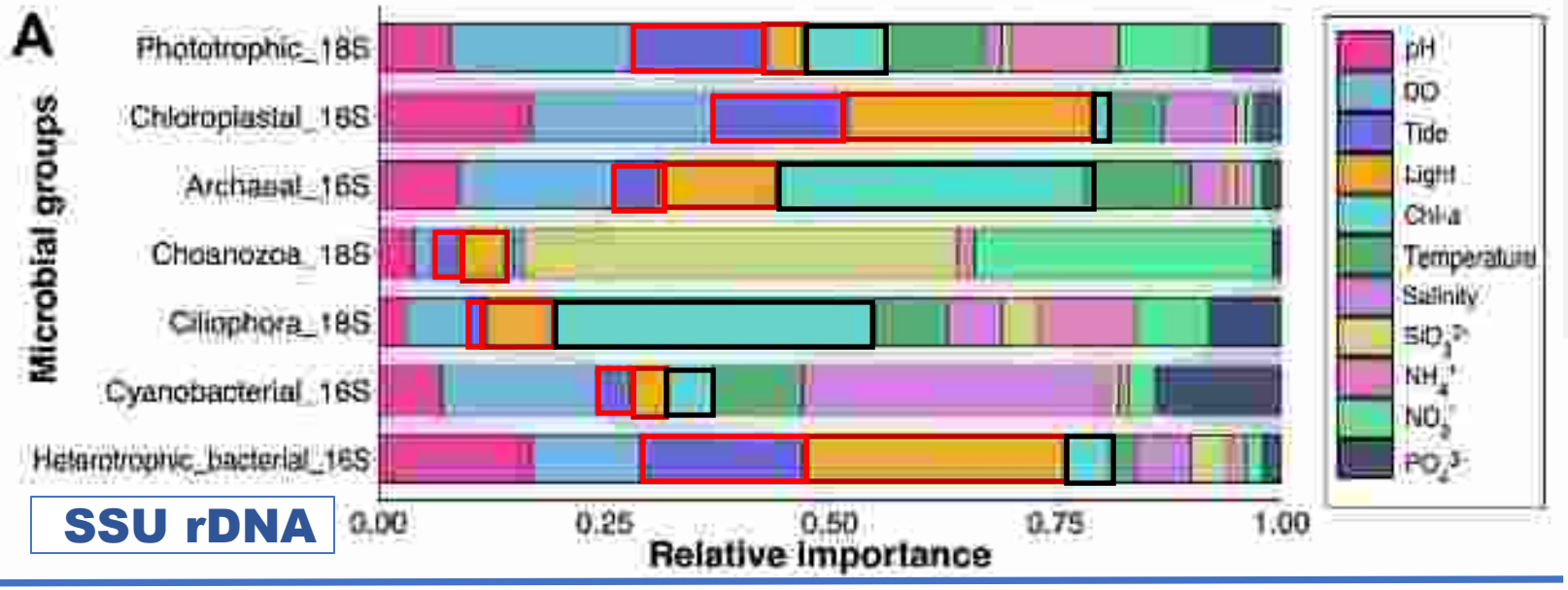




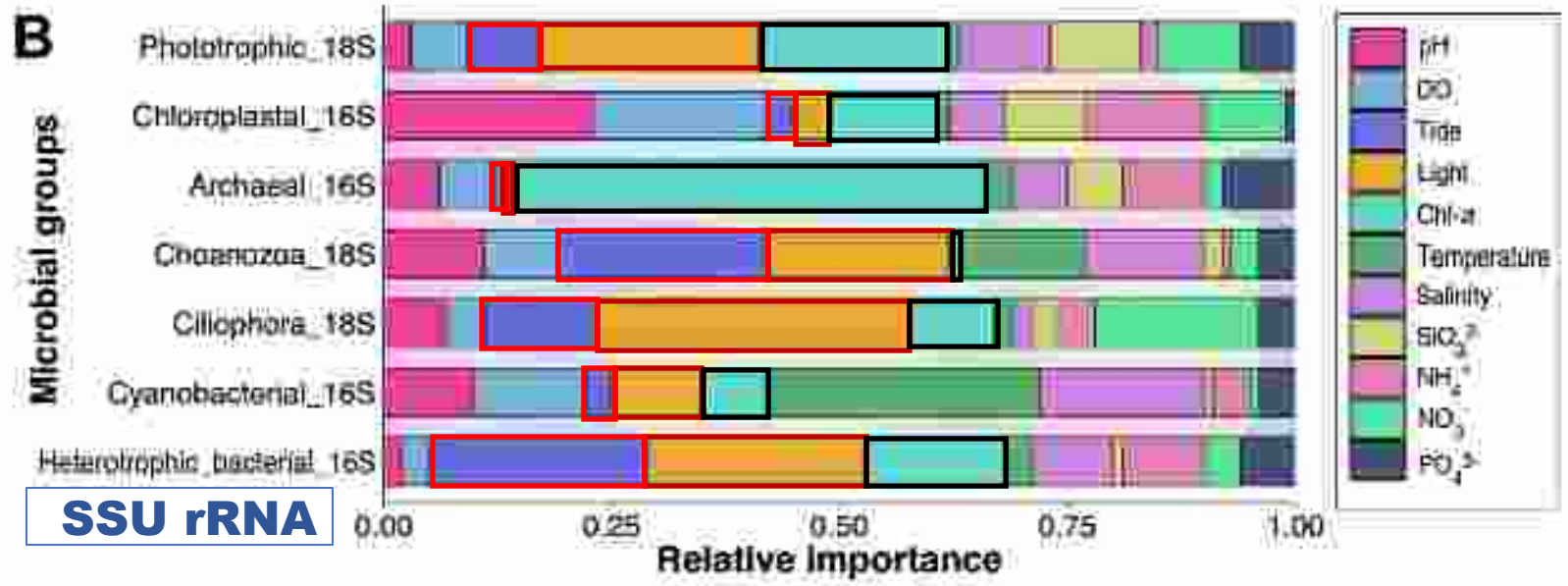
- Different microbes showed distinct rhythmic patterns peaking at different time points
- Phylogenetically close microbes tend to have similar rhythmic patterns, at either rDNA or rRNA levels
- RNA level rhythmic detection were more sensitive for SAR11, Flavo, Chaetoceros, ciliates
- DNA level rhythmic detection were more sensitive for picoeukaryotes, Synechococcus and copepods

Microbes of diel rhythms

海洋科学与工程系
Department of Ocean Science and Engineering



SSU rDNA



SSU rRNA

- Diel periods of light and tides might drive the rhythmic changes of chloroplast-bearing and heterotrophic microbes, particularly at the rRNA level
- Picocyanobacteria were mainly regulated by salinity, temperature, and nutrients.
- Chlorophyll a concentrations had a strong influence on the abundance of archaea and ciliates
- Light-dependent heterotrophy might be common for heterotrophic bacteria

Summary 1

To sum up：

- Diel rhythms are common in coastal microbes
- Differential rhythms can be detected at rDNA / rRNA levels
- Rhythms of protists and heterotrophic bacteria depend on light, tides, and primary productivity

Part 4

Rhythmic changes of viruses in Daya Bay

Viruses of diel rhythms

海洋科学与工程系
Department of Ocean Science and Engineering

Sampling Site



Environmental Parameters

Temperature, pH, Dissolved oxygen, Salinity and chlorophyll

Sampling preparation

72-h time series, with 2-h interval

One liter of water per sample

Filtered through 0.2/0.02 μm

Obtain Cellular/Viral Fraction

Isolate genetic material

Sequencing

Cellular Fraction

Metagenomic

Metatranscriptomic

Amplicon sequencing (SSU rRNA V4-5)

Viral Fraction

Viromics

Amplicon Sequencing (viral gene g23)

Data processing

Assembly (MetaSpades)

Detect viral contigs (DMC, VirSorter2)

Completeness/quality check (checkV)

Assign taxonomy (vContact2)

Detection of viral encoded metabolites (DRAMv)

Diel cycle of viral abundance (R package RAIN)

viral contigs as a reference (DMC, VirSorter2)

