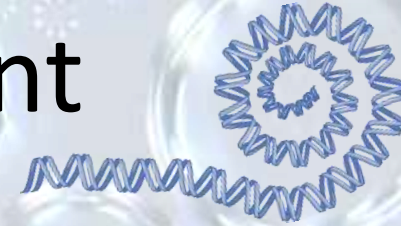
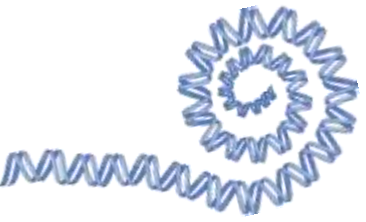


Molecular tools for implementing international ballast water regulations – verification of the ballast water treatment



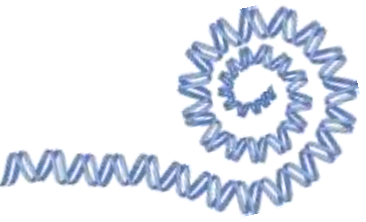
Anastasija Zaiko*, Susie Wood, Xavier Pochon, Laura Biessy, Olivier Laroche, Peter Croot, Eva Garcia-Vazquez

[* Anastasija.Zaiko@cawthron.org.nz](mailto:Anastasija.Zaiko@cawthron.org.nz)



Seaborn trade intensifies, ship sizes increase



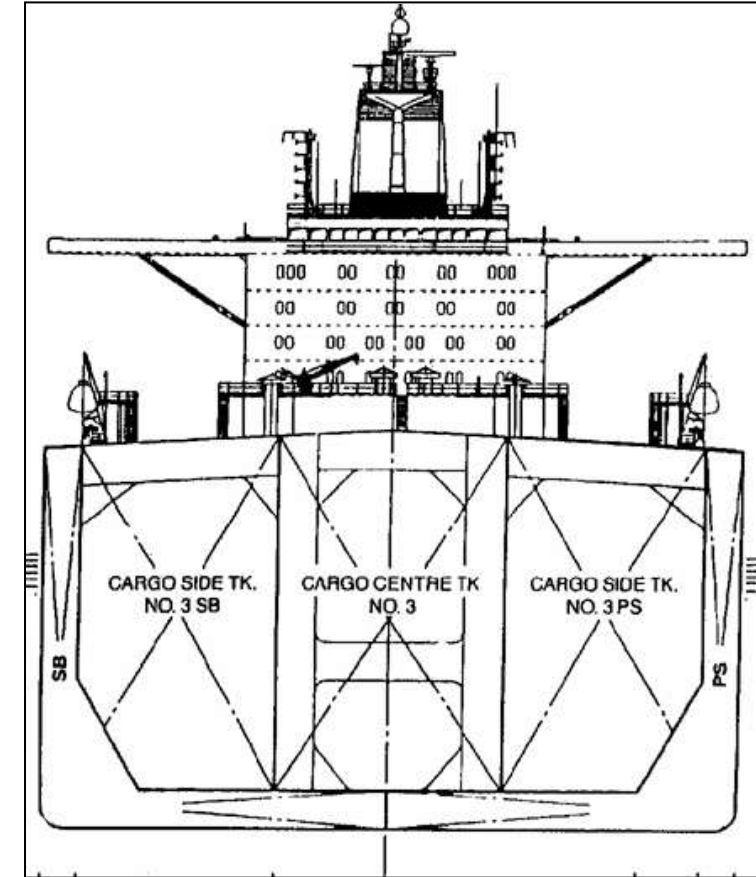


What do vessels carry besides cargo?

- **Ballast water**
(tens of thousands tons per ship)



To stabilize the unloaded cargo ship



BALLAST WATER CYCLE

① At source port



② During voyage



③ At destination port

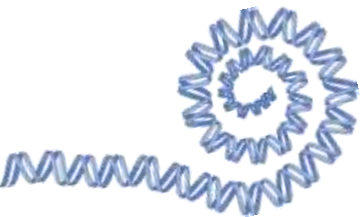


④ During voyage



SOURCE: GloBallast

- Ca. 3-5 billion tonnes of ballast water /year
- 7,000 and 10,000 different species of marine microbes, plants and animals per day



International Convention for the Control and Management of Ship's Ballast Water and Sediments

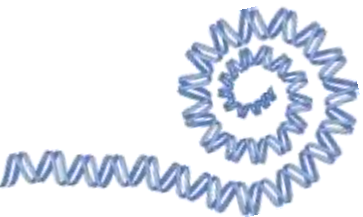


- Adopted by IMO in 2004
- Came into force on 8 September 2017
- Sets up ballast water quality standards at discharge

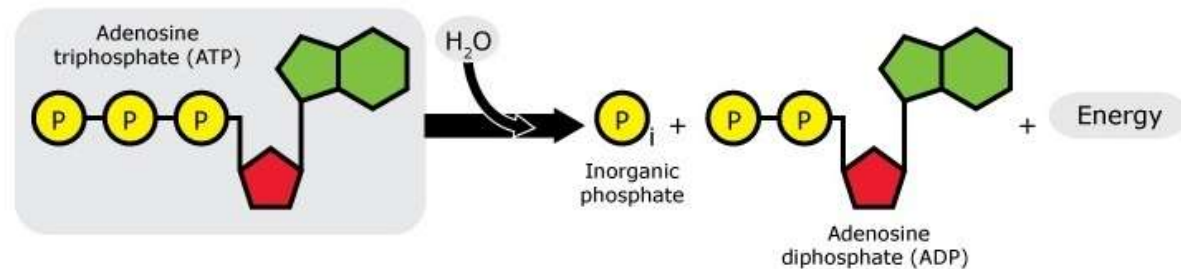
Key question: how to assess compliance to meet BWMC enforcement requirements?

