Diatoms metabarcoding: Intercalibration

Agnès Bouchez

BIOLAWEB

‘Ring Test’
Inter-laboratory test to compare wet lab protocols of diatom metabarcoding in view of a future standardisation

DNAqua-Net
COST ACTION

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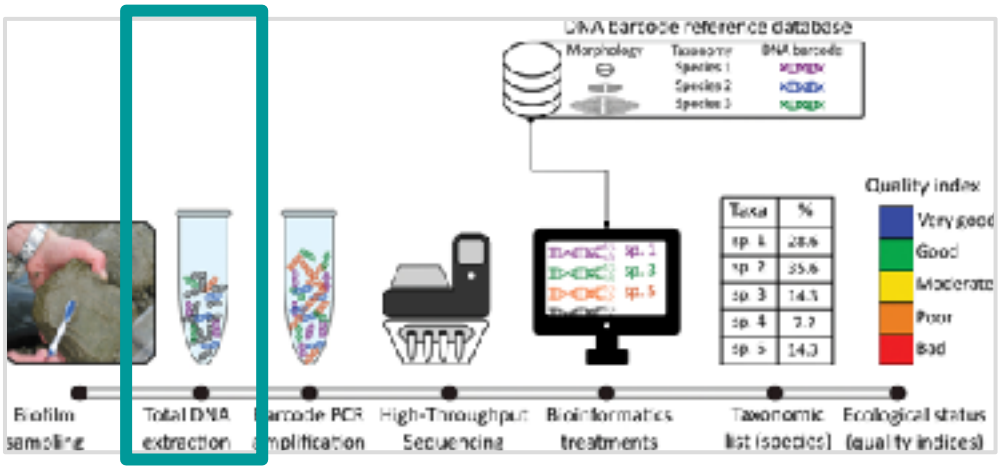
INRAE

SCIMABIO interface

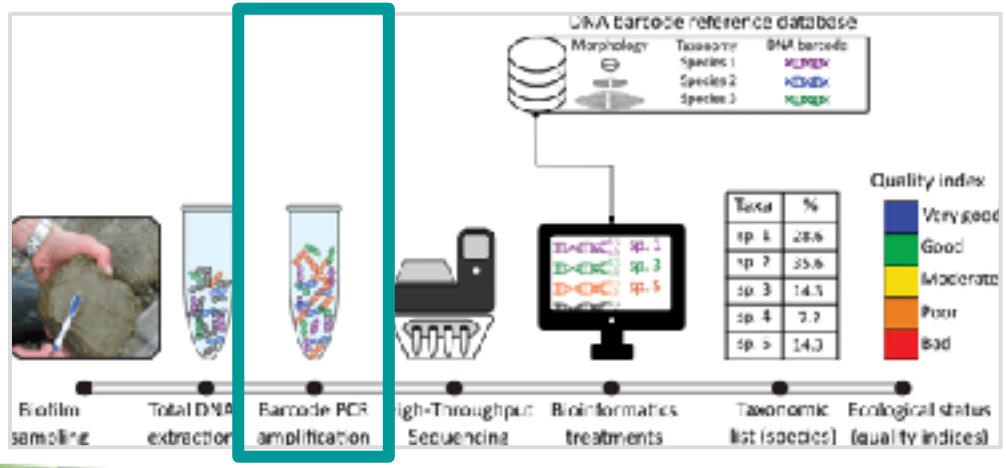
Funded by European Union

LACK OF STANDARDISED PROTOCOLS

- **DNA extraction**



- **Barcode amplification**



- **17 participants** *(A to Q)*
  - ➢ public/private
  - ➢ beginner/advanced

  + 1 Reference Laboratory (RL) - INRAE CARRTEL

- **1 set of controlled samples**
  - ➢ 2 environmental samples : **1 lake + 1 river**
  - ➢ 2 artificial samples : **1 synthetic community + 1 mock community**