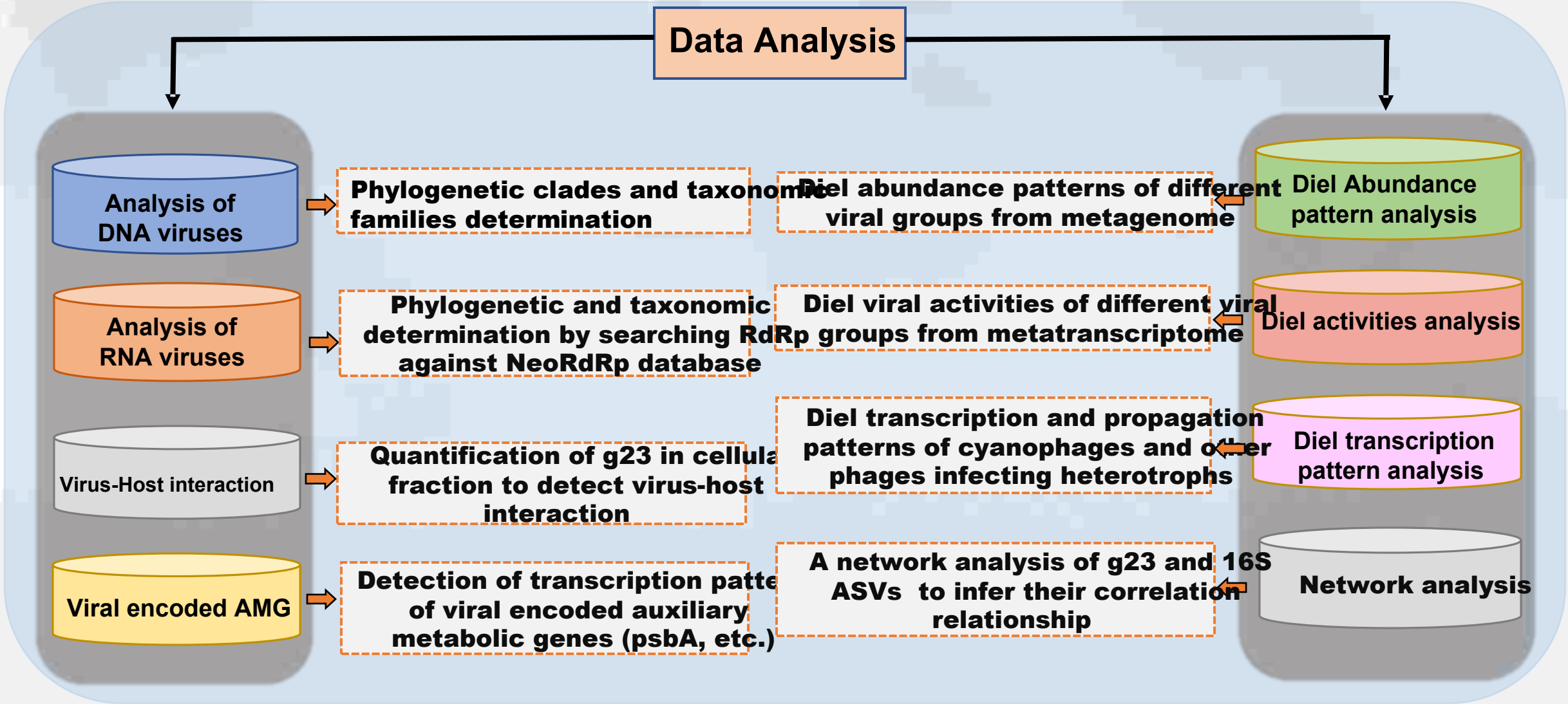
Mapping reads (coverM)

Aligned read (RPKM)