- discharge less than 10 viable organisms per ml in size range 10-50 μm
- restriction of numbers of indicator microbes (bacteria): *V. cholerae*, *E. coli*, *Enterococci*

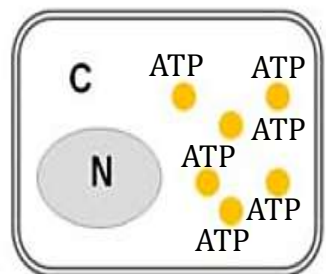




Adenosine triphosphate assays



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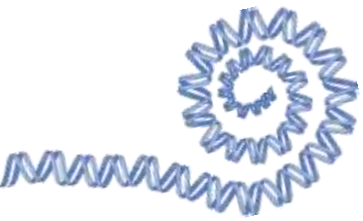
Cell lysis



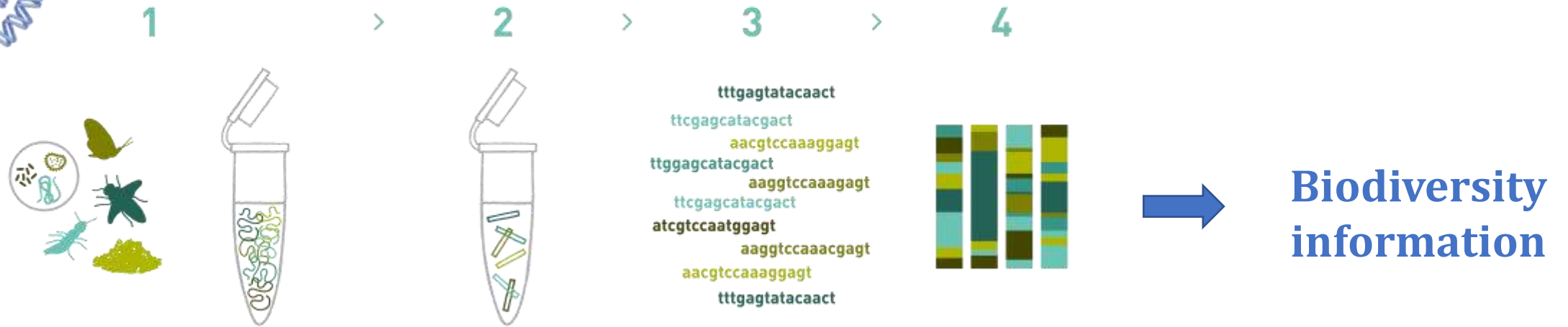
A proxy of living biomass

But: what does it mean in terms of BW biodiversity and how it relates to different groups of organisms?

BA FRACTION	COMPLIANT	CLOSE TO THE LIMIT	NON-COMPLIANT	FULL COMPLIANCE
≥50µm cATP ₅₀ (pg/m ³)	< 10000	10000 to 750000	> 750000	REPEAT ANALYSIS
≥1µm cATP ₁ (pg/ml)	< 1000	1000 to 5000	> 5000	NON COMPLIANCE
Bacteria cATP bact (pg/100ml)	< 1000	1000 to 5000	> 5000	



Metabarcoding



But: not quite quantitative, if going for DNA metabarcoding – how much bias is introduced by legacy signal?



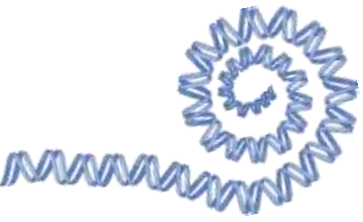
DNA (Deoxyribonucleic acid)



RNA (Ribonucleic acid)

→ 'Recent' biodiversity: signal from living organisms + 'legacy DNA'

→ 'Living' biodiversity: signal from living (biologically active) organisms (more complex and expensive)

