

Bringing the understanding of microbes in their natural environments: the relevance standardizing initiatives

Catarina Magalhães et al.

Meta_Microbial
Workshop



Photo credit: Catarina Magalhães

BIG PICTURE

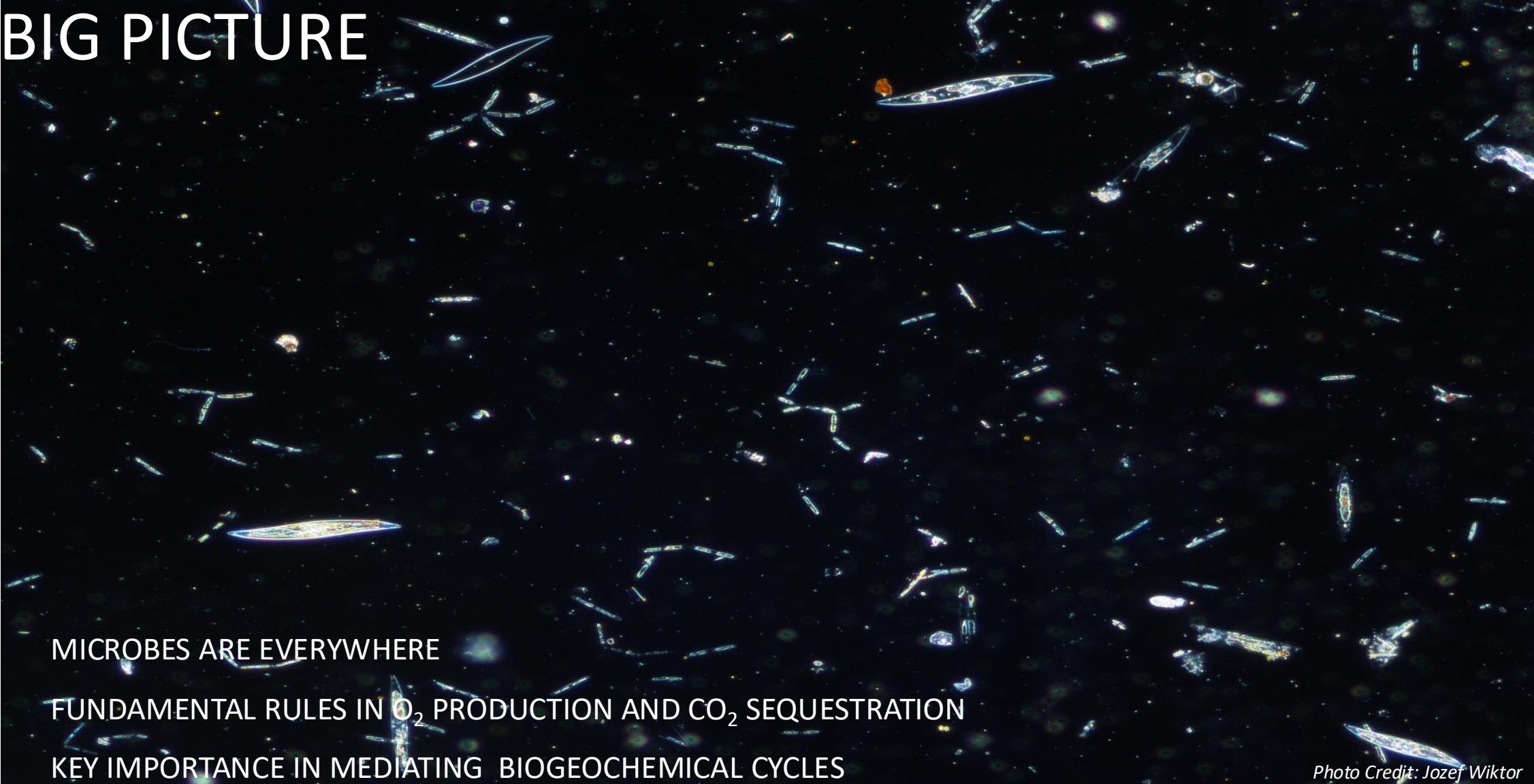


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Meta_Microbial
Workshop



BIG PICTURE



MICROBES ARE EVERYWHERE

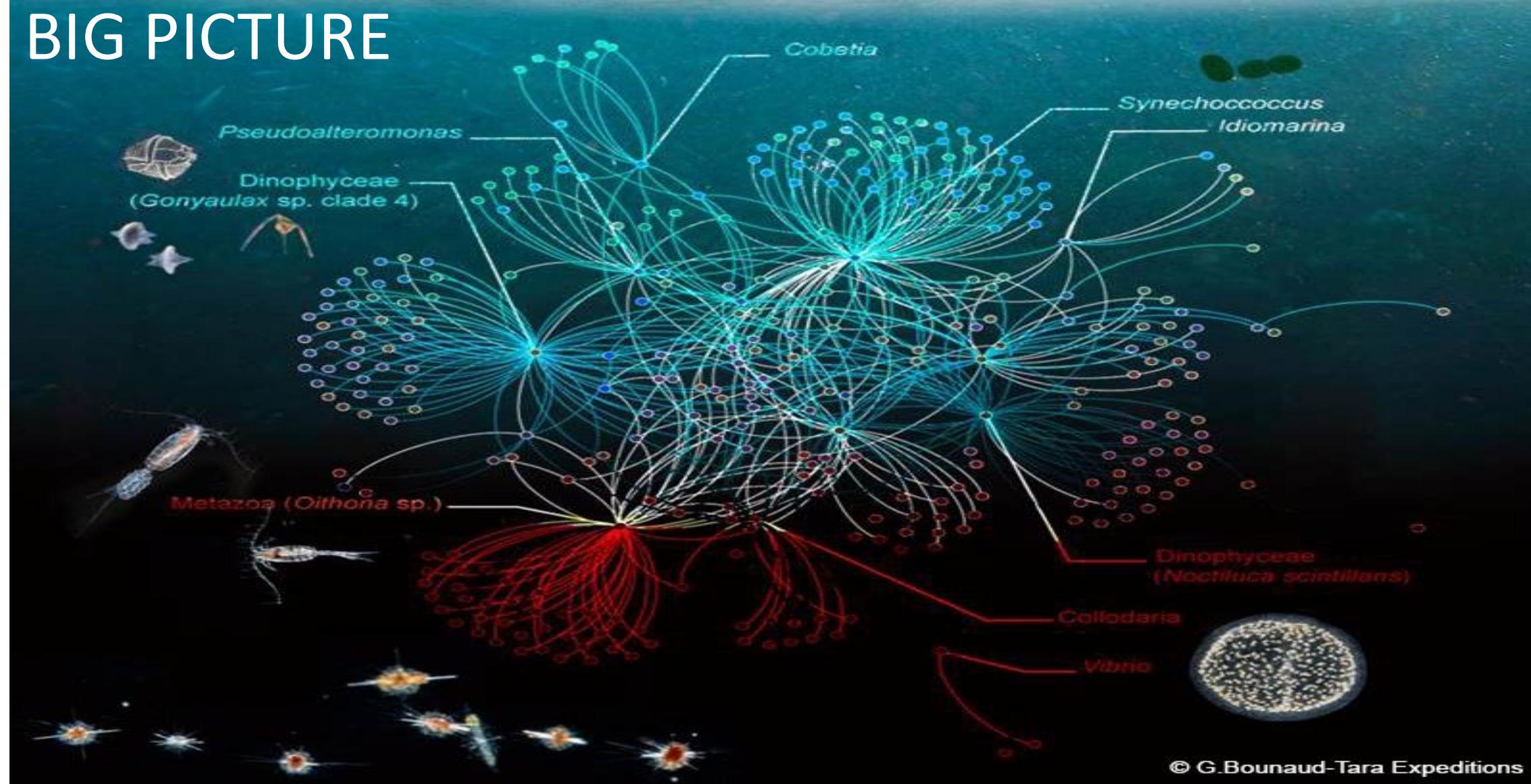
FUNDAMENTAL RULES IN O₂ PRODUCTION AND CO₂ SEQUESTRATION

KEY IMPORTANCE IN MEDIATING BIOGEOCHEMICAL CYCLES

Photo Credit: Jozef Wiktor

Meta_Microbial
Workshop

BIG PICTURE



Meta_Microbial
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Photo Credit: Maria Stenzel



Photo Credit: Elizabeth Jones



2017 Arctic Expedition | Photo Credit: Elizabeth Jones

OUR APROACH

STUDYING MICROBES IN THE
MARINE ENVIRONMENTS

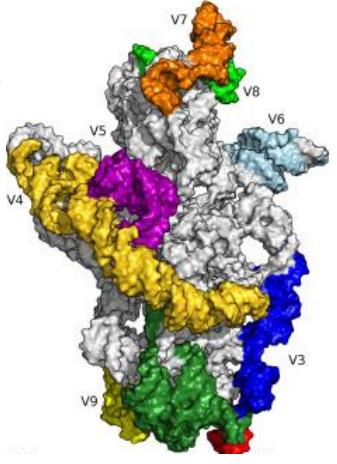
OCEANOGRAPHIC CAMPAIGNS

Meta_Microbial
Workshop

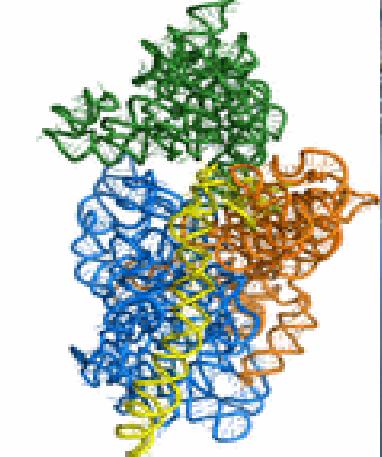
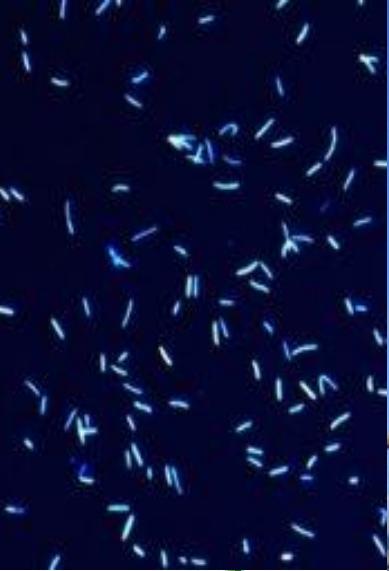








16S rDNA GENE | V4V5



18S rDNA GENE | V5



Shotgun Metagenomic
Metatranscriptomics
Sequencing



Photo credit: Catarina Magalhães

MICROBIOME MONITORING PROGRAMS



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ARCTIC OCEAN



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N-

ARCTIC PERMAFROST



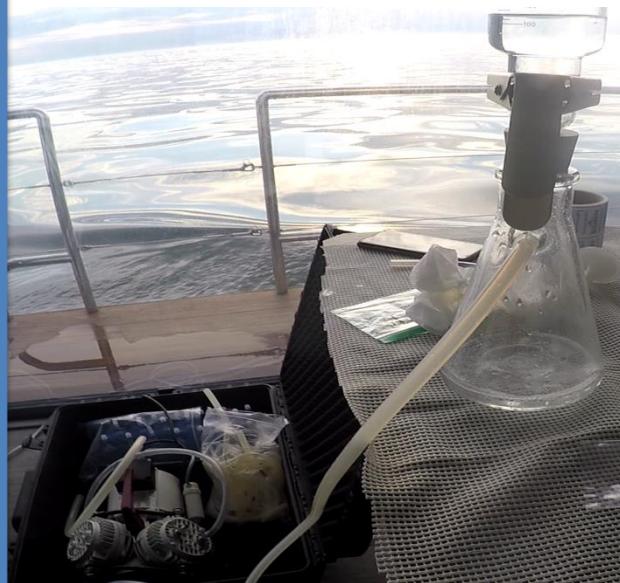
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CIIMAR - PORTUGAL

OSD74

ARCTIC OCEAN MONITORING PROGRAM

Complete sampling processing from historical data sets

Metabarcoding
Illumina and PacBio (16S rRNA and 18S rRNA)