- **2 targeted steps**
  - ➢ DNA extraction
  - ➢ Barcode PCR amplification

« **One for All** » Protocol transfer (PCR, Taq Takara LA, *rbc*L barcode)

- 4 DNA extracts sent by Ref lab
  - 17 laboratories: 1 PCR per sample
  - 1 Ref Lab (RL): 3 PCR per sample
- PCR products sent by participants back to RL
- RL proceeds: sequencing library prep / sequencing (platform) / bioinformatics

« **All for One** » Variability among protocols (DNA Extraction)

- 2 environmental samples (river/lake biofilm) sent by Ref Lab
  - 9 participants: 3 DNA extractions per sample
  - 1 Ref Lab (RL) : 3 DNA extractions per sample
- DNA extracts sent by participants back to RL
- RL proceeds: PCR / sequencing library prep / bioinformatics

« **One for All** » Protocol transfer (PCR, Taq Takara LA, *rbc*L barcode)

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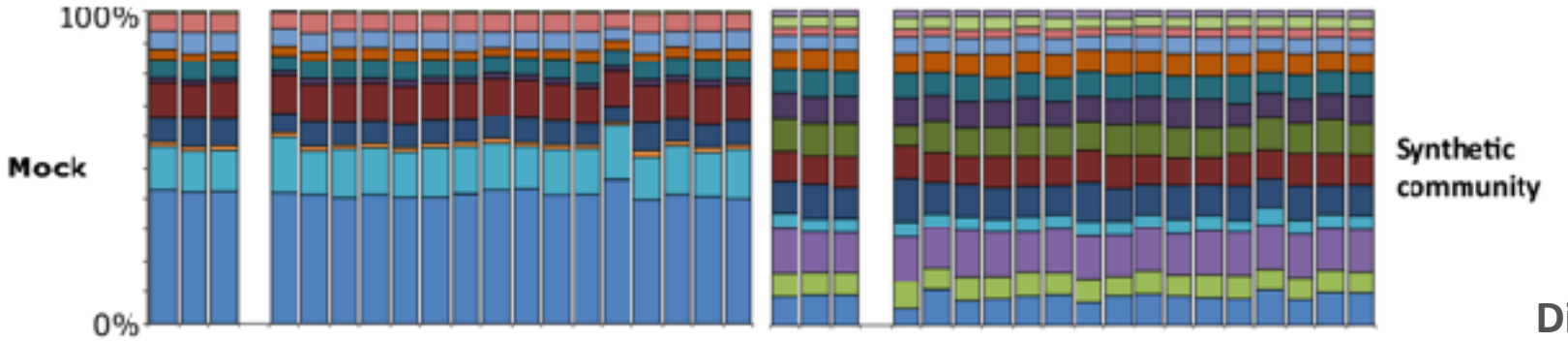