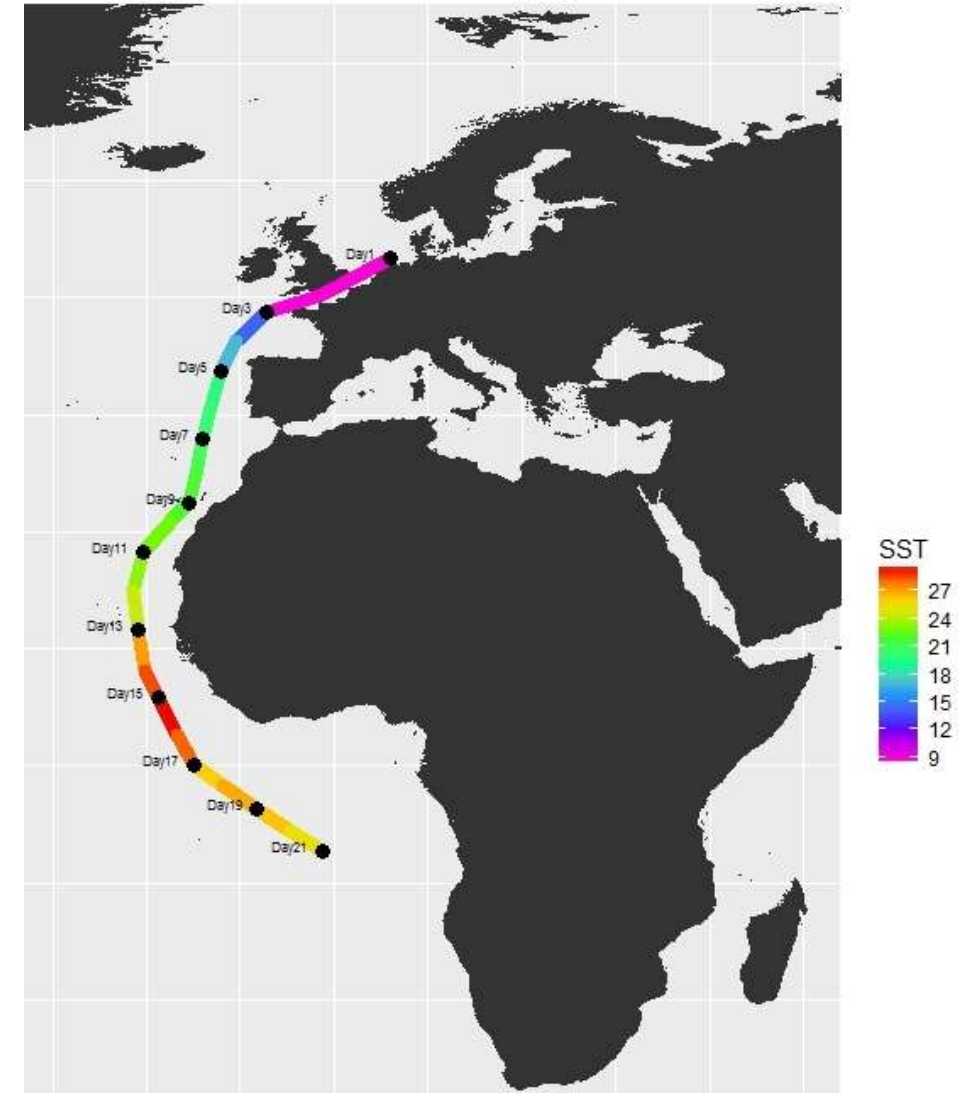
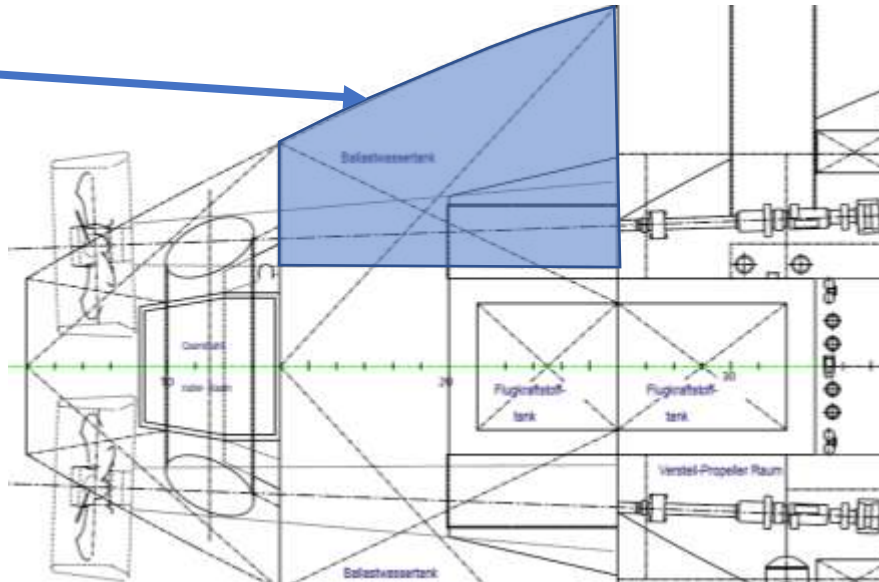


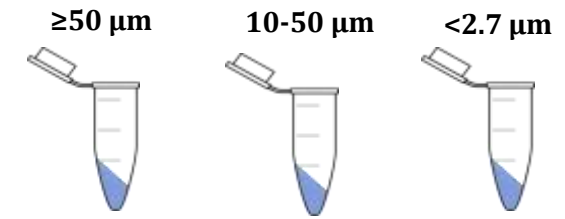
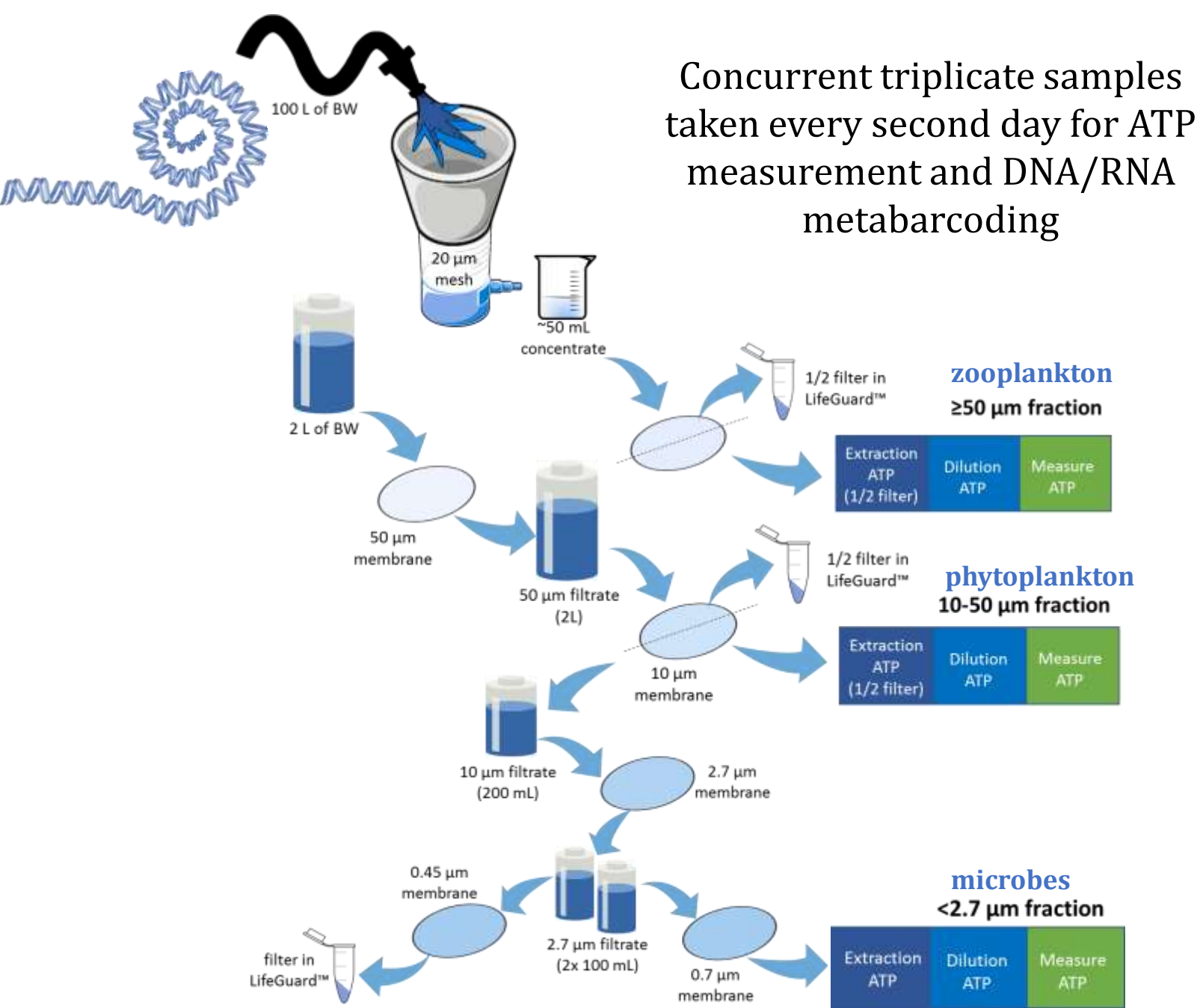
ATP measurements vs DNA/RNA metabarcoding:

