



Diatoms metabarcoding: Preservation & Storage

Agnès Bouchez

The INRAE logo is positioned at the bottom left of the slide. It consists of the letters "INRAE" in a bold, teal-colored, sans-serif font. The letter "E" is stylized with a circular element at its bottom right corner. The logo is partially overlaid by a large, abstract graphic of overlapping rounded hexagons in various shades of green and teal on the left side of the slide.

INRAE

The CARRTEL logo is located at the bottom right. It features the word "CARRTEL" in a bold, sans-serif font, with "CARR" in blue and "TEL" in green. To the right of the text is a circular icon containing a stylized mountain range and a blue wave. Below the main text, the full name of the center is written in a smaller, teal font: "CENTRE ALPAIN DE RECHERCHE SUR LES RÉSEAUX TROPHIQUES ET ÉCOSYSTÈMES LIMNIQUES".

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



Comparison of preservation methods and storage durations



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Advantages of DNA metabarcoding vs morphological analysis :

- ❖ Cost-effectiveness
- ❖ Reproducibility, comparability
- ❖ High-throughput analysis : potential to increase the number of monitored sites, the frequency of controls

Disadvantages :

- ❖ No standard protocols

E.g. : different methods of sample preservation are used –
no information about duration between sampling and sequencing



Aims

- ❖ Evaluate the impact of **preservation conditions** and **storage durations** of samples on the eDNA metabarcoding process
- ❖ Bring scientific and operational knowledge for coming standardisation at CEN level

How

- ❖ DNAqua-Net: workshop + Short-term scientific missions
- ❖ lead and HTS funding: INRAE France
- ❖ participants: France, Croatia, Spain, Sweden, Germany





5 contrasted European sites

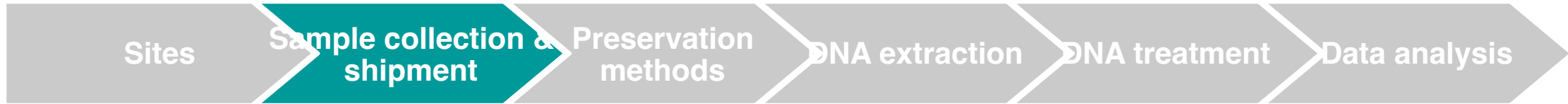
❖ 2 marine sites

- Cádiz bay (Spain)
- Dubrovnik bay (Croatia)

❖ 4 river sites

- Oligotrophic alpine river (France)
- Mesotrophic river (Spain)
- Eutrophic river (Germany)
- Humic river (Finland)





Marine samples

- ❖ Phytoplankton
- ❖ Water column filtration
- ❖ Sample preservation as filters

Freshwater samples

- ❖ Benthic biofilm
- ❖ Stones scraping
- ❖ Sample preservation as pellet or biofilm suspension



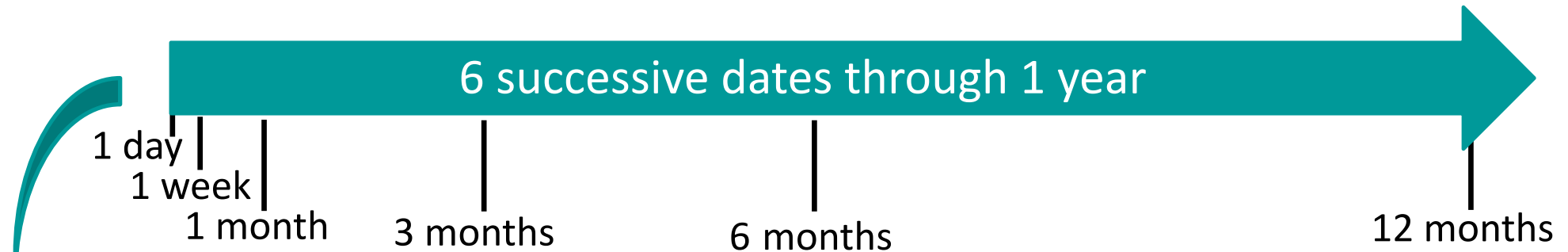


3 preservation methods

- ❖ Cryo-preservation (-80°C marine samples / -20°C freshwater)
- ❖ + 4°C + Ethanol
- ❖ - 20°C + Home-made « RNA later »