Metagenomics

2015 2016 2017 2018 2019 2020 2021 2022 2023 2024

10 Years of Complete Microbial
Genomic Data

DATA /SAMPLES ARCHIVES



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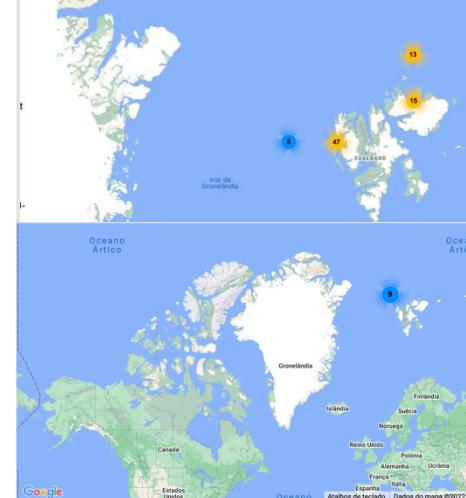
Classification



root:Environmental:Aquatic:Marine:Oceanic

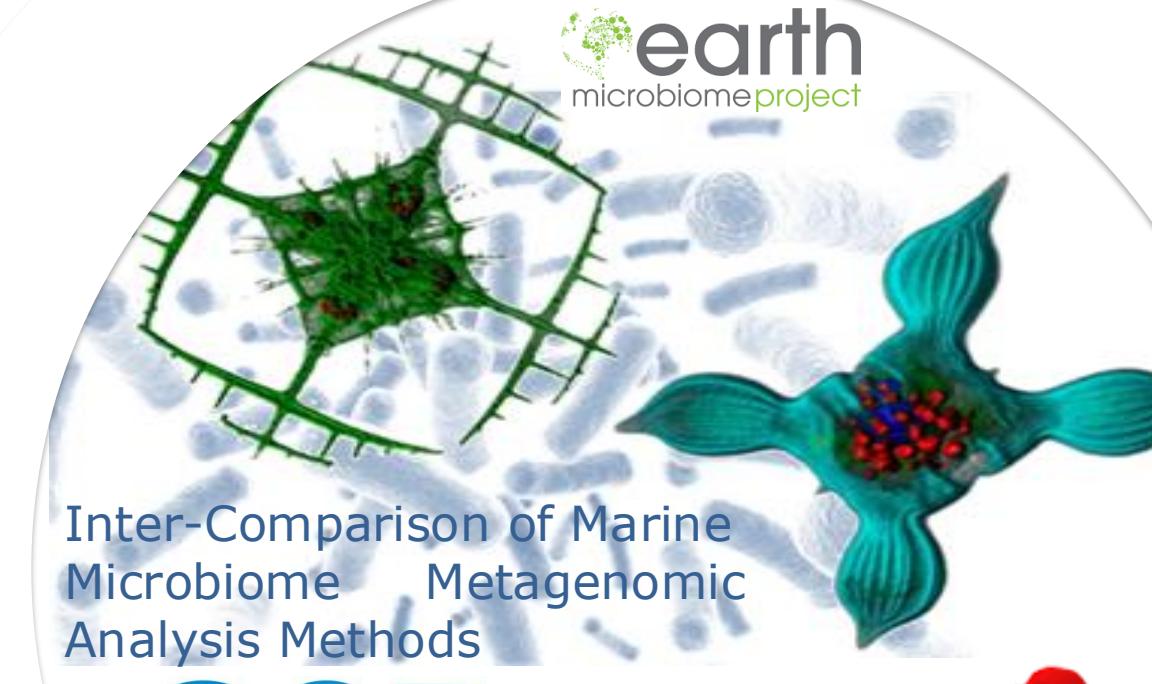
Description

One of the main concerns about the Arctic Ocean has been the changing sea ice regime with a reduction in the summer sea ice extent and a shift in dominance from thicker, perennial multiyear ice towards thinner, first-year ice. As the dietary basis of marine food webs and central players of biogeochemical cycles, microbial communities play an irreplaceable role when evaluating the ecological impact of the Arctic's thinner ice regime. During the Norwegian young sea Ice cruise 2015 (N-ICE2015), that took place in drifting pack ice north of Svalbard between January-June 2015, seawater was collected, at 5, 20 or 50, 250 m depth in 9th March, 27th April and 16th June, together with physical and biogeochemical data. Through the massively parallel sequencing of environmental DNA (metagenomics) we expect to get a snapshot of the Arctic's microbiome structure, key functions and dynamics through the dark-light transition.

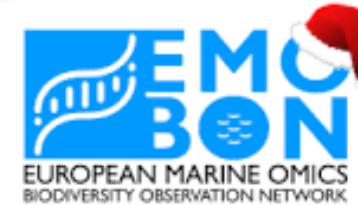


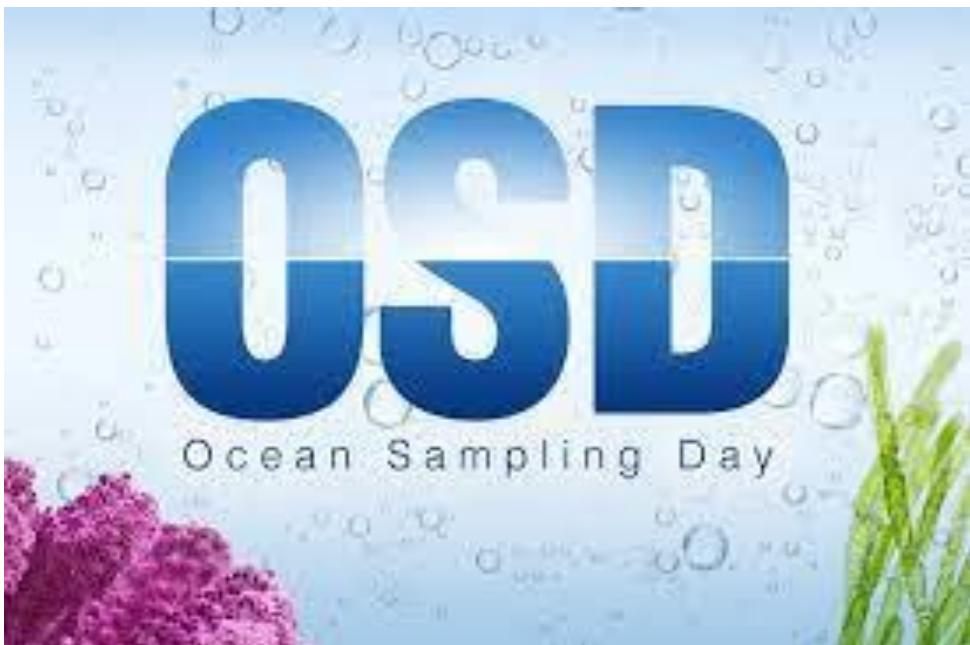
STUDYING MICROBES IN THE ENVIRONMENT

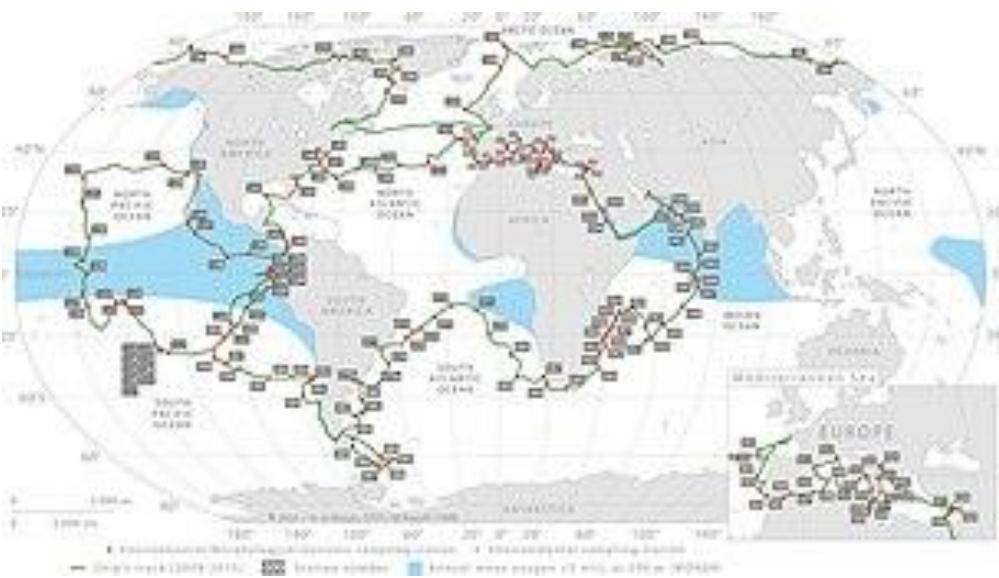
MICROBIOME STANDARDIZED INITIATIVES



Inter-Comparison of Marine
Microbiome Metagenomic
Analysis Methods









OSD

Ocean Sampling Day

WHOLE WATER FILTRATION

STERIVEX FILTERS

VOLUME OF WATER VARIES
BETWEEN 1L – 20L

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TARA OCEANS


WATER FILTRATION BY SIZE-FRACTIONATION

MEMBRANE FILTERS

VOLUME OF WATER VARIES
BETWEEN 10L – 100L

Volume of water sampled
0.5L, 1L, 2L, 3L, 5L, 7L,
10L, 100L...

Environmental Microbiology | Published: 08 January 2019

Diversity and Composition of Pelagic Prokaryotic and Protist Communities in a Thin Arctic Sea-Ice Regime

António Gaspar G. de Sousa, Maria Paola Tomasin, Pedro Duarte, Mar Fernández-Méndez, Philipp Assmy, Hugo Ribeiro, Jaroslaw Surkot, Ricardo B. Leite, José B. Pereira-Leal, Luís Torgo & Catarina Magalhães

Microbial Ecology 78, 388



Frontiers in Microbiology

2080 Accesses | 23 Citations

Volume 12 - 2021 | https://doi.org/10.3389/fmicb.2021.624071

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Abstract

One of the most prominent regime with a reduction multiyear ice towards the likely to impact microbial biogeochemical cycles. In Svalbard, seawater samples mesopelagic (250 m) deep physical and biogeochemical communities. Through the amplicon and metageno

Depth Profile of Nitrifying Archaeal and Bacterial Communities in the Remote Oligotrophic Waters of the North Pacific



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Javier J. González-González

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² Faculty of Science and Technology, University of Salford, Salford, United Kingdom
³ International Research Institute for Marine Sciences, University of Salford, Salford, United Kingdom
⁴ Department of Biological Sciences, University of Salford, Salford, United Kingdom
⁵ School of Biological Sciences, University of Salford, Salford, United Kingdom

Nitrification is the process by which inorganic nitrogen is converted into organic forms that the ecosystem can use. Previous studies have shown that oligotrophic waters contain relatively low concentrations of nitrifying bacteria.

Get citation

Appl Environ Microbiol. 2013 Jan; 79(1): 177–184.

doi: 10.1128/AEM.02155-12

PMCID: PMC3536108

PMID: 23087033

Relationship between Abundance and Specific Activity of Bacterioplankton in Open Ocean Surface Waters

Dana E. Hunt,^a Yajuan Li,^a Zackary I. Johnson,^b Maia M. Miguez,^c and Michael S. Azam^a

► Author information |

Associated Data

► Supplementary I

ABSTRACT

Marine microbial communities in pelagic ecosystems are composed of many different species within genetically diverse lineages. These specific activities (i.e., metabolic functions) identify locations and times where specific processes are occurring. By analyzing 1.6 million sequences from a representative sample of the ocean, we found a relationship between the abundance and specific activity of bacterioplankton in open ocean surface waters. We found that the abundance of bacterioplankton was positively correlated with their specific activity, suggesting that the abundance of bacterioplankton is influenced by their specific activity. This study provides new insights into the relationship between the abundance and specific activity of bacterioplankton in open ocean surface waters.