compare and cross-validate their performance in the context of the BWMC



Aft ballast tank
(70 m³) filled
with sea water off
the German coast



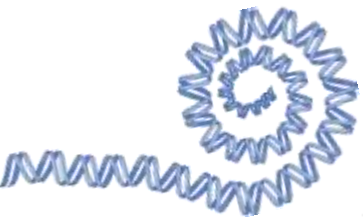


DNA/RNA co-extraction

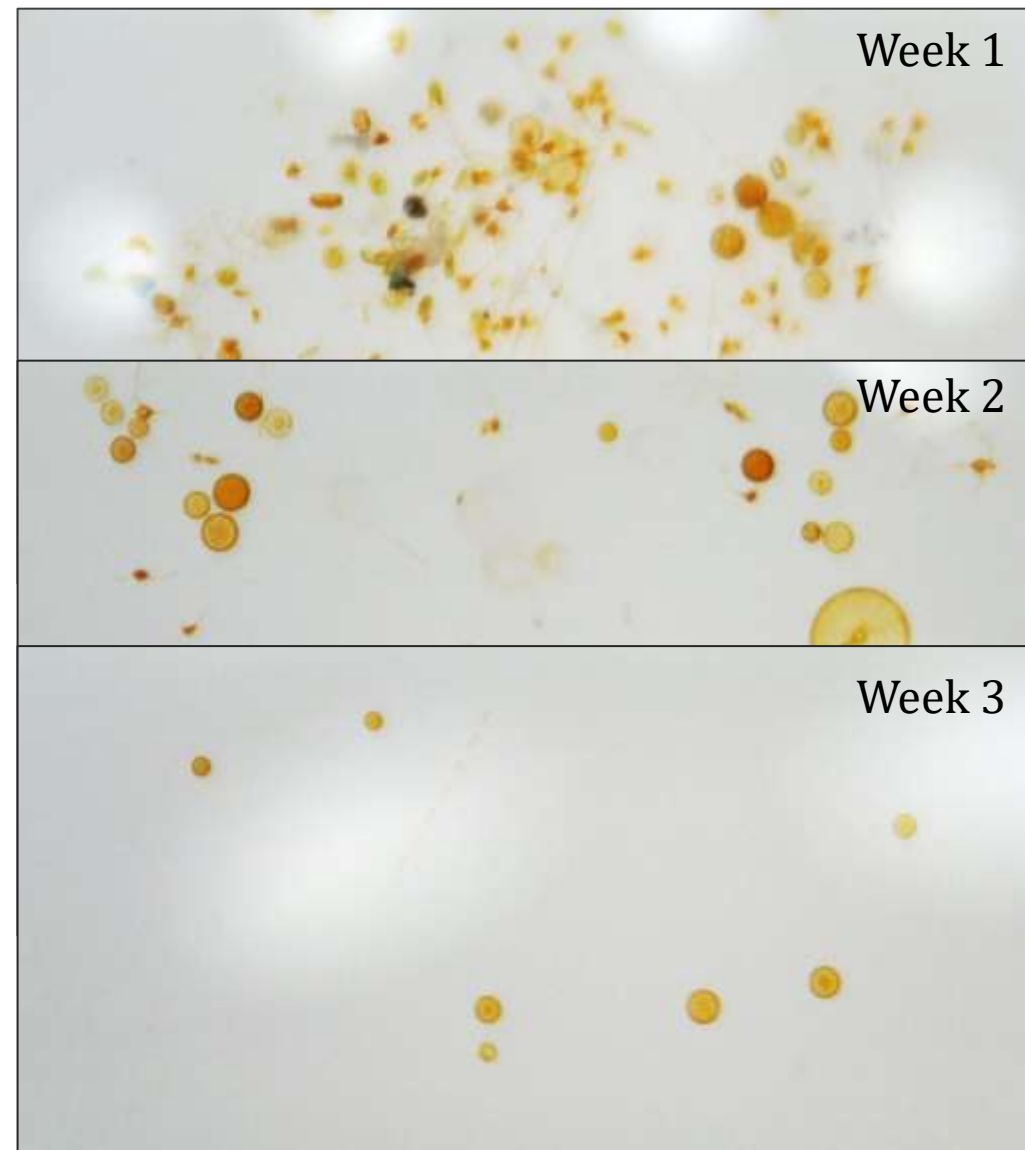
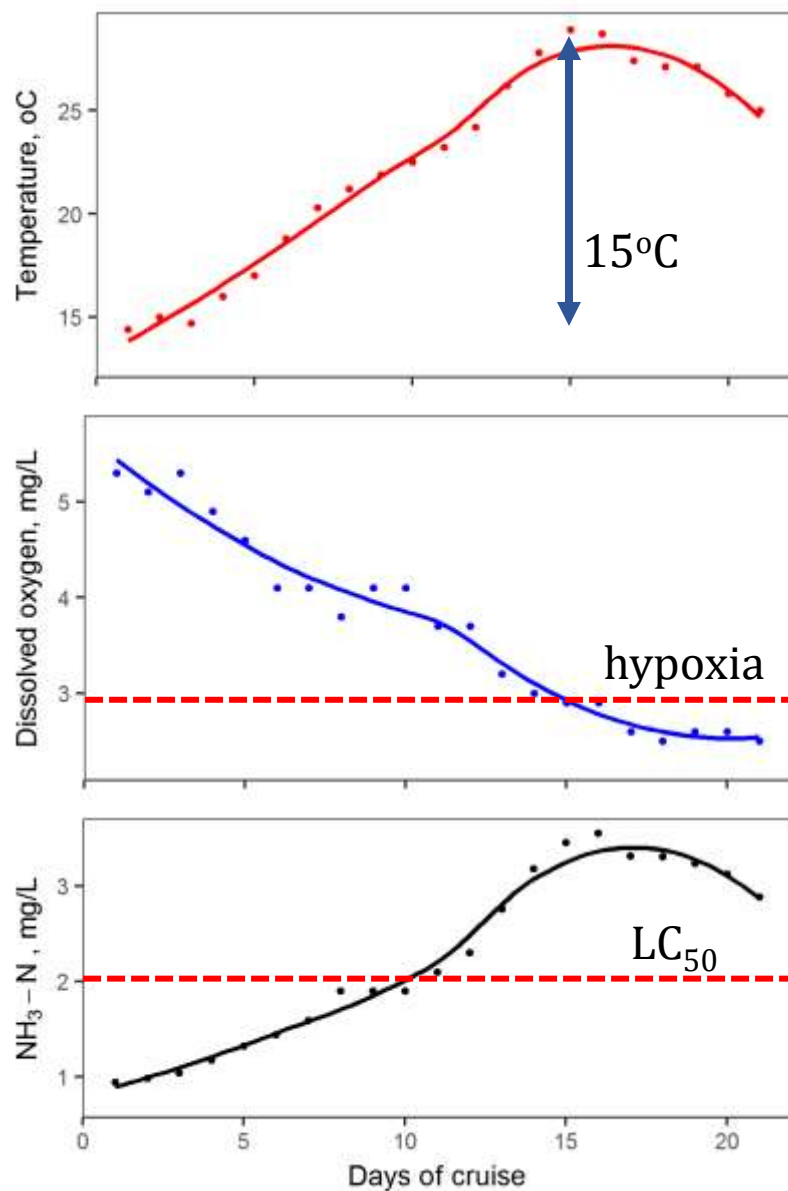
16S rRNA (bacteria) and
18S rRNA (eukaryotes)
metabarcoding