❖ NucleoSpin Soil Kit - Macherey Nagel (*Vasselon et al. 2017*)

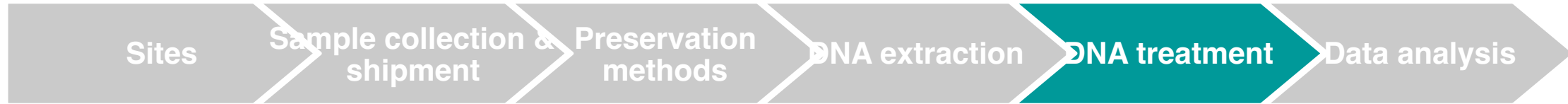


* 6 sites

* preservation methods

* 2 DNA extract replicates (per site and per method)

= 216 DNA samples

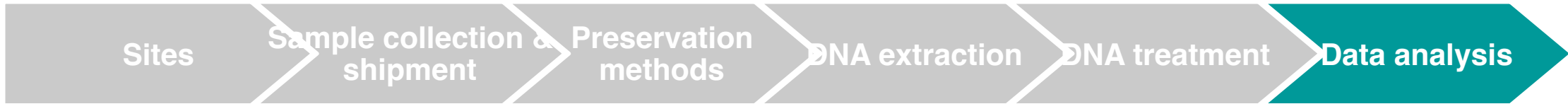


DNA quality and quantity

- ❖ Quality - 260/280 nm ratio - Nanodrop[®]ND-1000
- ❖ Quantity - DNA concentration (ng/μL) - Quant-iT[™] PicoGreen[®] dsDNA assay kit

