Case study: Unveiling the seasonal dynamics of protist communities in tropical coastal ecosystems using long-read metabarcoding

BIO9905MERG1 – Bioinformatics for Environmental Sequencing

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Genomics and Ecology of Eukaryotes Lab (GEEK Lab)
8 April 2025

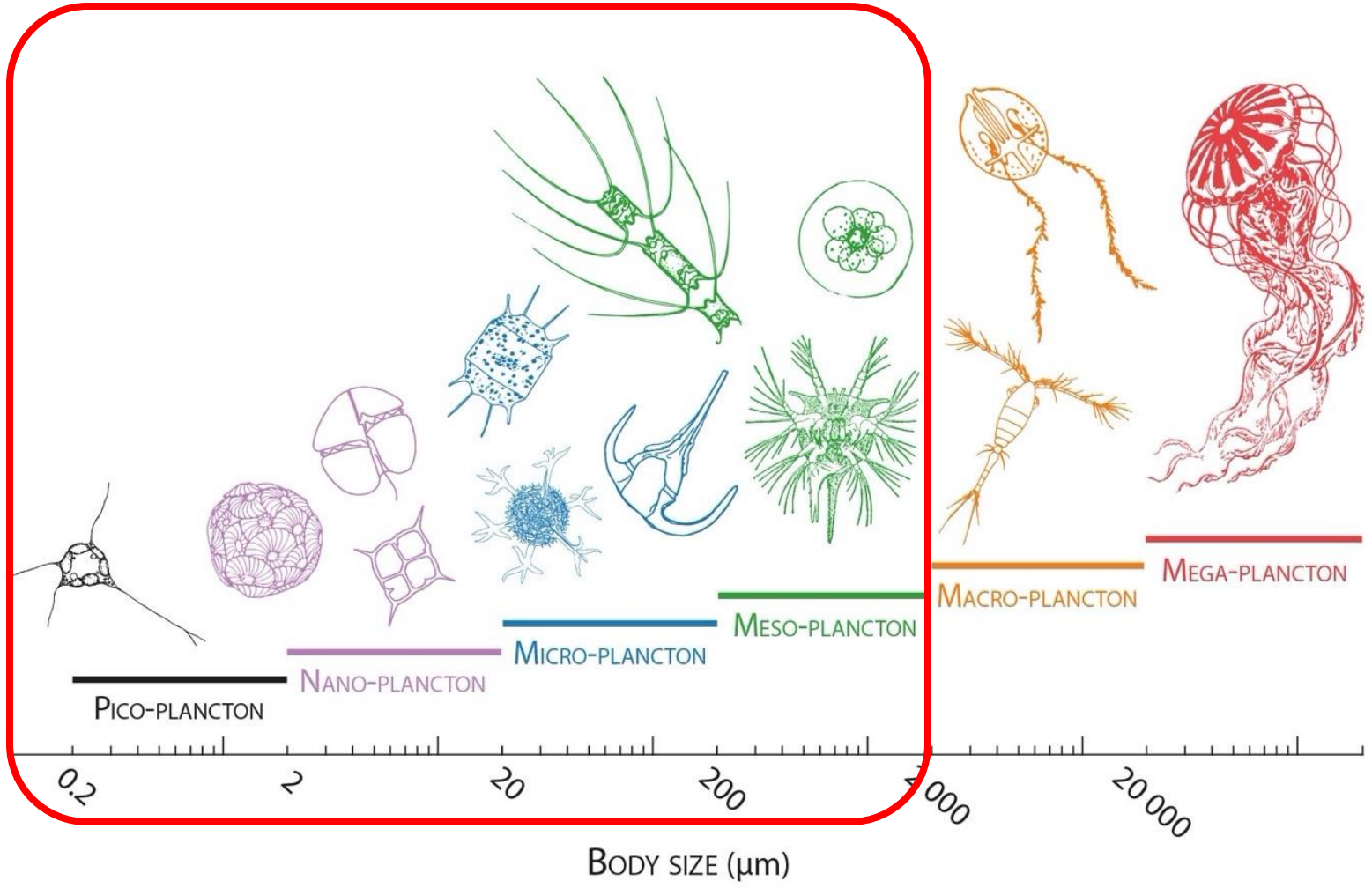
About me

- Postdoctoral research fellow at University of Oslo, Norway
- PhD at Nanyang Technological University, Singapore

My research

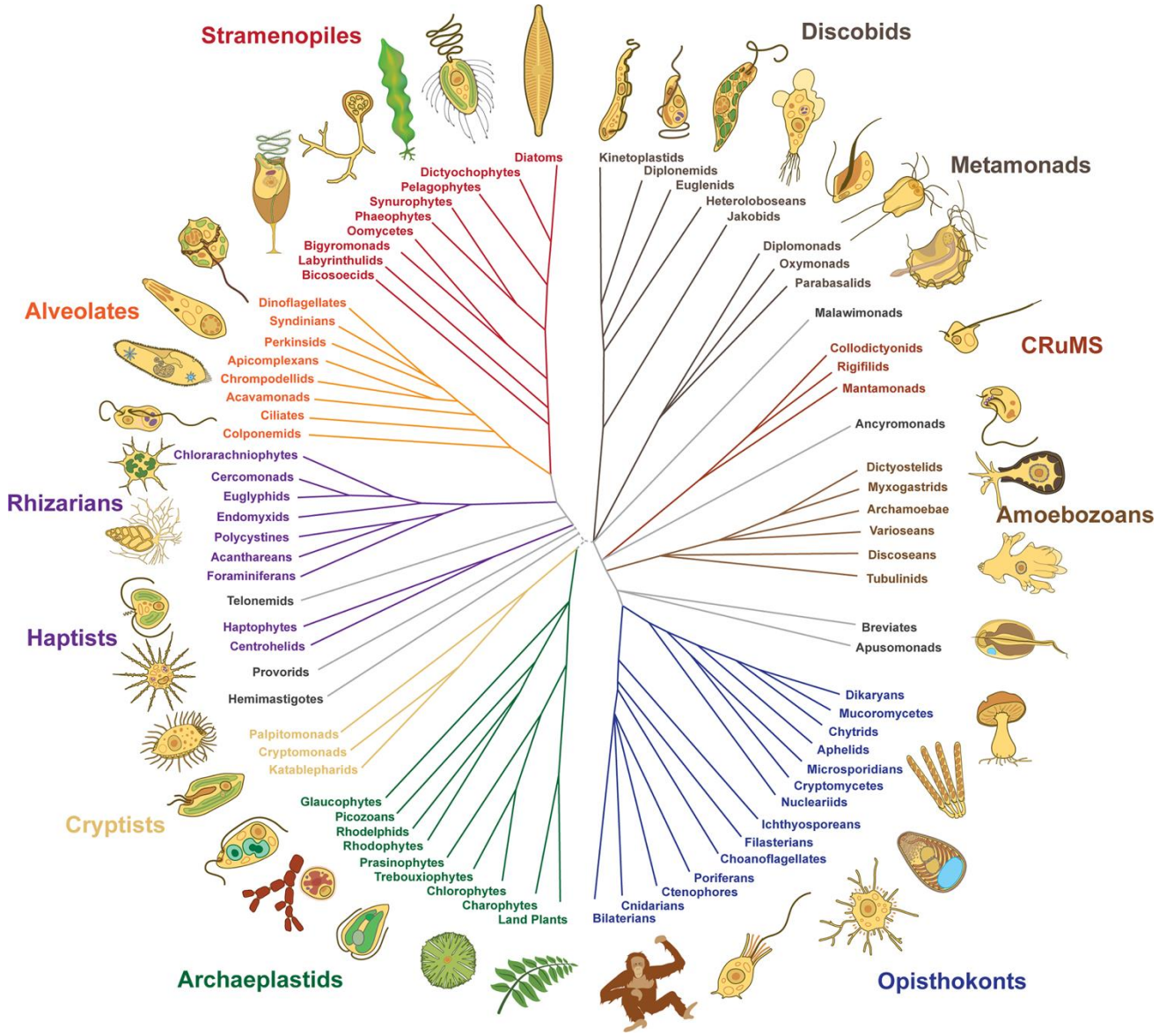
- Marine planktonic microbial eukaryotes (**protists**)
- Biological carbon pump – carbon fixation and carbon export
- DNA metabarcoding

Protist communities are diverse in **size**



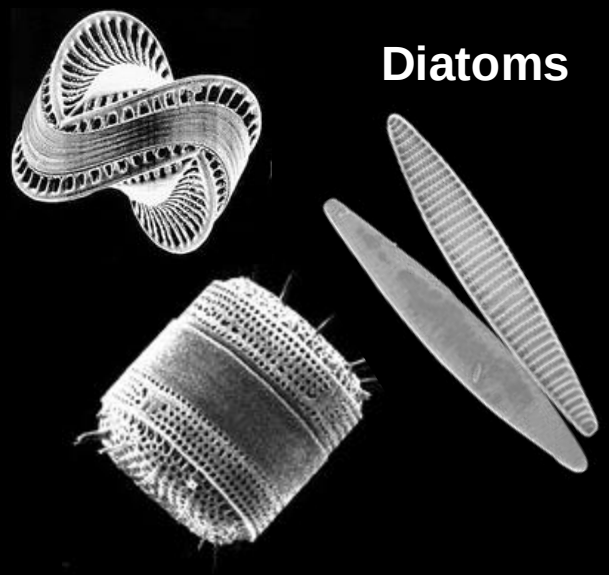
Biard (2016)

Protist communities are **taxonomically** diverse

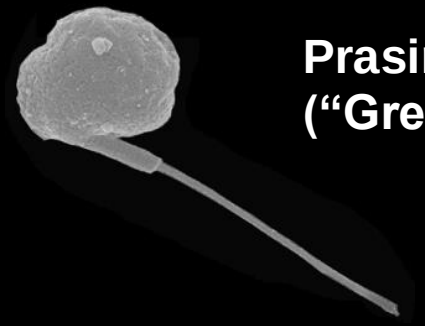


Keeling & Eglit (2023)

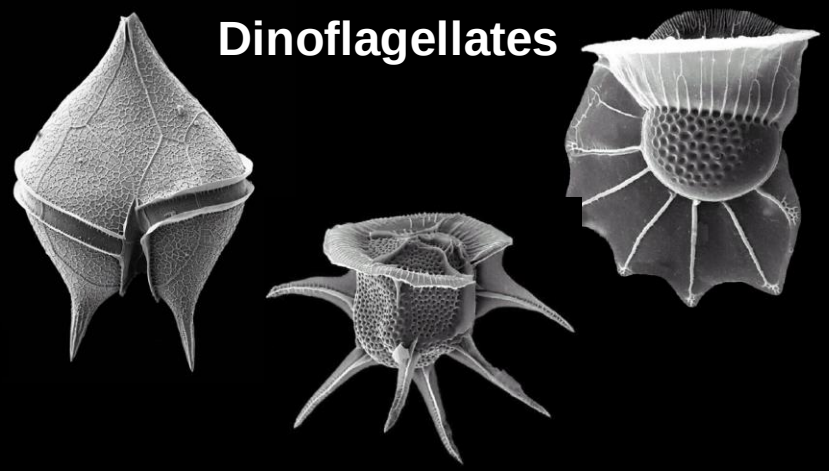
Ecologically significant groups of coastal protists



Diatoms

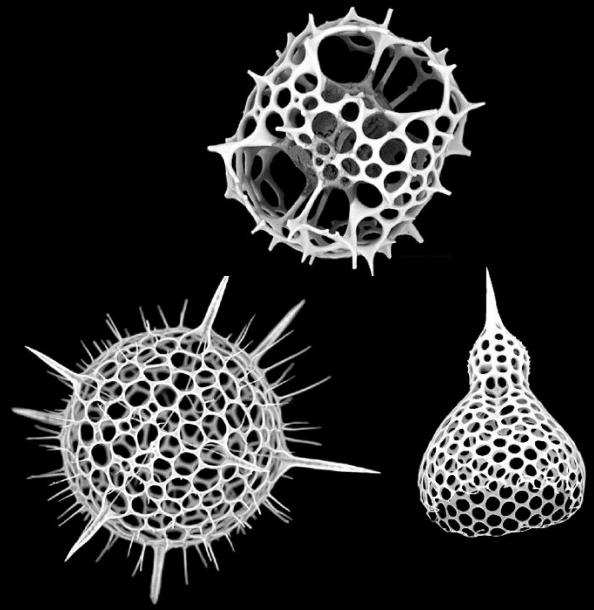


Prasinophytes (“Green Algae”)