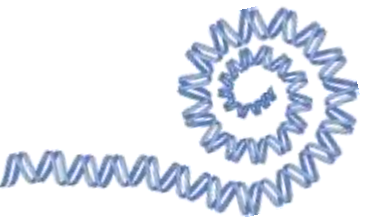
Additionally:

- Physico-chemical parameters of the BW monitored daily (T, DO, N-NH₃)
- Microscopy photos taken for reference (≥50 µm fraction)



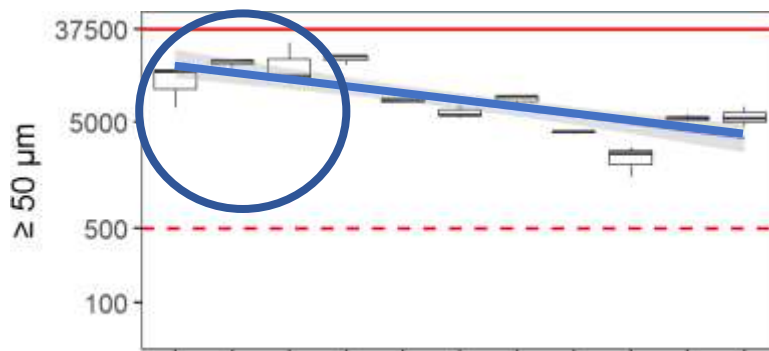
Physico-chemical conditions and microscopy screening