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Article | Open access | Published: 04 May 2022

Influence of nutrient supply on plankton microbiome biodiversity and distribution in a coastal upwelling region

Chase C. James, Andrew D. Barton, Lisa Zeigler Allen, Robert H. Lampe, Ariel Rabines, Anne Schulberg, Hong Zheng, Ralf Goericke, Kelly D. Goodwin & Andrew E. Allen

Nature Communications 13, Article number: 2448 (2022) | Cite this article

7582 Accesses | 7 Citations | 198 Altmetric | Metrics

► A Publisher Correction to this article was published on 18 May 2022

► This article has been updated



2022

TYPE OF FILTERS

Cartridge membrane filters
“STERIVEX FILTERS”, with
a pore size of 0.22 /0.45 µm

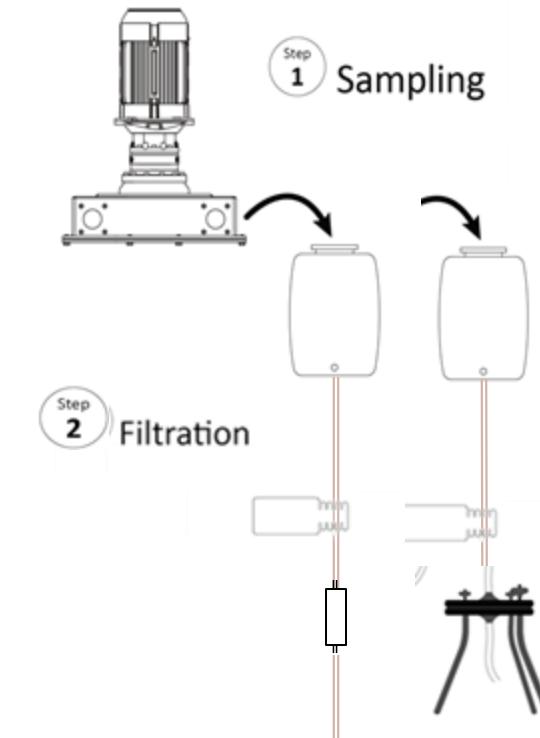


Flat membrane filters
that can differ in the
material and pore size
(polyethersulfone,
polycarbonate, cellulose,
etc.)



FILTRATION METHODS

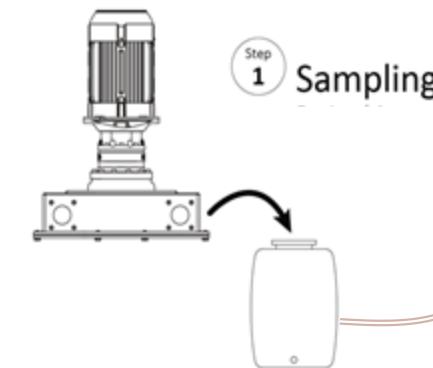
WHOLE WATER FILTRATION



Sterivex or membrane Filter

0.22 μm

FILTRATION BY SIZE FRACTIONATION



Membrane Filter

20 μm , 3 μm or 8 μm , 0.22 μm

Step 2 Pre-filtration
47 mm in-line filter holder
20 μm

Step 3 Filtration
142 mm tripod
0.3/0.8 μm

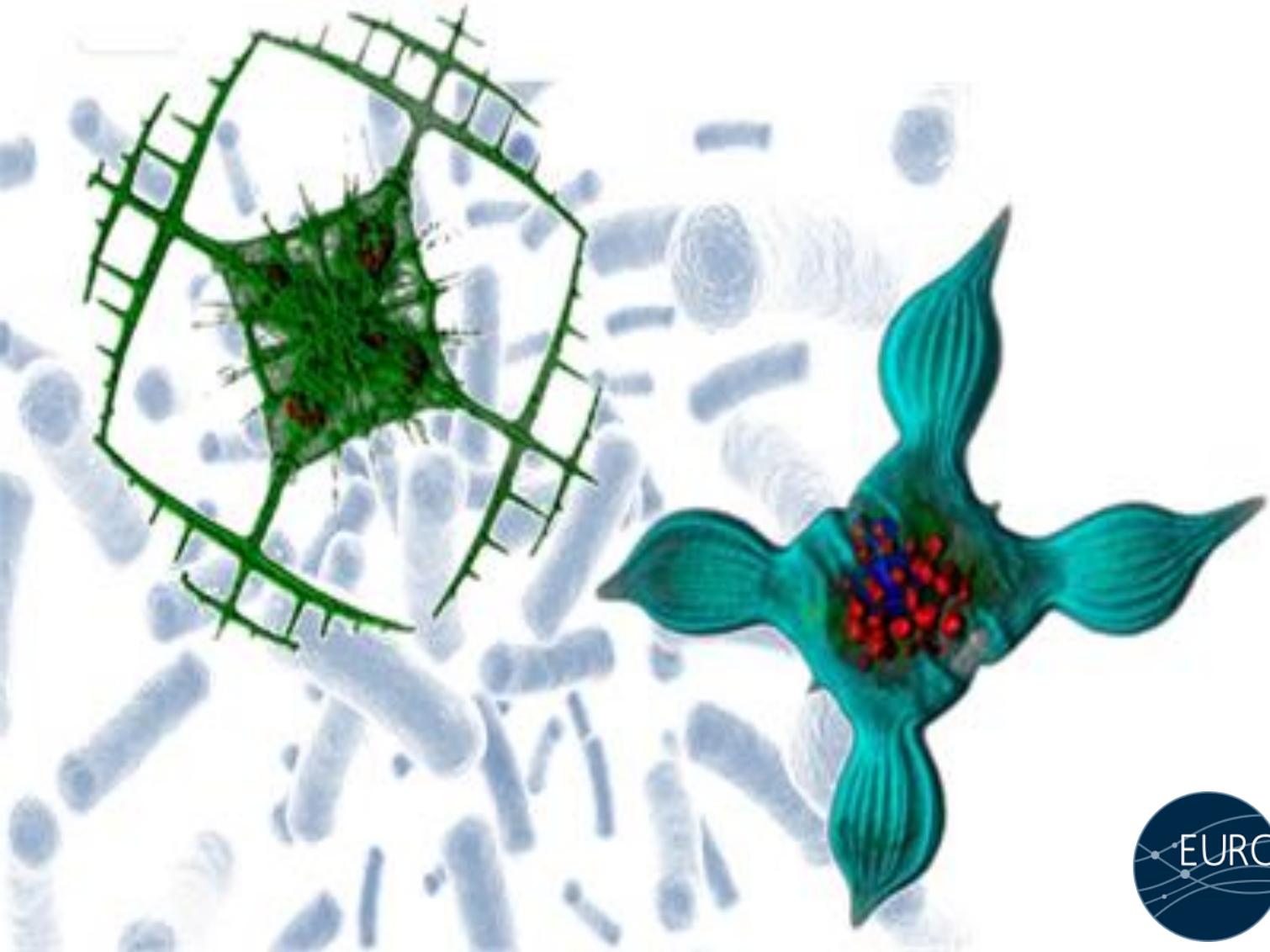
Step 3 Filtration
142 mm tripod
0.22 μm

As a consequence of the multiple methodologies used for studying marine microbiomes, we now have an enormous amount of data available in international archives that we do not know if it is comparable.

Therefore, we thought it could be incredibly useful to produce a new dataset that will allow us to compare the protocols used in global marine microbiome initiatives.

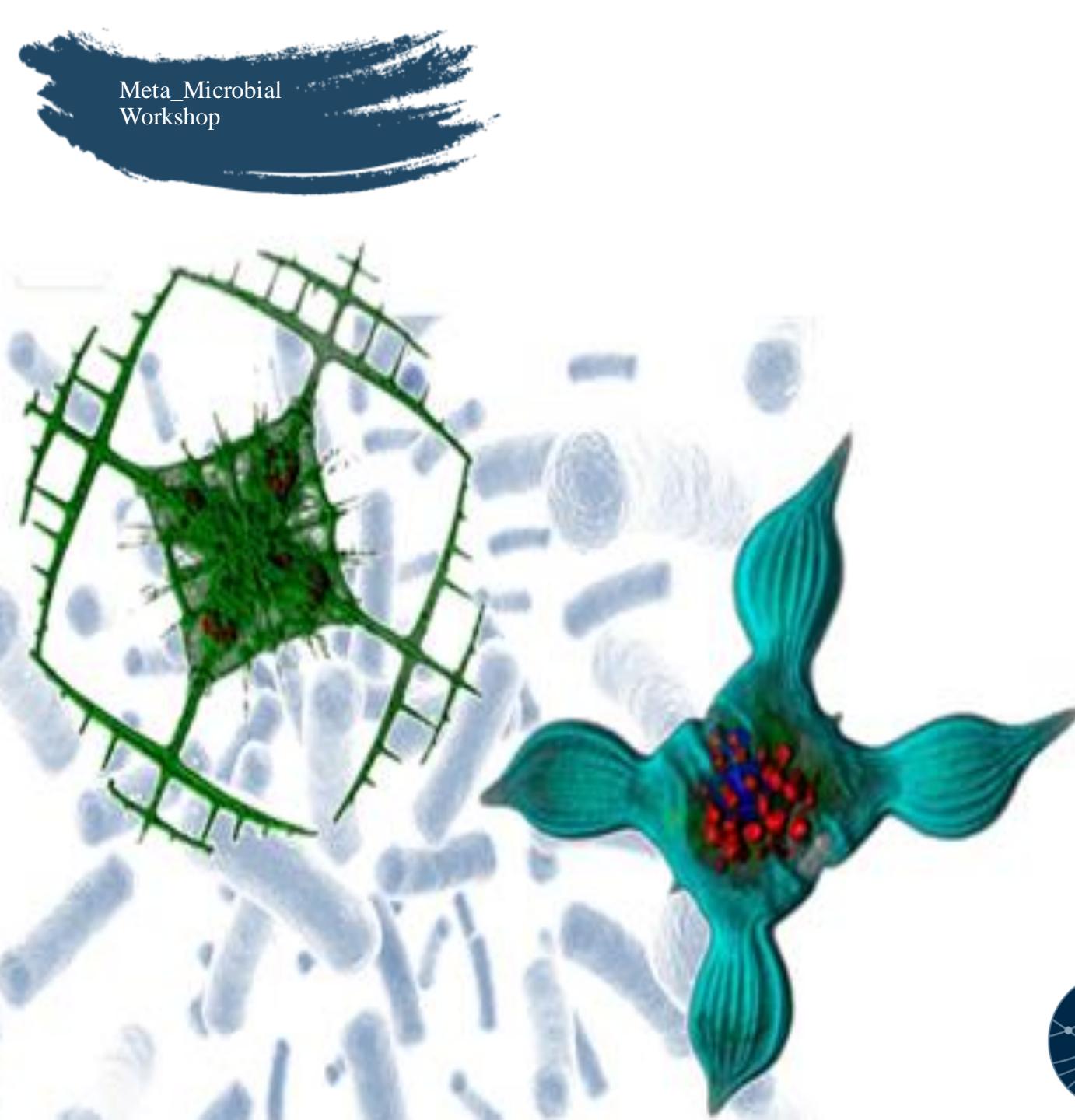
Meta_Microbial
Workshop

Inter-Comparison of Marine Microbiome Metagenomic Analysis Methods

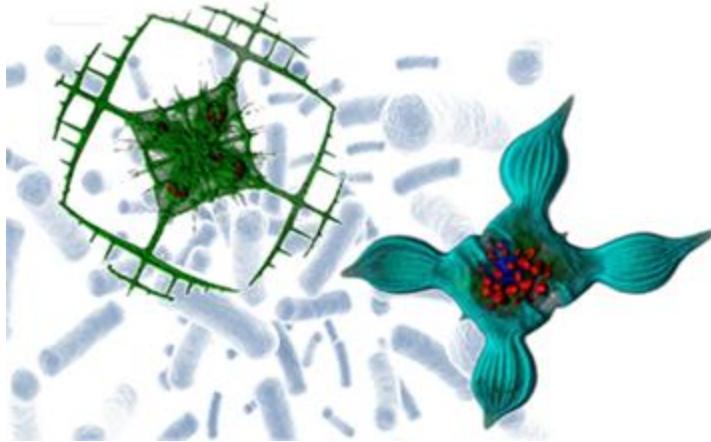


EMOSE INITIATIVE

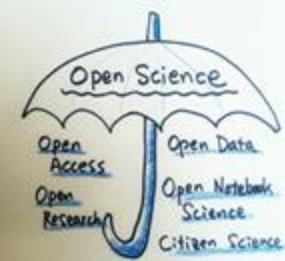
Scientific objective of Review methods used in global ocean initiatives that use samples for metabarcoding and metagenomics microbial studies.