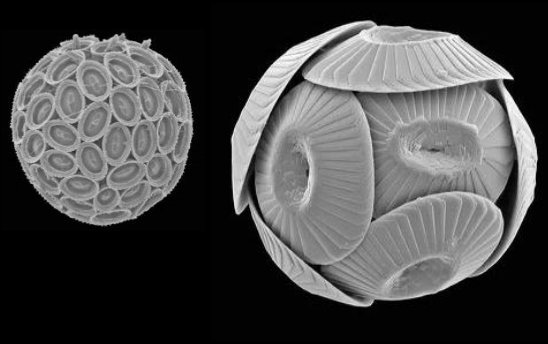


Dinoflagellates

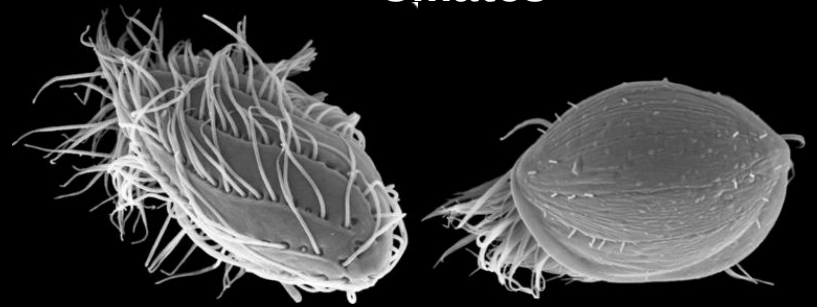
Radiolaria



Haptophytes

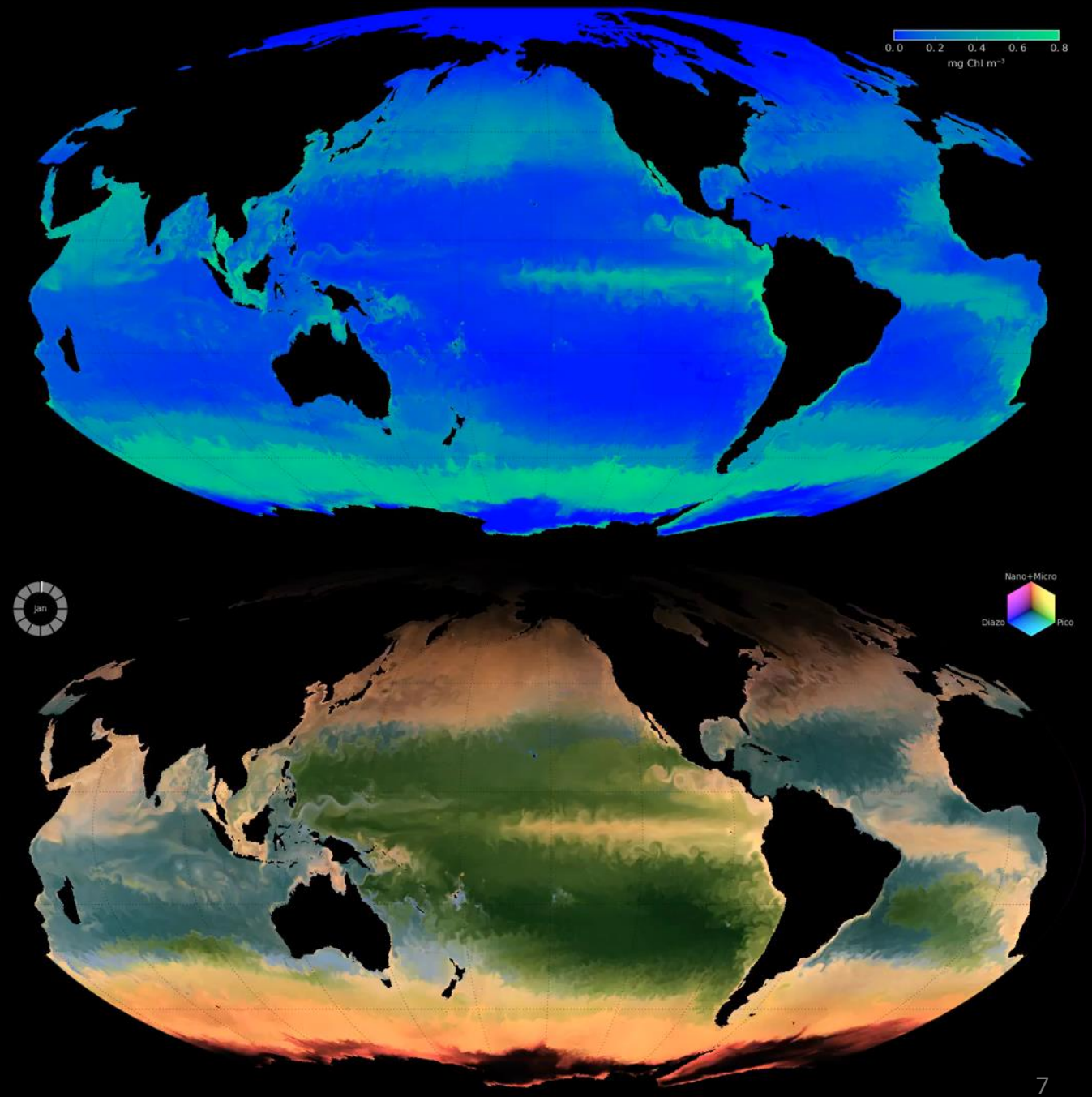


Ciliates



Tragin, M. *et al*. Perspectives of Phycology. 2016
Sandin M. M. protocols.io. 2018
Weinisch, L. Microbial Ecology. 2019

Climate seasonality affects the marine plankton community

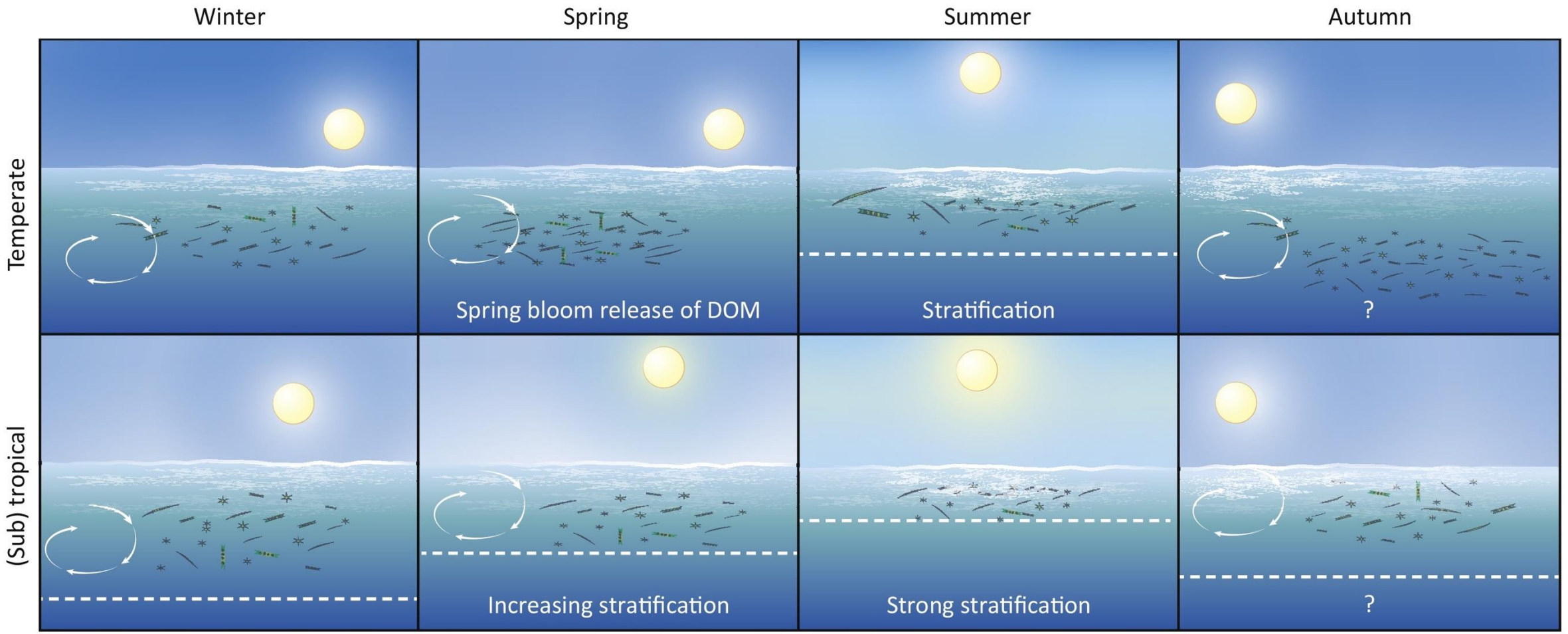


https://darwinproject.mit.edu/media-library/

Time series sampling

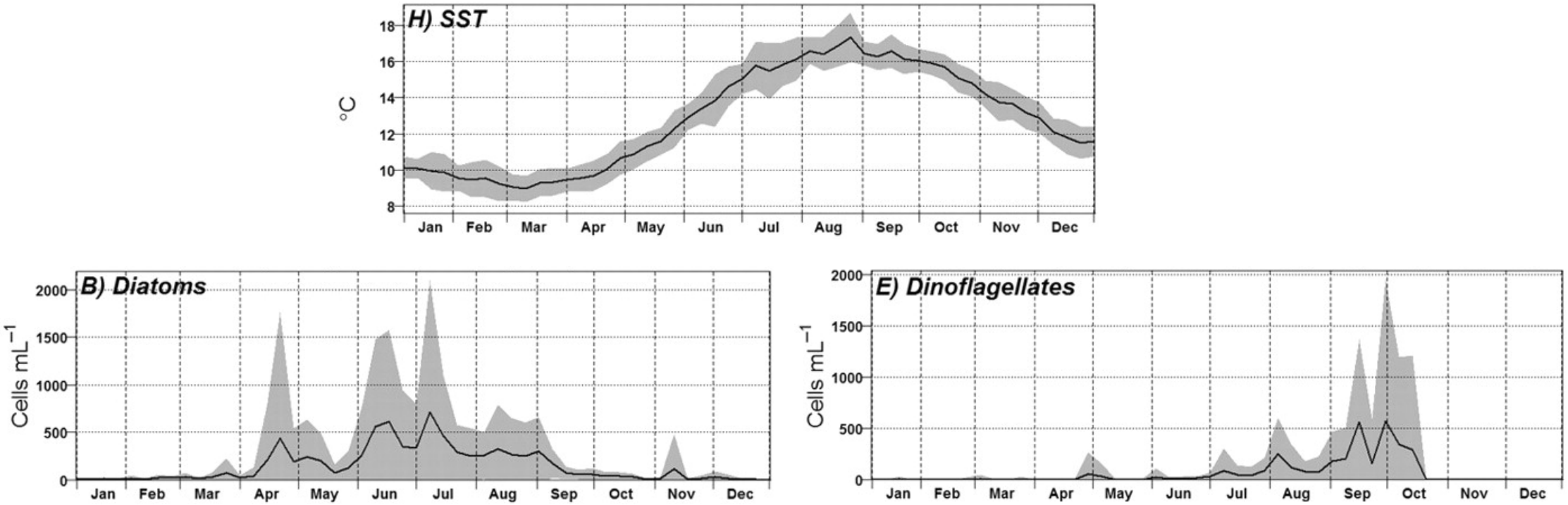
- Repeated sampling and measurements
  - Hourly
  - Daily
  - Weekly
  - Monthly
- Different methods to characterize the community
  - Microscopy
  - Flow cytometry cell counting
  - Chlorophyll *a*/photosynthetic pigment
  - DNA metabarcoding
- Temporal dynamics
  - Yearly (seasons), daily
  - Long-term
- What biotic and abiotic factors that drive the marine plankton community variation?

Temperate regions have strong seasonality



Bunse, C. and Pinhassi, J. Trends in Microbiology. 2017.

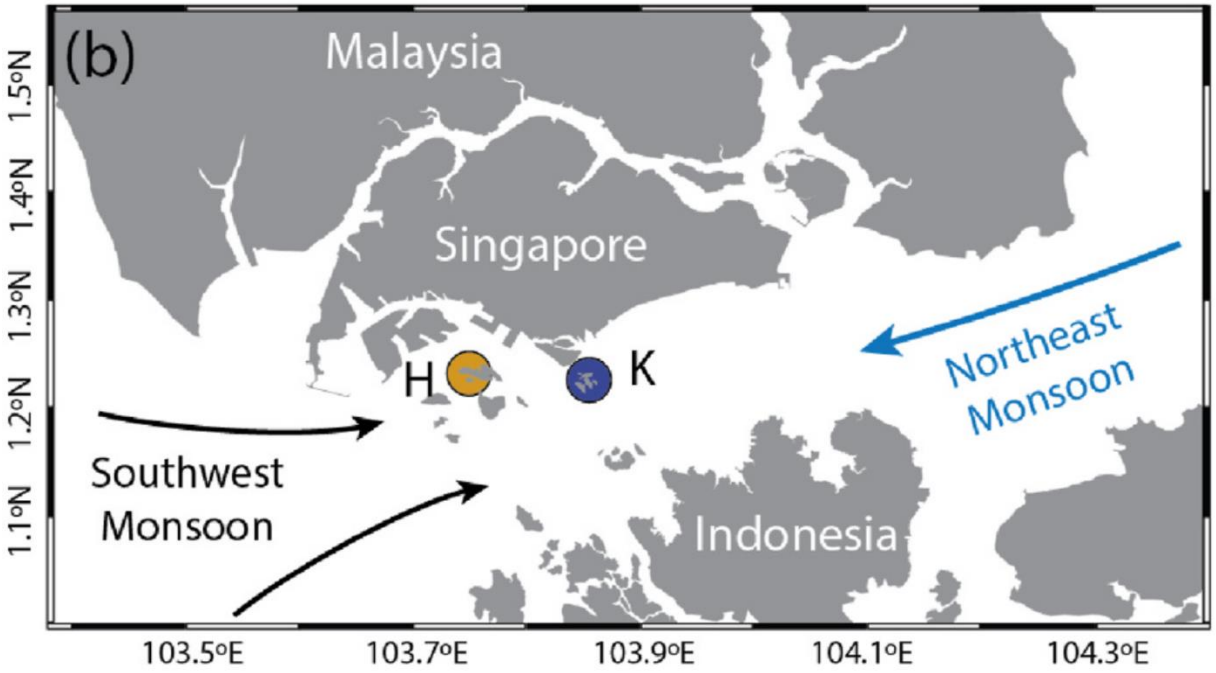
Temperate regions have strong seasonality

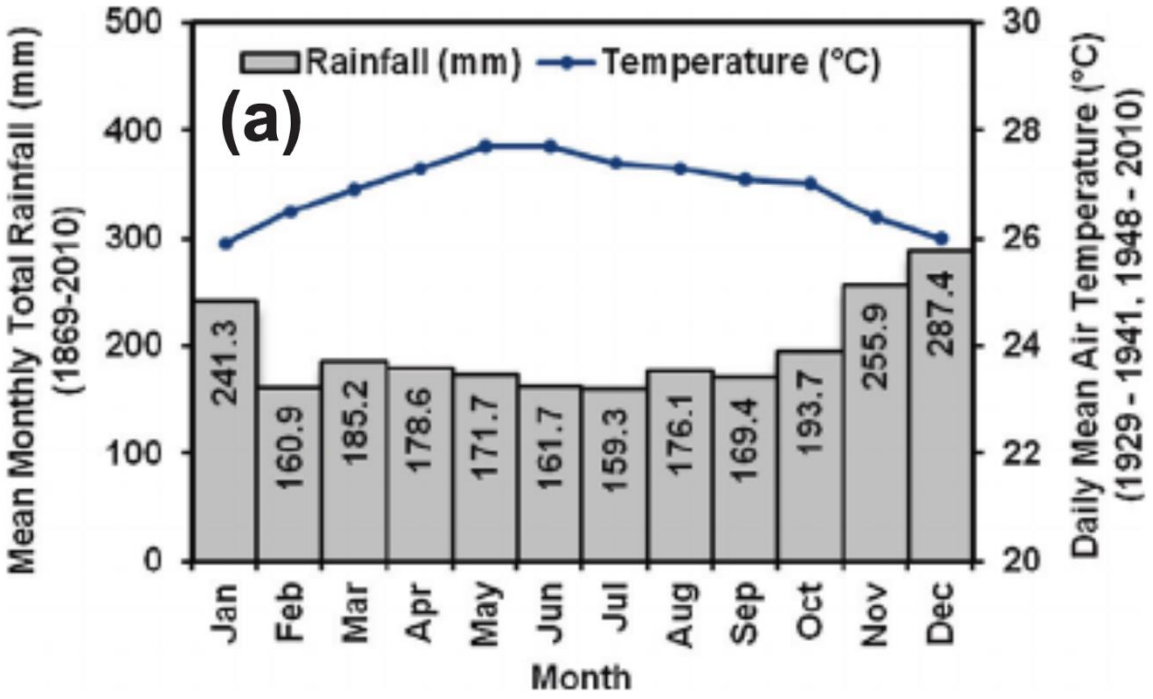


Widdicombe C. E., et al. 2010. Journal of Plankton Research.

What about tropical regions?

