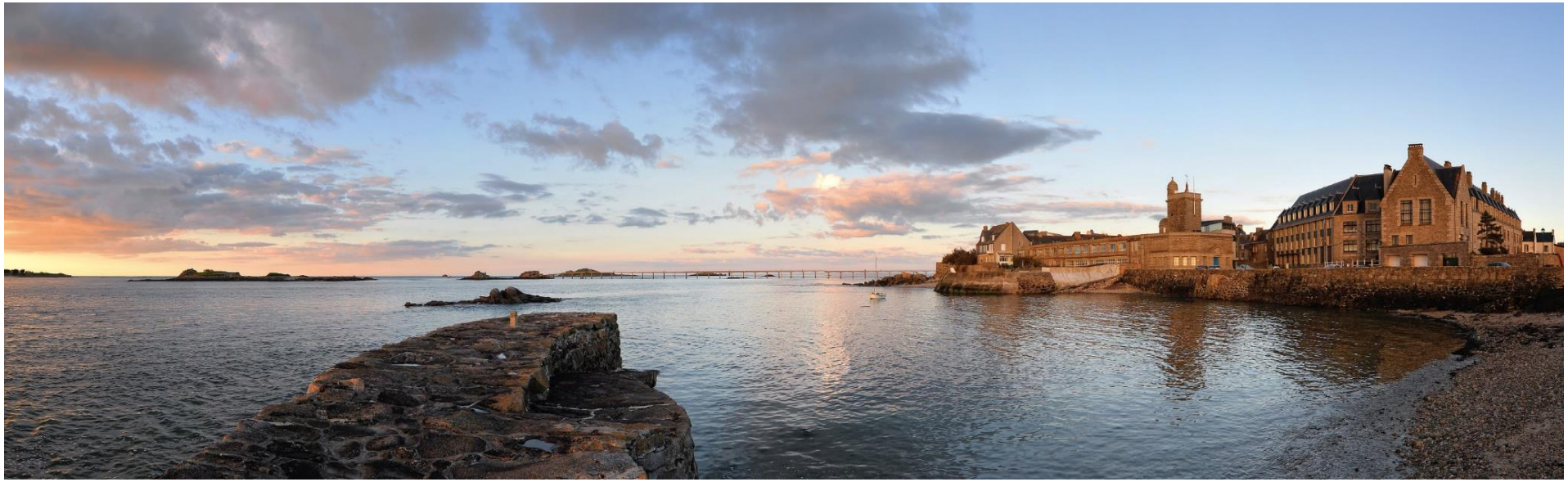
CNRS • SORBONNE UNIVERSITÉ
Station Biologique de Roscoff

Cryopreservation and recovery of a complex hypersaline microbial mat community



Michele Grego

Roscoff Culture Collection (RCC)

Cryopreservation

Single micro-organisms

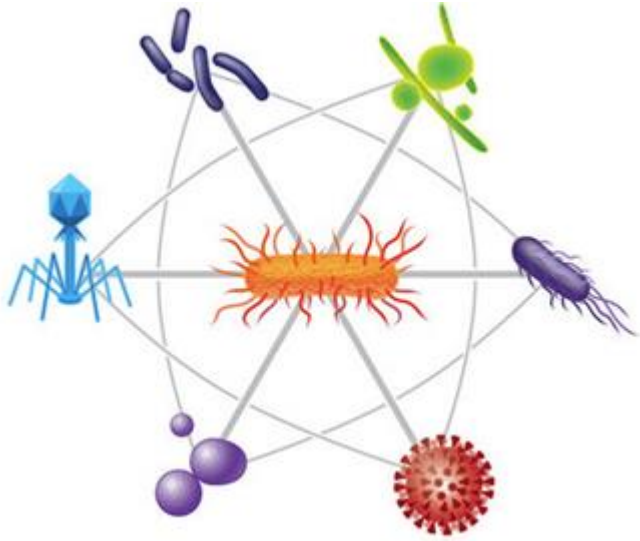
- ✓ Bacteria species relatively easy
- ✓ Problems with cyanobacteria and especially microalgae
- ✓ Advance in cryobiology resulted in development of successful protocols

Preservation of complex microbial communities from environmental samples

- ✓ Sampling from remote and less accessible ecosystems (deep sea sediments, drilling cores)
- ✓ High sampling costs

Preservation of complex microbial communities from environmental samples

- ✓ Sampling from remote and less accessible ecosystems (deep sea sediments, drilling cores)
- ✓ High sampling costs
- ✓ Re-analyze with new technology
- ✓ New insight in microbial interactions



Preserve the taxonomic and functional diversity and reconstruct the three dimensional structure

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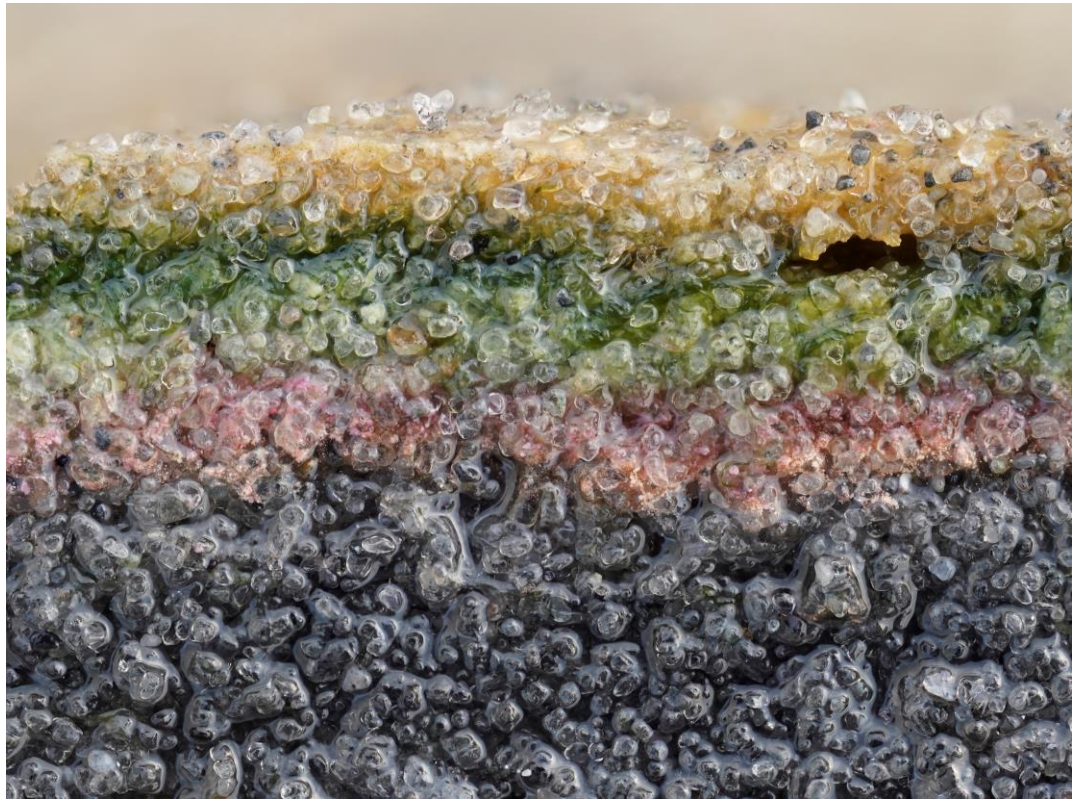
Hypersaline Microbial Mat