Inter-Comparison of Marine Microbiome Metagenomic Analysis Methods



Step1 CONCEPT DESIGN



Step2 BANYULS EXPERIMENT



Step 3 MASSIVE SEQUENCING



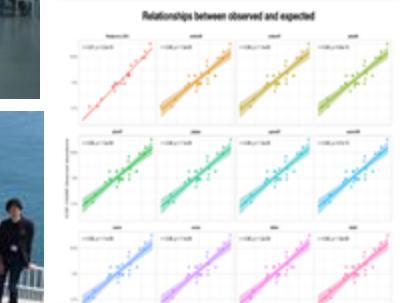
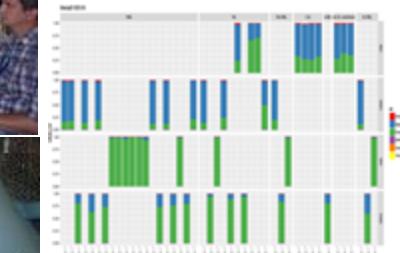
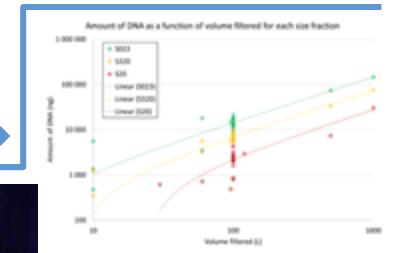
METABARCODING
METAGENOMICS



Step 4 HACKATHON



Step 5 EMOSE DATA SET & PAPER



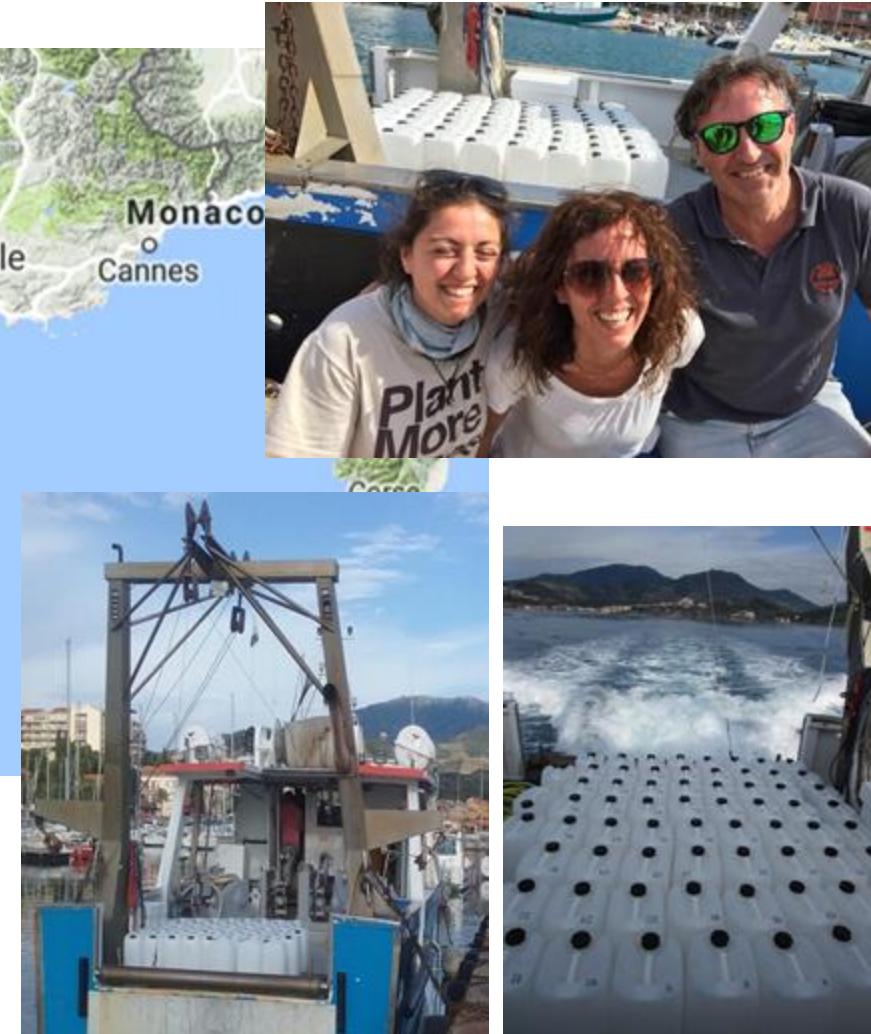
Summary of EMOSE activities

- Does filtration volume matters?
- Does the use of different filters make a difference?
- What is the effect of size-fractionation/whole water on results?

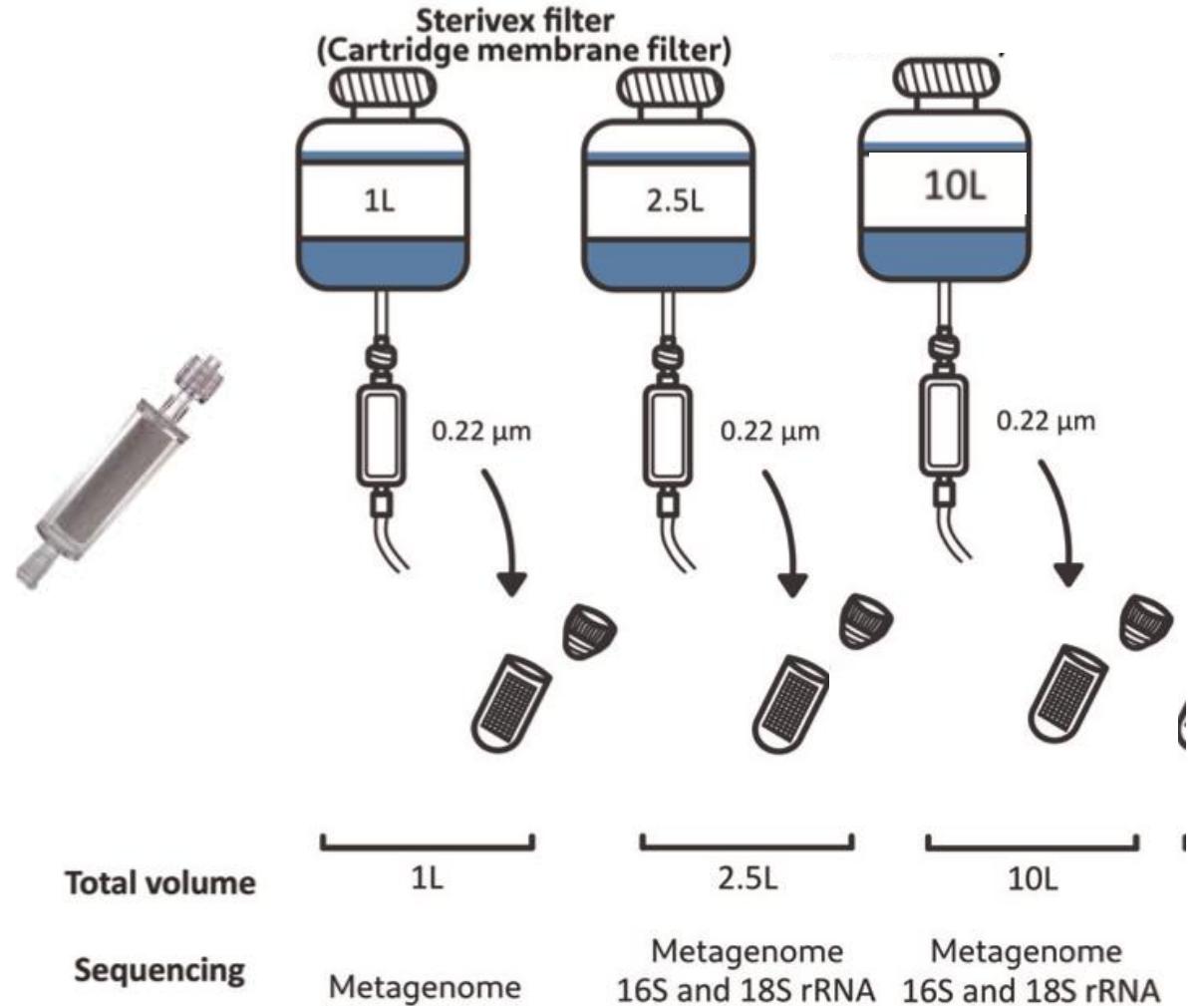
Generate samples



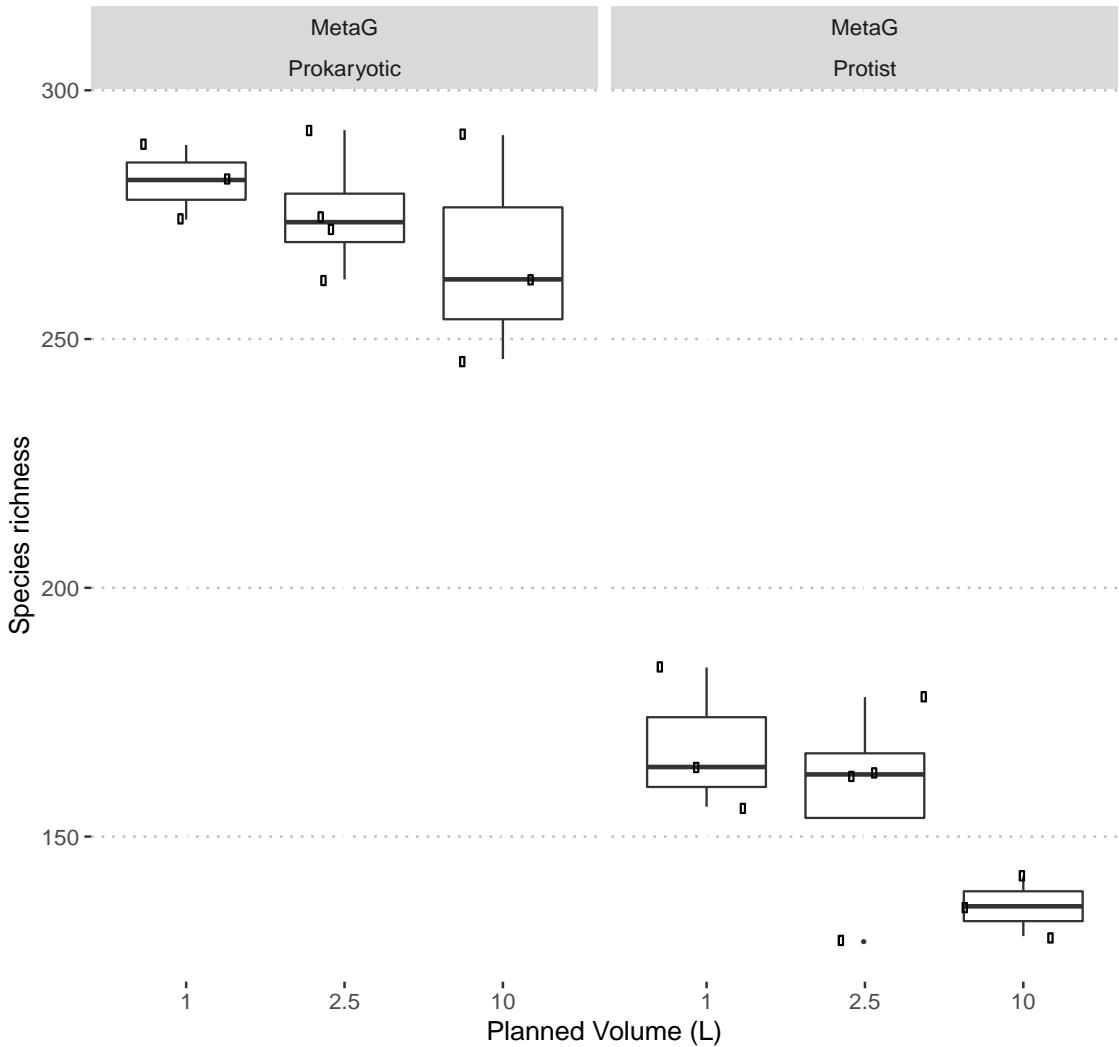
BANYULS SUR-MER
European Marine Observatory
Sola monitoring station (50 m deep)
Surface water sampling (>5000L)