Tropical coastal ecosystem – Singapore Strait

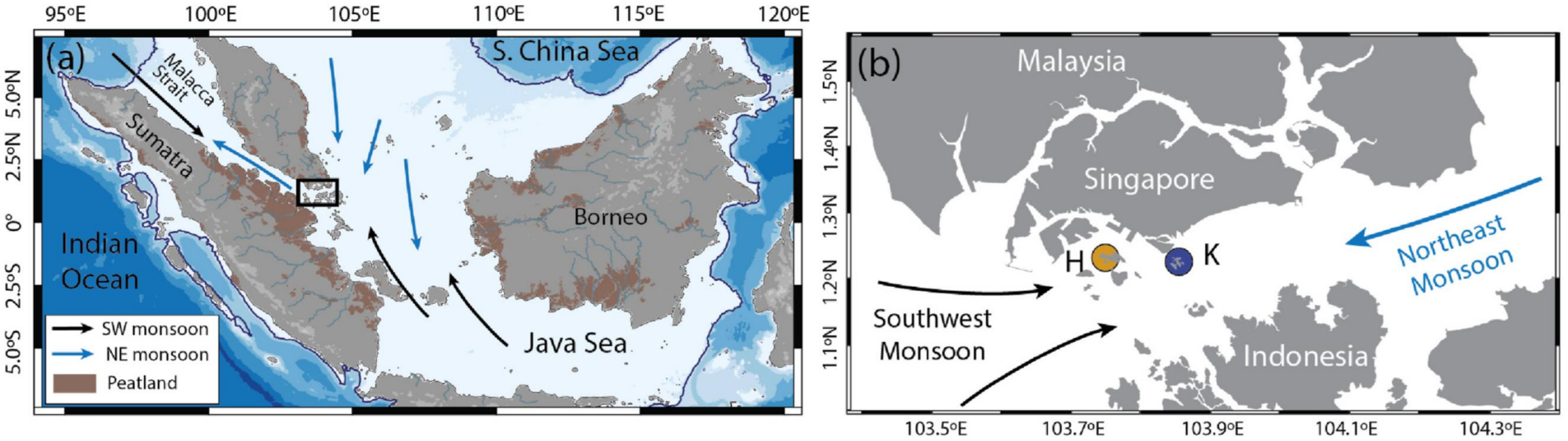




Martin et al., 2022. Estuarine, Coastal and Shelf Science

Behera, M. R., et al., 2013. Marine Pollution Bulletin.

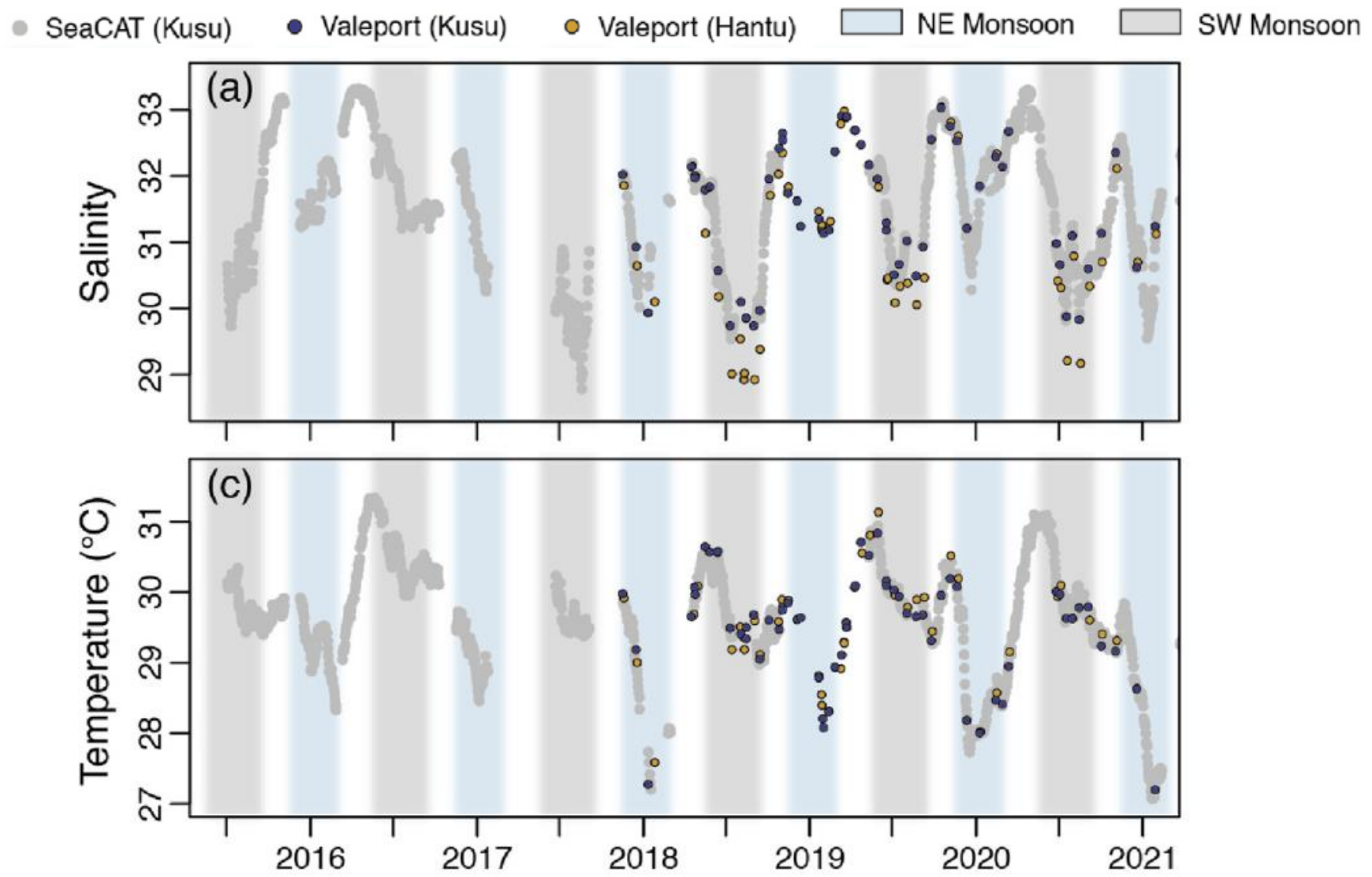
Monsoon seasons in the Singapore Strait



Monsoon season	Duration
Northeast monsoon	15 Nov to end Feb
Intermonsoon 1	01 Mar to 15 May
Southwest monsoon (coastal darkening)	15 May to 15 Sept
Intermonsoon 2	15 Sept to 15 Nov

Martin et al., 2022. Estuarine, Coastal and Shelf Science

Monsoon seasons in the Singapore Strait

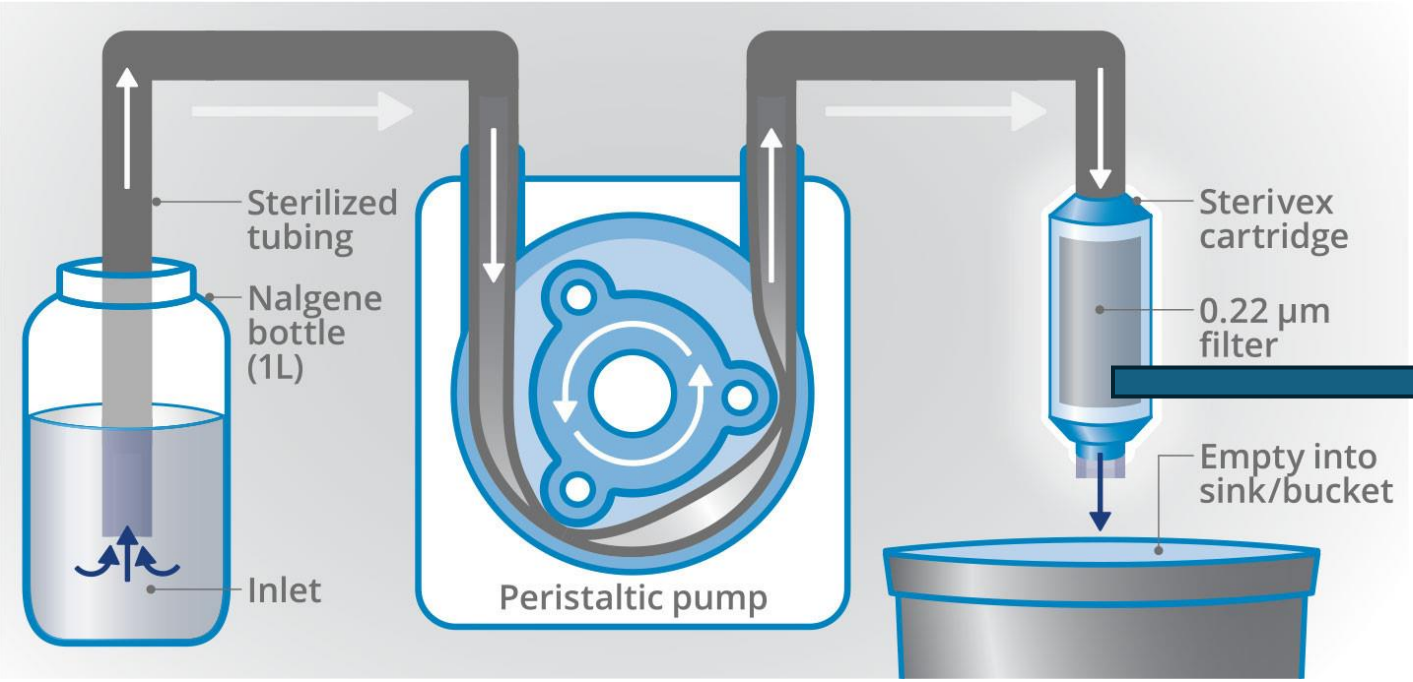


Martin et al., 2022. Estuarine, Coastal and Shelf Science

Is the tropical coastal protist community seasonal?

Monthly sampling to collect surface seawater

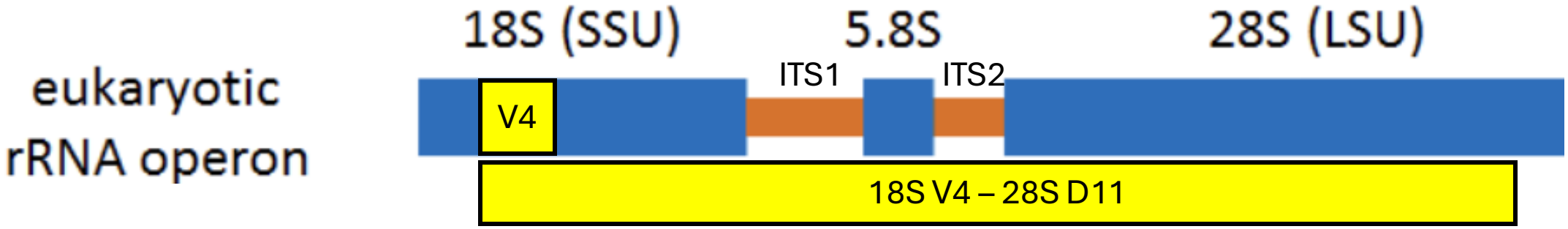
- Five-year time-series
  - Feb 2018 to Dec 2022



DNA extraction and sequencing

Galaska, M.P., et al. 2023. *Oceanography*

Long-read metabarcoding



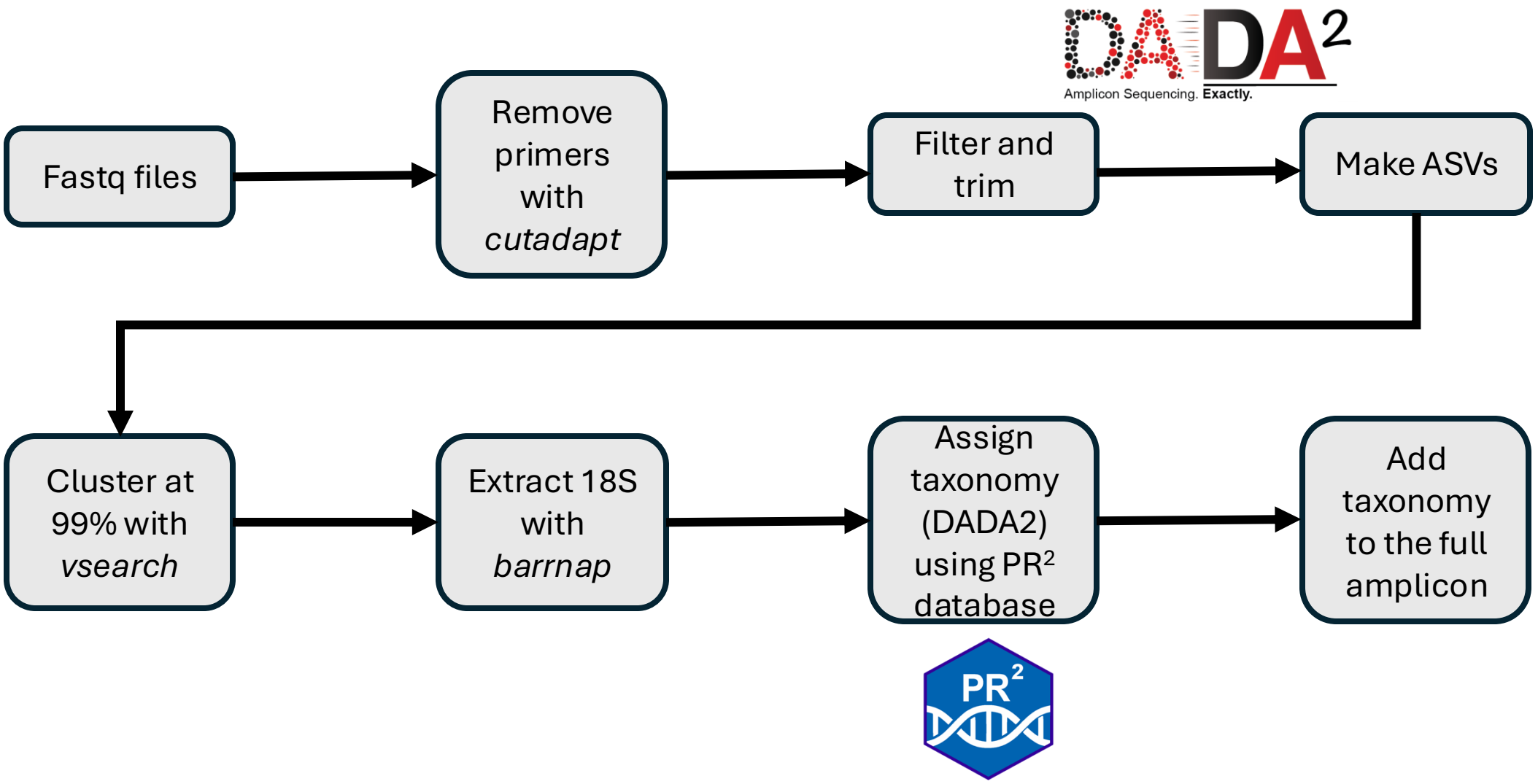
- Increased genetic resolution (400 bp to 5000 bp)
- Differentiate between closely related clades