Downstream Analysis

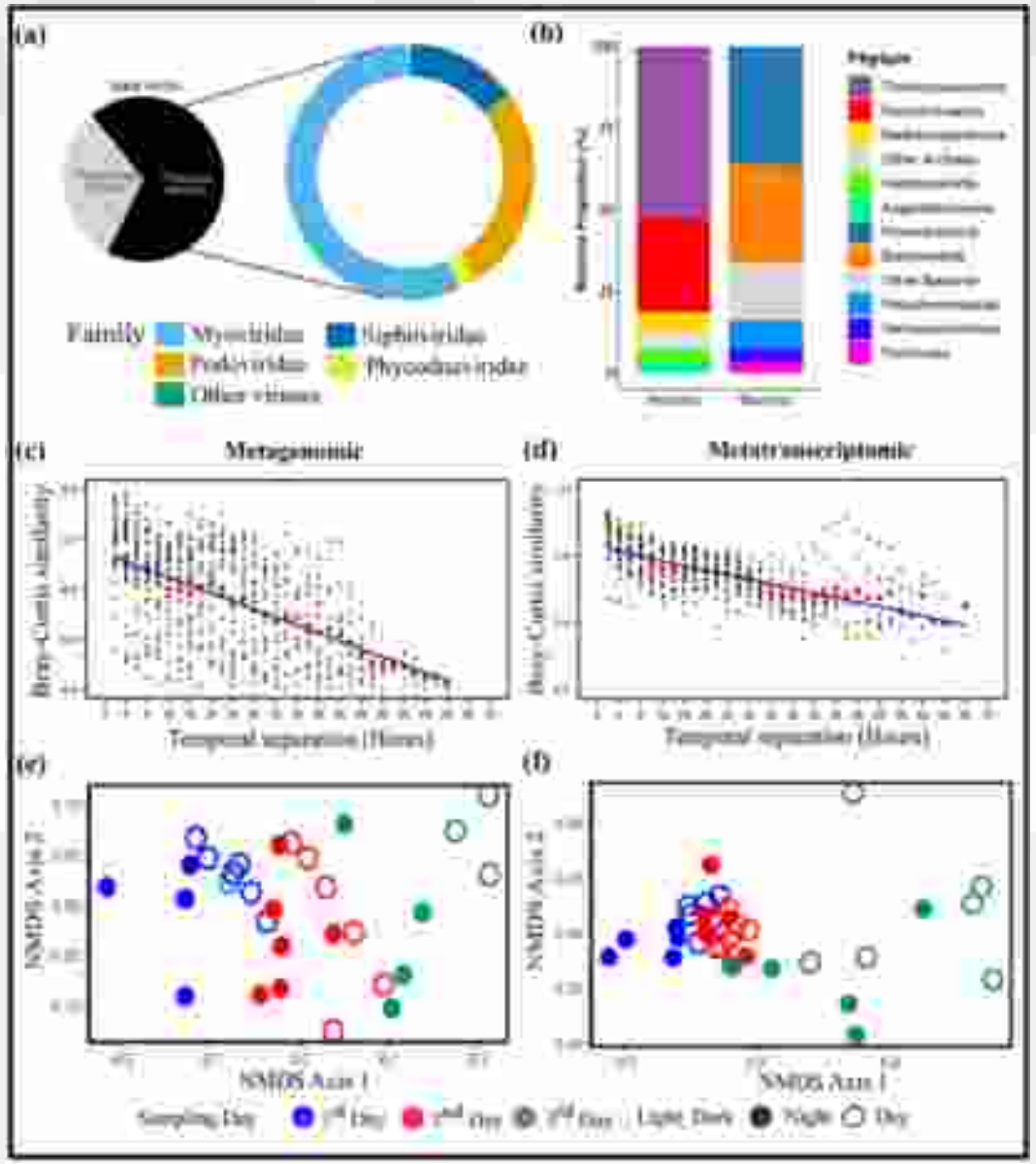
Viral data analysis

海洋科学与工程系
Department of Ocean Science and Engineering



Diel dynamics orchestrate viral communities

海洋科学与工程系
Department of Ocean Science and Engineering

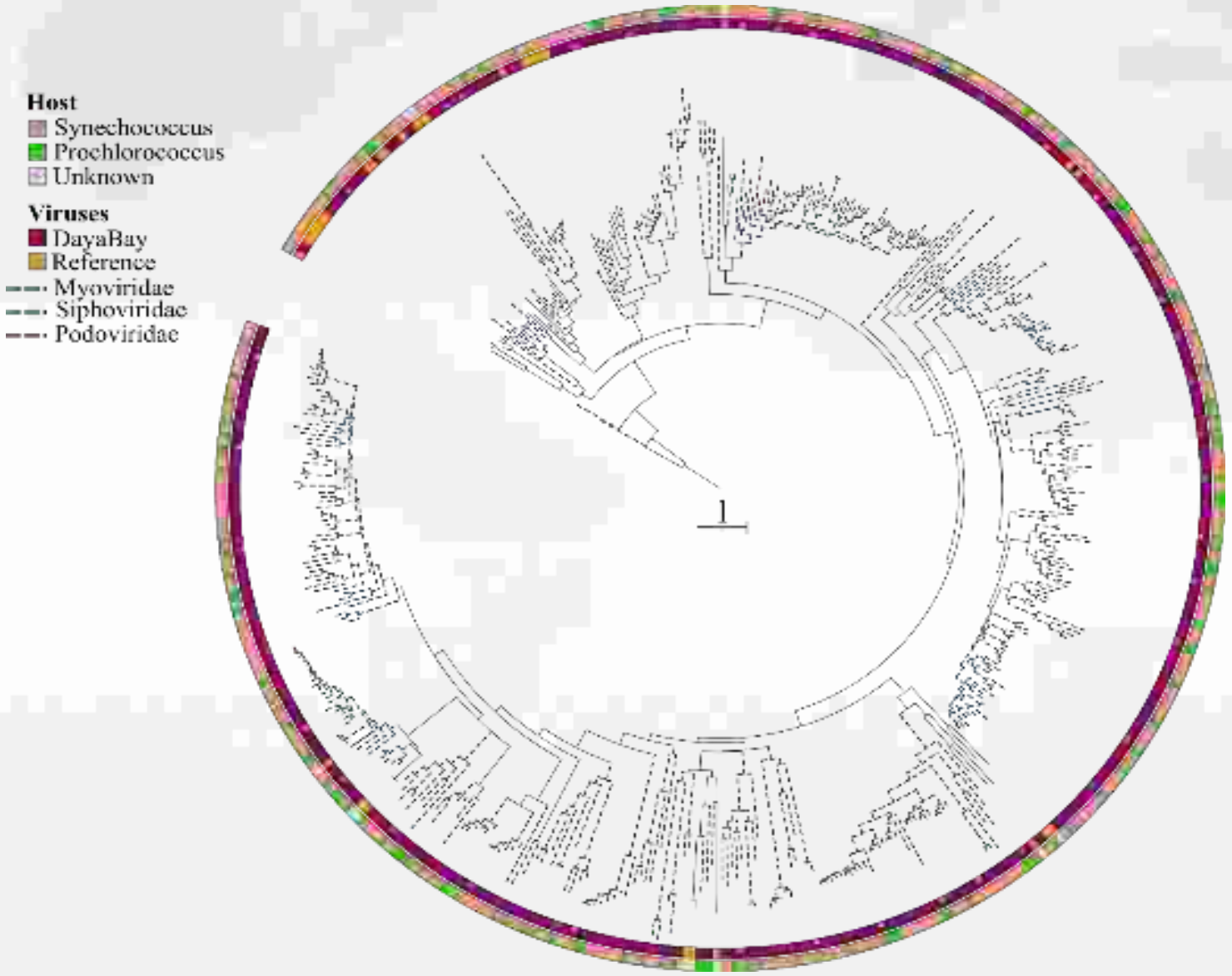


Temporal dynamics of viral communities at Daya Bay

- 22,151 viral Operational Taxonomic Units (vOTUs) were predicted.
- Of these, 68.4% were classified into viral families, with Myoviridae dominating, followed by Podoviridae and Siphoviridae.
- The majority of classified viruses, 70% of which had a predicted host, target bacterial hosts, primarily Proteobacteria and Firmicutes.
- A smaller proportion of viruses were predicted to infect archaeal hosts, including Thermoplasmatota, Nanoarchaeaota, and Methanobacterota
- Temporal analysis revealed significant diel variations in the actively infecting viral forms.
- Peak community similarity occurred at 24-hour intervals (Bray-Curtis), and non-metric multidimensional scaling (NMDS) analysis demonstrated distinct clustering patterns correlated with sampling days and light conditions.
- These findings highlight the strong influence of diel cycles on viral community assembly, regardless of daily shifts in community composition.

Phylogeny of diel cyanophages

海洋科学与工程系
Department of Ocean Science and Engineering

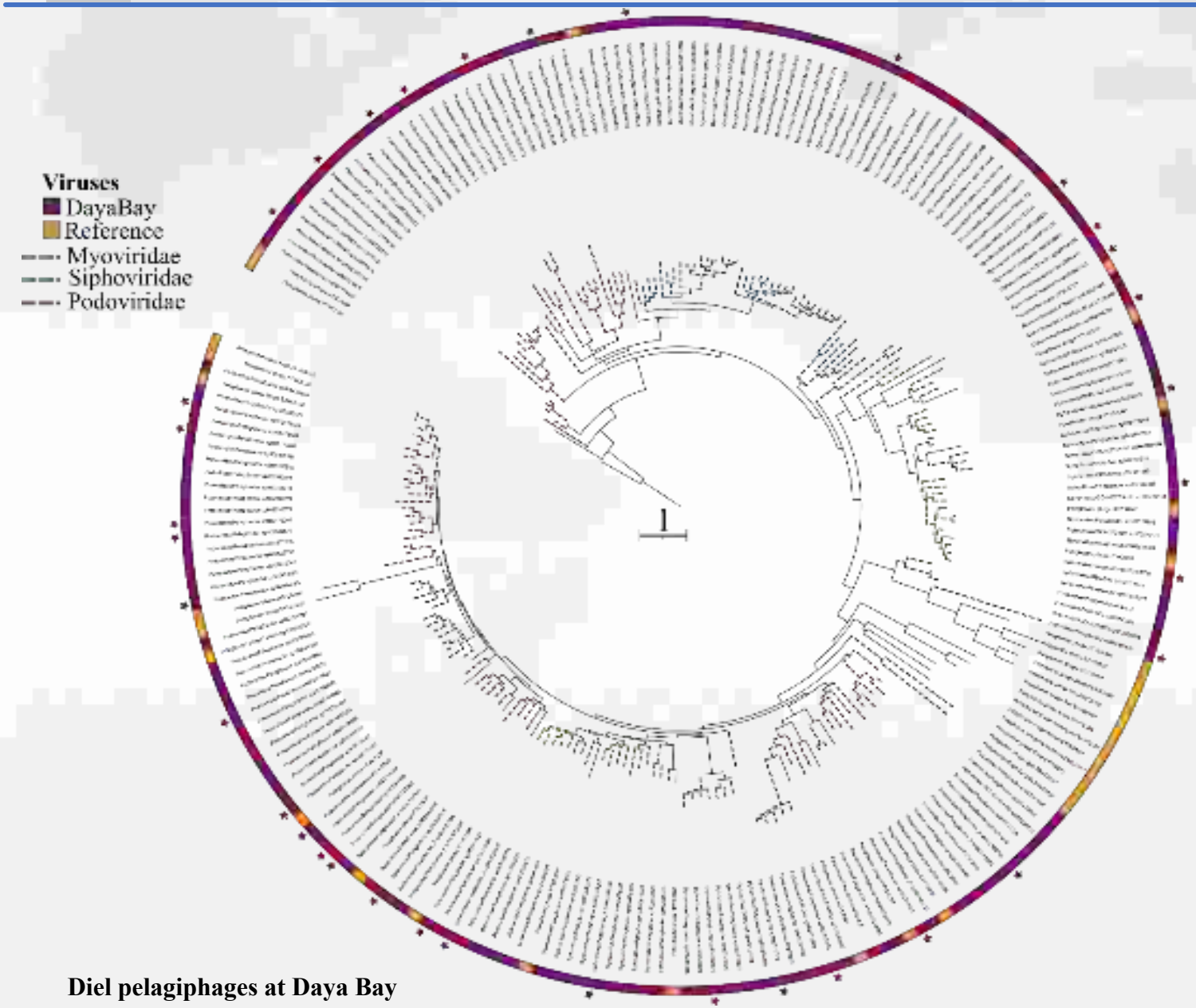


Diel cyanophages at Daya Bay

- Unrooted phylogenetic trees of Cyanophages (n = 464 from this study, plus 50 similar matches from RefSeq proteins) based on large terminase (TerL) sequences.
- Phages exhibiting significant diel patterns were highlighted with a star.
- Most of the diel cyanophages belong to the family Myoviridae.
- The hosts of diel cyanophages are mainly *Synechococcus*, and a few are *Prochlorococcus*