Diatom community composition is homogeneous between participants

- For mock/synthetic controlled samples

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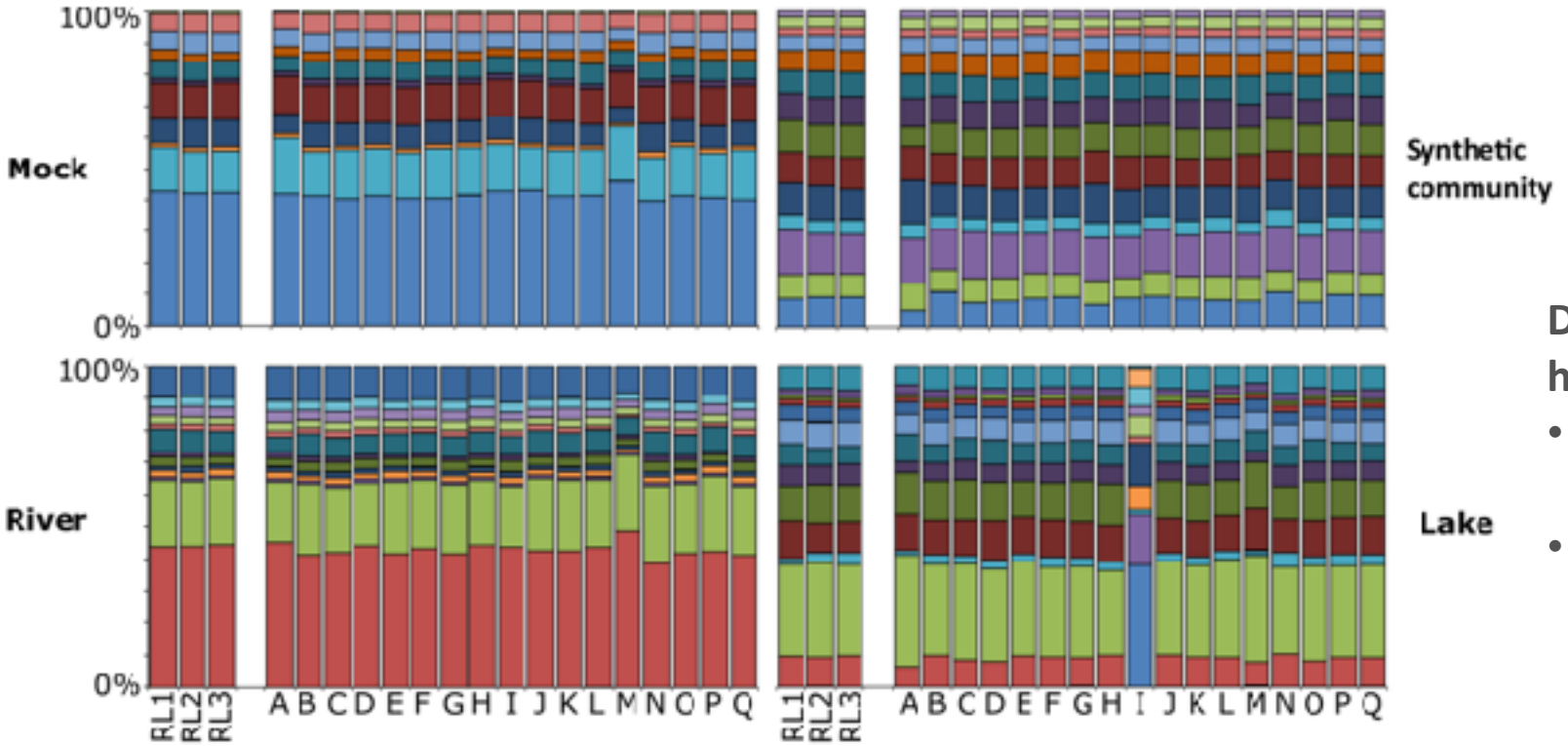