Covered tomorrow by Embla Stokke

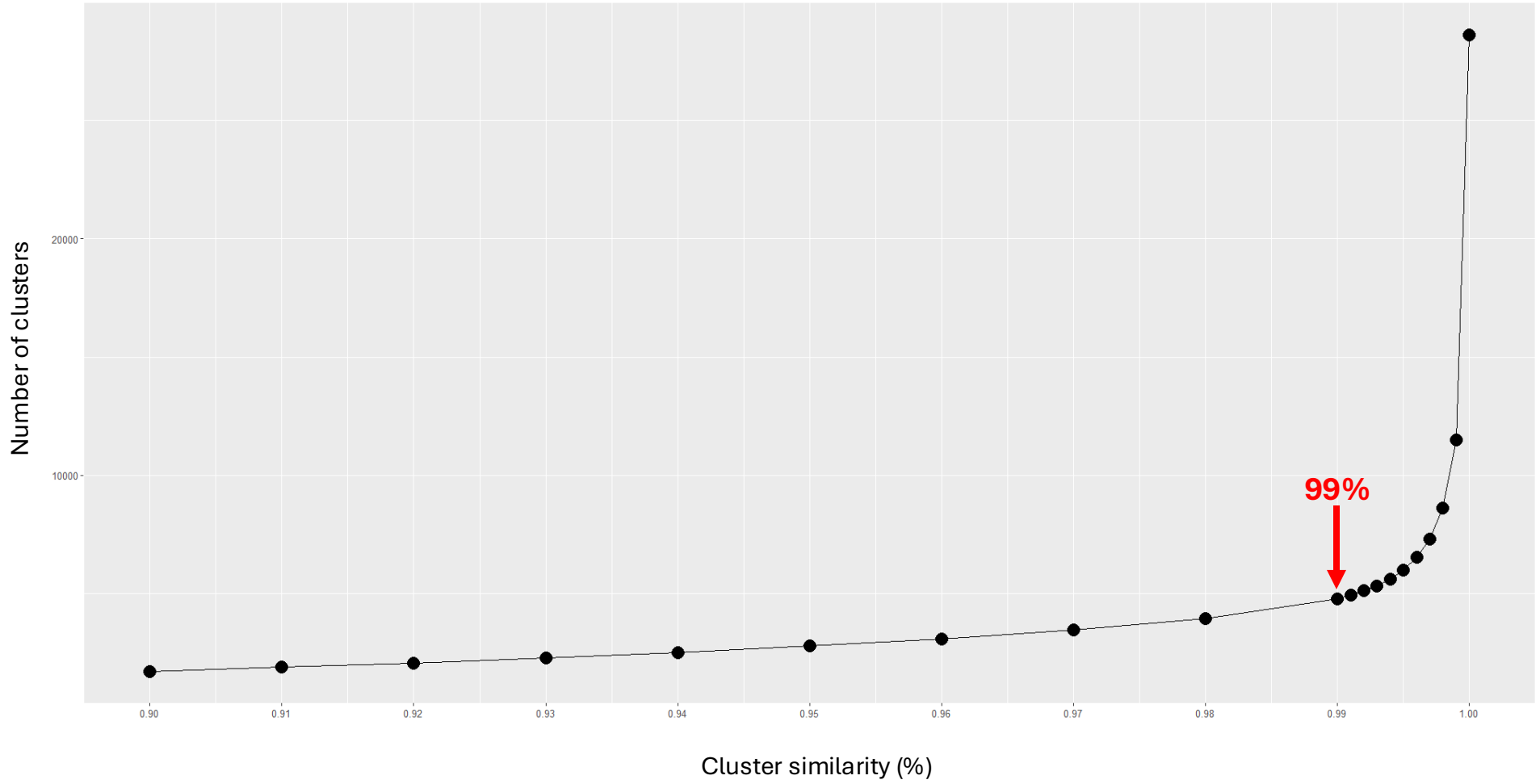
Clarence Sim
Ph.D., GEEK Lab

Adapted from http://www.sixthresearcher.com/amplicon-sequencing-and-high-throughput-genotyping-metagenomics/