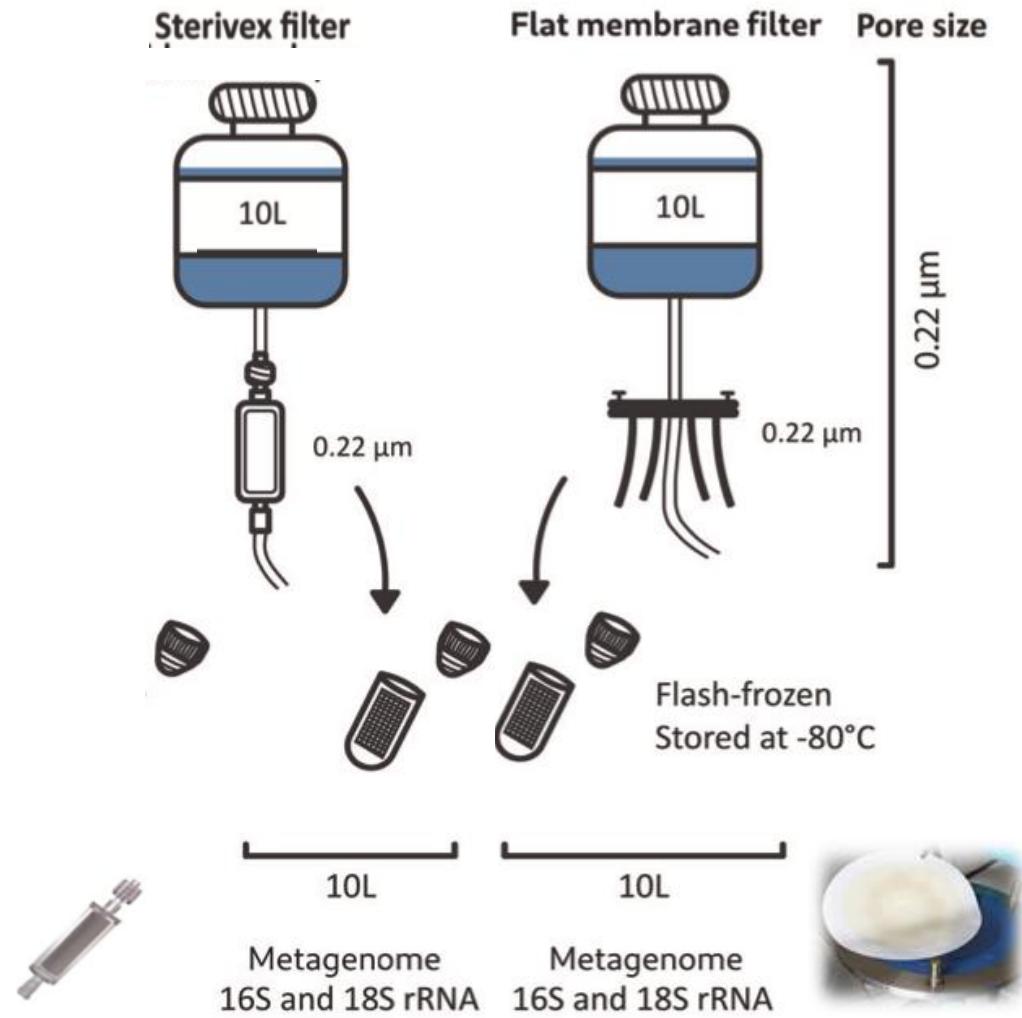


Whole water filtration (single filter)

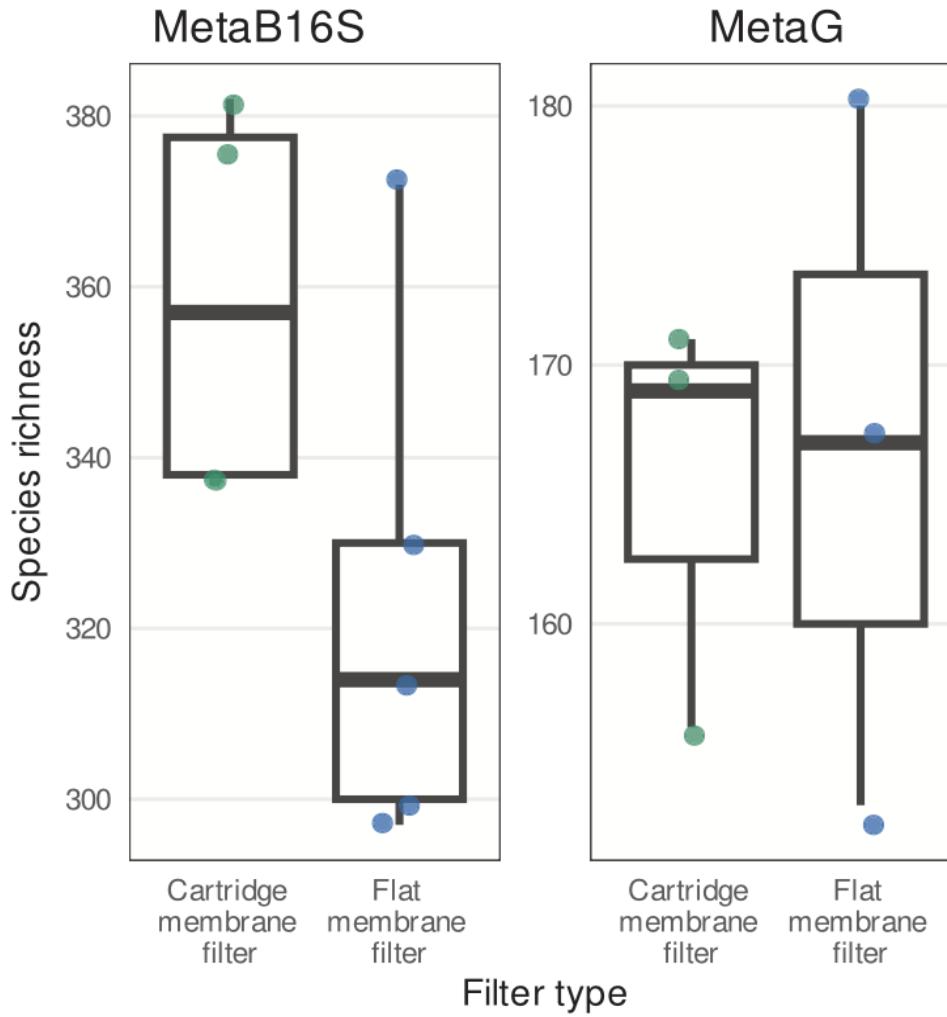


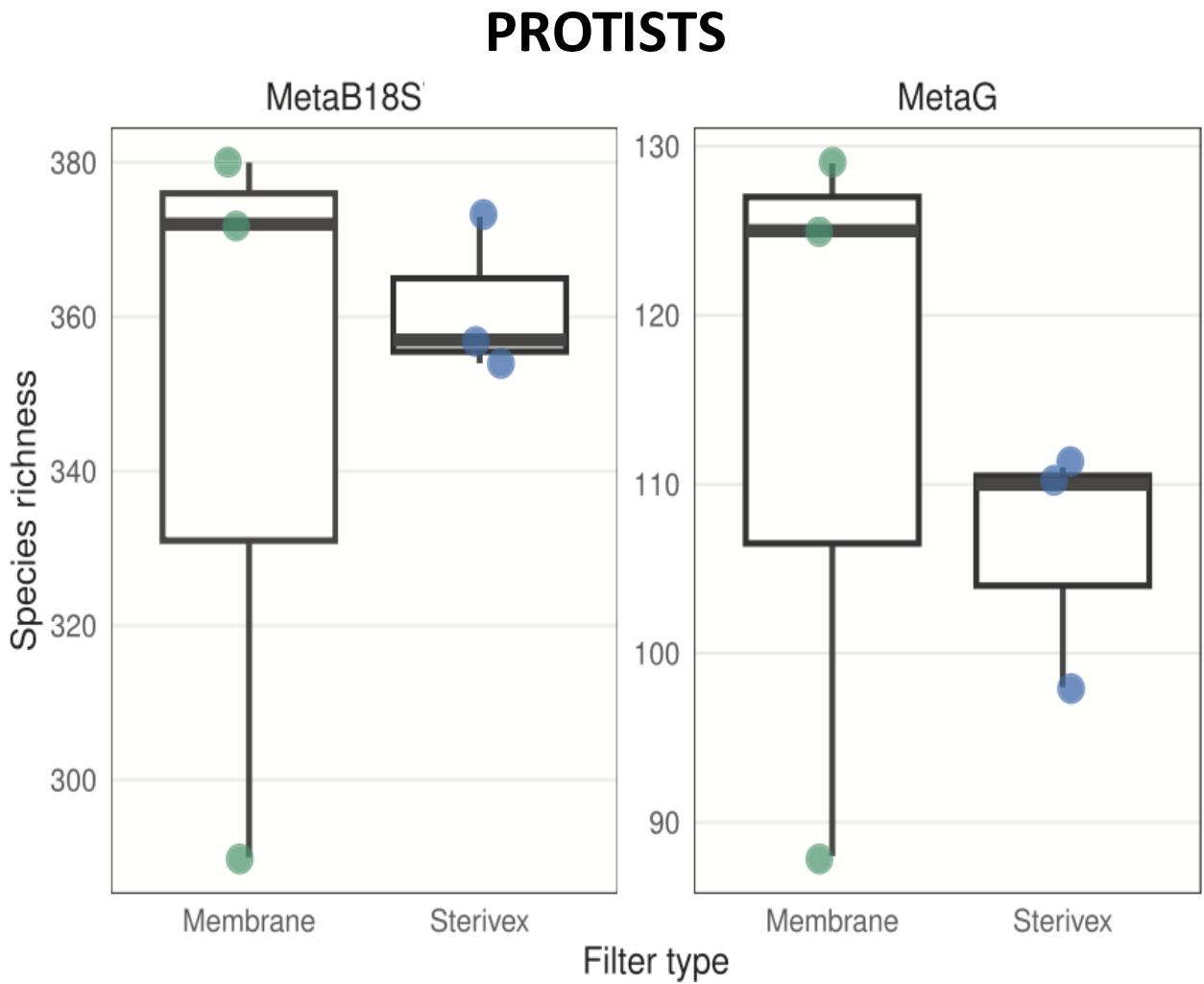
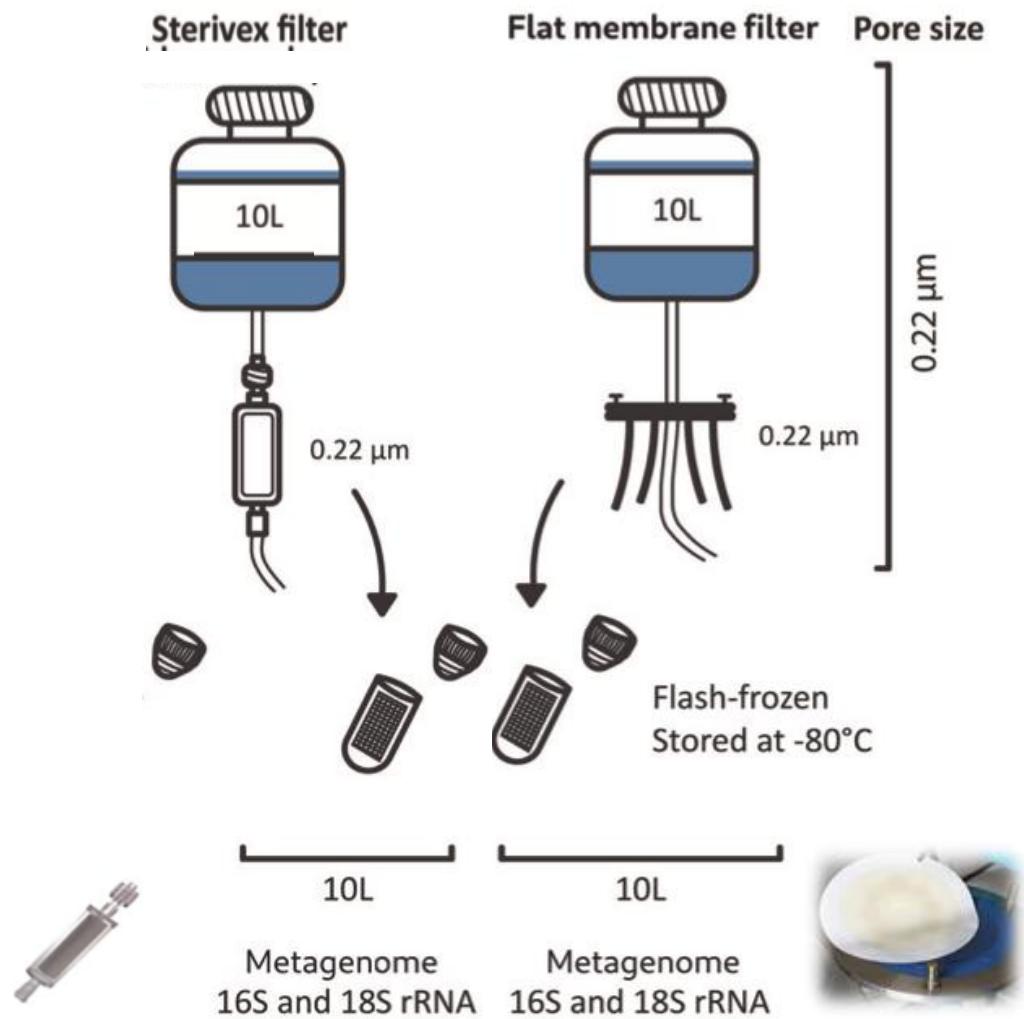
Small volumes, sterivex