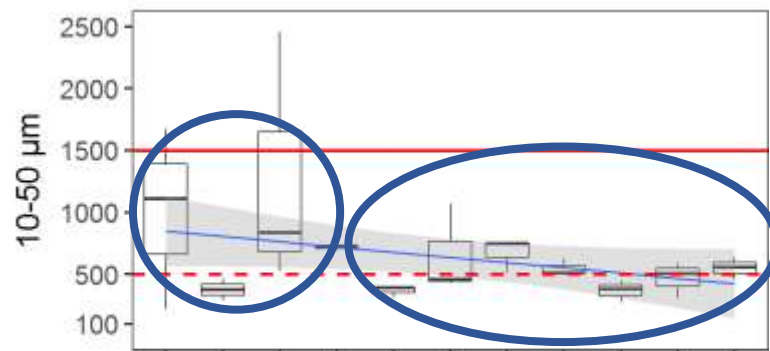


Dynamics of the ATP signal, pg/m³

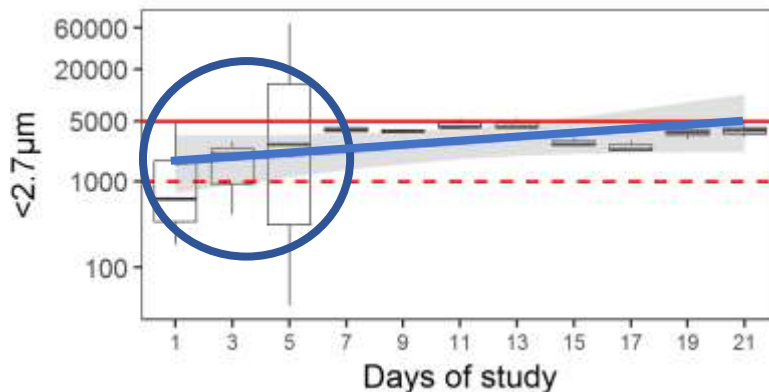
≥50 μm fraction →



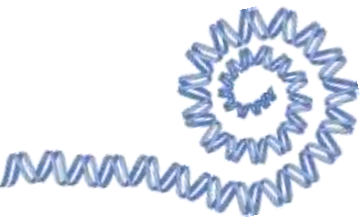
10-50 μm fraction →



<2.7 μm fraction →

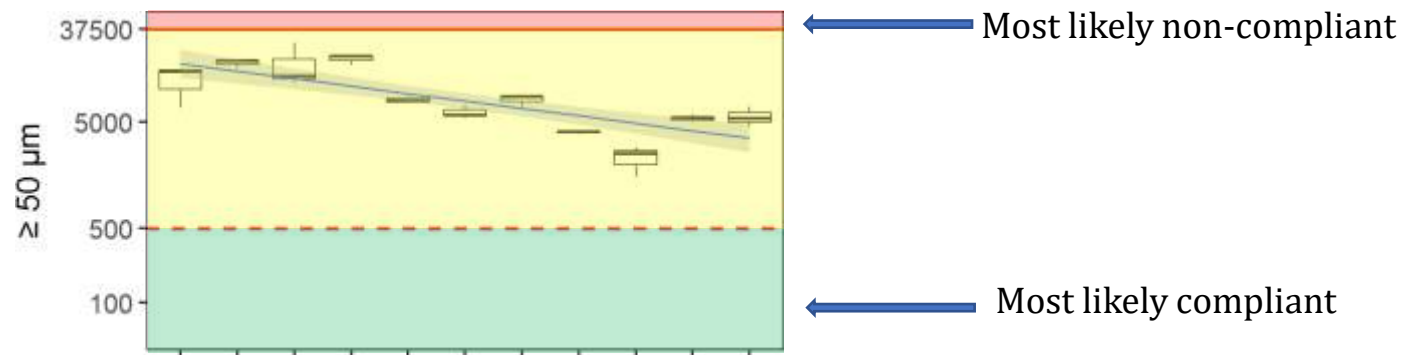


- high variance between replicates
- negative trend in the larger fraction
- stable signal in medium fraction
- increase in the smallest fraction