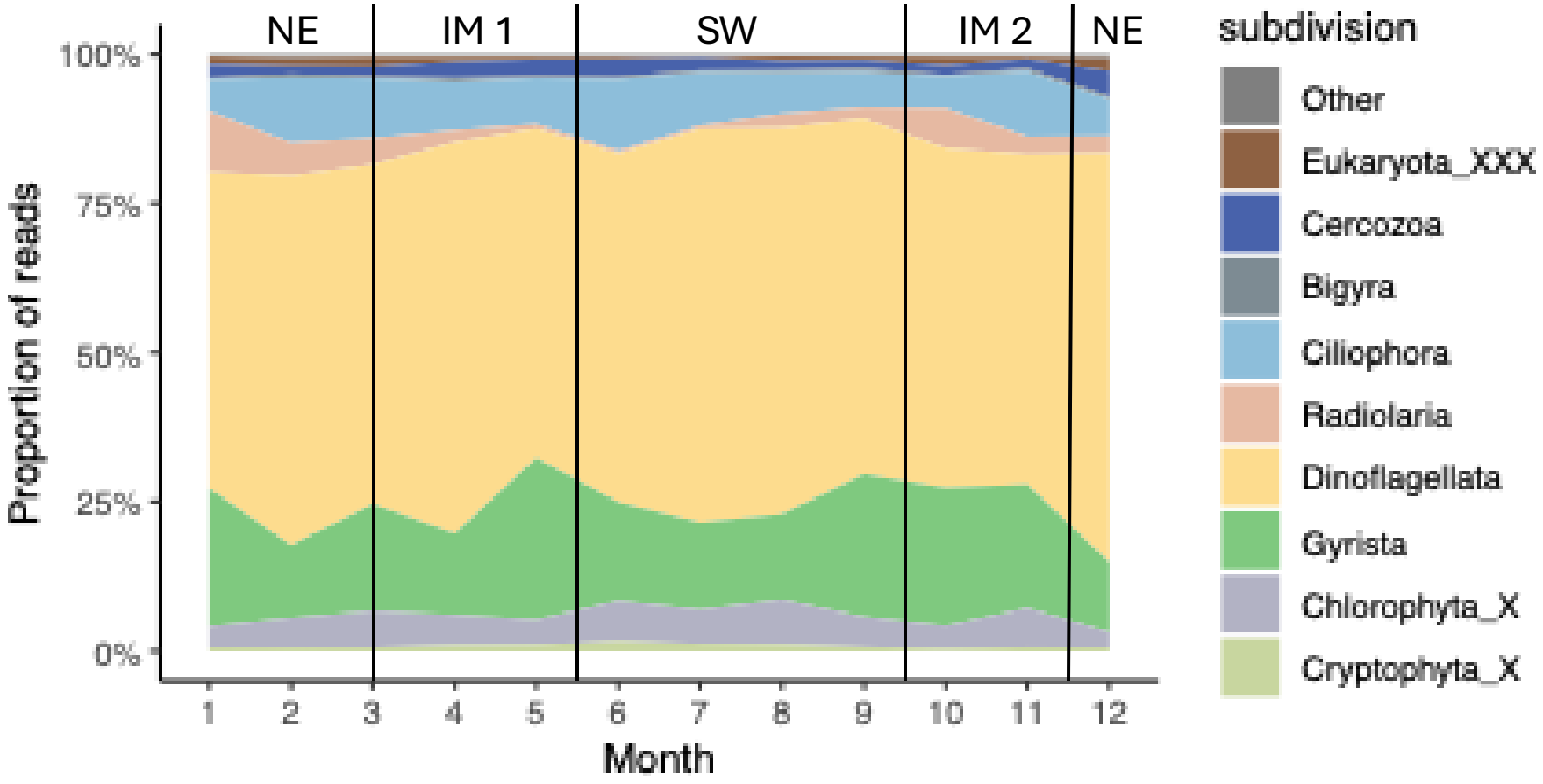
Bioinformatic process



Bioinformatic process – clustering ASVs

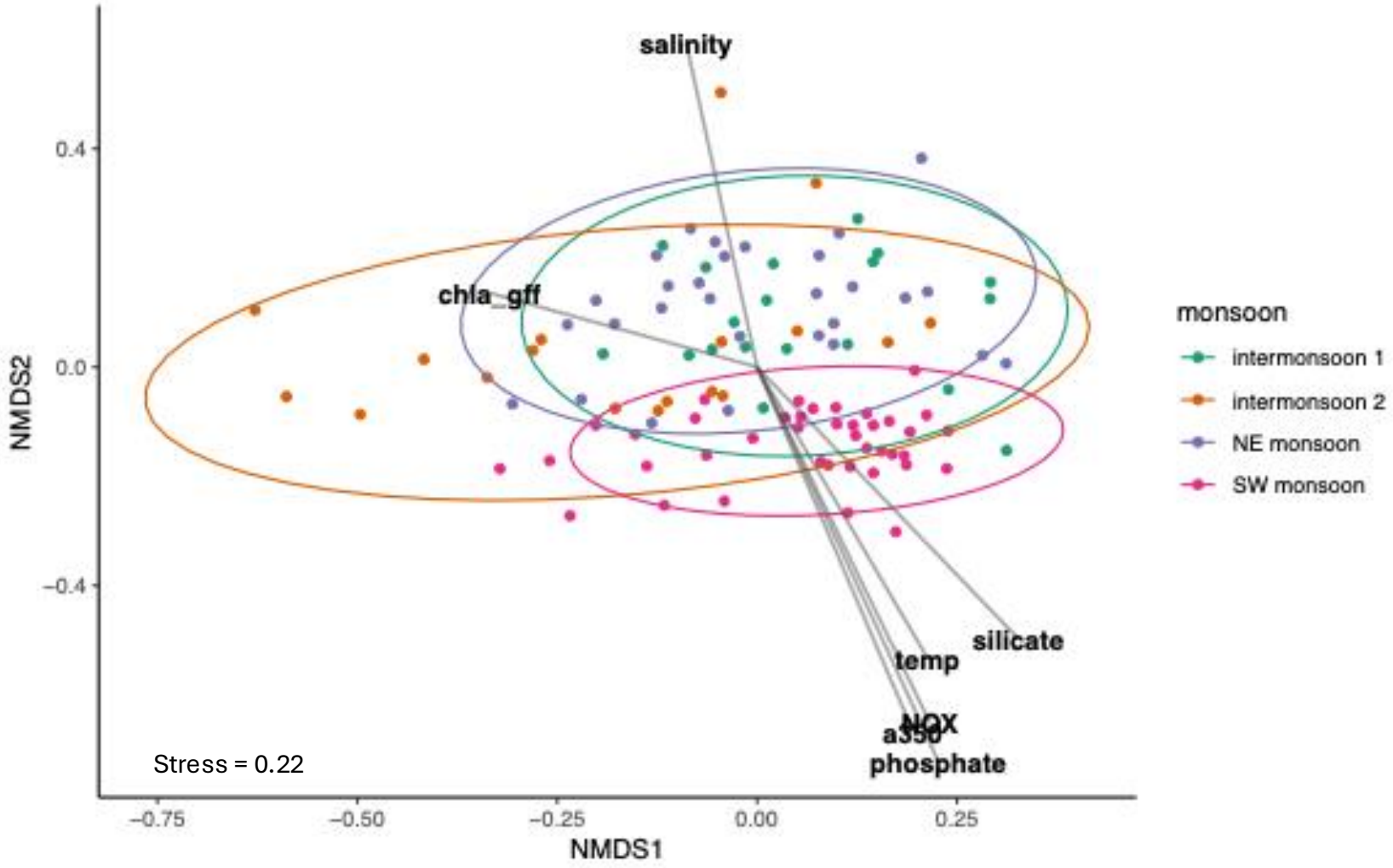


Overall protist community

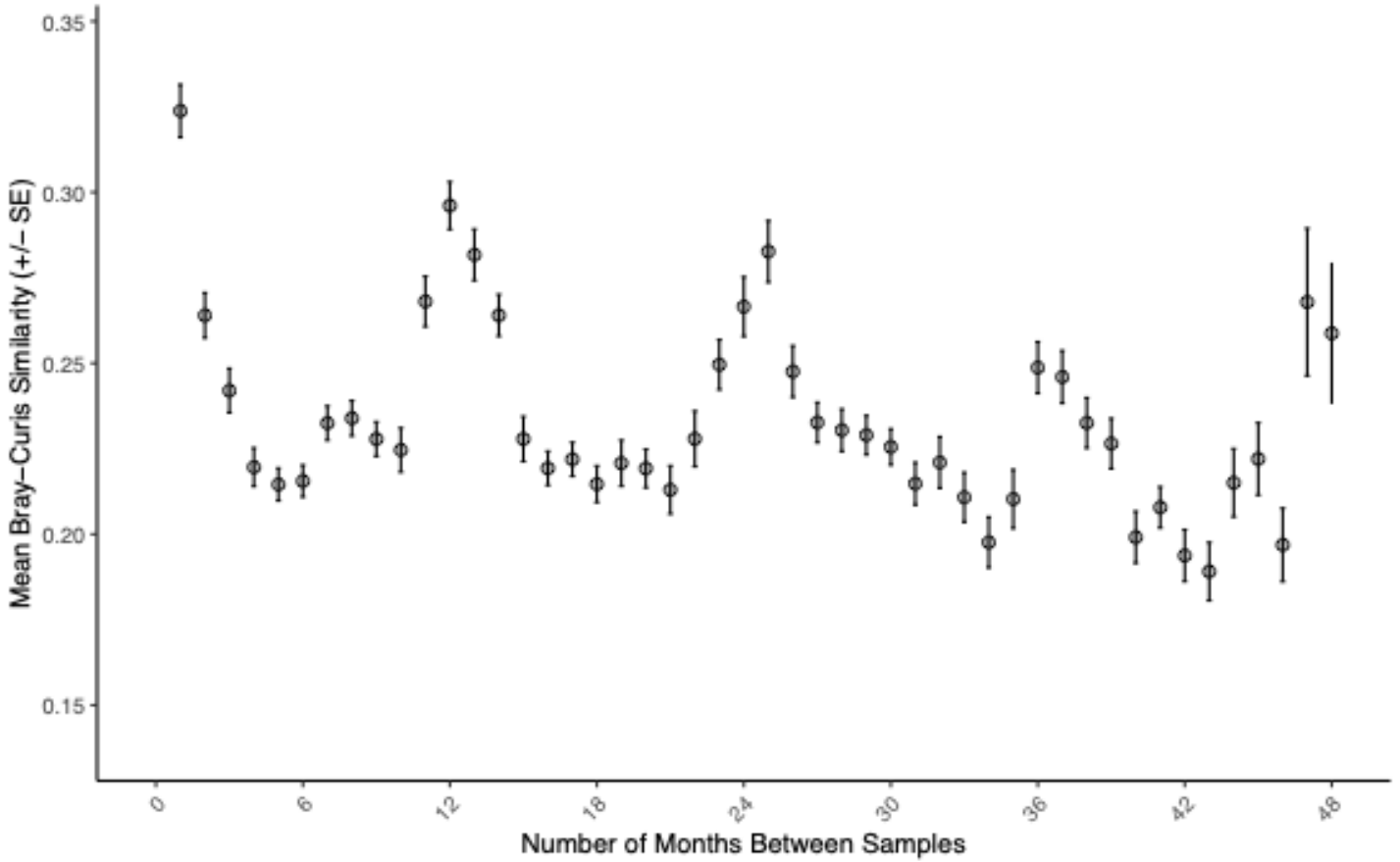


Mostly stable community

Southwest monsoon community is distinct from other seasons



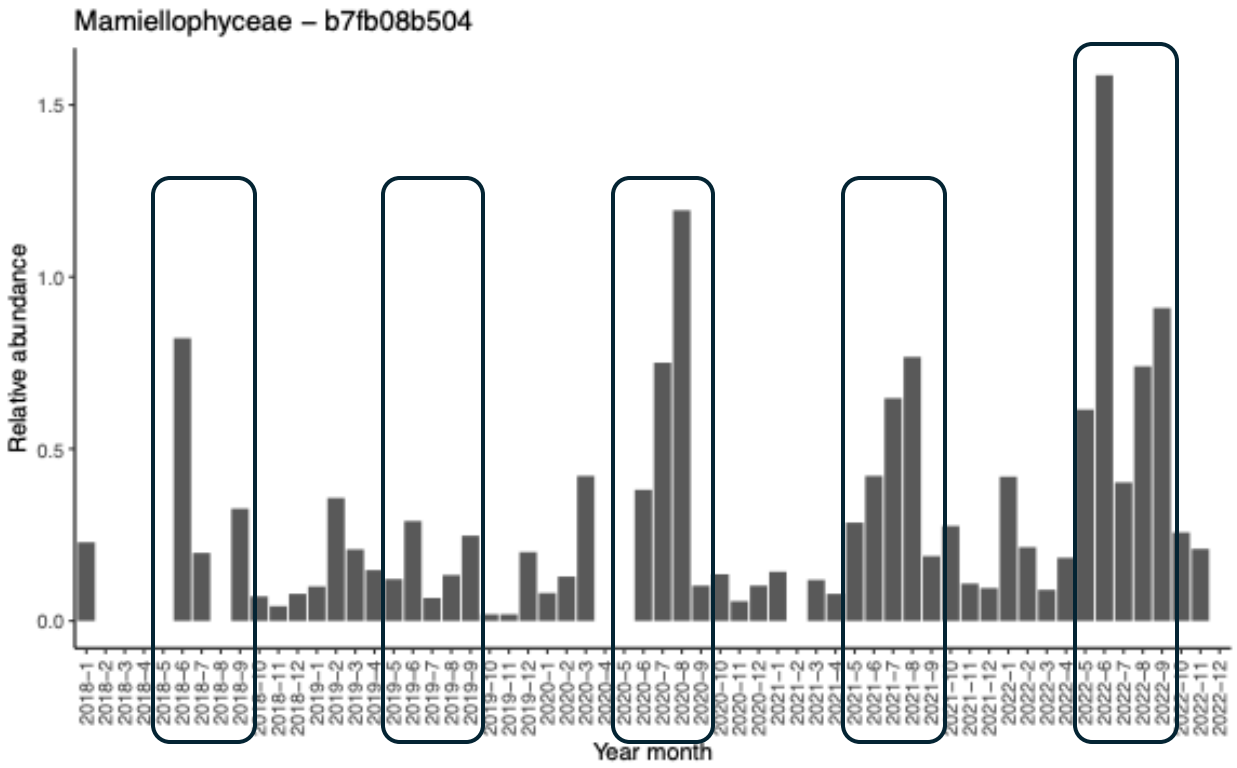
Is the protist community seasonal?



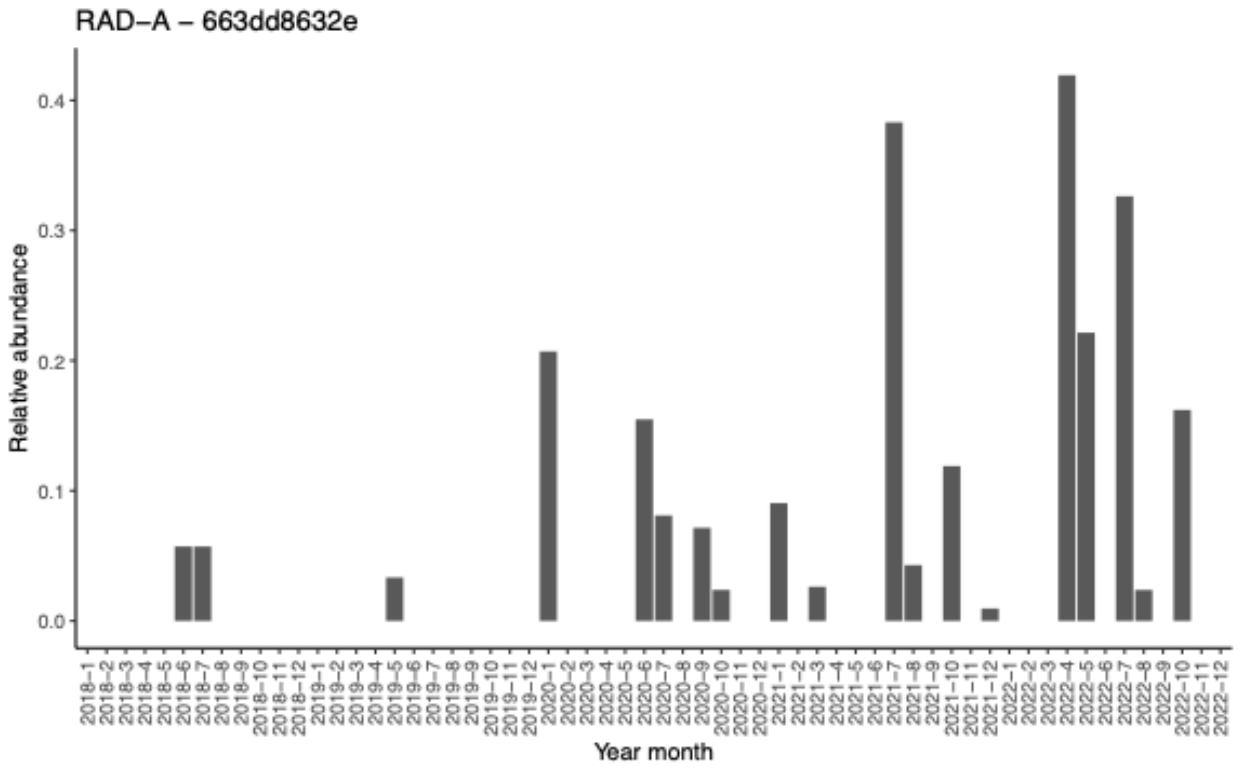
- Comparing the similarity (Bray-Curtis) of the community at different months sampled
- Mantel test; R=0.1556, p< 0.001
- Significant but weak correlation

How can we differentiate between seasonal and non-seasonal ASVs?

Seasonal



Non-seasonal



Missing months: 2018-2 to 2018-5, 2018-8, 2020-4, 2020-5, 2021-2

Recurrence Index from Giner et al. (2018)

Received: 11 May 2018 | Revised: 8 October 2018 | Accepted: 30 October 2018

DOI: 10.1111/mec.14929

FROM THE COVER

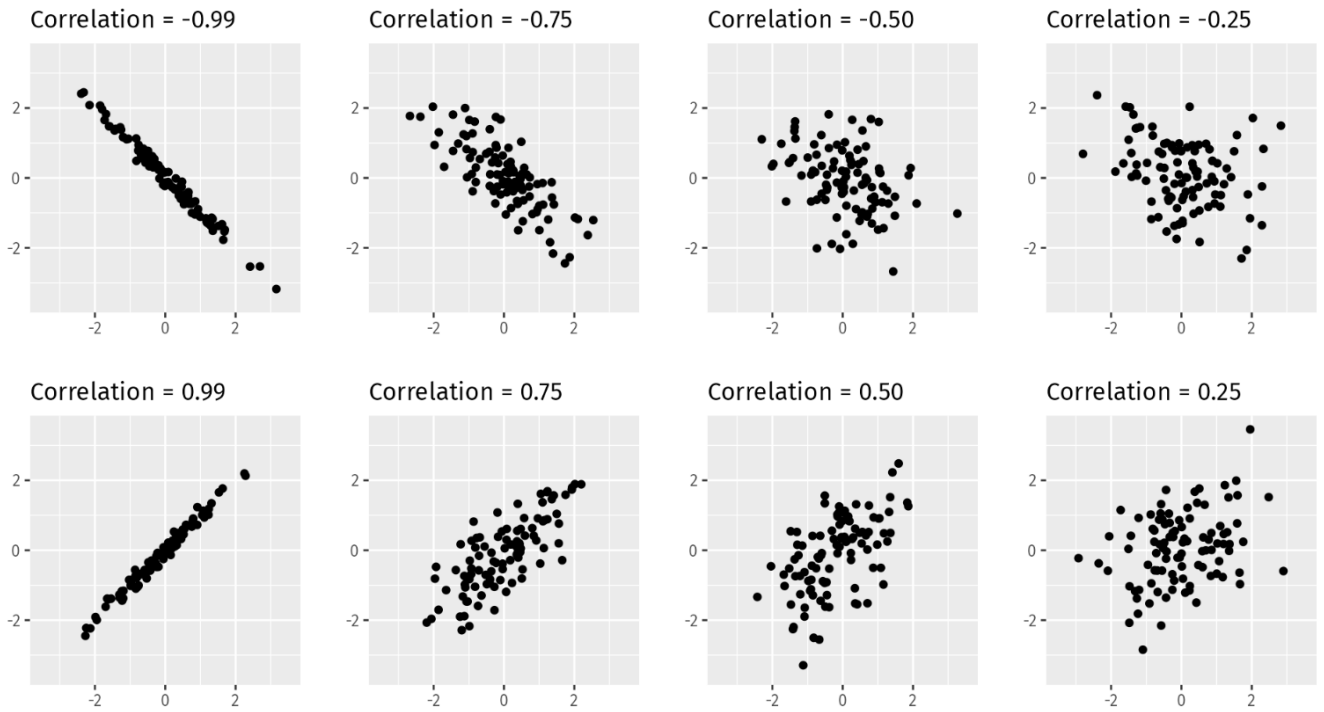
WILEY MOLECULAR ECOLOGY

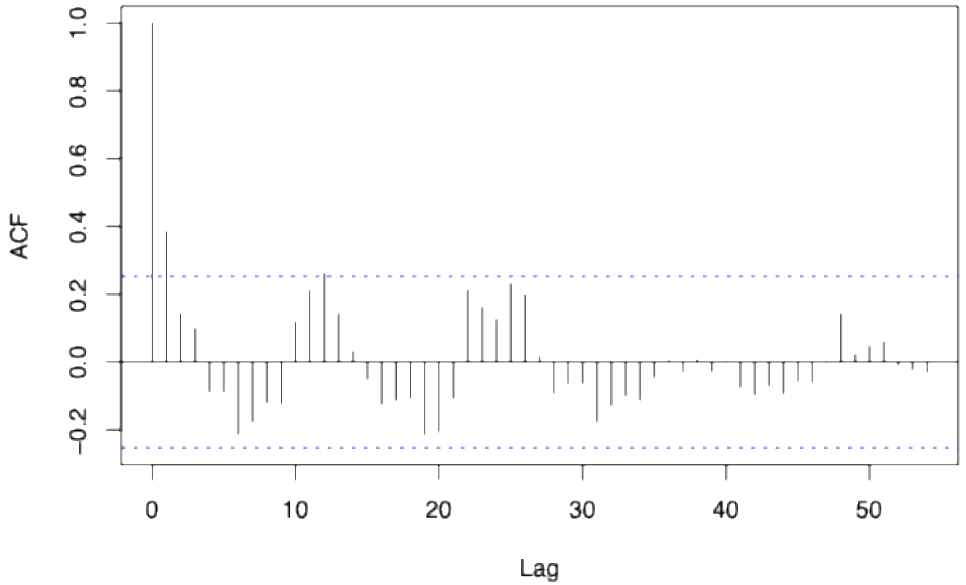
Quantifying long-term recurrence in planktonic microbial eukaryotes

Caterina R. Giner[1] | Vanessa Balagué[1] | Anders K. Krabberød[2] | Isabel Ferrera[1] | Albert Reñé[1] | Esther Garcés[1] | Josep M. Gasol[1] | Ramiro Logares[1,2] | Ramon Massana[1]

Autocorrelation

- Linear relationship between lagged values of a time series
- Correlation between the relative abundance of an ASV against its lagged values (1 month apart, etc.)
- At every time interval…