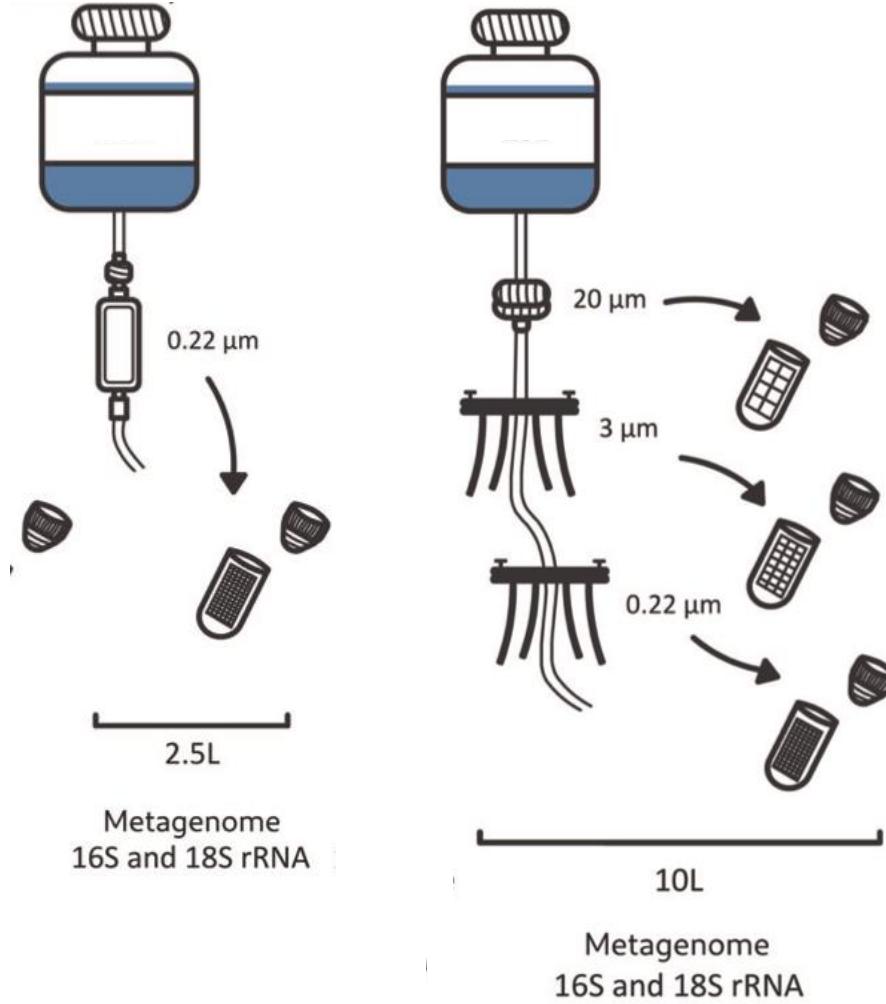
PROKARYOTES



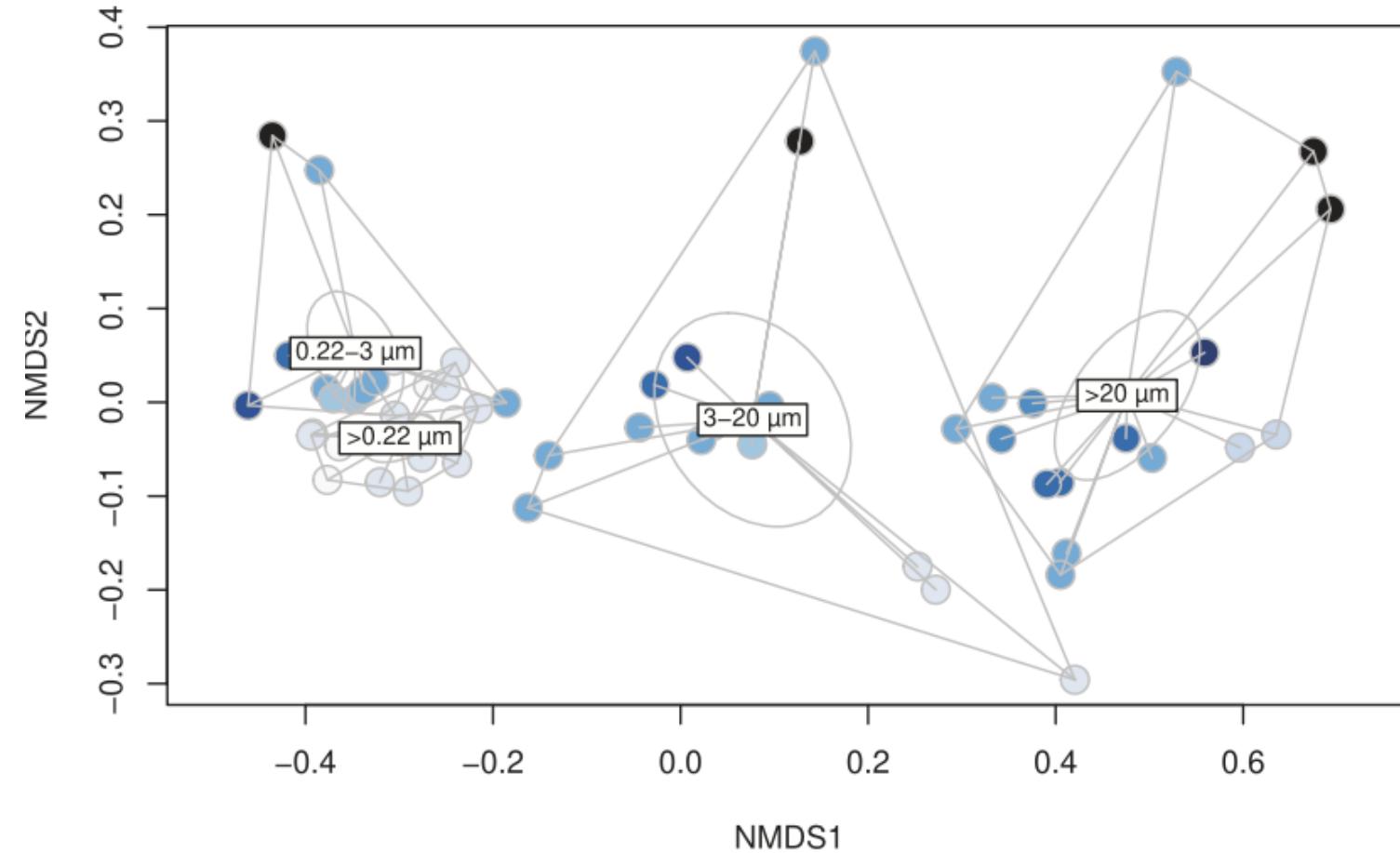


FILTER PORE SIZE

PROKARYOTES

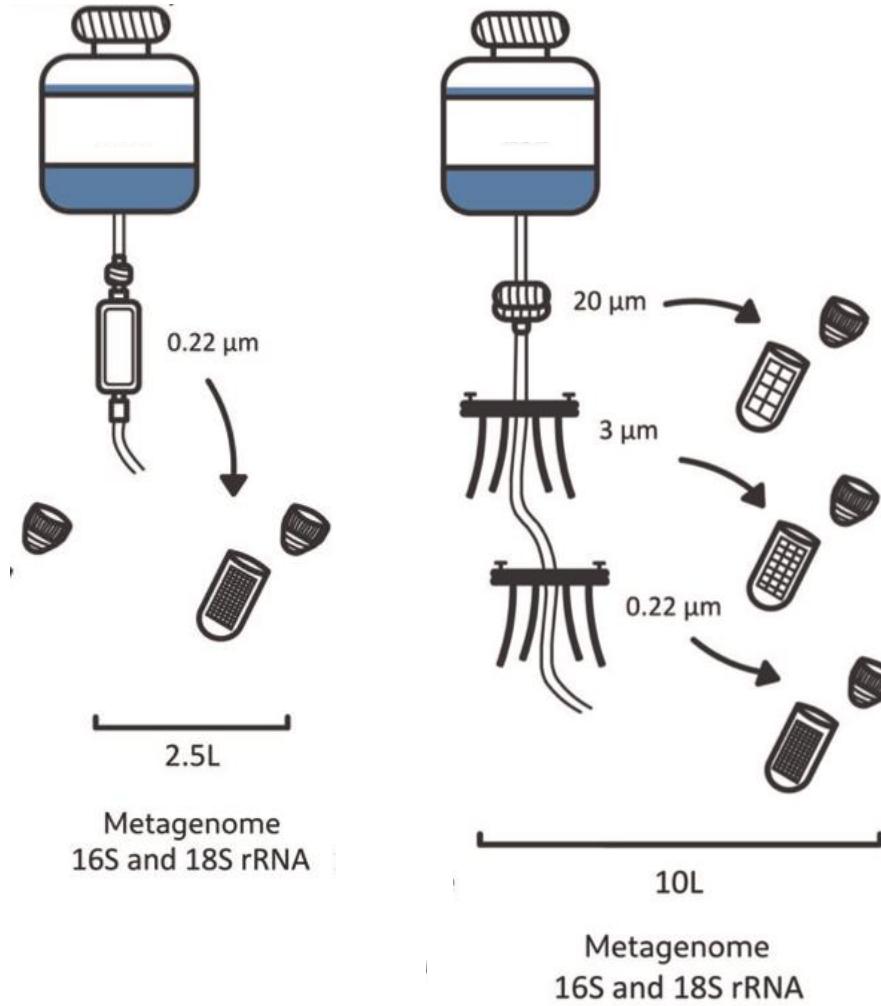


Prokaryotes, MetaB16S

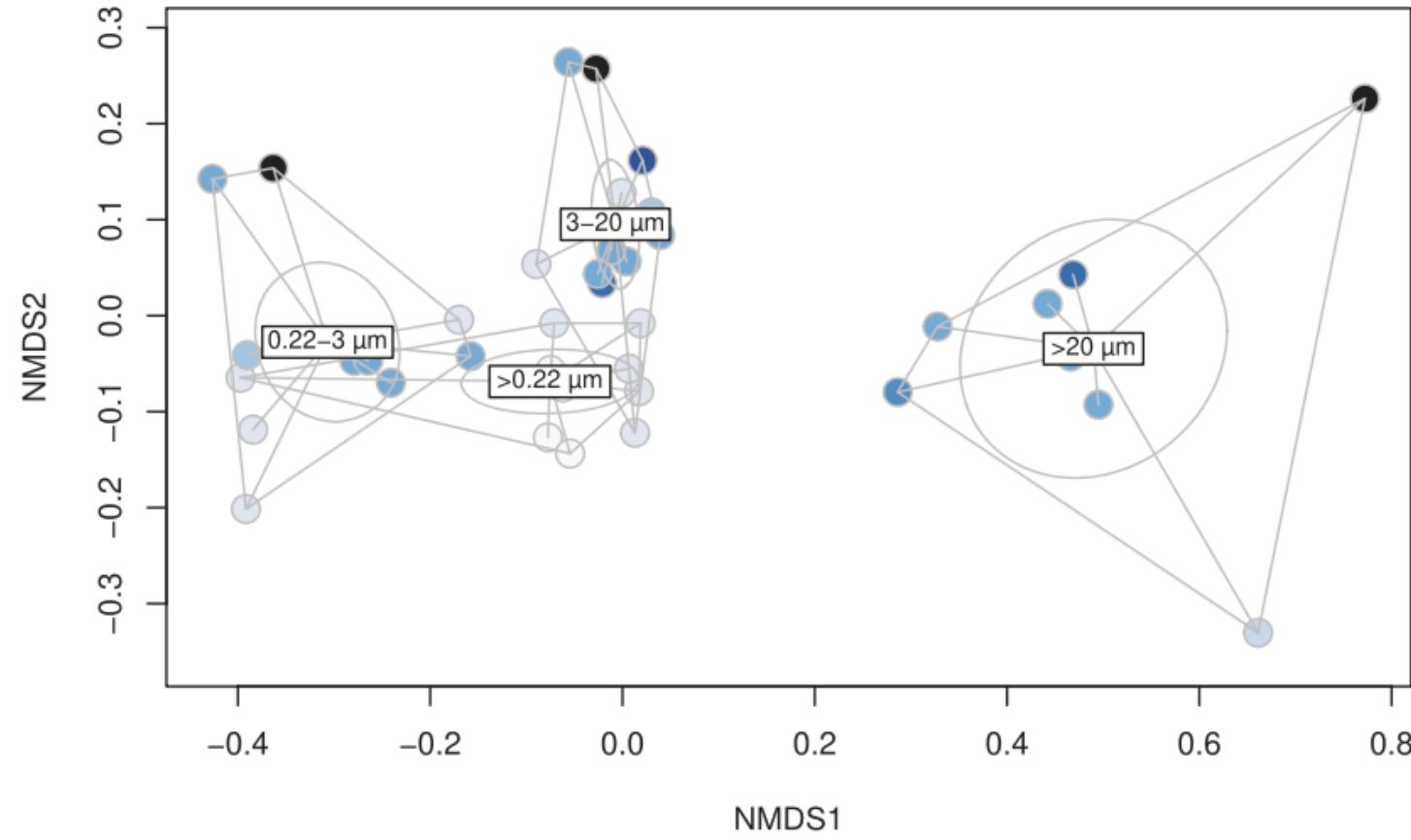


FILTER PORE SIZE

PROTISTS



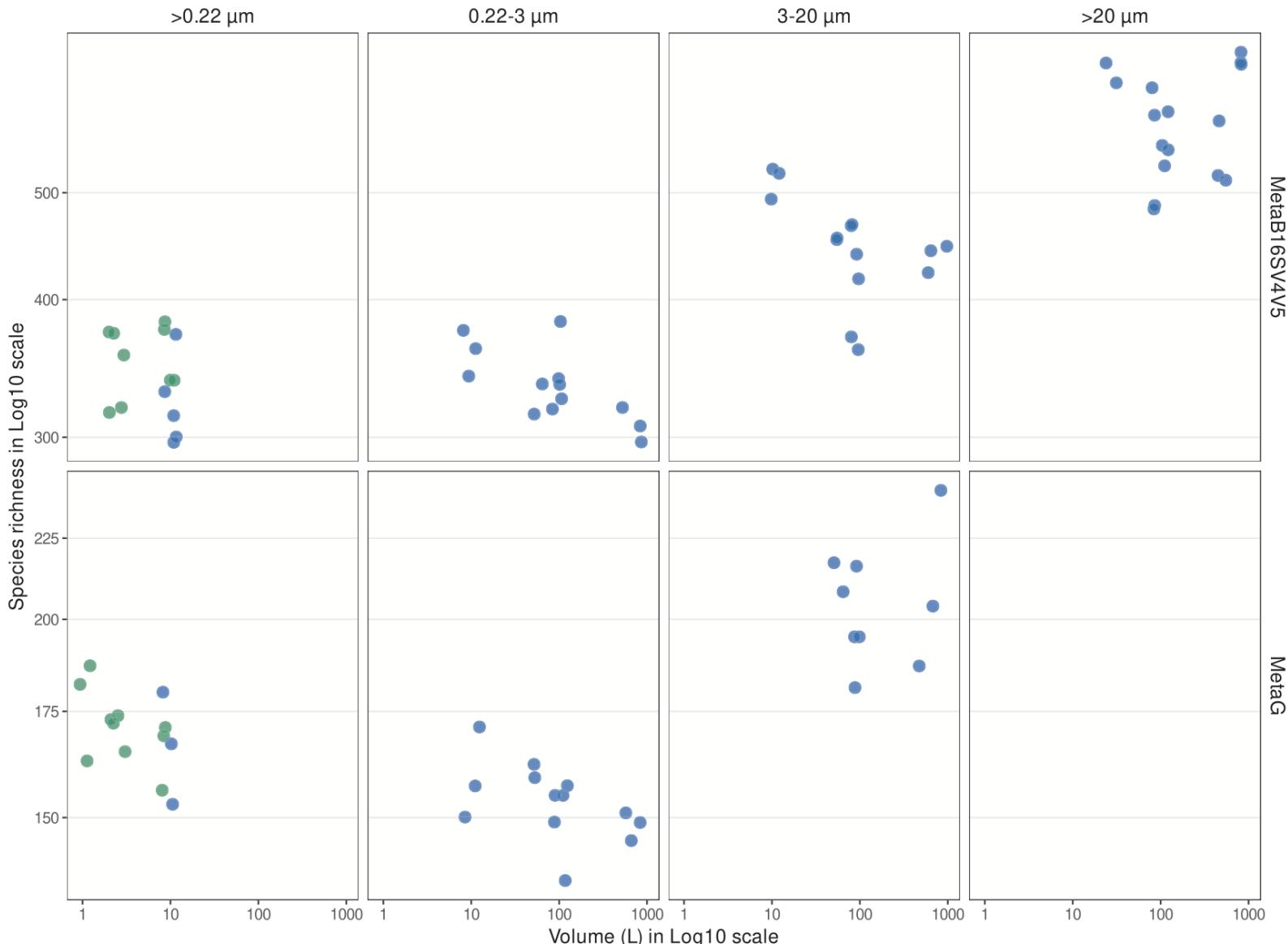
Protists, MetaB18S



Results Overview

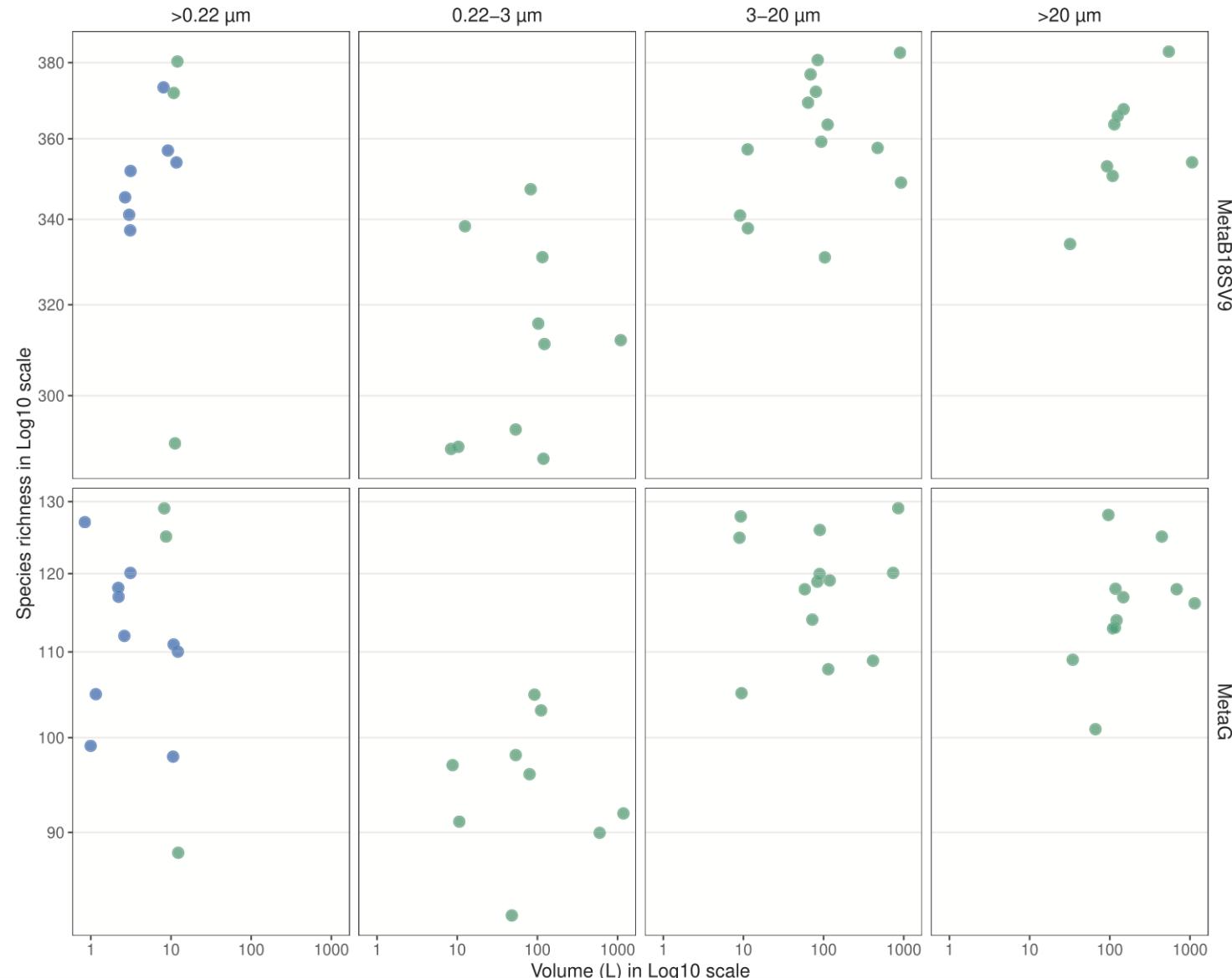


Filter ● Cartridge membrane filter ● Flat membrane filter



PROKARYOTIC
RICHNESS

Results Overview



**PROTISTS
RICHNESS**

RICHNESS PER TAXONOMY GROUP



EUROMARINE

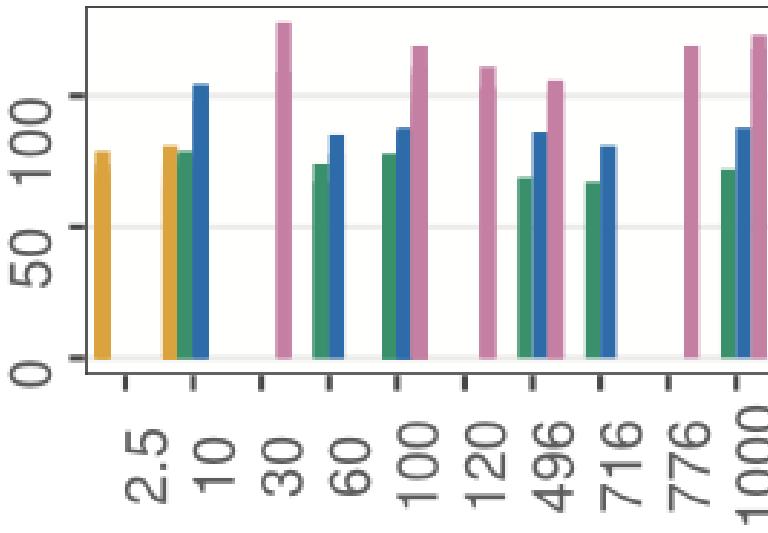


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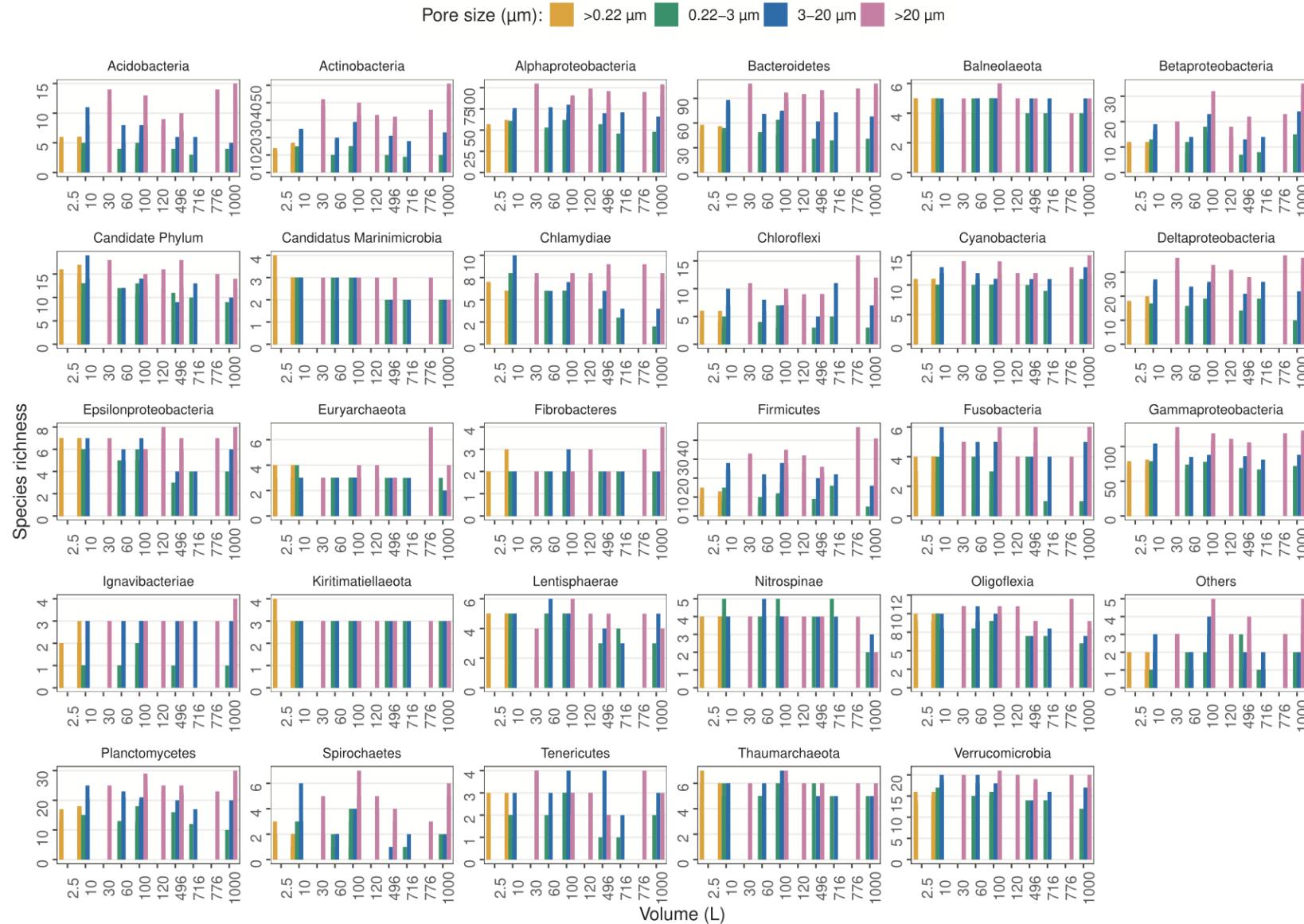
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PROKARYOTES

Gammaproteobacteria



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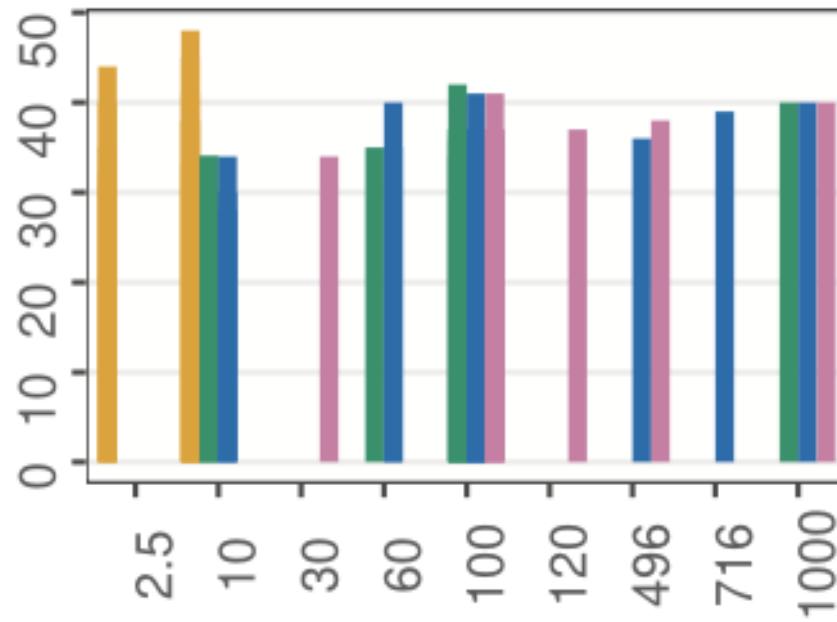


RICHNESS PER TAXONOMY GROUP

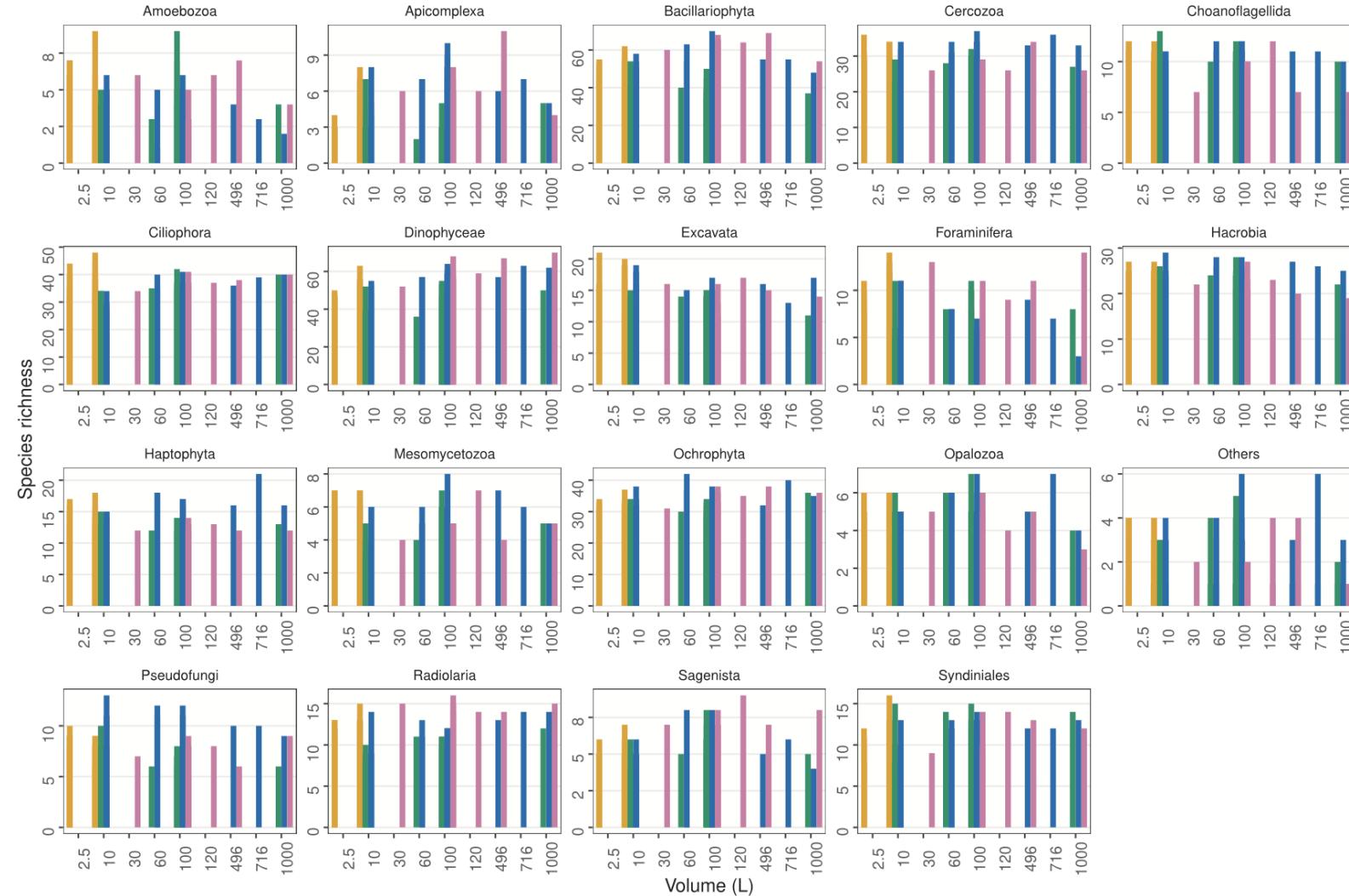


PROTISTS

Ciliophora



Pore size (μm): █ >0.22 μm █ 0.22–3 μm █ 3–20 μm █ >20 μm



Sharing the data

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MGYS00001935

EMOSE (2017) Inter-Comparison of Marine Plankton Metagenome Analysis Methods

[Overview](#) [Analysis summary](#)

Last updated: Tue Sep 20 2022

Classification



root:Environmental:Aquatic:Marine



Description

This study includes experiments from the following five sequencing strategies: 1) Shotgun DNA sequencing; 2) Amplicon sequencing after 18S amplification by PCR using 1391F/EukB primer set. Library were constructed according to Illumina Library protocol without any sizing; 3) Amplicon sequencing after 16S amplification by PCR using 515F/926R primer set. Library were constructed according to Illumina Library protocol without any sizing; 4) Amplicon sequencing after 16S amplification by PCR using 515F/926R primer set. Library