Diatom community composition is homogeneous between participants

- For mock/synthetic controlled samples
- For environmental samples

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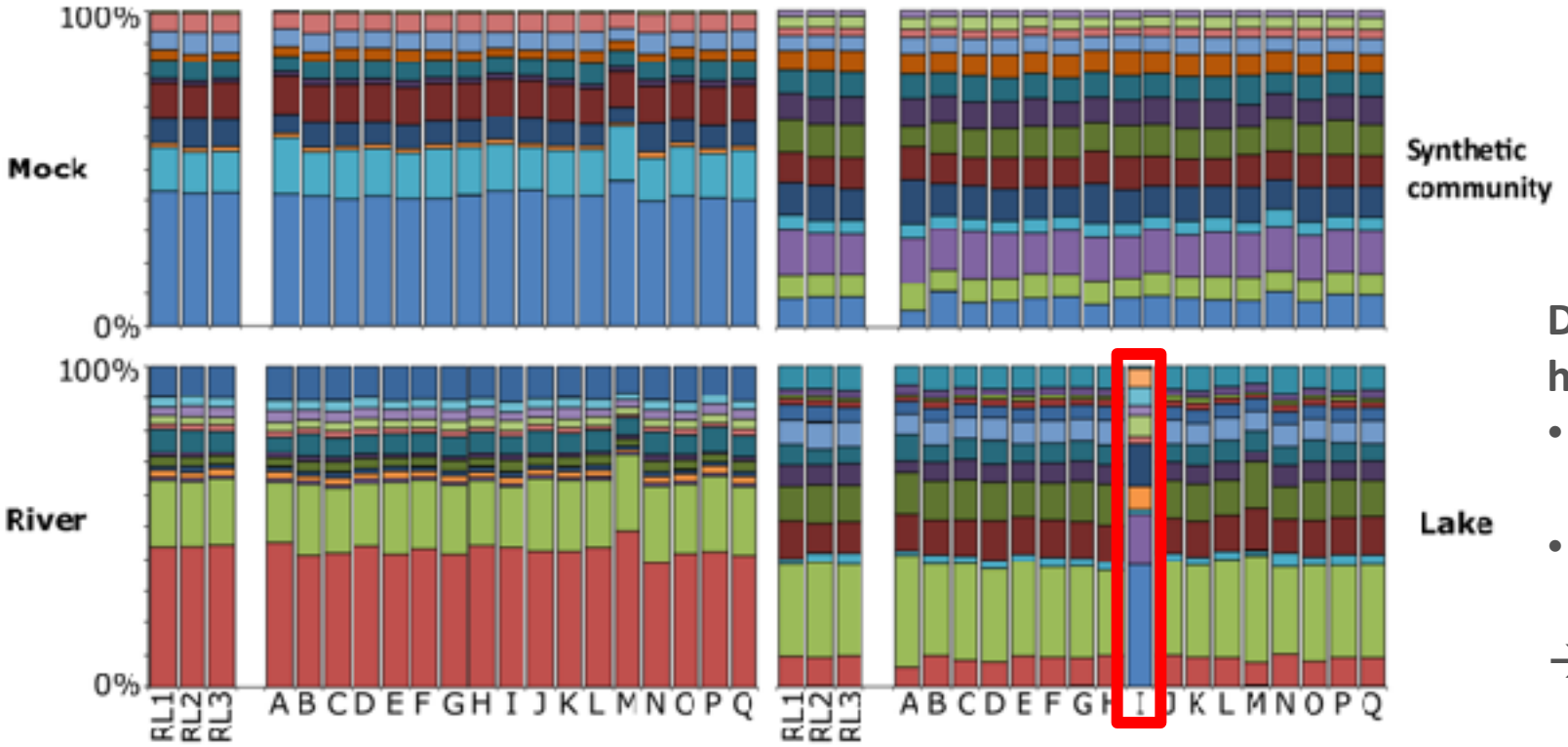

Diatom community composition is homogeneous between participants

- For mock/synthetic controlled samples
- For environmental samples

→ **Protocol is easily transferable**

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« **All for One** » Variability among DNA Extraction protocols

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Participant	**DNA extraction Method**
RL	Macherey-Nagel NucleoSpin Soil
F	GenElute/home-made
D	Macherey-Nagel Nucleospin Plant Mini Kit
P	Qiagen Biofilm
O	Qiagen Powerlyzer Powersoil
I	Qiagen/home-made
J	Qiagen DNeasy Plant Mini Kit
K	Qiagen DNeasy Plant Mini Kit
B	Qiagen DNeasy PowerSoil
N	Machery-Nagel Nucleospin Soil kit with a precellys machine