Phylogeny of diel pelagiphages

海洋科学与工程系
Department of Ocean Science and Engineering

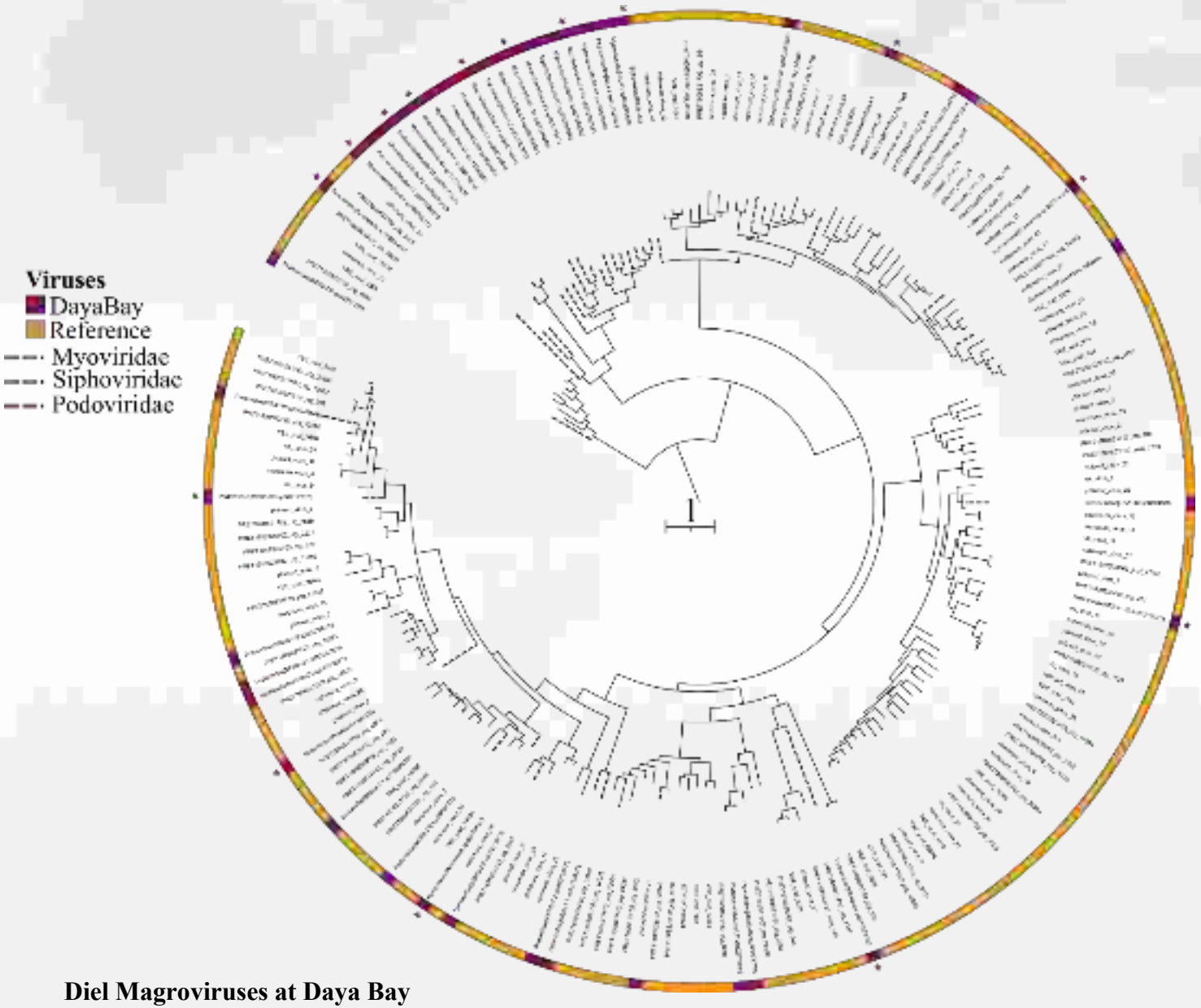


Diel pelagiphages at Daya Bay

- Unrooted phylogenetic trees for (b) Pelagiphages (n = 178 from this study, plus 35 similar matches from NCBI RefSeq sequences) based on DNA polymerase A (DNApolA) sequences.
- Phages exhibiting significant diel patterns were highlighted with a star.
- Most of the diel pelagiphages belong to the families Podoviridae and Siphoviridae.