DNA metabarcoding: diatom assemblage

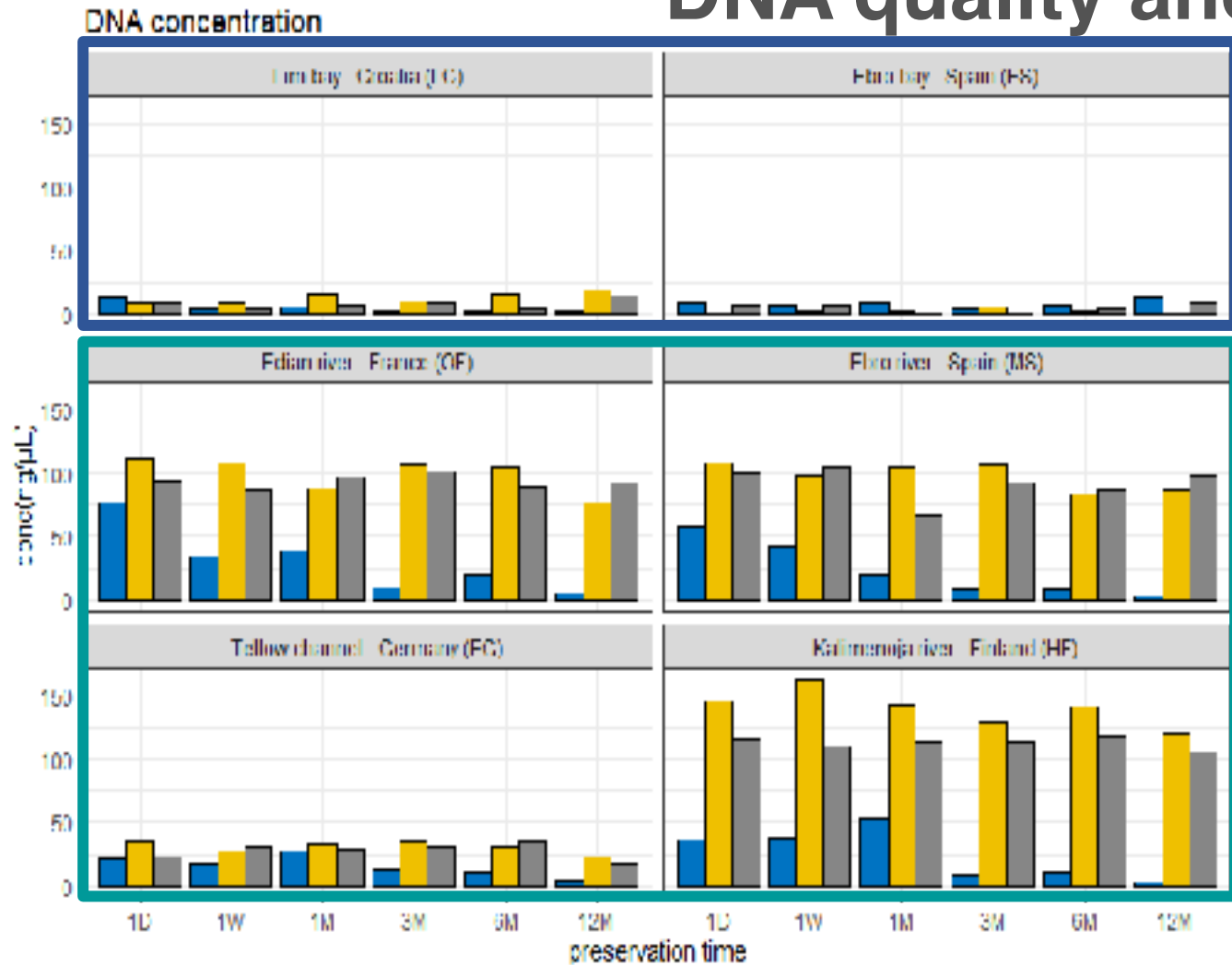
- ❖ PCR with *rbcL* chloroplastic gene (312 bp)
- ❖ Library preparation and sequencing: Illumina MiSeq paired-end sequencing kit (V2, 250 bp × 2) (GeT-PlaGe, Auzeville, France)



DNA quality and quantity

marine

freshwater



- No observed impact on DNA quality
- [DNA marine] < [DNA freshwater]
- Freshwater samples in ethanol (ET) had significantly lower [DNA] than FR & RL