Guerande, France 2016

Microbial Mat

- ✓ Three dimensional structures
- ✓ Micro-organisms embedded in EPS (Extracellular Polymeric Substances)
- ✓ Often driven by photosynthetic communities
- ✓ High biodiversity in just 5mm



© Wim van Egmond

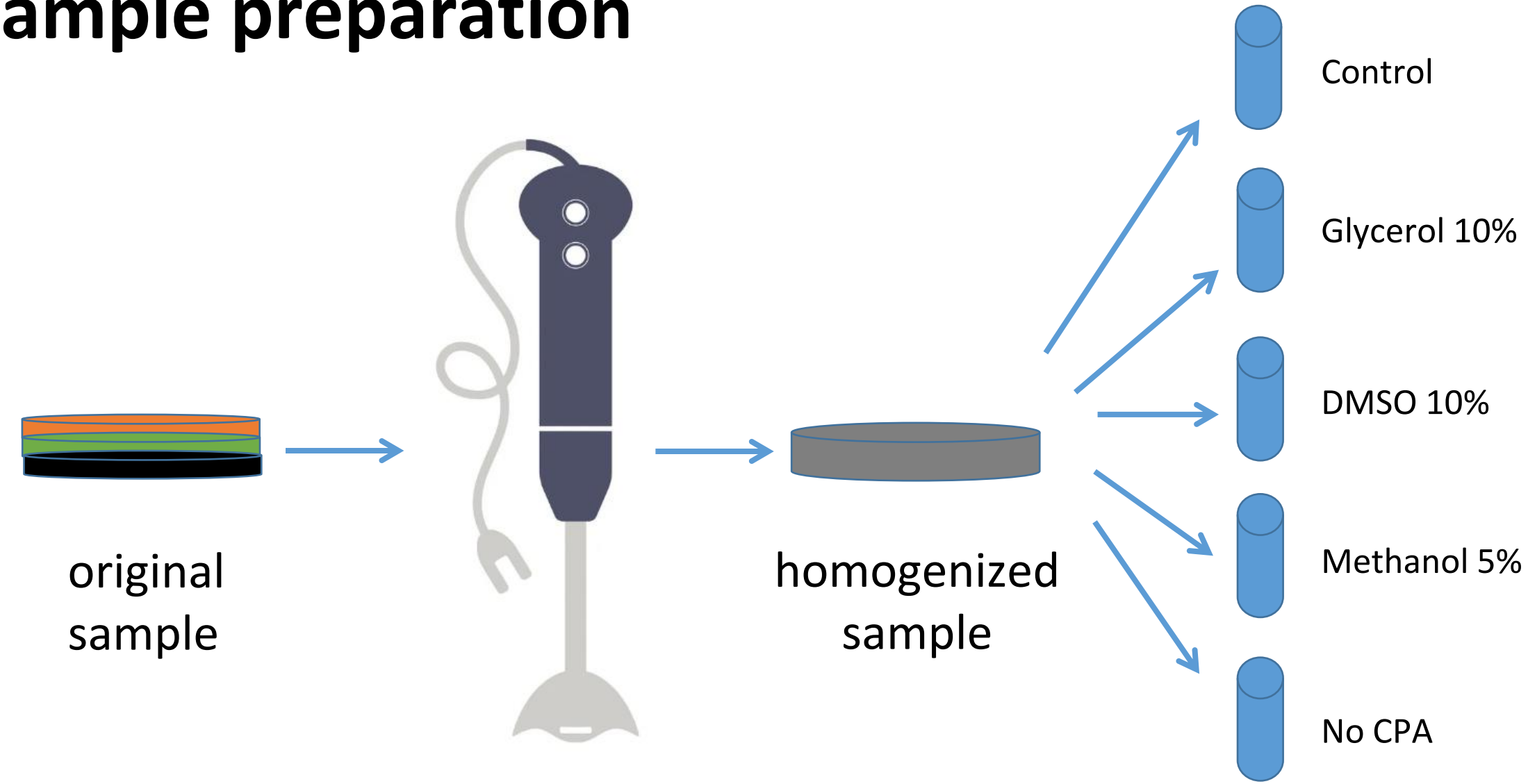
Cyanobacteria & Diatoms

Heterotrophic Bacteria

Purple Sulphur Bacteria

Sulphate Reducing Bacteria