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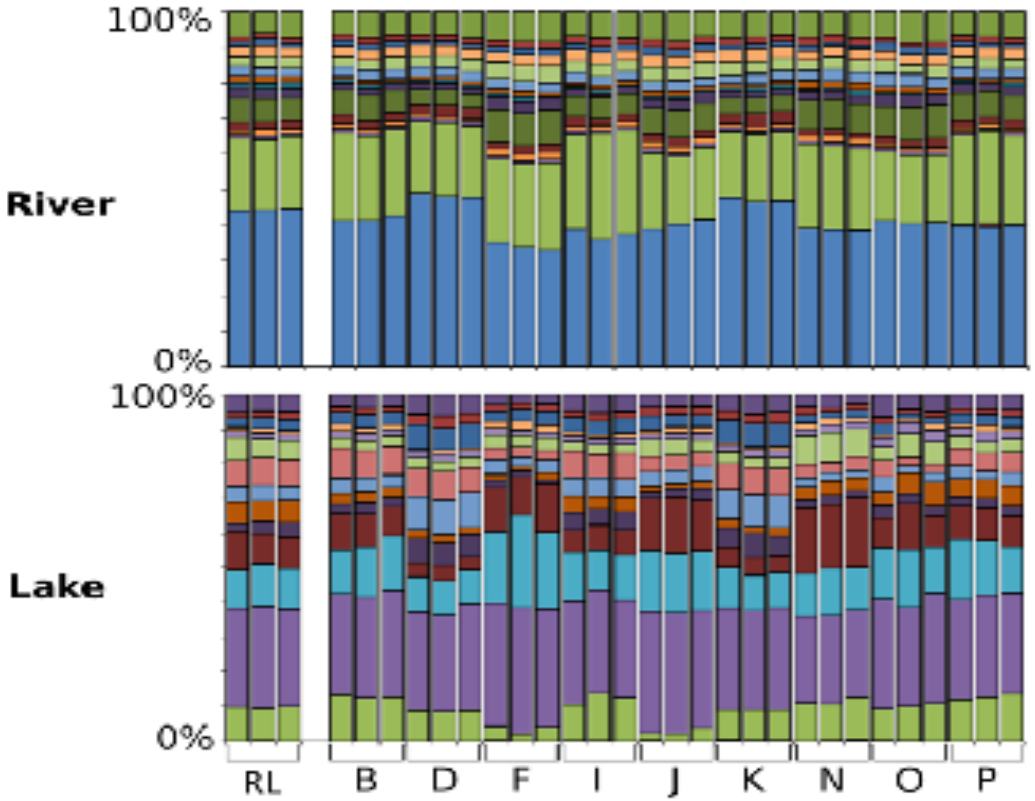

- Similar species detected
- Variability on relative abundances (changes in sp. %)