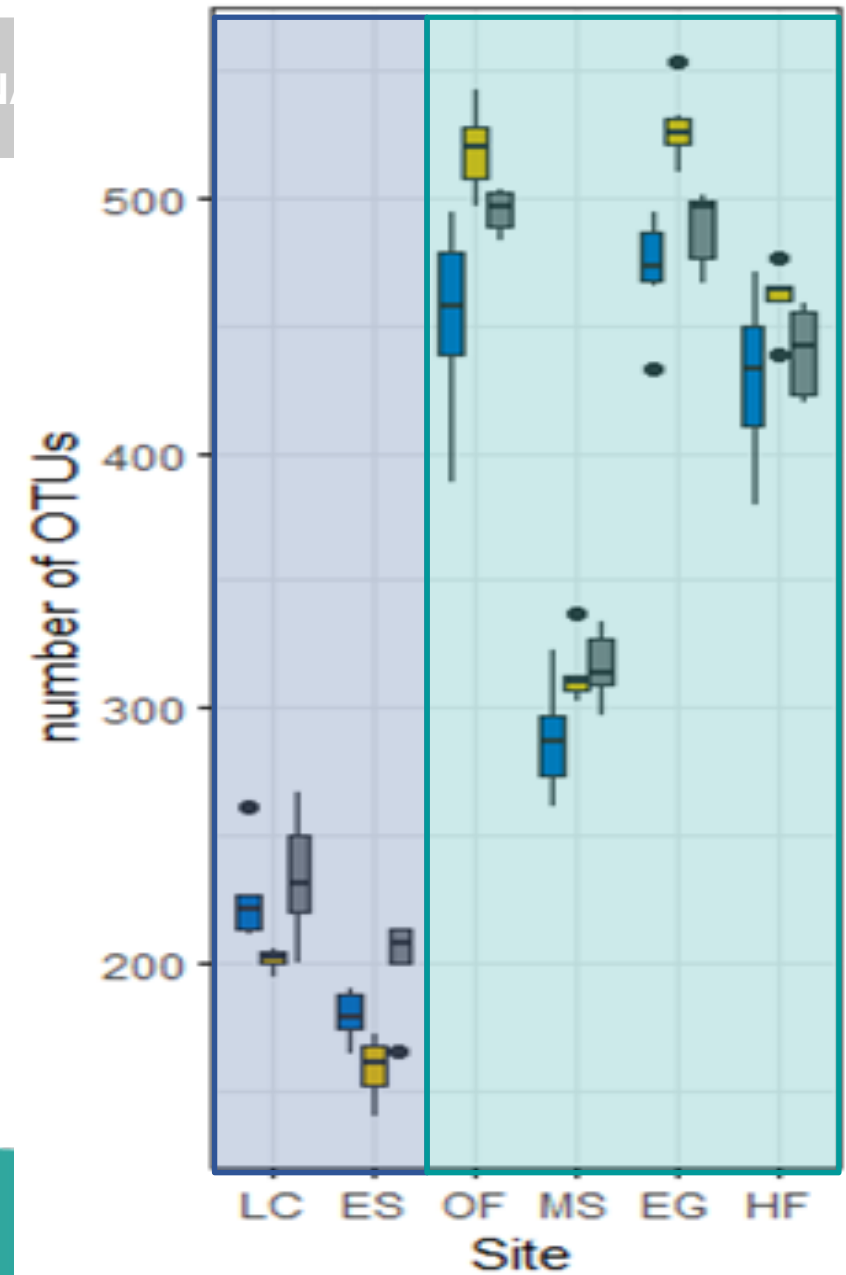


Diatom community diversity

- ❖ Freshwater sites have higher:
 - read nb
 - **OTU richness**
 - diversity index values (Shannon)

- ❖ Preservation methods have no significant impact on:
 - read numbers
 - **OTU richness**
 - diversity index values (Shannon)