Phylogeny of diel magroviruses

海洋科学与工程系
Department of Ocean Science and Engineering



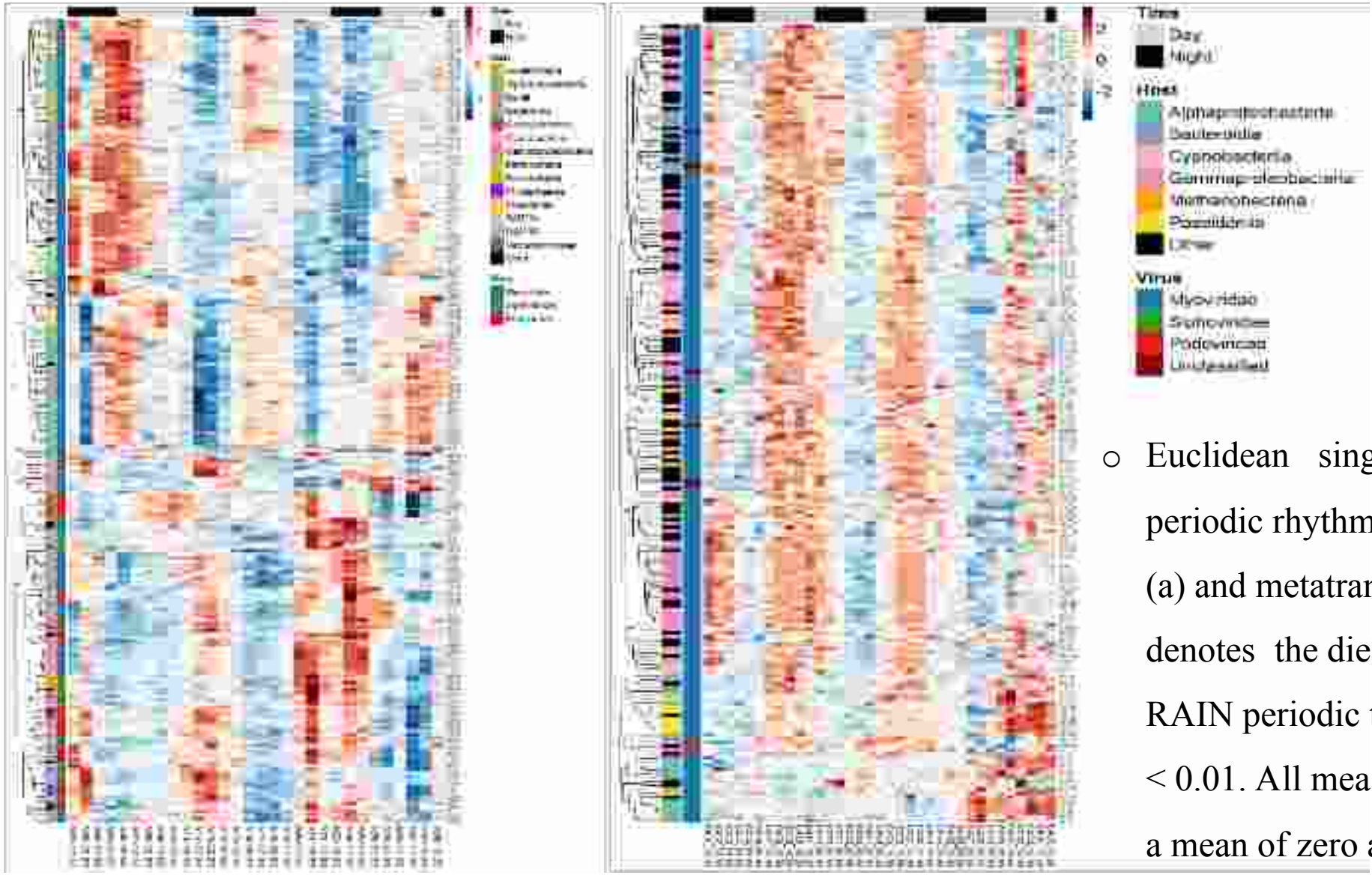
Diel Magroviruses at Daya Bay

- Unrooted phylogenetic trees for Magroviruses (n = 47 from this study, plus 152 similar matches from NCBI RefSeq sequences) based on DNA polymerase B (DNApolB) sequences.
- Phages exhibiting significant diel patterns were highlighted with a star.
- Most of the diel pelagiphages belong to the families Podoviridae and Myoviridae.

Diel dynamics of coastal viruses

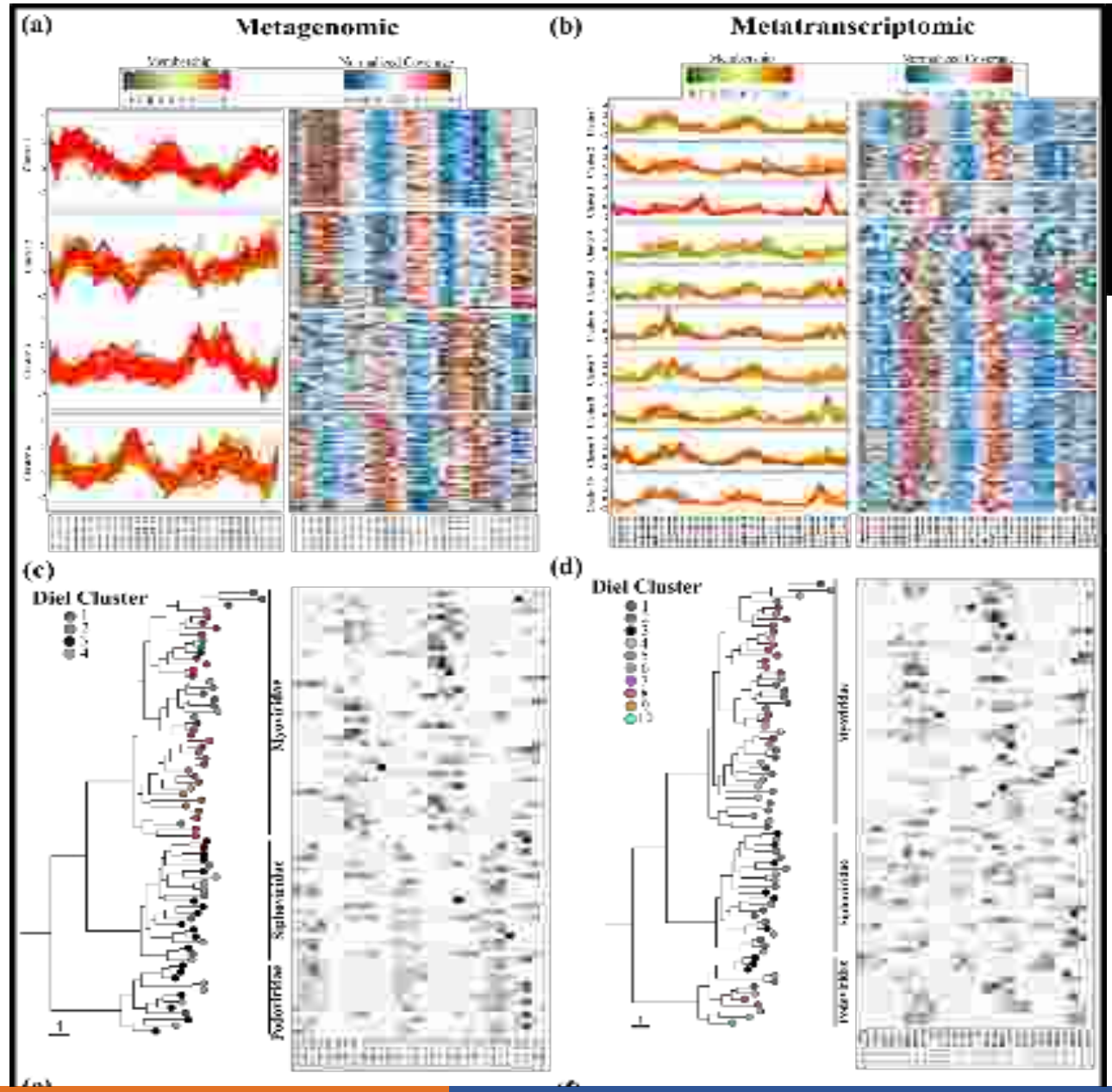
(a) Metagenomic abundance

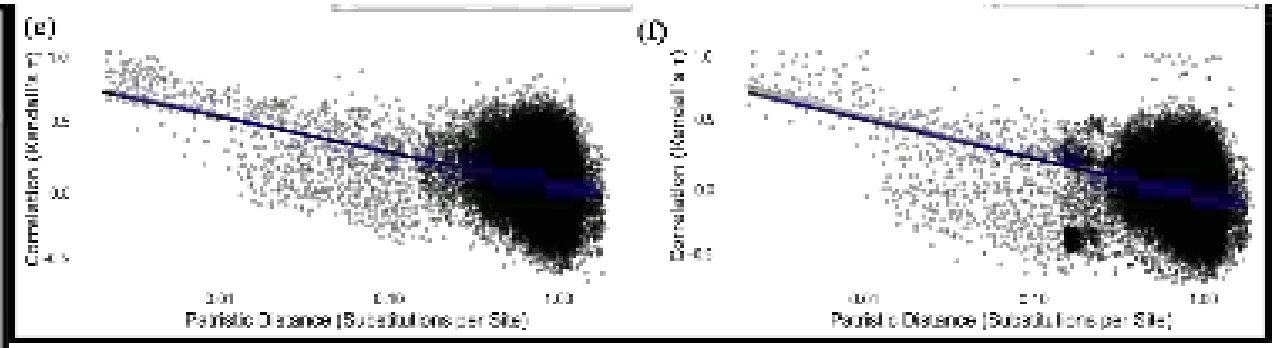
(b) Metatranscriptomic abundance



- Euclidean single cluster analysis of diel periodic rhythms in vOTUs in metagenome (a) and metatranscriptome (b). The heatmap denotes the diel vOTUs determined by the RAIN periodic test with the corrected P value < 0.01. All measurements were scaled to have a mean of zero and a variance of one.