Anaerobic bacteria

Sample preparation



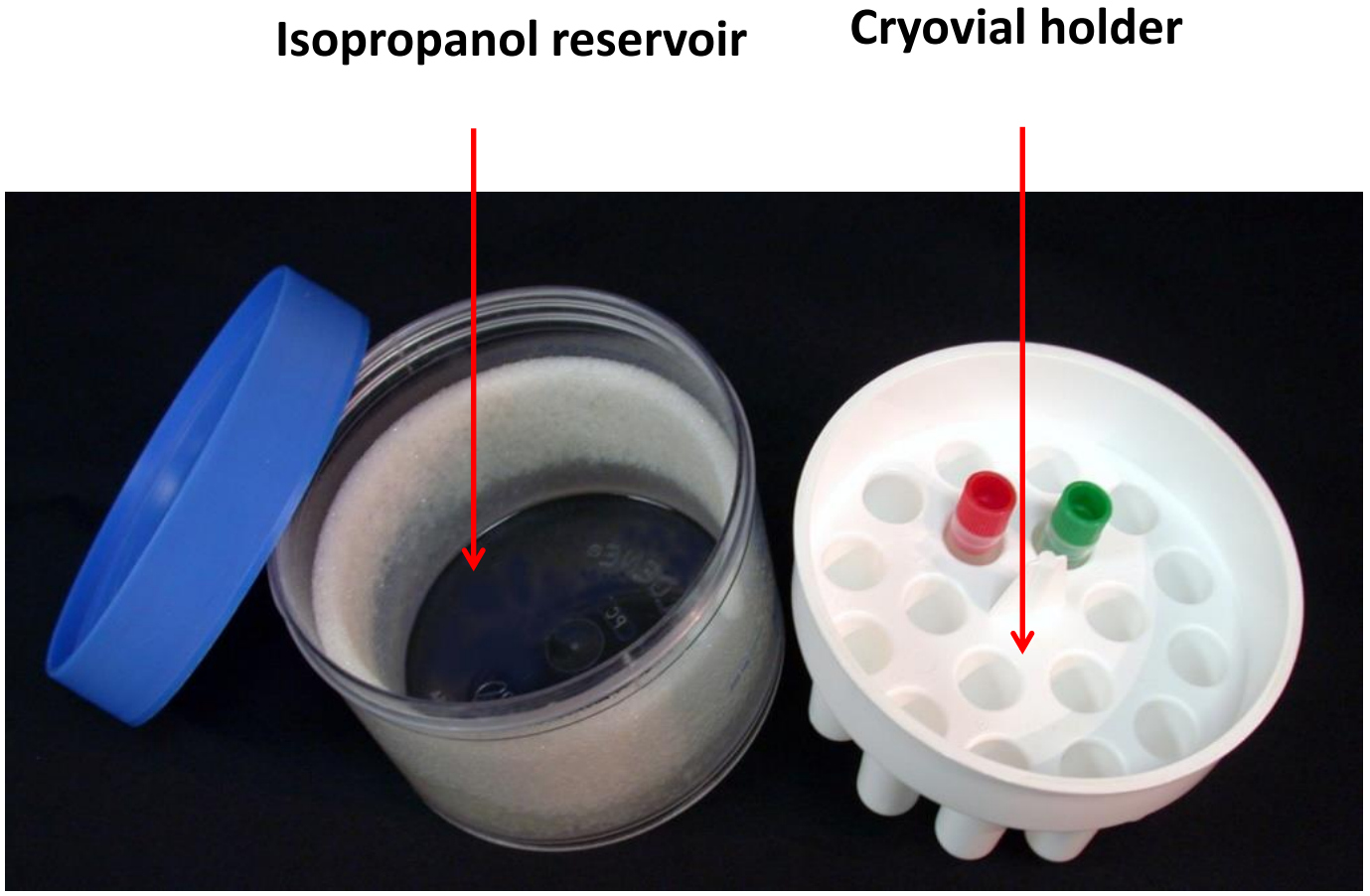
Two-step freezing methodology

Mr. Frosty

- ✓ 1st -80°C

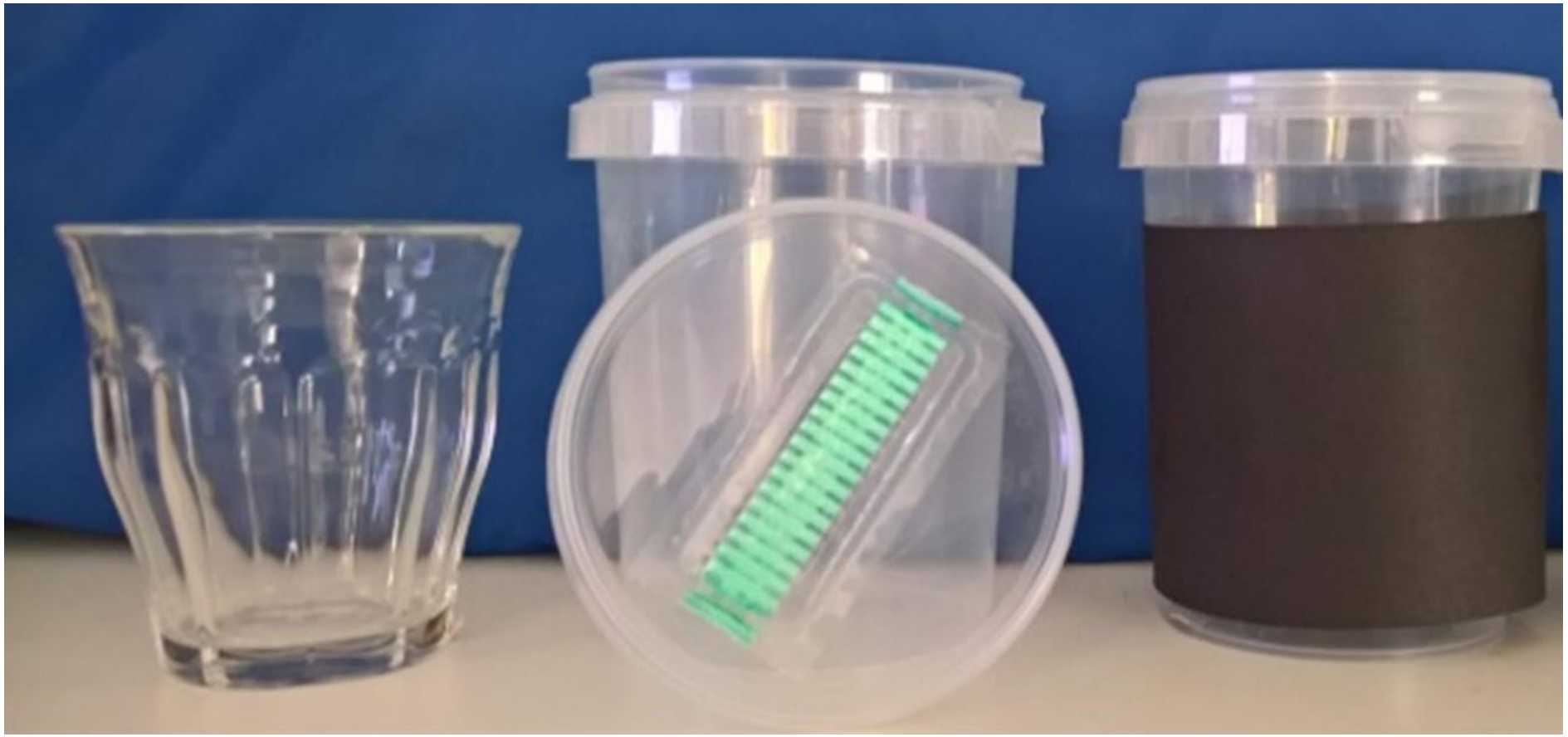
Freezing rate -1°C/min

- ✓ 2nd -150°C

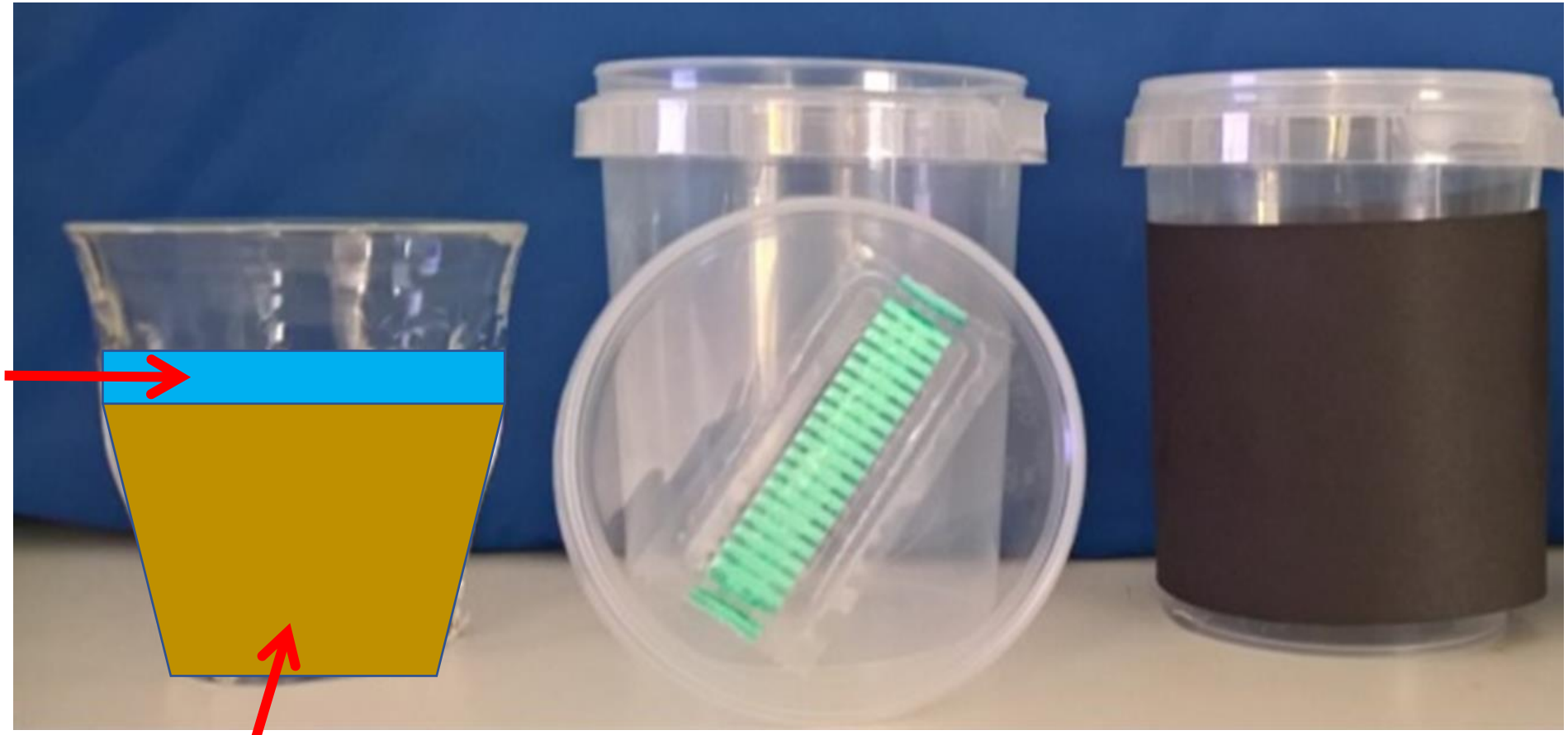


Mr. Frosty

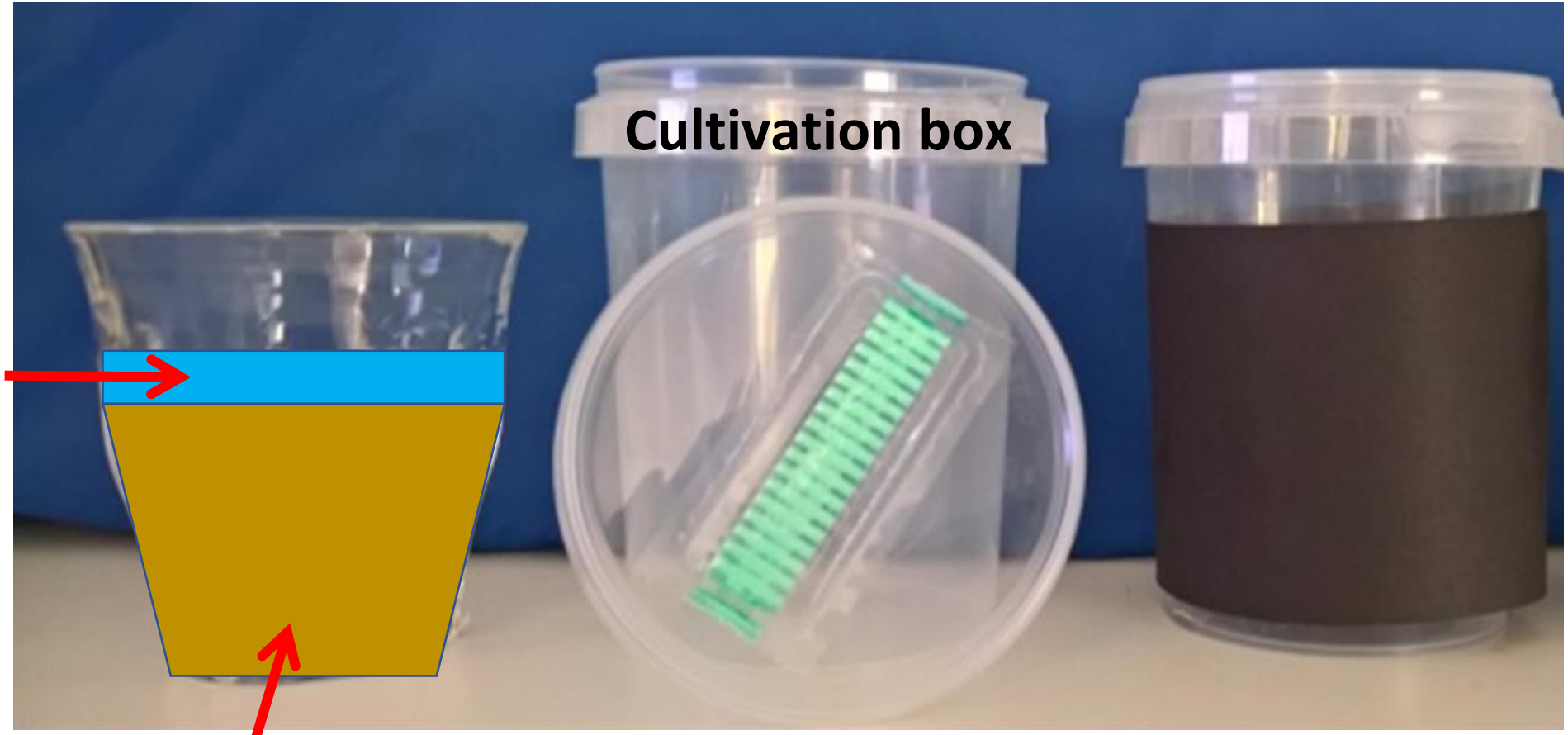
Microbial mat recovery



Microbial mat recovery



Microbial mat recovery



ASW 13% w/v

filtered sand

gas exchange filter (0.2µm)
(SacO2 microbox)

shielding of light from side

Important reminder

✓ Centrifugation or filtration could not be use to separate/remove the CPAs from the cells post-thawing

✓ Possible loss of fragile and small species

✓ Carry-over of the CPAs

Incubation

- Total darkness for 24h
- Semi darkness for 48h
- 37°C
- Light cycle 14L/10D
- 120 days