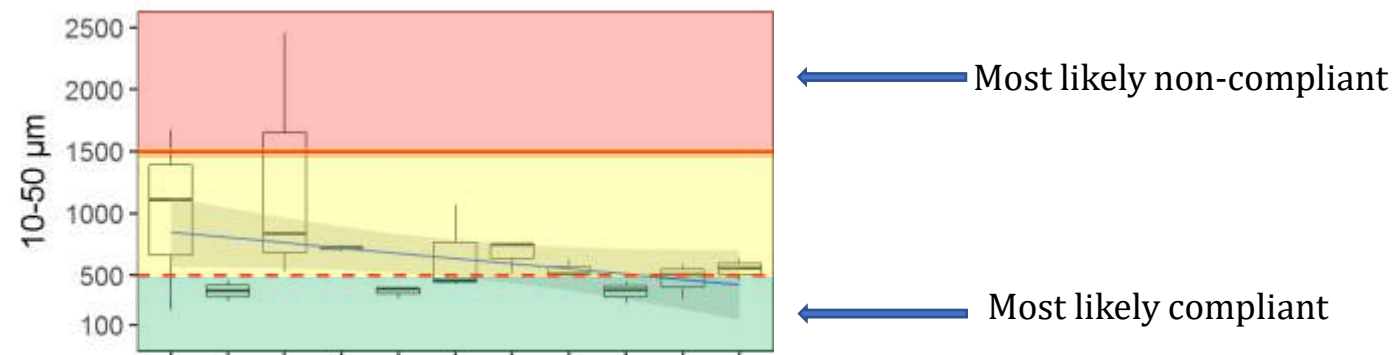


Dynamics of the ATP signal, pg/m³

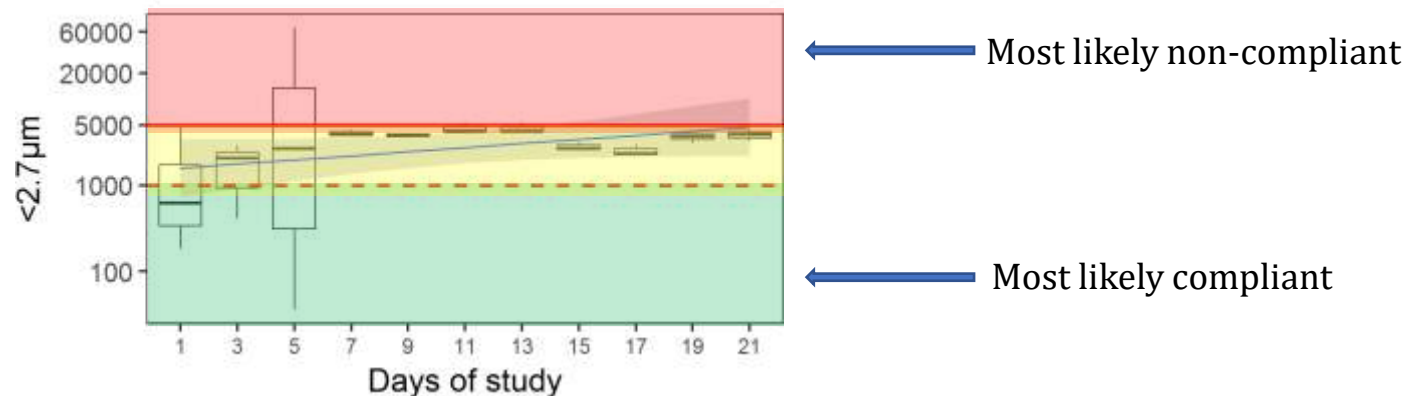
≥50 μm fraction →

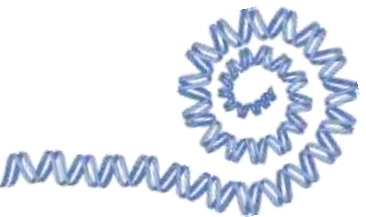


10-50 μm fraction →



<2.7 μm fraction →

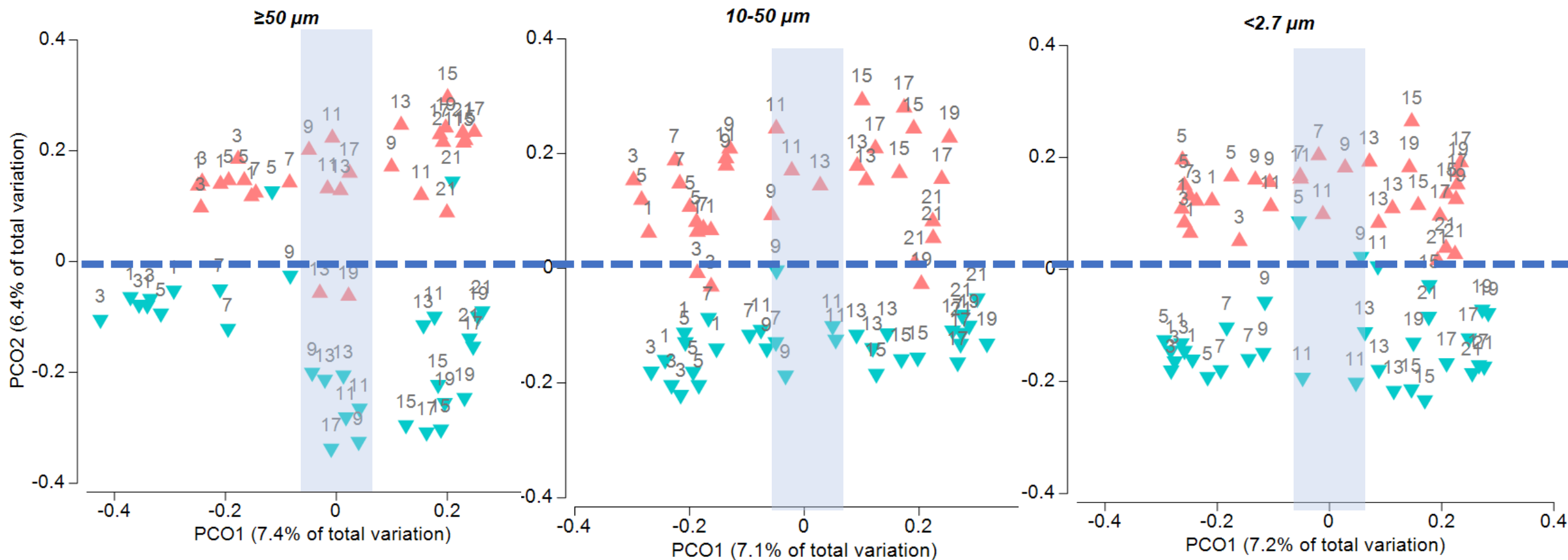


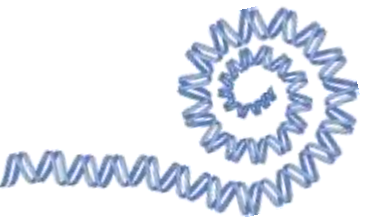


Biodiversity of BW: multivariate analysis (log-transformed OTUs, modified Gower similarity matrix)