method  ET  FR  RL





Sites

Sample collection & shipment

Preservation methods

DNA extraction

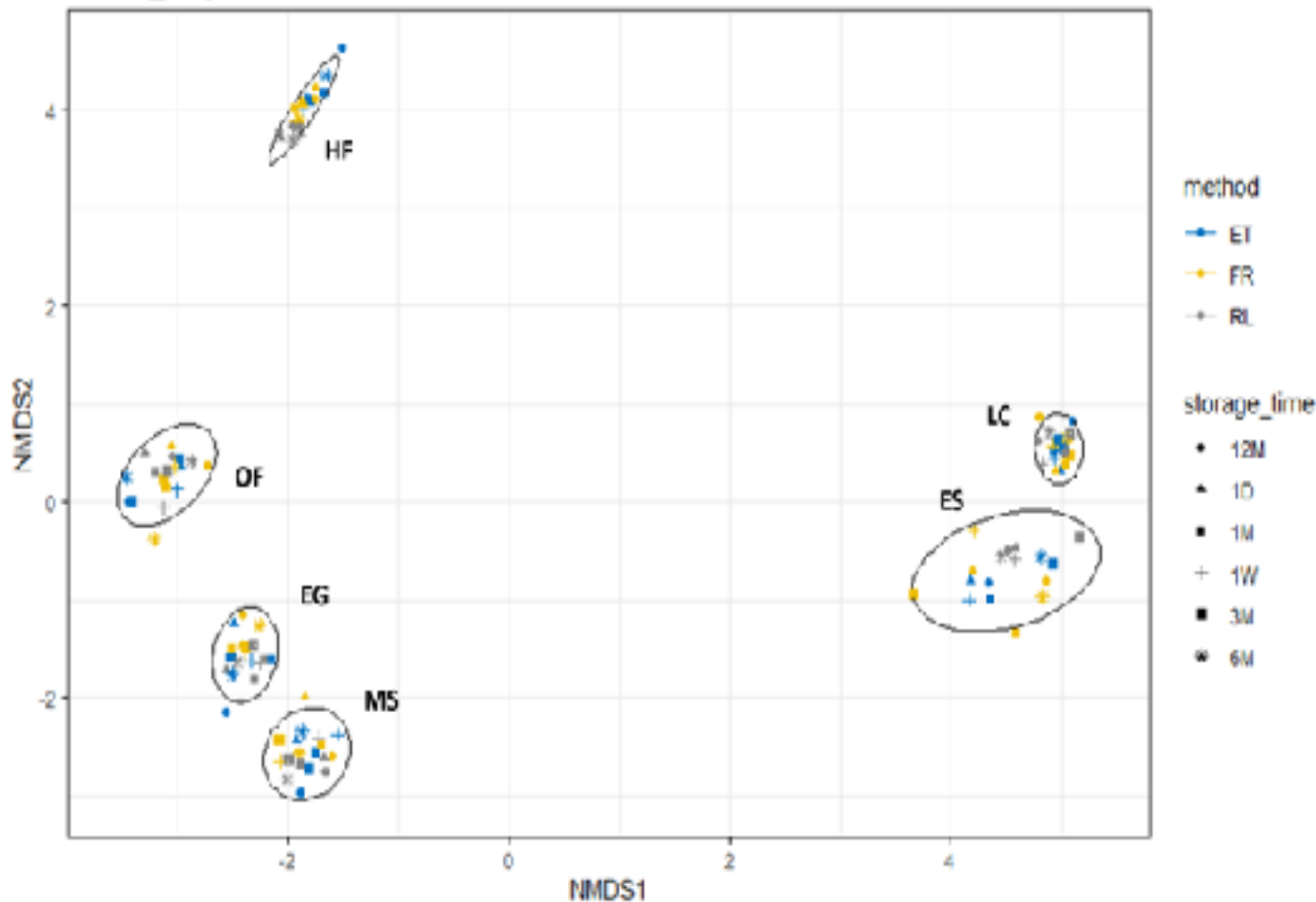
DNA treatment

Data analysis

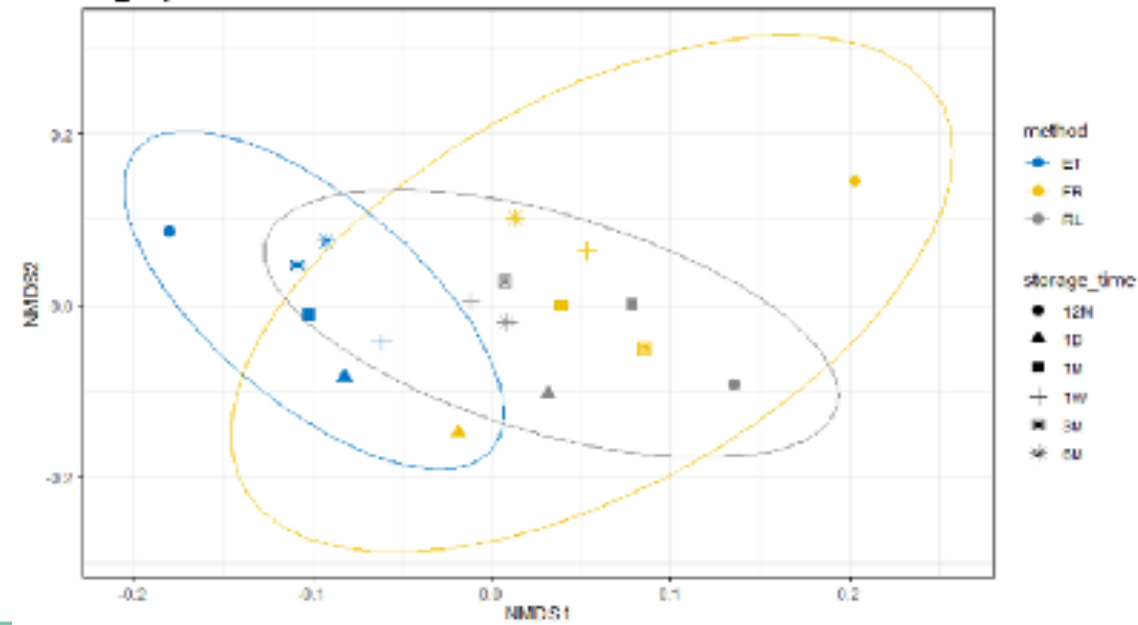
Diatom community composition

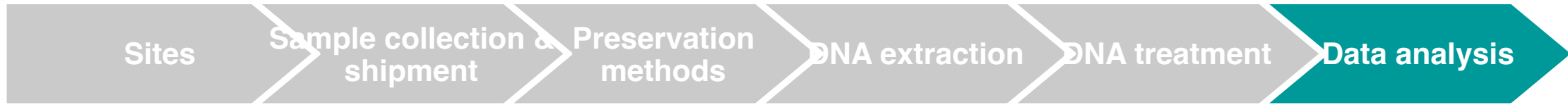
- Sampling sites have main effect
- Site-by-site analysis:
 - Storage duration has no effect
 - Preservation method has a significant effect at all sites