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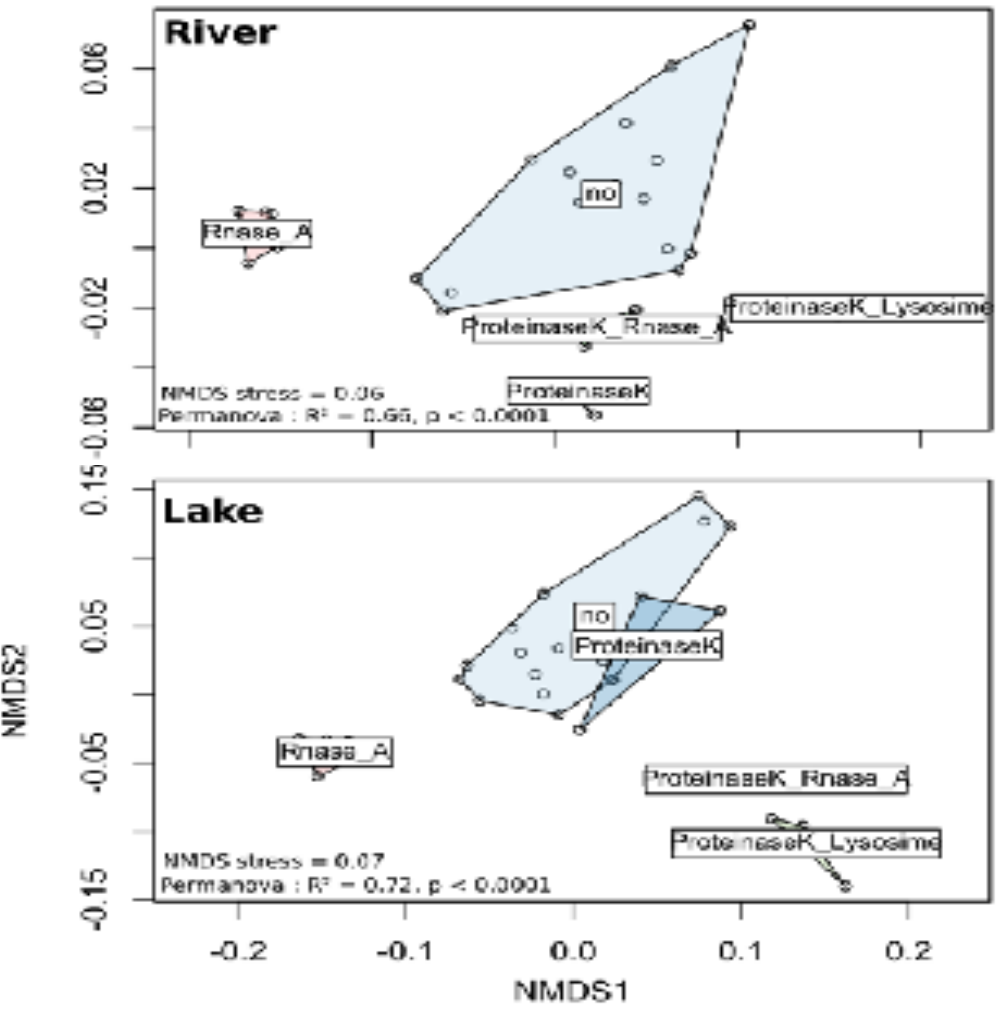

The variability in species % is due to:

- Processing lab
- DNA extraction method (lysis step)

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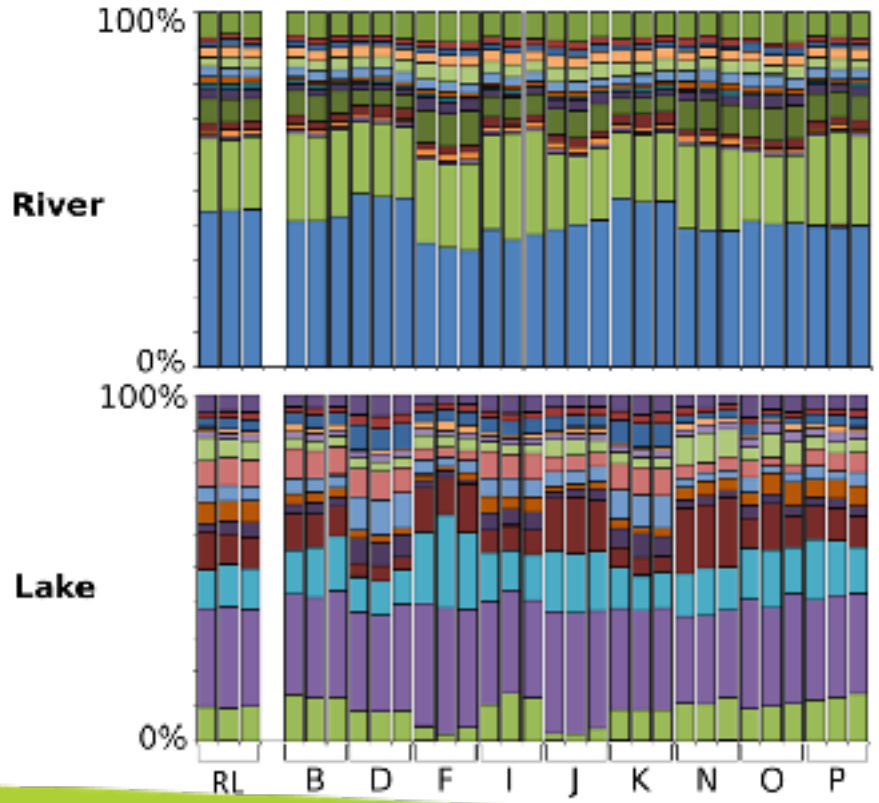