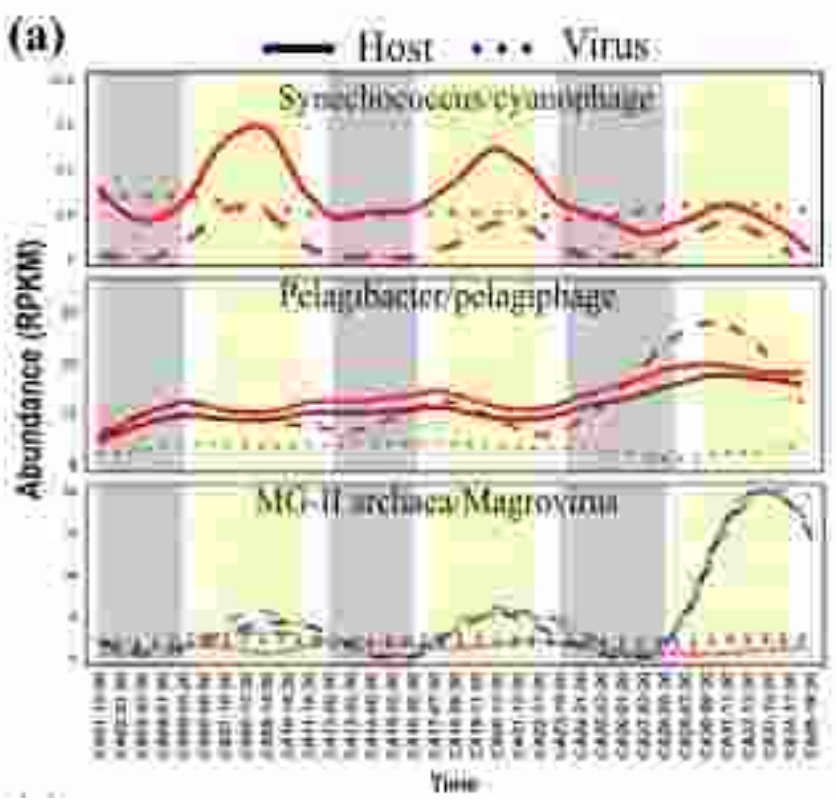
Phylogenetic proximity determines viral diel dynamics

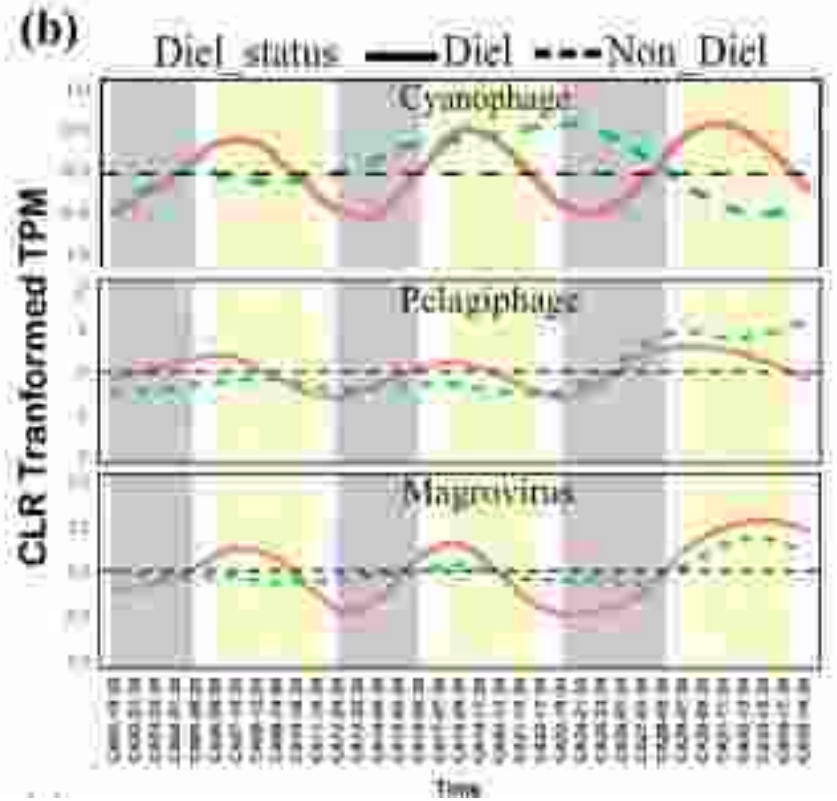




- 14.48% (3,209 vOTUs) and 437 vOTUs (1.97%) at the metagenomic and metatranscriptomic levels, respectively, exhibited significant diel fluctuations and circadian rhythms.
- 45 vOTUs were pelagiphages infecting Pelagibacter (SAR11 clade), 5 vOTUs were cyanophages targeting Synechococcus and Prochlorococcus, 20 vOTUs were found to infect archaea, including Thermoplasmatota and Nanoarchaeota.
- Interestingly, phylogenetic analysis revealed a significant link between viral evolutionary relatedness and diel dynamics. For example, closely related viral strains, based on T4 g23 gene, exhibited similar diel abundance and activity patterns (Figure 2c-d).
- Quantitatively, we observed a significant negative correlation between phylogenetic distance and the similarity of diel abundance (Figure 2e) and transcriptional activity profiles.

Tight virus-host coupling

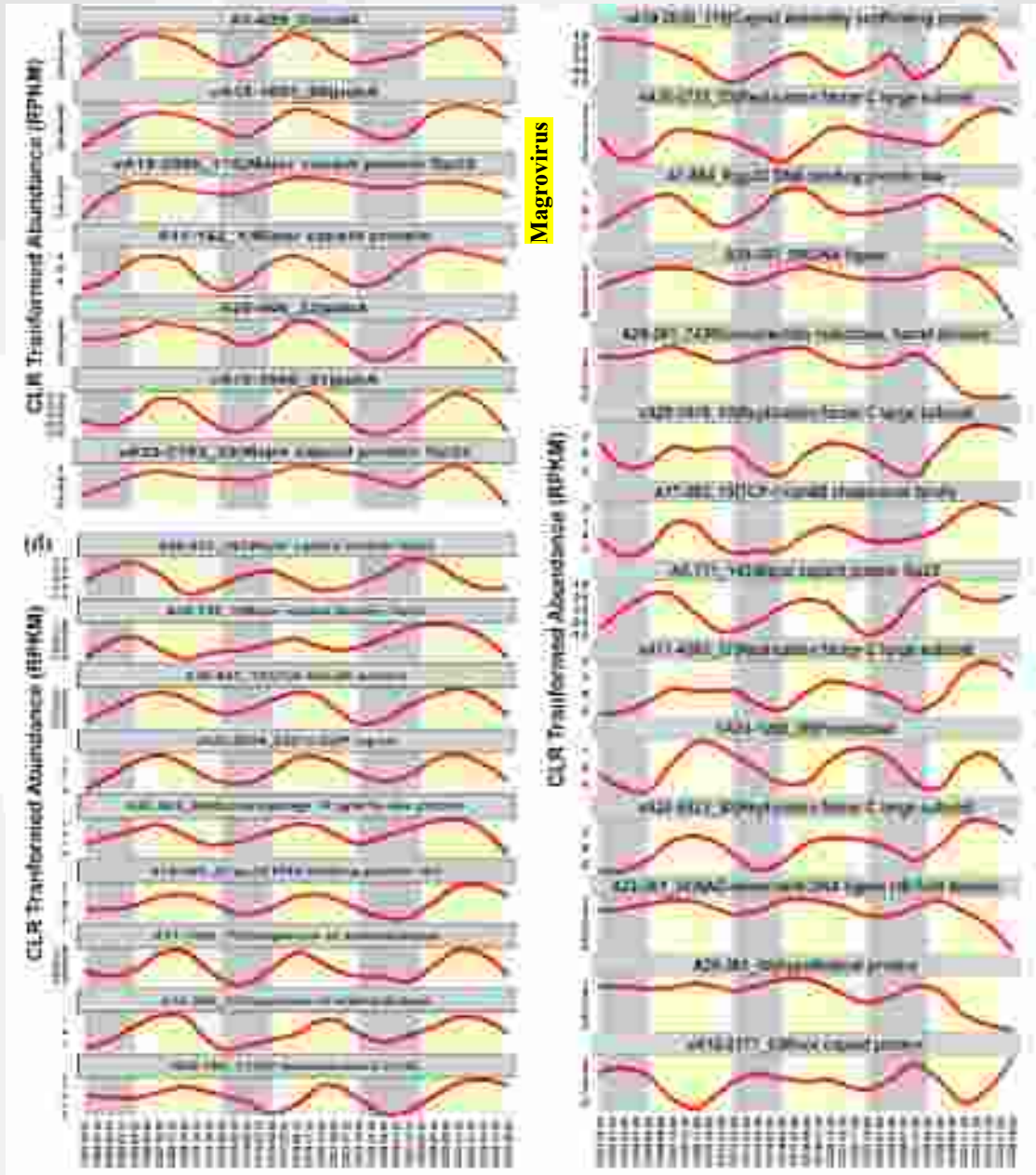




- A general mirroring of viral transcription with the diel activity of their putative hosts was observed, underscoring the tight interconnectedness of viral and host dynamics in marine ecosystems.
- Cyanophages showed tight synchronization with their hosts activities
- Peligiphages showed weak synchronization with their hosts
- Magroviruses peaked during daylight hours while their host peaked at night.