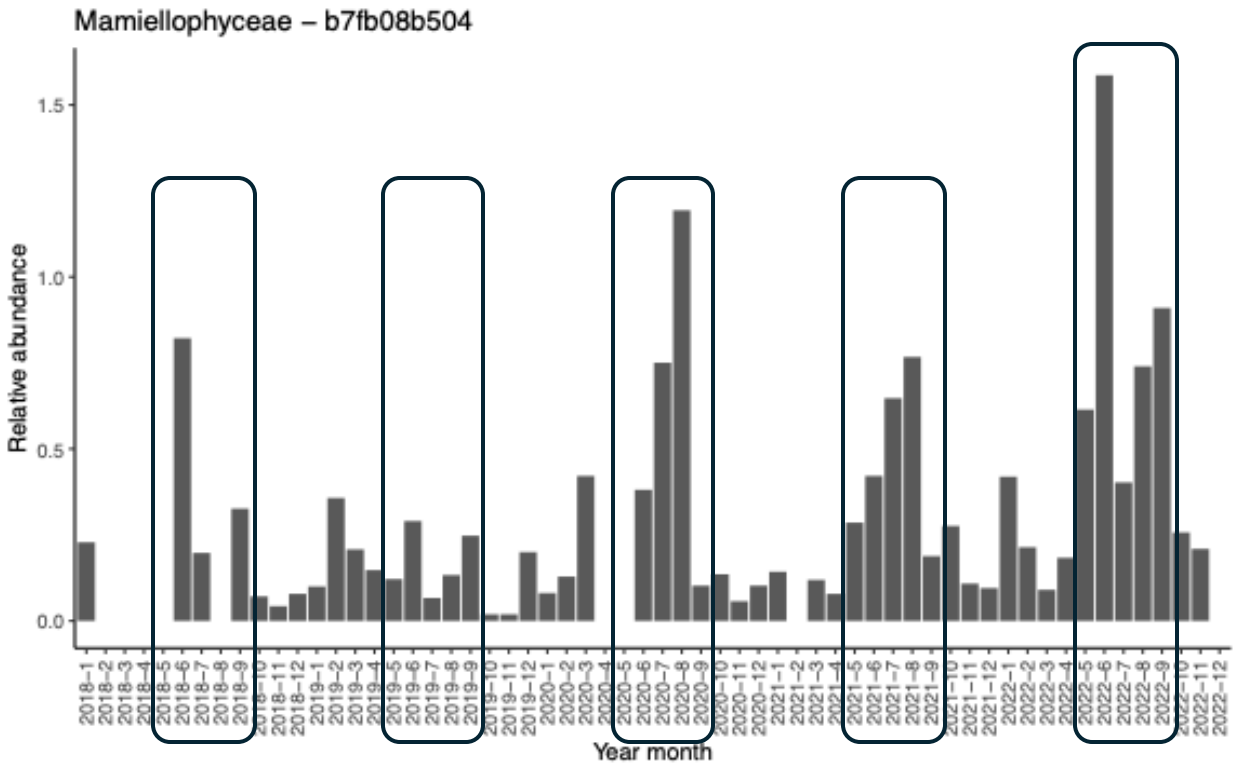




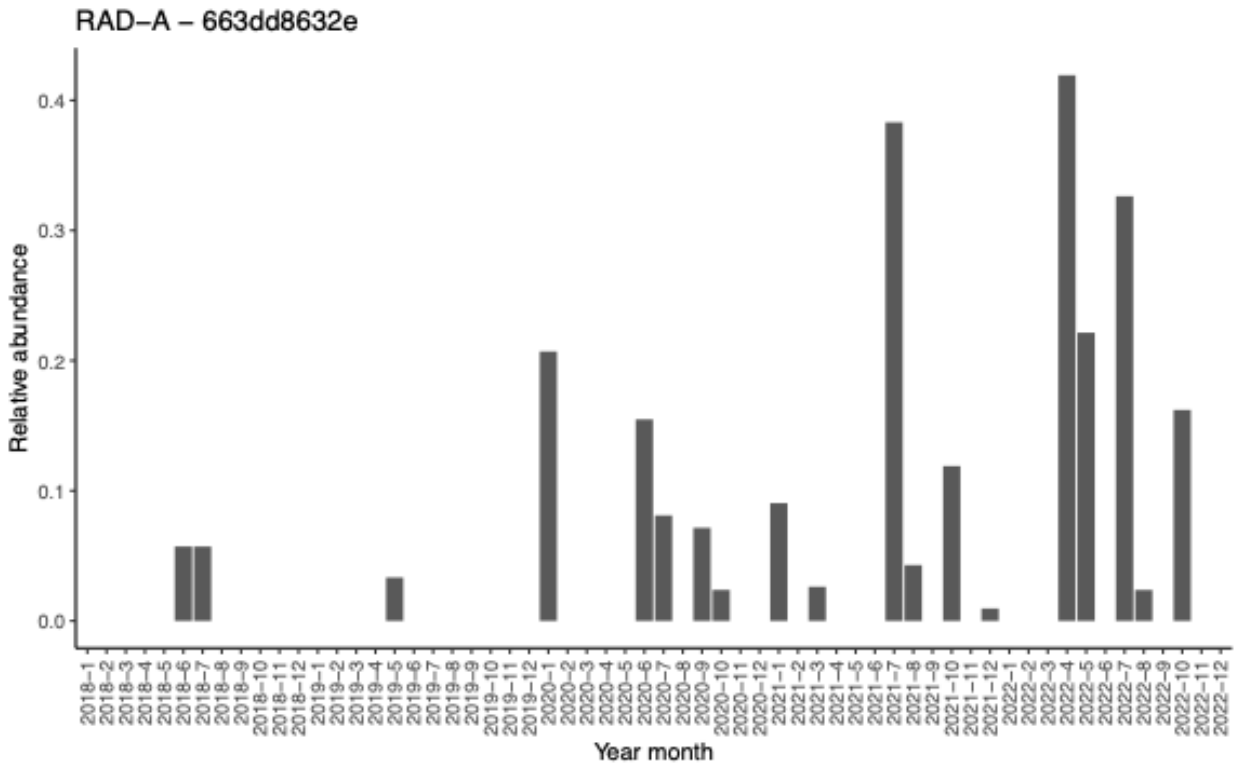
Hyndman and Athanasopoulos. Forecasting: Principles and Practice (2nd ed)

How can we differentiate between seasonal and non-seasonal ASVs?

Seasonal

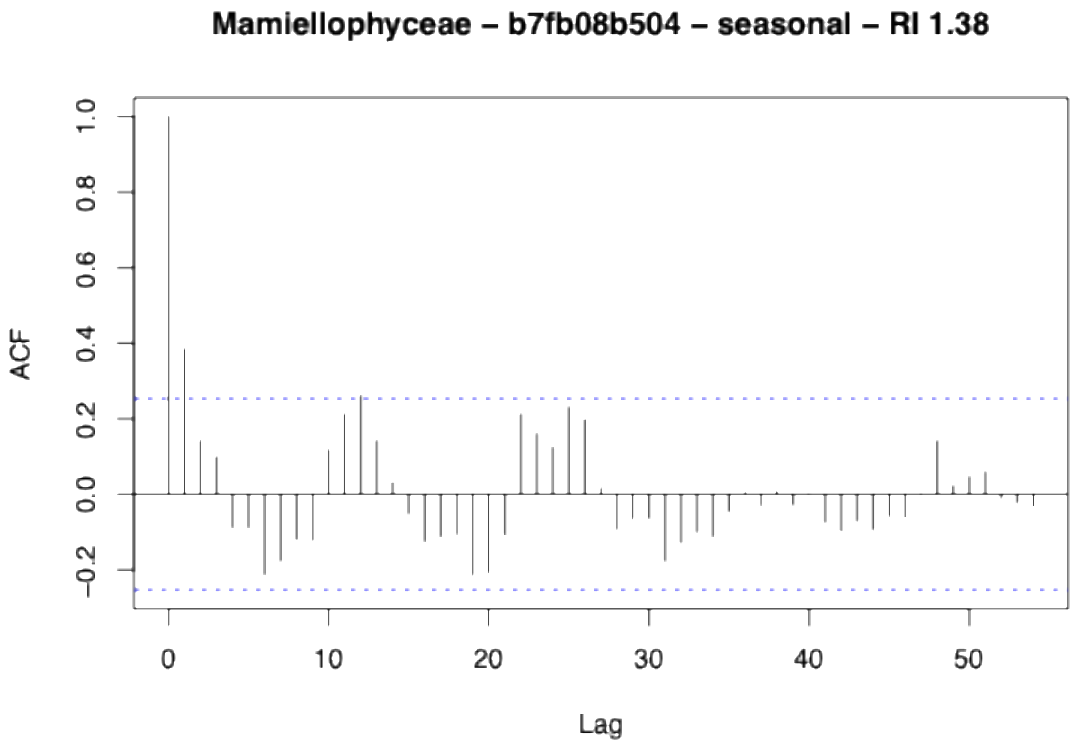


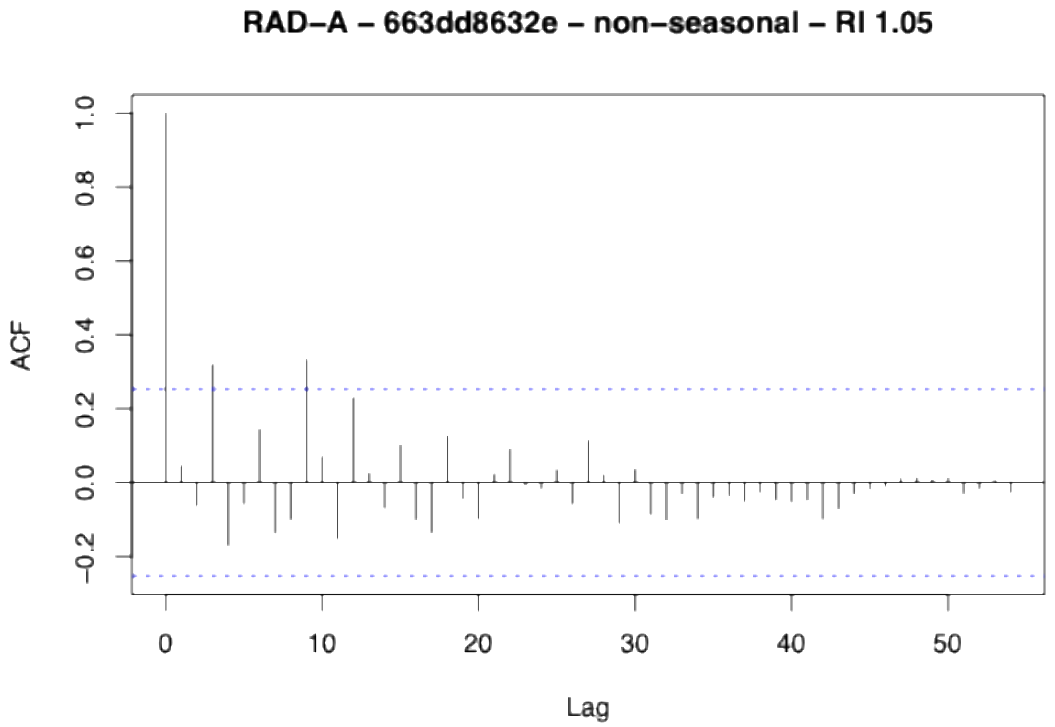
Non-seasonal



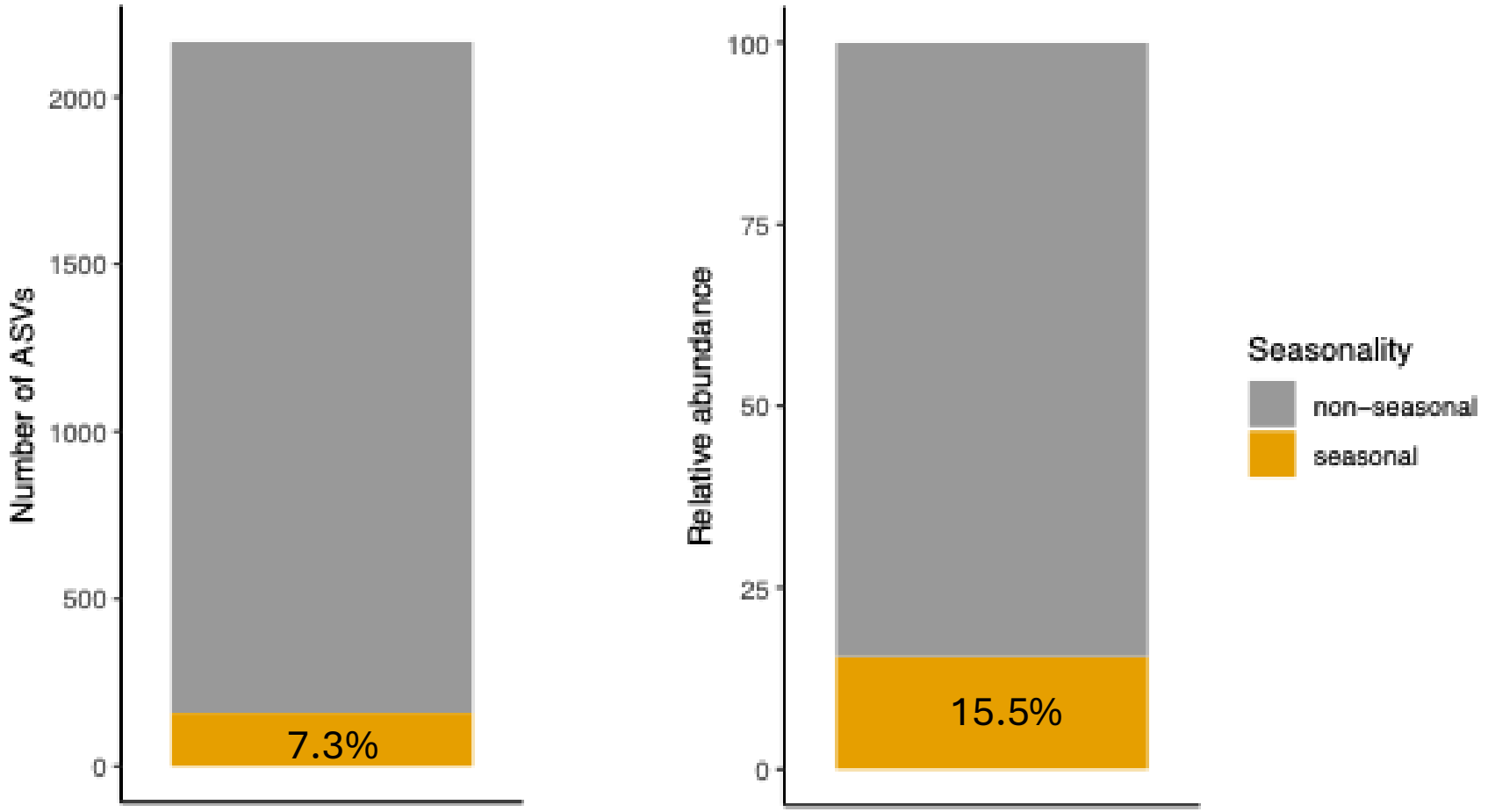
Missing months: 2018-2 to 2018-5, 2018-8, 2020-4, 2020-5, 2021-2

Using autocorrelation to calculate the Recurrence Index





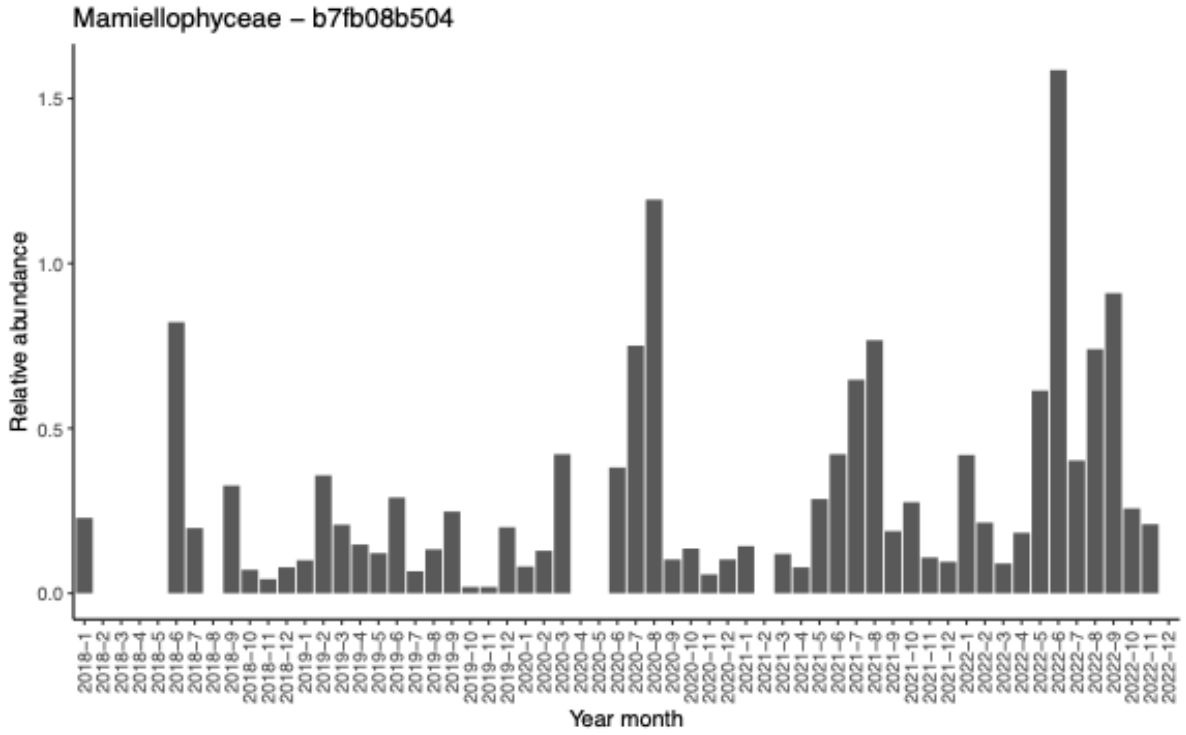
A small proportion of the community is seasonal