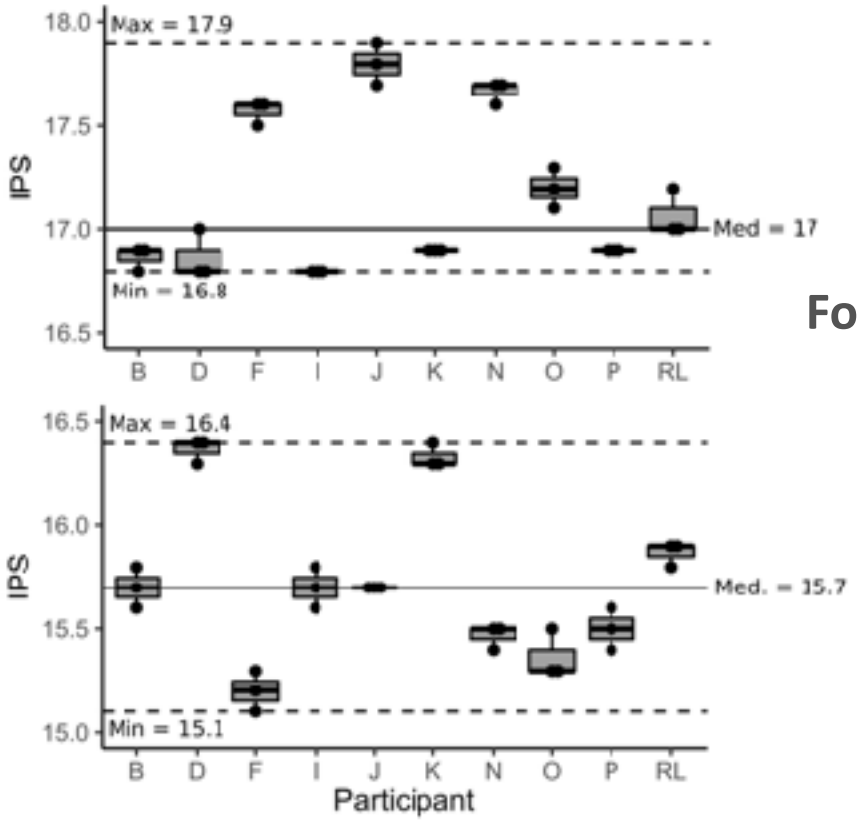

For Ecological Quality Assessment:

- No impact of sp % changes on IBD
- Moderate impact on IPS

→ **All protocols can be used**

NATIONAL INSTITUTE OF BIOLOGY

SOUTHERN CALIFORNIA COASTAL WATER RESEARCH PROJECT

EPA United States Environmental Protection Agency

ID-GENE ecodiagnostics

IRTA INVESTIGACIÓN Y TECNOLOGÍA AGROALIMENTARIAS

Environment Agency

Centre for Ecological Research Hungarian Academy of Sciences

DNAqua-Net COST ACTION

SUPSI

Thanks to all participants !

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…paper coming soon (hopefully)

BG BM Botanischer Garten & Botanisches Museum Berlin

Naturalis Biodiversity Center

SLU SCIENCE AND EDUCATION FOR SUSTAINABLE LIFE

INRAE

CARRTEL CENTRE ALPIN DE RECHERCHE SUR LES RÉSEAUX TROPHIQUES ET ÉCOSYSTÈMES LIMNIQUES

IRB

Cyprus University of Technology

SCIMABIO interface science-management interface for biodiversity conservation

GeoBioTec GeoBioSciences, GeoTechnologies and GeoEngineering

INRA Transfert

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Questions ?