- Distinct viral strategies in Daya Bay, “Kill-the-Winner” and “Piggyback-the-Winner” models were observed.
- Specifically, cyanophages and pelagiphages appear to employ the "Kill-the-Winner" strategy, where viral abundance increases with host population size.
- Conversely, viruses infecting MGII Euryarchaeota may adopt the "Piggyback-the-Winner" strategy, exhibiting increased abundance even as host populations decline, potentially indicating a shift towards lysogeny under specific conditions

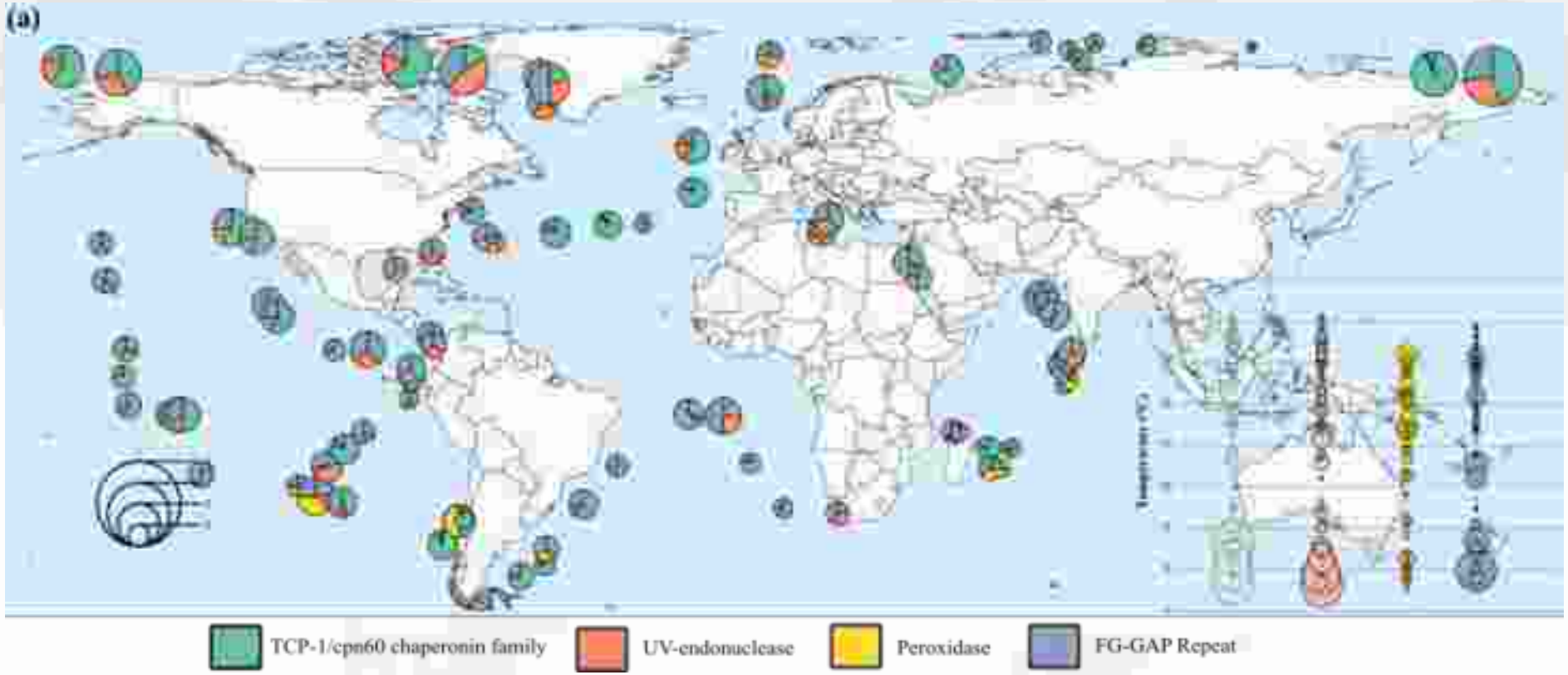
Diel orchestration of key viral processes



- 30 dominant genes with significant diel expression patterns were identified.
- DNA replication and virion assembly genes consistently peaked during daylight hours, in synchrony with the host's peak photosynthetic activity.
- Structural protein genes, including those encoding the T4-like major capsid protein Gp23, minor capsid proteins, and the capsid assembly scaffolding protein, exhibited distinct diel profiles, peaking sequentially in the early morning, at noon, and in the afternoon, respectively.
- DNA replication and repair genes (e.g., replication factor C large subunit, *dnaN*, gp23, DNA ligase) peaked between morning and noon.
- FG-GAP (phenylalanyl-glycyl, glycyl-alanyl-prolyl) repeat proteins, exhibited peak transcription during daylight hours.
- Several stress-related genes, such as chaperones (e.g., TCP-1/cpn60 family proteins found in archaeal viruses and chaperones in pelagiphages), UV-endonuclease UvdE, and peroxidase, showed significant diel patterns.
- The expression time of genes encoding peroxidase peaks later in the afternoon (16:30), coinciding with the peak generation of HOOH, which leads to cumulative oxidative stress. This late-afternoon peak in peroxidase activity is strategically timed to detoxify accumulated ROS and alleviate oxidative damage during a period of maximal environmental stress.

Biogeography of diel-regulated viral proteins

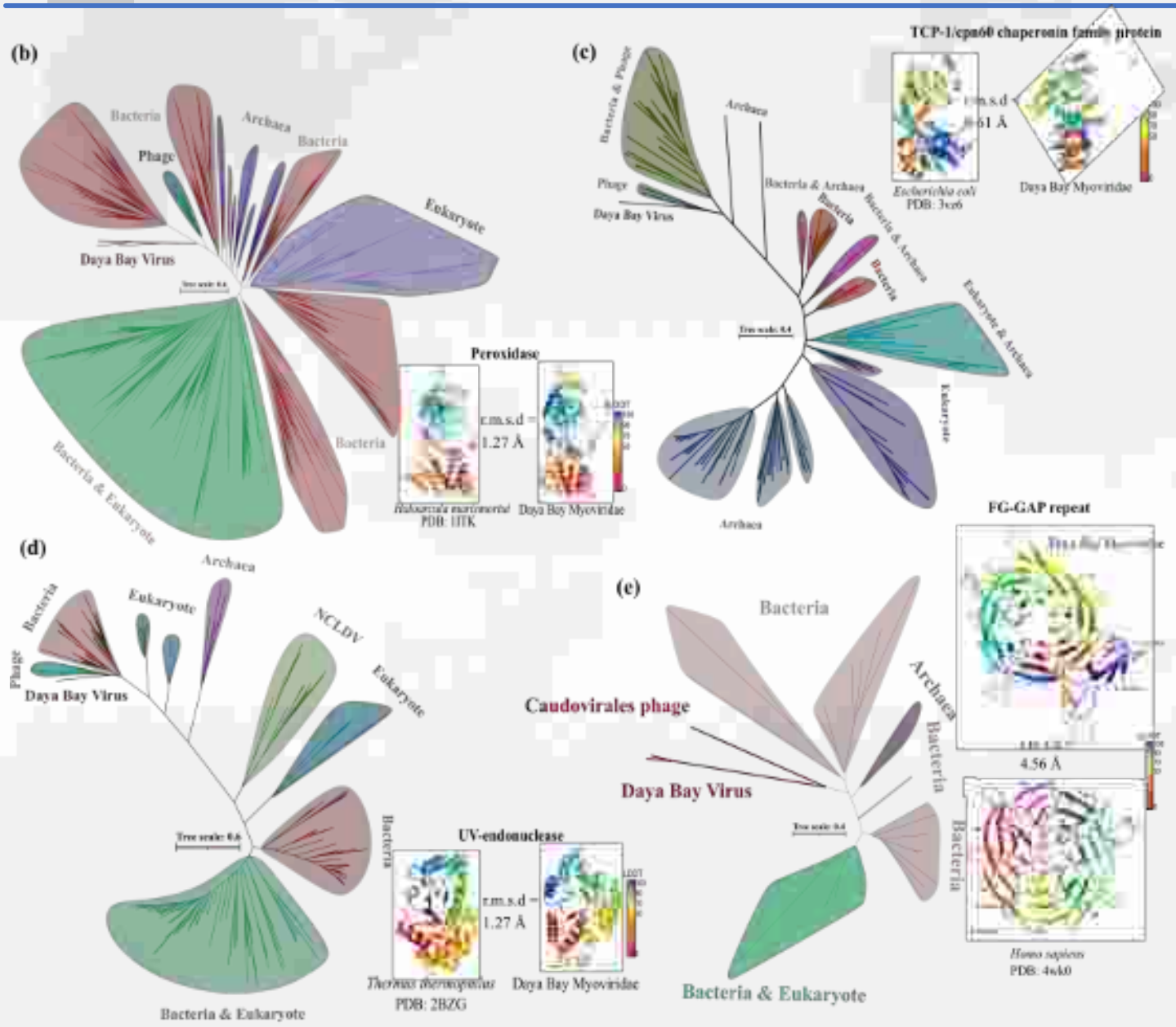
海洋科学与工程系
Department of Ocean Science and Engineering