were constructed according to Illumina Library protocol with a sizing step selecting the 450-650 bp fragments; and 5) Amplicon sequencing after 16S amplification by PCR using 515F/926R primer set. Library were constructed according to Illumina Library protocol with a sizing step selecting the 650-850 bp fragments.



www.nature.com/ismecomms

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Inter-comparison of marine microbiome sampling protocols

Francisco Pascoal  ^{1,2}, Maria Paola Tomasino ¹, Roberta Piredda ³, Grazia Marina Quero ⁴, Luís Torgo ⁵, Julie Poulain ⁶, Pierre E. Galand  ⁷, Jed A. Fuhrman  ⁸, Alex Mitchell ⁹, Tinkara Tinta ¹⁰, Timotej Turk Dermastia ¹⁰, Antonio Fernandez-Guerra  ¹¹, Alessandro Vezzi  ¹², Ramiro Logares  ¹³, Francesca Malfatti ¹⁴, Hisashi Endo  ¹⁵, Anna Maria Dąbrowska  ¹⁶, Fabio De Pascale  ¹², Pablo Sánchez  ¹³, Nicolas Henry  ^{17,18}, Bruno Fosso ¹⁹, Bryan Wilson ²⁰, Stephan Toshchakov  ²¹, Gregory Kevin Ferrant ²², Ivo Grigorov ²³, Fabio Rocha Jimenez Vieira ²⁴, Rodrigo Costa  ^{25,26}, Stéphane Pesant  ⁹  and Catarina Magalhães  ^{1,2} 

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Research on marine microbial communities is growing, but studies are hard to compare because of variation in seawater sampling protocols. To help researchers in the inter-comparison of studies that use different seawater sampling methodologies, as well as to help them design future sampling campaigns, we developed the EuroMarine Open Science Exploration initiative (EMOSE). Within the EMOSE framework, we sampled thousands of liters of seawater from a single station in the NW Mediterranean Sea (Service d'Observation du Laboratoire Arago [SOLA], Banyuls-sur-Mer), during one single day. The resulting dataset includes multiple seawater processing approaches, encompassing different material-type kinds of