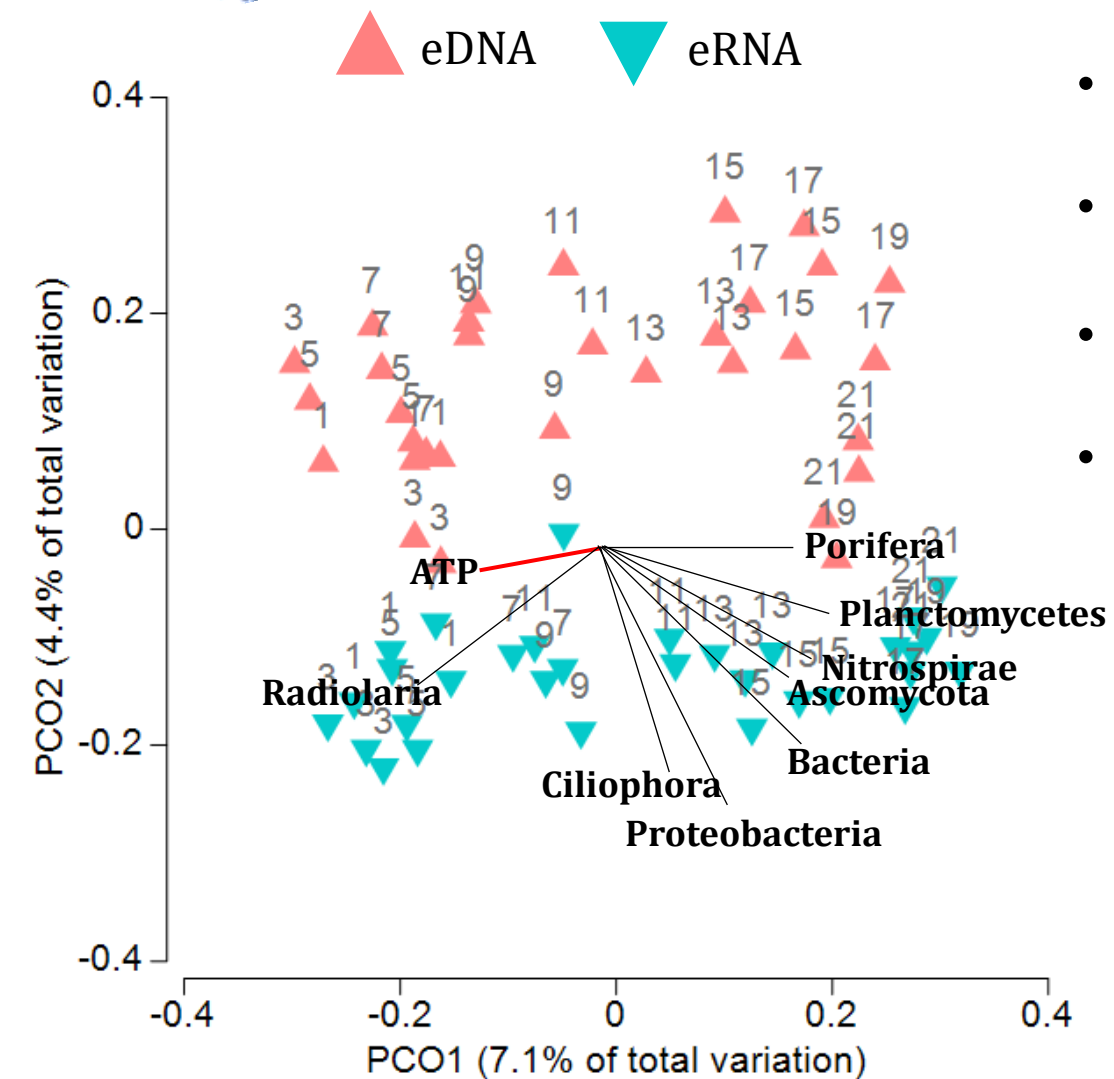
▲ eDNA

▼ eRNA

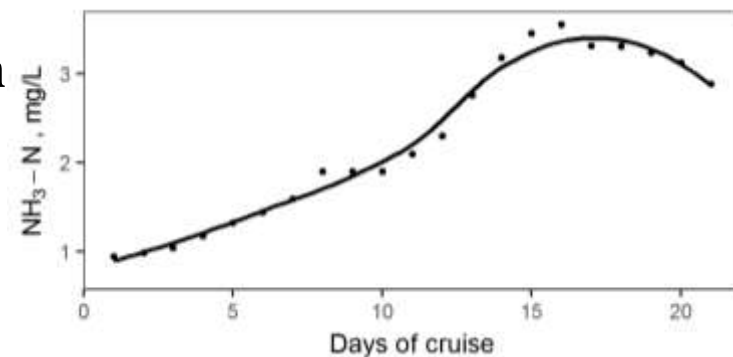




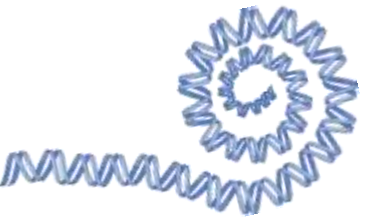
Phyla, highly correlated with 2 first axes: 10-50 μm fraction example



- No phyla were highly associated exclusively with DNA
- Radiolaria associated with RNA in earlier samples
- Porifera associated with later samples of DNA and RNA
- A bunch of phyla associated with later samples of RNA
 - Protists, fungi and bacteria feeding on decaying material
 - Ammonia-utilizing bacteria

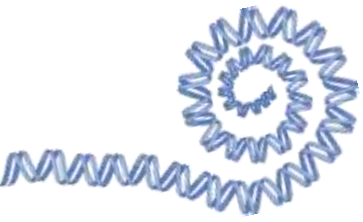


- No collinearity of ATP signal with any specific taxa

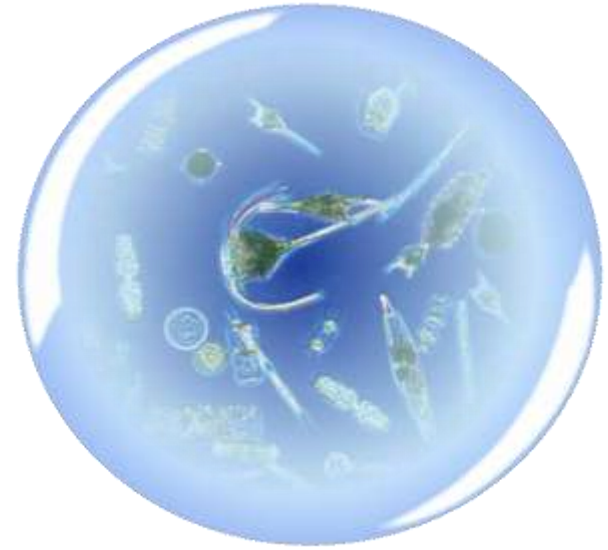


Summary and conclusions

- Both methods provided complementary information: **tiered analytical approach for BW tests?**
- High variability among replicate samples: **well-designed sampling needed**
- No reliable numbers for detailed compliance tests as required by the BWMC
- **Reconsider BWMC standards and introduce molecular compliance criteria?**



Thank you!



Acknowledgements

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- AWI, NOSOAT 2016