NMDS_bray



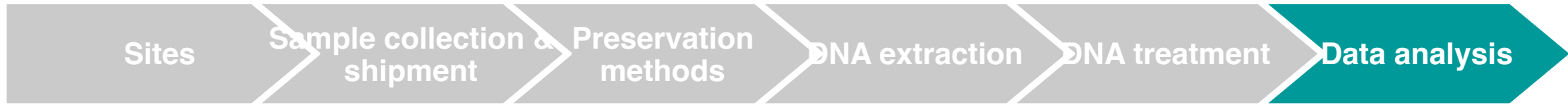
OF_bray





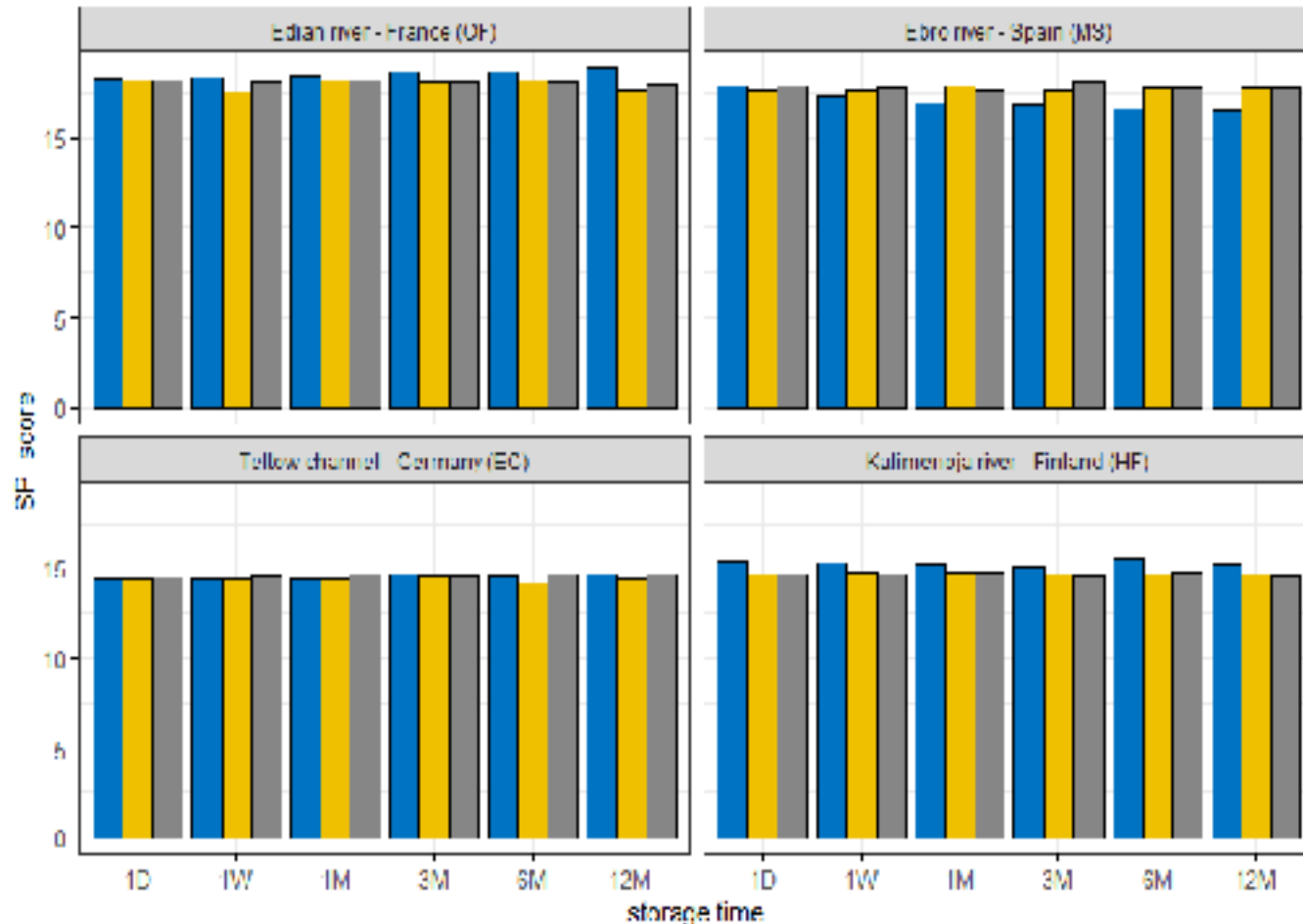
Are some taxa differentially detected?

- ❖ Community changes are mainly due to:
 - changes in relative abundances for abundant taxa
 - changes in presence-absence for low-abundant taxa
- ❖ Overall number of taxa detected ≈ 300 taxa/sample:
 - 81% of taxa detected by all 3 preservation methods
 - <5% of taxa detected by only 1 method
- ❖ Rare taxa were mostly method-specific and usually appeared and disappeared over time without any obvious pattern.



Ecological quality (freshwater sites)

Specific pollution-sensitivity index



IPS scores based on:

- OTUs at species (73%) / genus (19%) levels
- read abundances

- ❖ IPS values were very stable:
 - whatever the preservation meth.
 - whatever the storage duration

method
EI
FR
RI



For biomonitoring purposes (biodiversity and/or ecological quality indices):

Overall robustness

Ethanol preservation of freshwater samples

- ✓ Lower [DNA], no impact on community composition / IPS
- ✓ Ethanol is an operational method for field campaigns and storage
- ✓ Even in the “worst case” (ethanol / 1-year preservation): richness, diversity, IPS were not affected

For detection of low-density species

Some differences for OTUs inventories -> due to changes in low-abundant taxa

Preservation/duration has to be well thought

Need for operational standards





Recommendations for the preservation of environmental samples in diatom metabarcoding studies

Ana Baricevic¹, Cécile Chardon², Maria Kahlert³, Satu Maaria Karjalainen⁴, Daniela Maric Pfannkuchen¹, Martin Pfannkuchen¹, Frédéric Rimet², Mirta Smodlaka Tankovic¹, Rosa Trobajo⁵, Valentin Vasselon^{2,6}, Jonas Zimmermann⁷, Agnès Bouchez²

<https://doi.org/10.3897/mbmg.6.85844>



Questions ?



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