Different types of seasonality

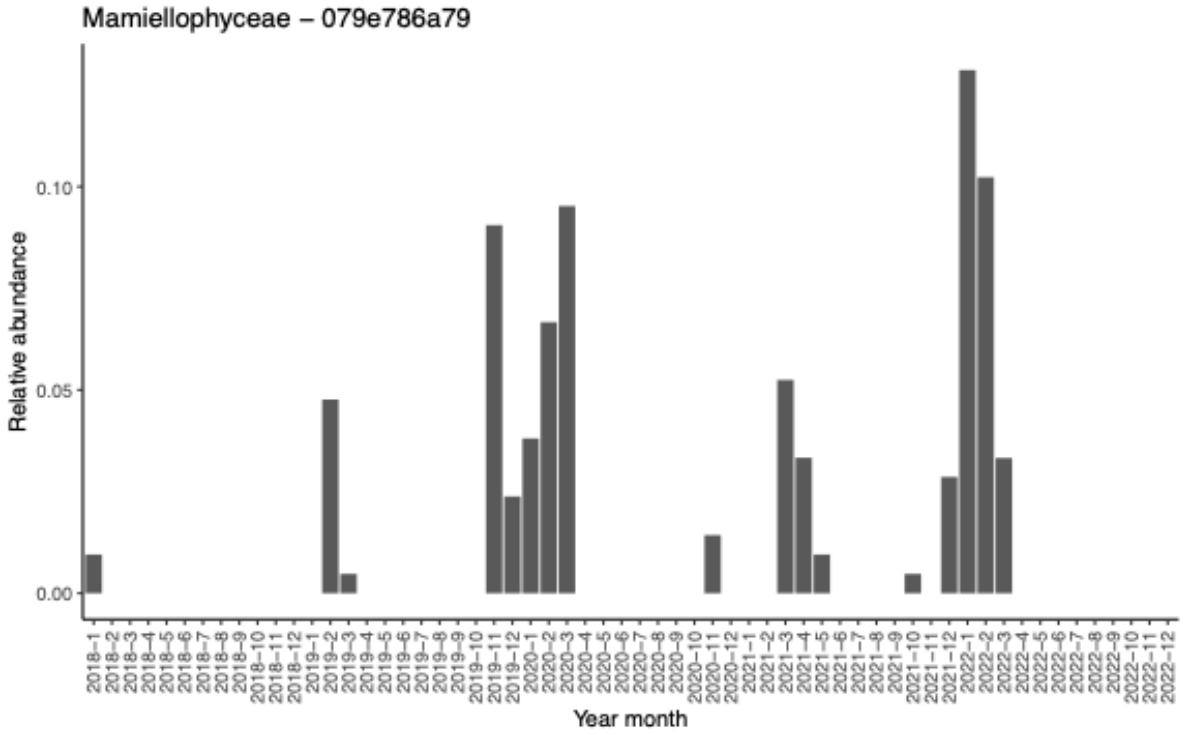
Long-term

Mantoniella sp.



Pulses

Micromonas bravo



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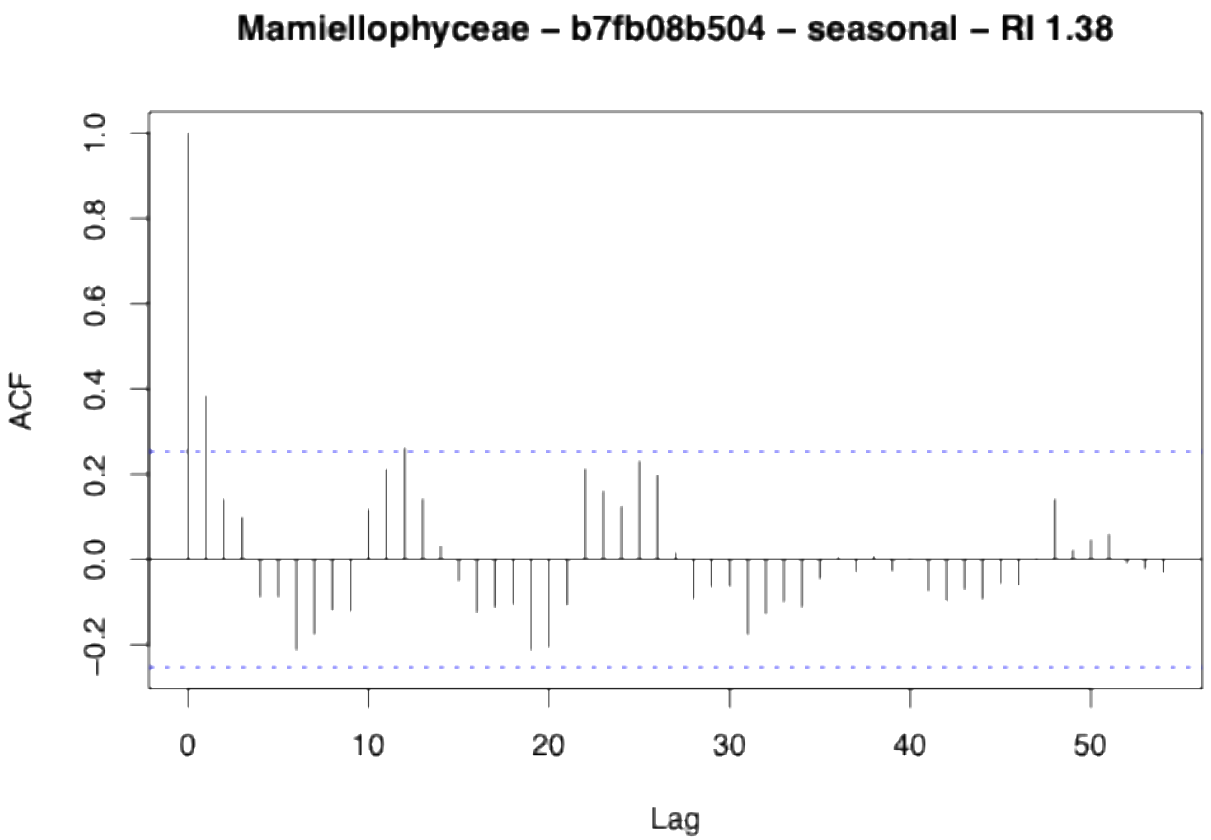

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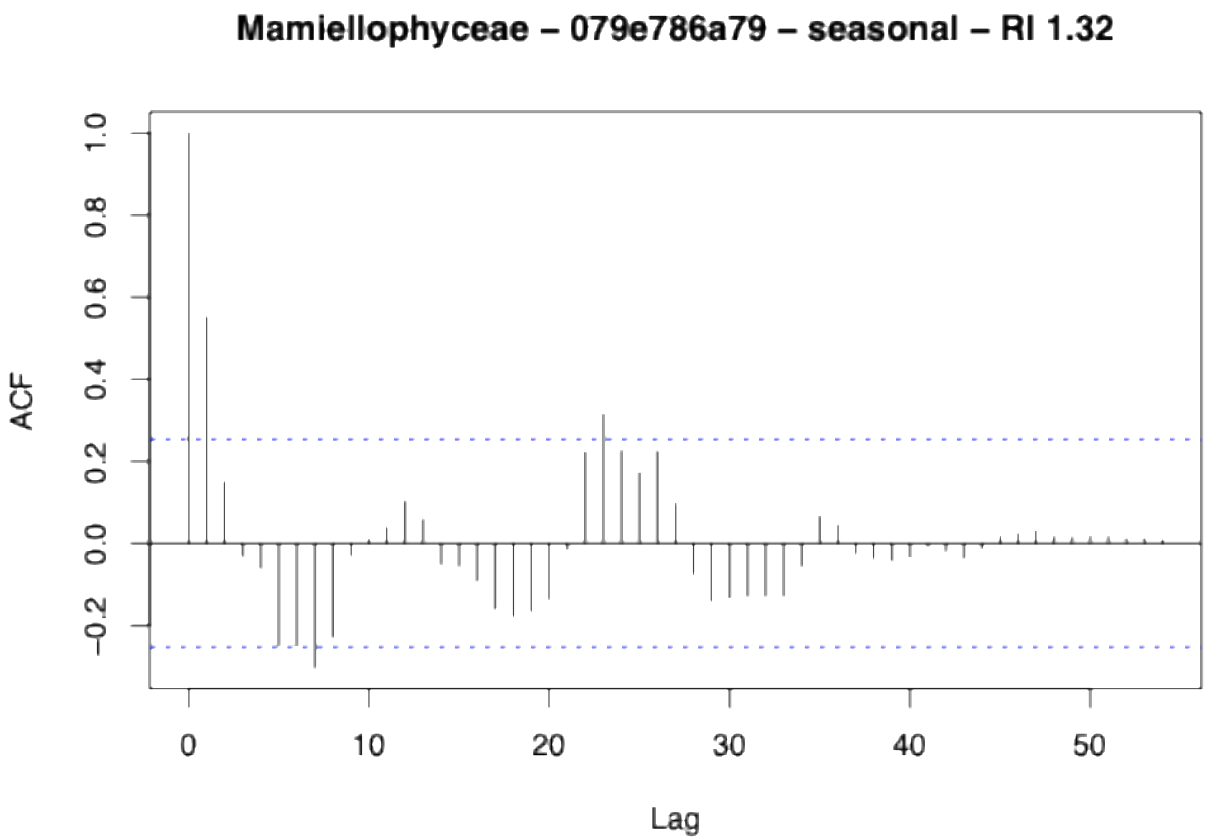
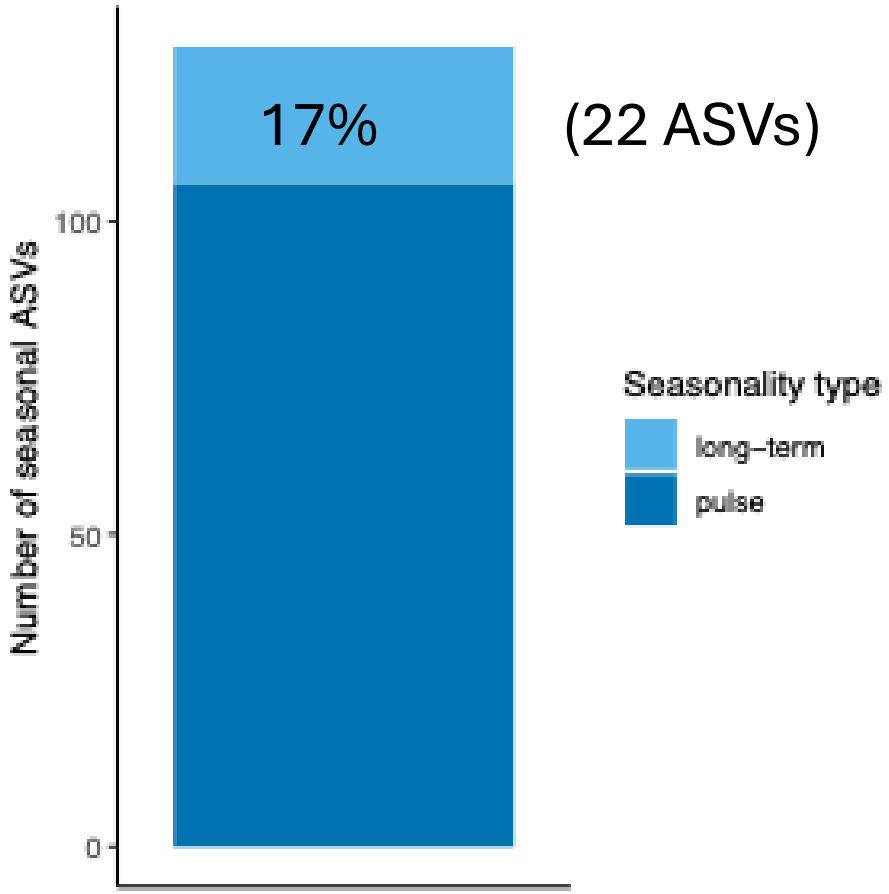
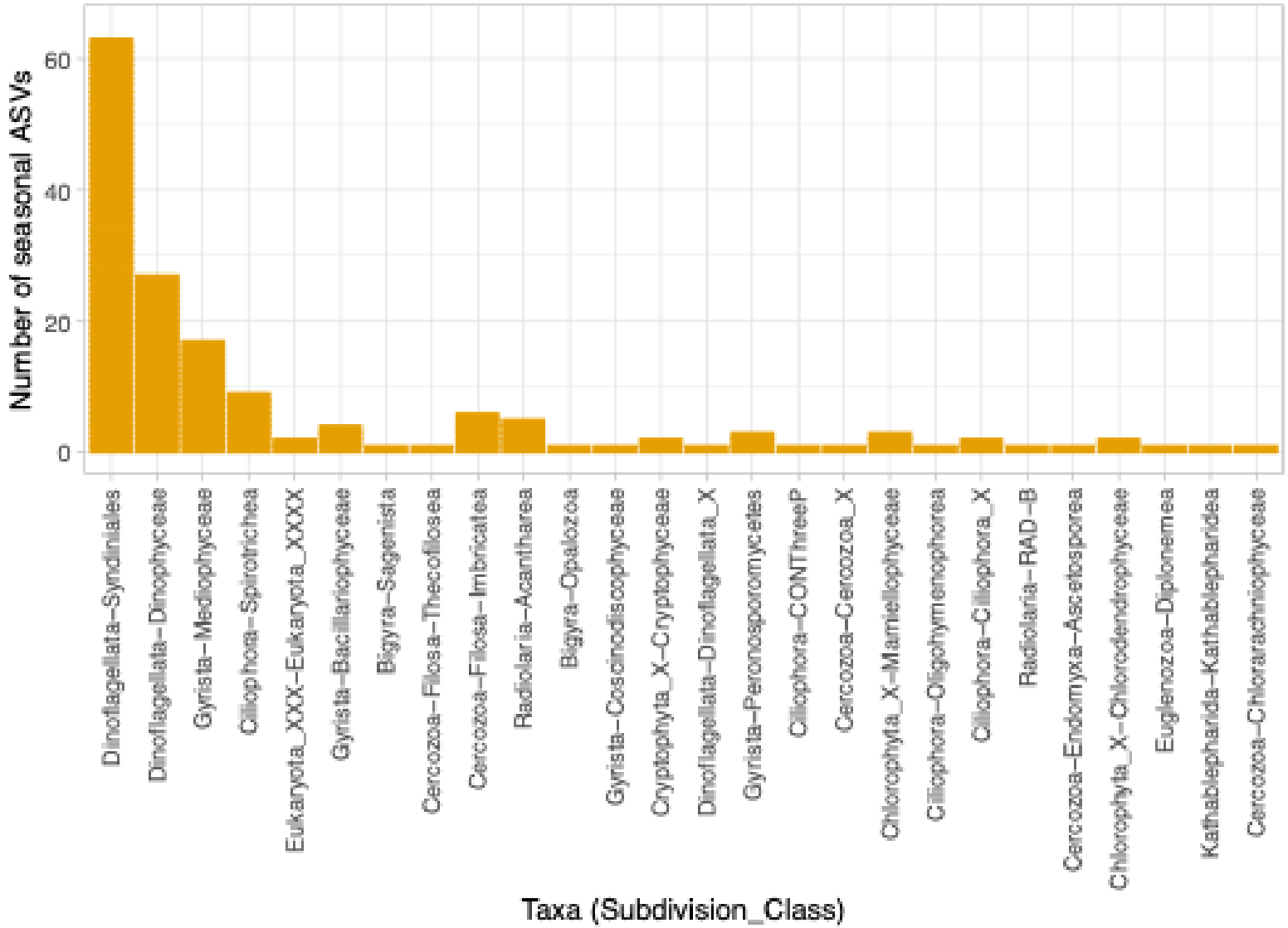