Sampling strategy

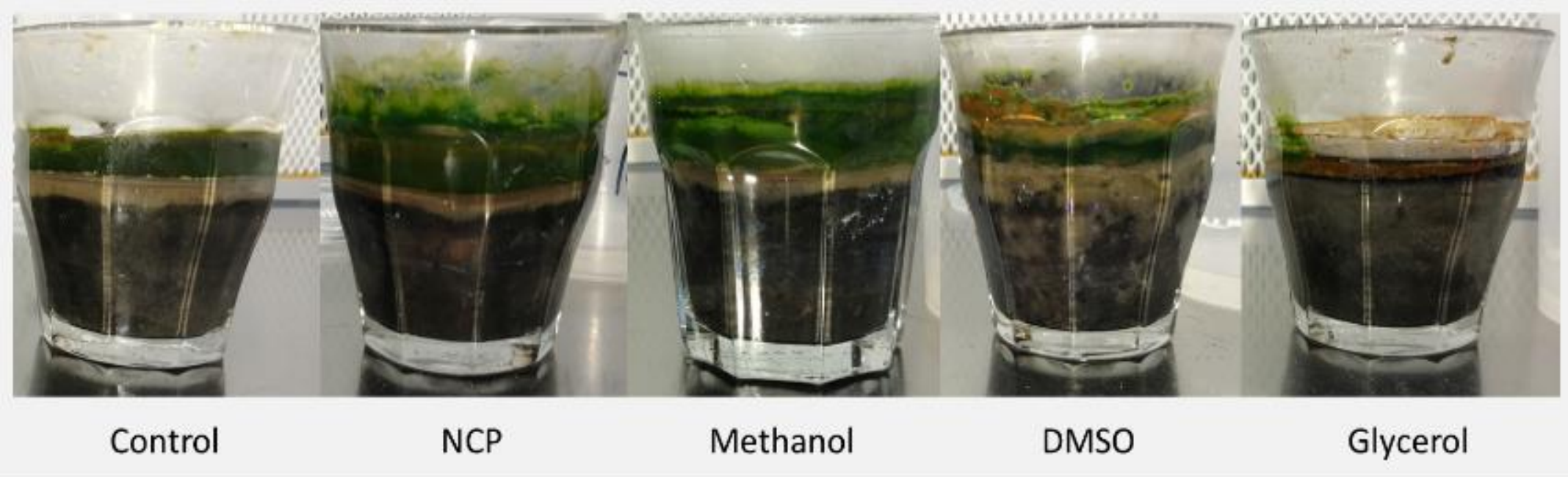
Cultivation
120 days

DNA
extraction

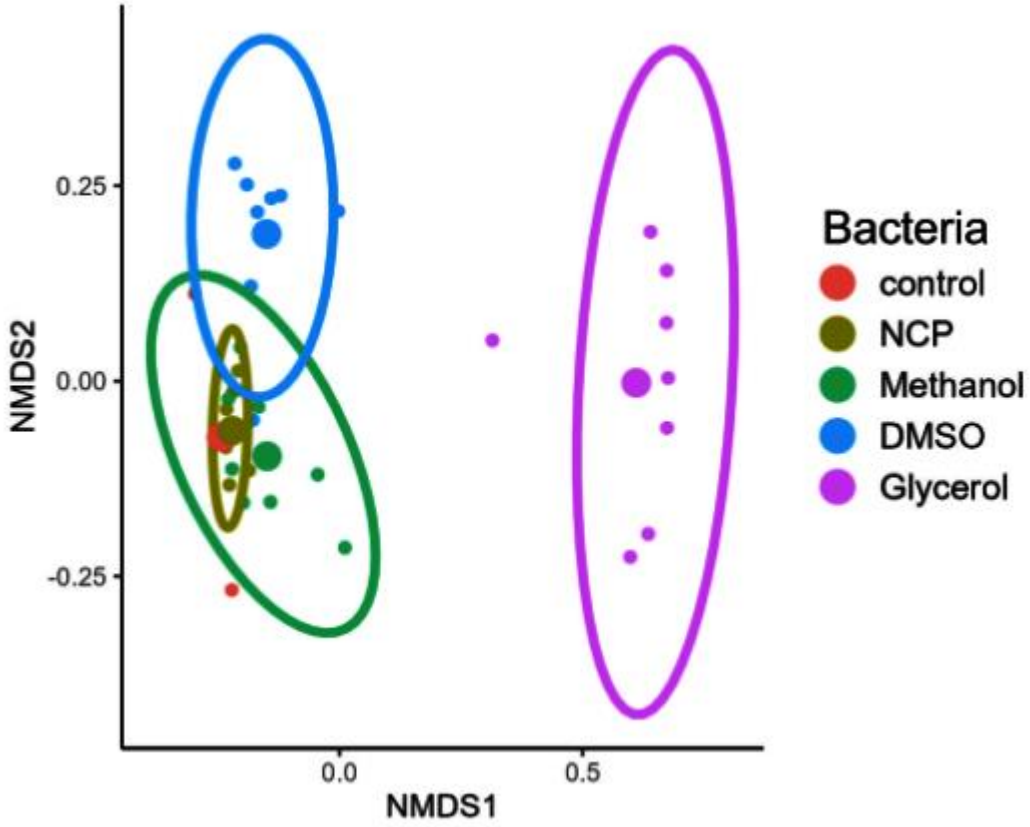
Illumina amplicon
sequencing

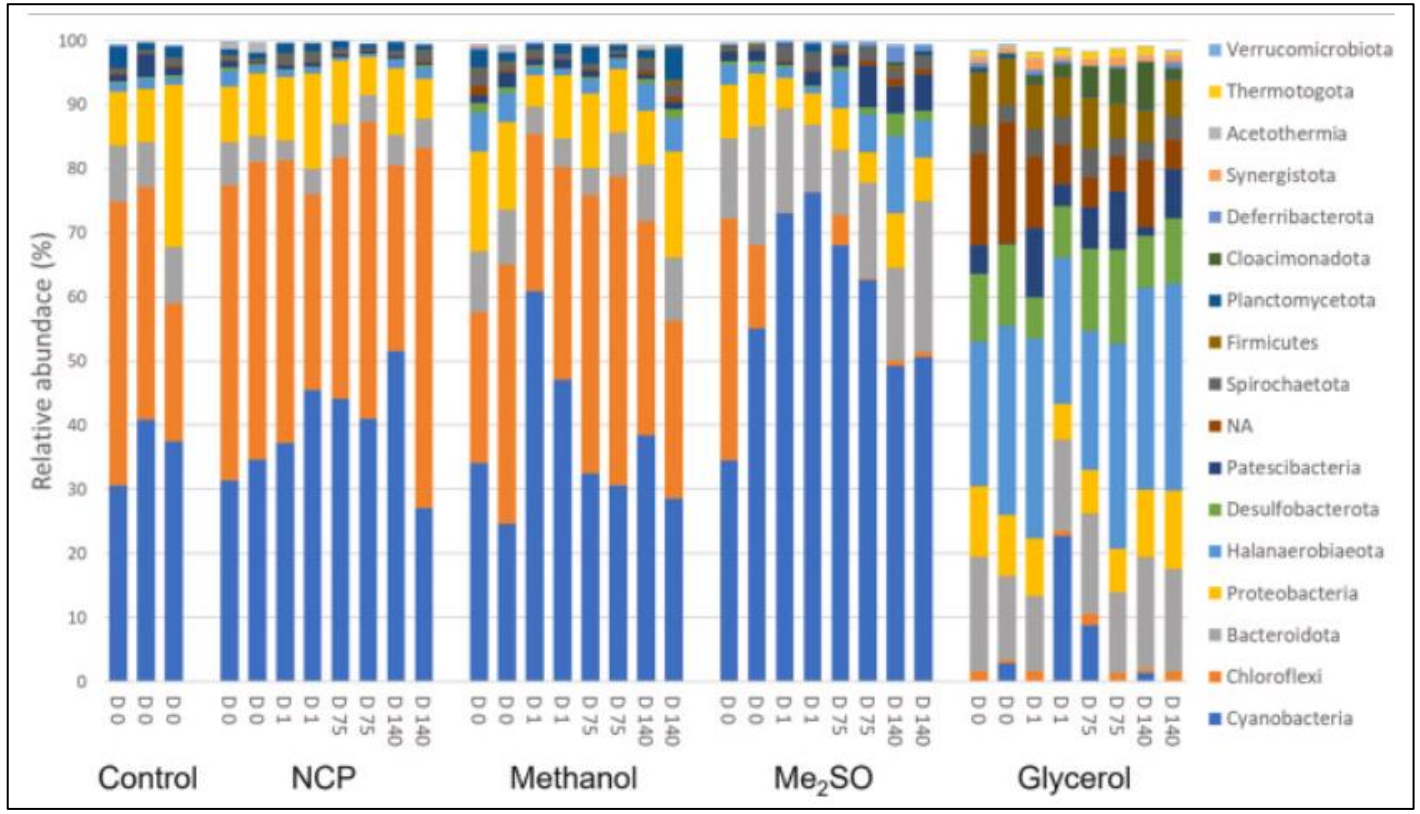
1cm

- 16S rRNA
  341F
  806R
- 18S rRNA
  Euk-F
  Euk-R

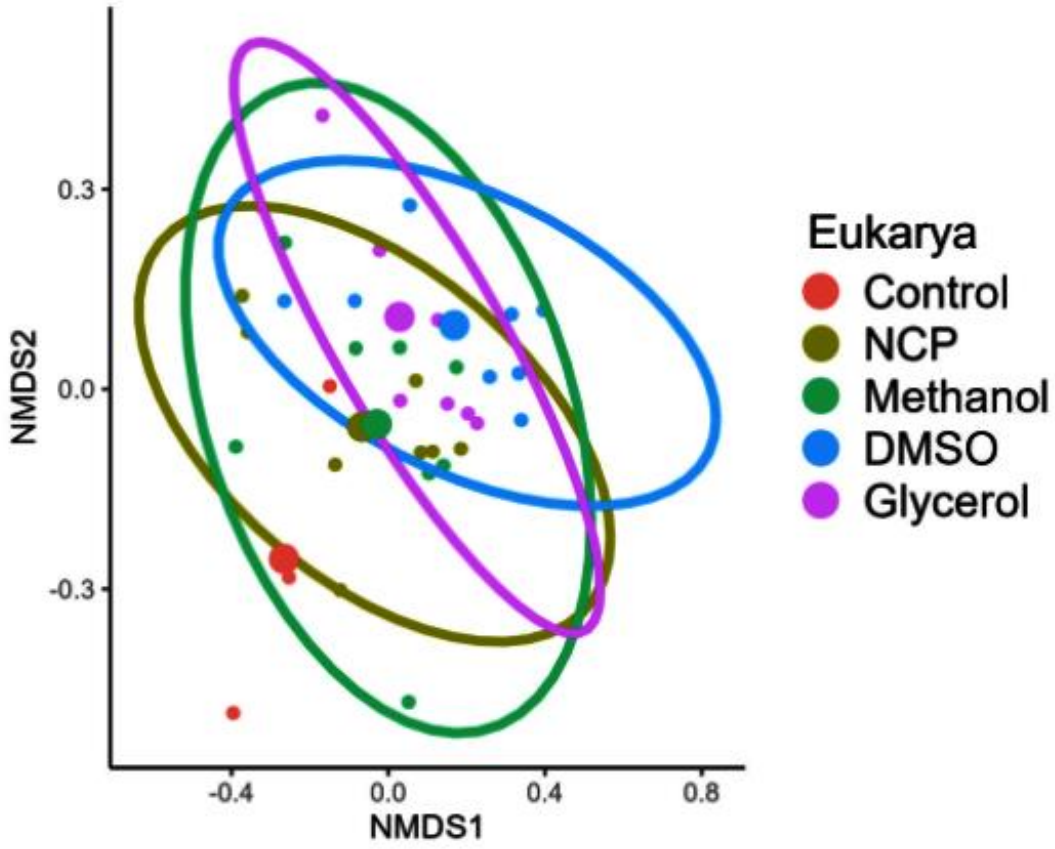

Control
NCP
Methanol
DMSO
Glycerol

Bacterial community composition





Eukaryote community composition



- Difficulties in sequencing procedure
- Even if less clear, similar results to Bacteria
- DMSO and glycerol less similar to the control

- Successfully maintained the structural integrity of the microbial mat upon revival with MetOH and NCPA
- We cannot say anything about the efficiency of the different CPAs on the cryopreservation step itself
- **BUT!!!** Re-establishment of the mat was negatively affected by carry over of DMSO and glycerol
- NCPA treatment suggests a potential role for EPS as a protectant
- NCPA probably not suitable for non-EPS containing samples

ELSEVIER

Cryobiology

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Henk Bolhuis ✉, Michele Grego [1] ✉

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ASSEMBLE
ASSOCIATION OF EUROPEAN MARINE BIOLOGICAL LABORATORIES EXPANDED

SIMBA
SUSTAINABLE INNOVATION OF MICROBIOME APPLICATIONS IN THE FOOD SYSTEM

MICROBE project

MICROBE
the Microbiome
Biobanking (RI)
Enabler

Cryopreservation
of complex microbial aquatic
communities

So…

HELMHOLTZ MUNICH

CABI

DSMZ

INRAE

EMBL

AIT AUSTRIAN INSTITUTE OF TECHNOLOGY

SORBONNE UNIVERSITÉ

THANKS TO….

Dr. Henk Bolhuis

THANKS TO….