filters (cartridge membrane and flat membrane), three different size fractionations ($>0.22\text{ }\mu\text{m}$, $0.22\text{--}3\text{ }\mu\text{m}$, $3\text{--}20\text{ }\mu\text{m}$ and $>20\text{ }\mu\text{m}$), and a number of different seawater volumes ranging from 1 L up to 1000 L. We show that the volume of seawater that is filtered does not have a significant effect on prokaryotic and protist diversity, independently of the sequencing strategy. However, there was a clear difference in alpha and beta diversity between size fractions and between these and "whole water" (with no pre-fractionation). Overall, we recommend care when merging data from datasets that use filters of different pore size, but we consider that the type of filter and volume should not act as confounding variables for the tested sequencing strategies. To the best of our knowledge, this is the first time a publicly available dataset effectively allows for the clarification of the impact of marine microbiome methodological options across a wide range of protocols, including large-scale variations in sampled volume.

ISME Communications; <https://doi.org/10.1038/s43705-023-00278-w>

<https://www.nature.com/articles/s43705-023-00278-w>



TAKE HOME MESSAGE

- Microbiome standardizing initiatives are essential to guaranty comparable marine microbiome data (e.g between monitoring programs).
- The EMSO data set will help researchers to design their own sampling campaigns for specific target microbiome organisms.
- The metabarcoding and metagenomic comparison from EMOSE initiative will help to understand limitations in comparing archived data from studies using different sampling protocols.
- There is more to be tested for the purpose of standardization of protocols in the future, for example, eDNA extraction protocols, primers used, sequencing strategies, etc