- FG-GAP repeat genes were predominantly concentrated in the tropical Pacific and Indian Oceans, with scattered presence in the Atlantic, suggesting adaptation to warm, oligotrophic waters
- Peroxidase genes showed a broader distribution across various latitudes, notably in the Mediterranean Sea, Levantine Sea, and Indian Ocean, indicating their widespread role in ROS detoxification across diverse marine environments
- The TCP-1/cpn60 chaperonin family exhibited a clear preference for polar and subpolar regions, with the highest relative abundances in the Arctic, Antarctic, and Southern Oceans, highlighting their functional role in adaptation to cold, demanding environments.
- Conversely, UV-endonuclease genes were more abundant in tropical and subtropical regions, particularly in the Indian Ocean and t he tropical Pacific, aligning with high solar UV radiation levels and emphasizing their importance in DNA repair

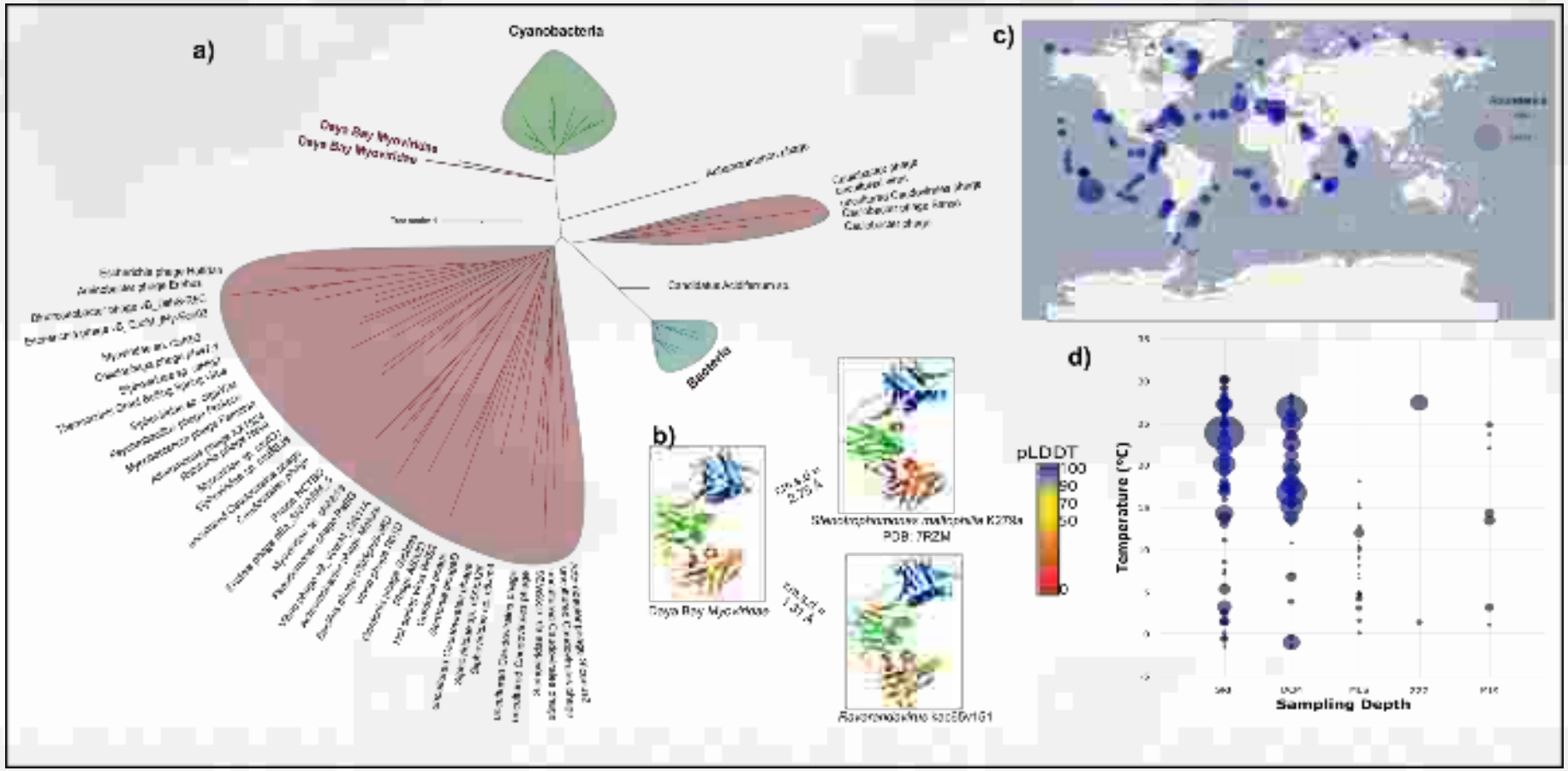
Diel-regulated viral proteins

海洋科学与工程系
Department of Ocean Science and Engineering



- Viral proteins exhibited closer phylogenetic affiliations to specific bacterial groups or other viral sequences, while remaining distantly related to archaeal and eukaryotic homologs. This pattern supports the idea that viruses acquired these genes through HGT, potentially from bacterial hosts or co-infecting phages.
- Comparative structural analysis with experimentally determined homologs revealed significant conservation for viral peroxidase (RMSD 0.74 Å), UV-endonuclease (RMSD 1.27 Å, and TCP-1/cpn60 chaperonin family protein (RMSD 0.61 Å). These low RMSD values strongly suggest that the overall protein structures are conserved and, by inference, similar functional mechanisms are expected.

Diel-regulated viral proteins



- The *dnaN* gene in a dominant Myoviridae vOTU is particularly significant, as this AMG, encoding the DNA Polymerase III beta subunit (the 'sliding clamp'), has thus far been found in only a limited number of sequenced cyanophages, such as P60.

- The presence of *dnaN* provides substantial benefits for the infecting phage by dramatically increasing the processivity of DNA replication, ensuring rapid and efficient synthesis of progeny viral genomes within the host cell.
- This strategy allows the phage to potentially override or augment the host's own replication machinery, thereby accelerating its life cycle and maximizing virion production, especially during periods of high host metabolic activity.

Summary 2

To sum up：

- Diel rhythms shape viral dynamics, community composition, host interactions, and gene expression
- Viral abundance and activity are synchronized with host physiology and environmental cues
- Novel diel genes associated with circadian regulation were identified, such as UV-endonuclease, peroxidase, and chaperones, etc.

南方科技大学
SOUTHERN UNIVERSITY OF SCIENCE AND TECHNOLOGY



Thank you for your attention!

海洋科学与工程系
Department of Ocean Science and Engineering