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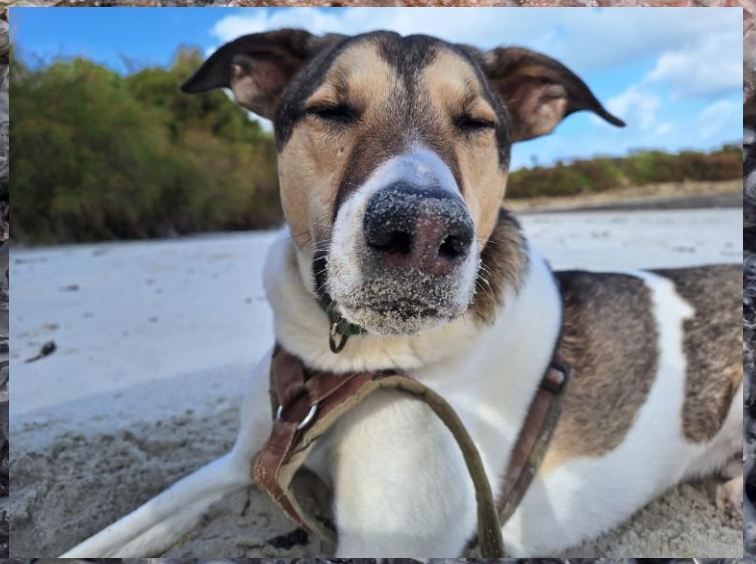
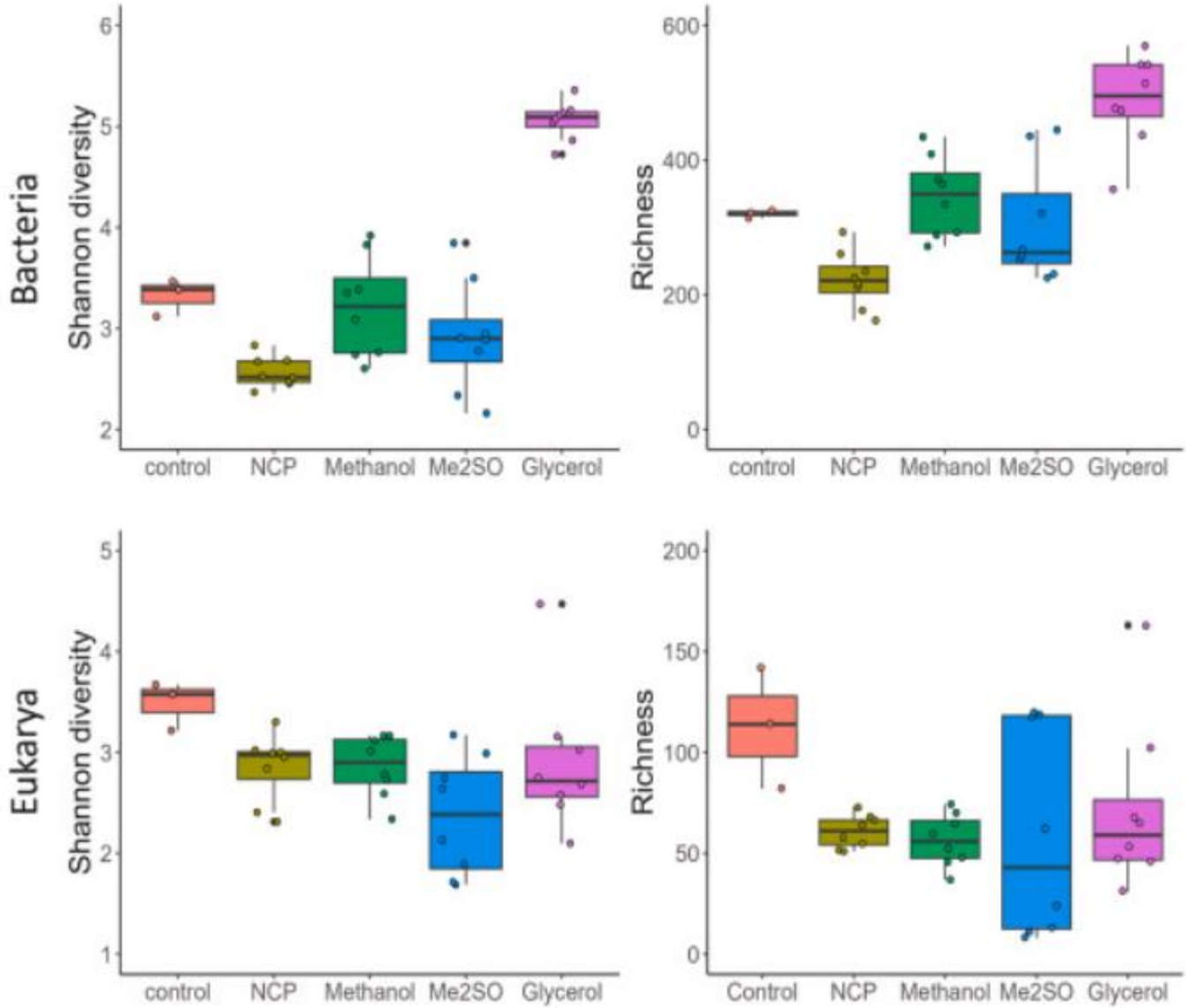
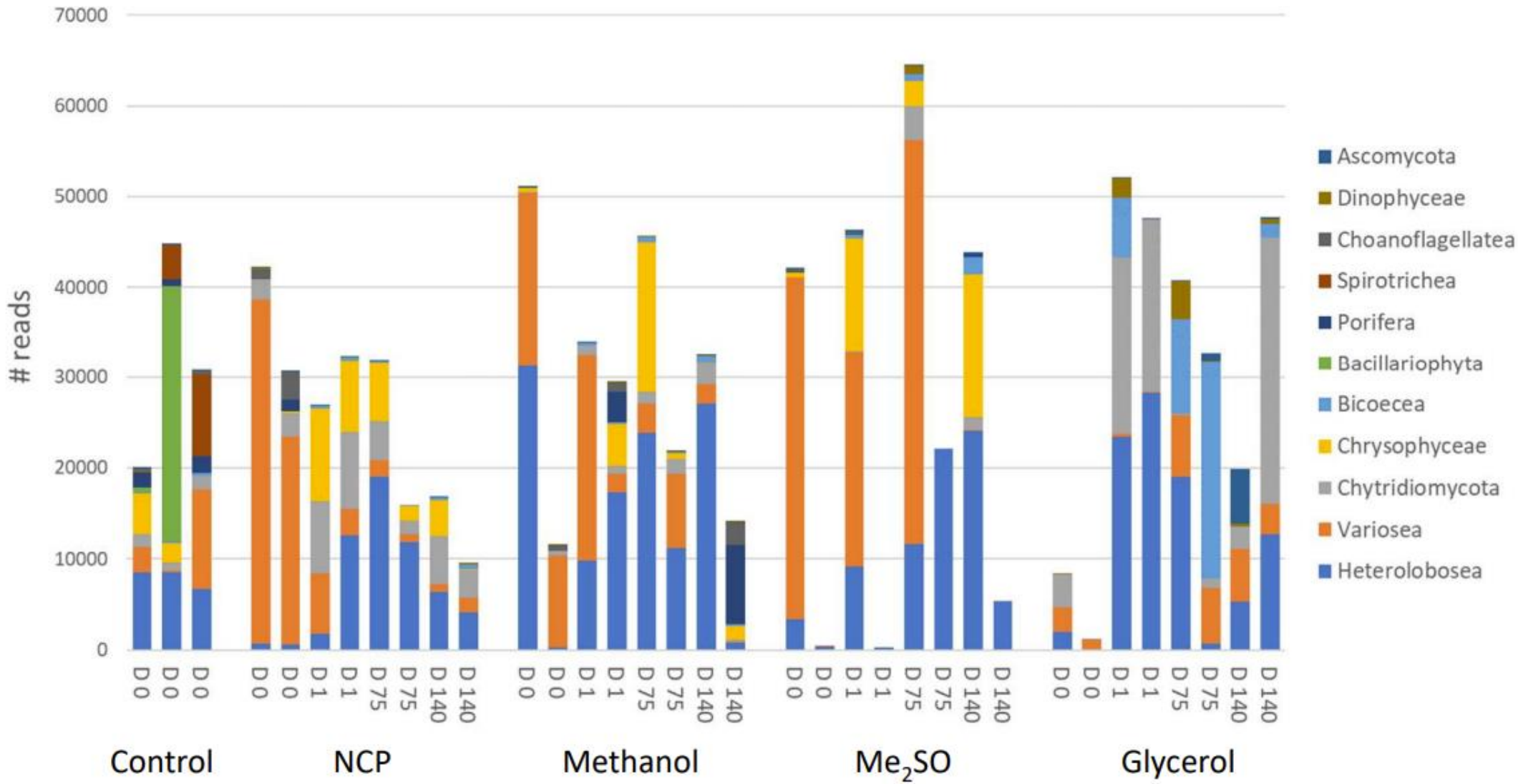
Thank you!




Bacteria
Shannon diversity
6
5
4
3
2
control
NCP
Methanol
Me2SO
Glycerol
Richness
600
400
200
0
control
NCP
Methanol
Me2SO
Glycerol
Eukarya
Shannon diversity
5
4
3
2
1
control
NCP
Methanol
Me2SO
Glycerol
Richness
200
150
100
50
0
Control
NCP
Methanol
Me2SO
Glycerol


70000
60000
50000
40000
30000
20000
10000
0
reads
D 0
D 1
D 75
D 140
Control
NCP
Methanol
Me2SO
Glycerol
Ascomycota
Dinophyceae
Choanoflagellatea
Spirotrichea
Porifera
Bacillariophyta
Bicoecea
Chrysophyceae
Chytridiomycota
Variosea
Heterolobosea