Seasonal ASVs: Long term vs pulses

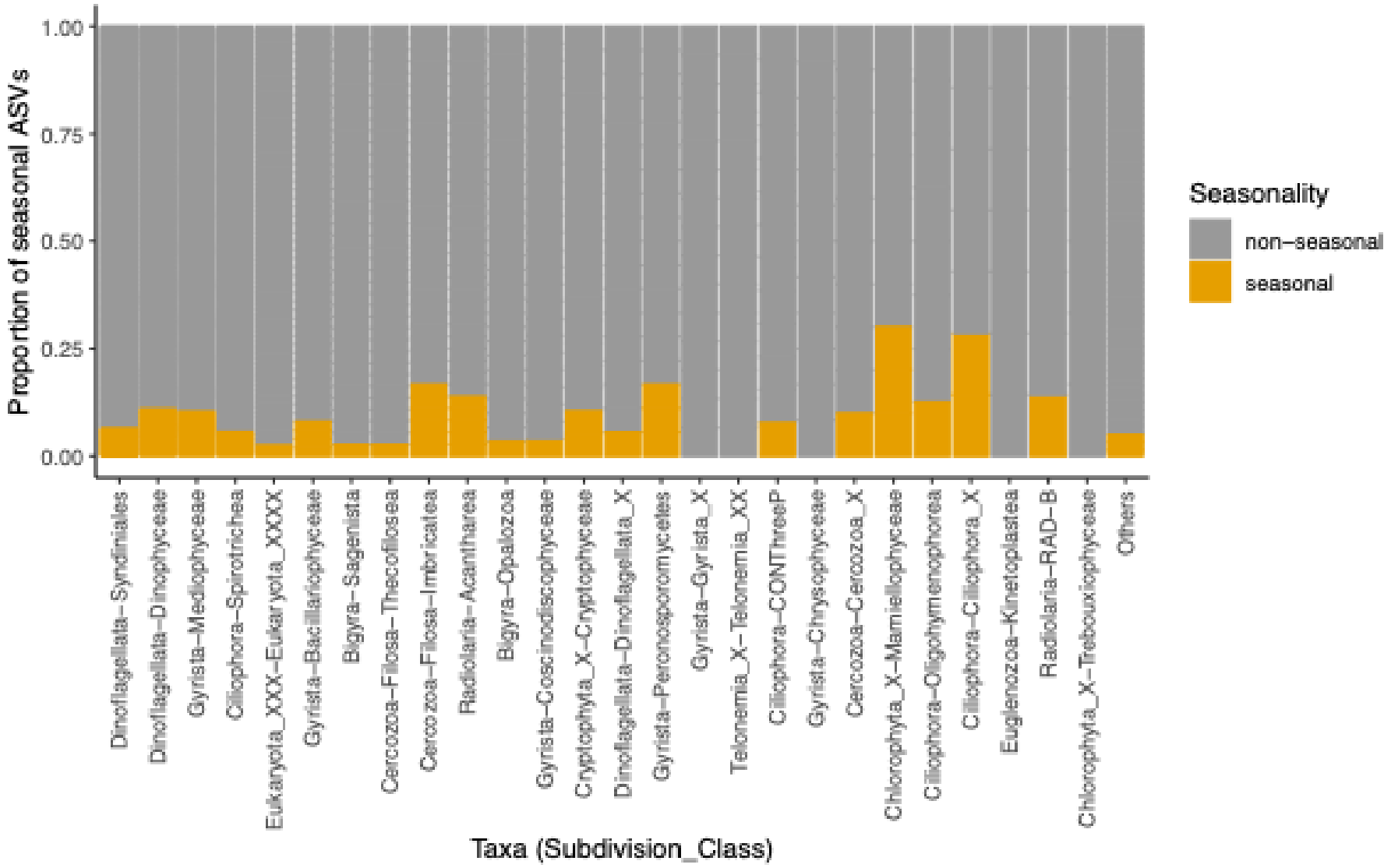
- Long term ASVs: present in more than ¾ of the samples



More abundant taxa have more seasonal ASVs

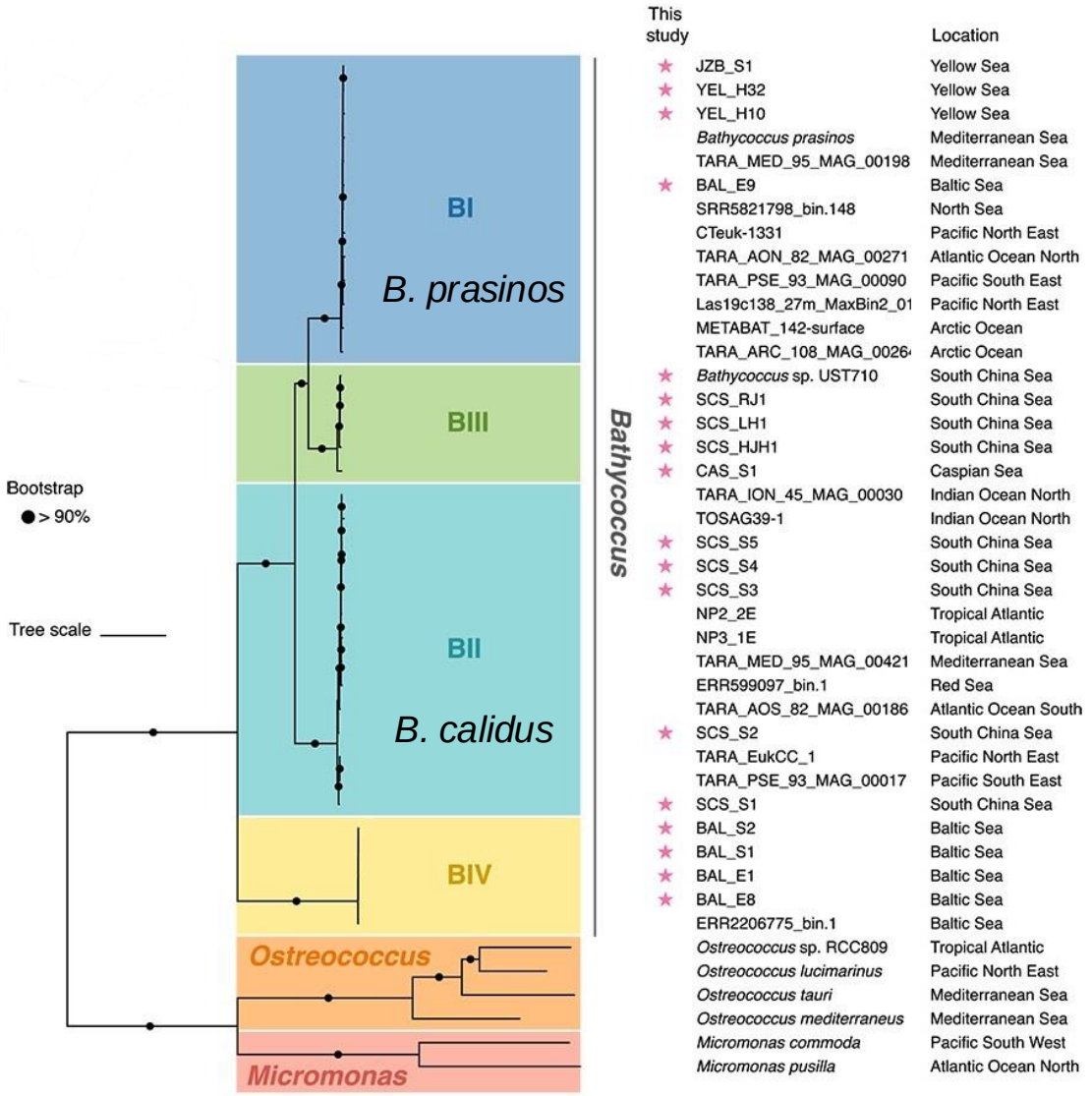


Seasonality does not dominate in any one taxa



Why long-read metabarcoding?

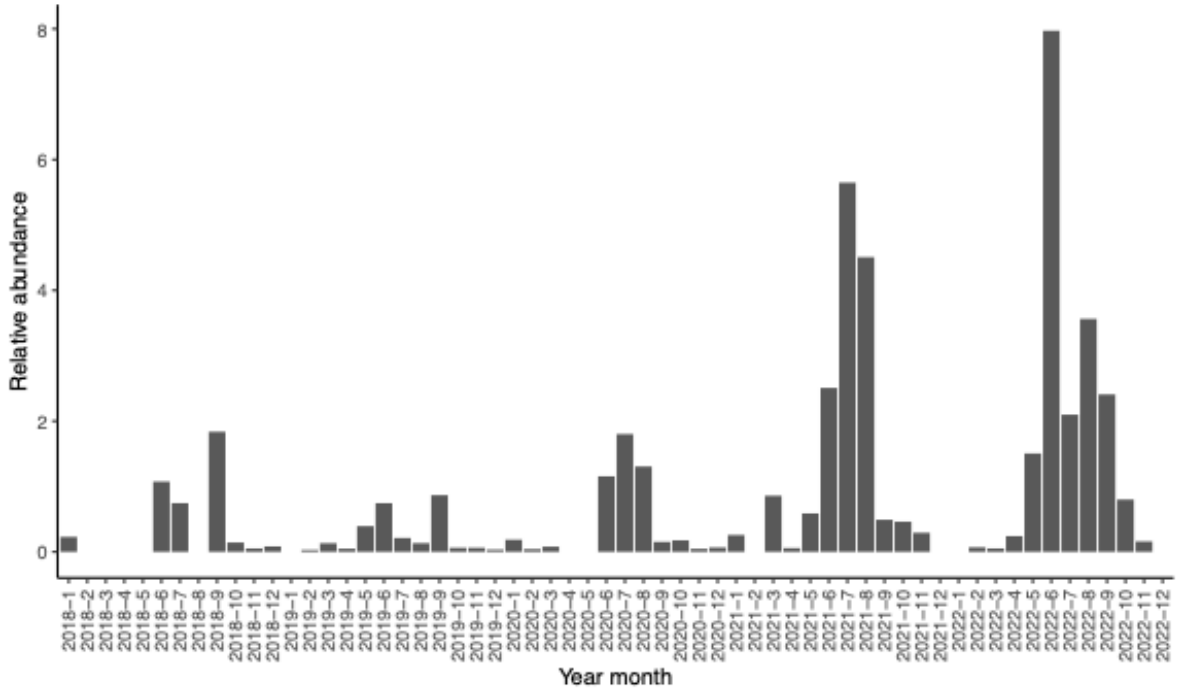
Bathycoccus

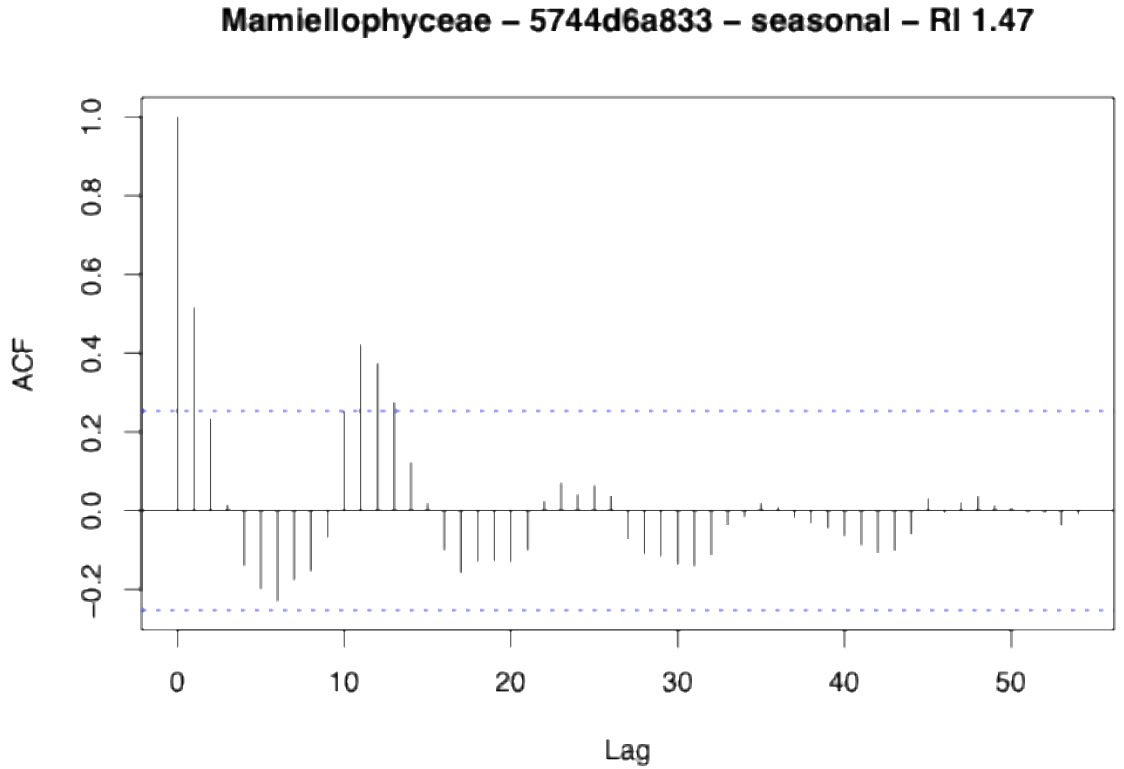


Xu, Y., et al. 2024. ISME.

Newly proposed clade present in the Singapore Strait

Bathycoccus sp., Clade III





Key takeaways

- The overall community has very little seasonality
- Identify seasonality for a small proportion of ASVs
- Understanding at a lower taxonomic level might help us to better identify the causes of plankton blooms (HABs, *Noctiluca scintillans*) in Singapore coastal waters
- Better classification of the plankton community with long-read metabarcoding

Acknowledgements

- Adriana Lopes dos Santos (UiO)
- Clarence Sim (NTU)
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- Rae Chua
- Patrick Martin (NTU)
- Woo Oon Yee (NTU)

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