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# Webinar on eDNA

## Task 2.3.1 “Novel technologies and approaches”

Organized by DACC (Catalonia)
and Iiris Kallajoki (OT)

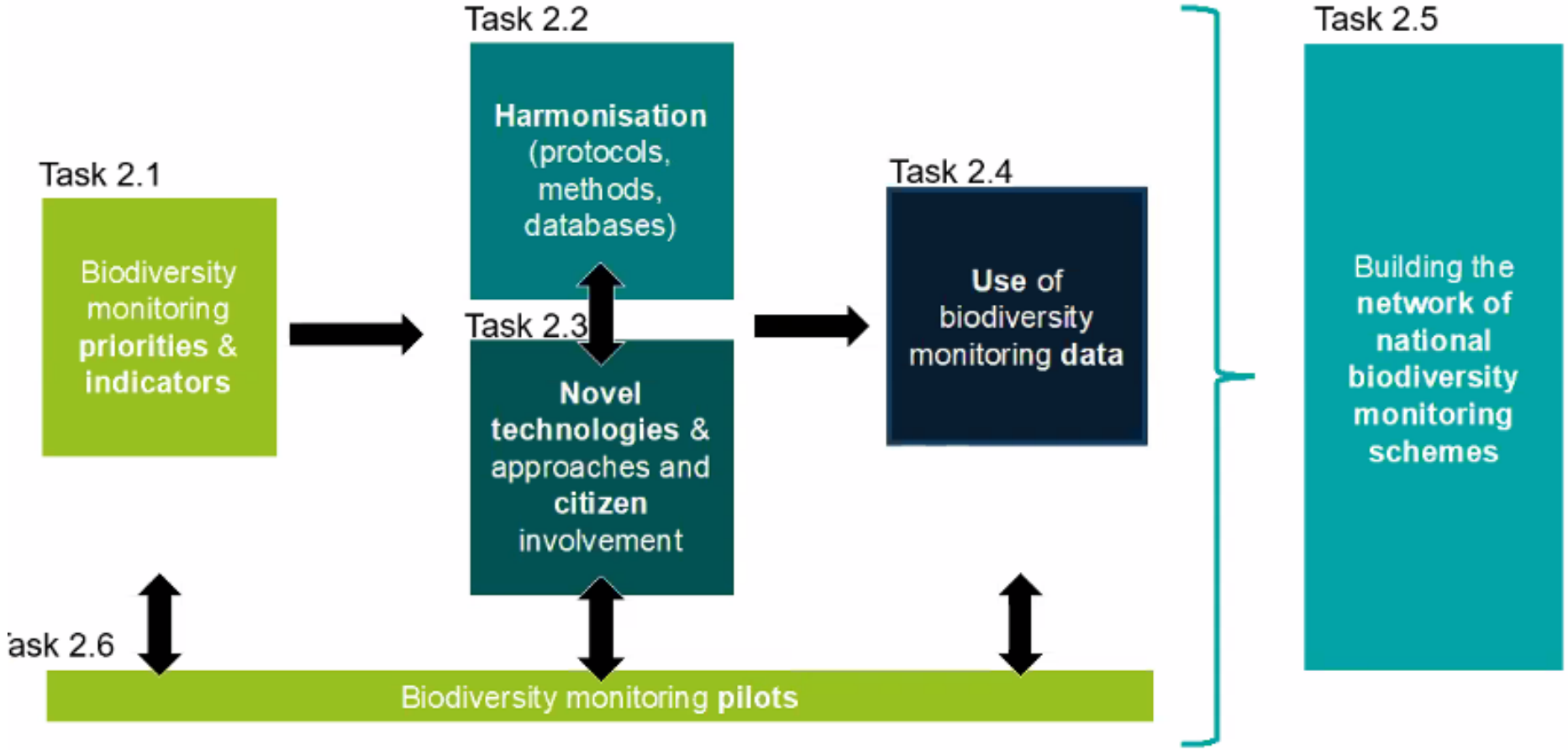
December 18th 2024

# About Biodiversa+

Biodiversa+ is the European Biodiversity Partnership supporting excellent research on biodiversity with an impact on policy and society.

**Workpackge 2**: Set up a network of harmonised schemes to improve monitoring of biodiversity and ecosystem services across Europe

# Biodiversa+ Workpackage 2, Task 2.3.1”Novel technologies and approaches”

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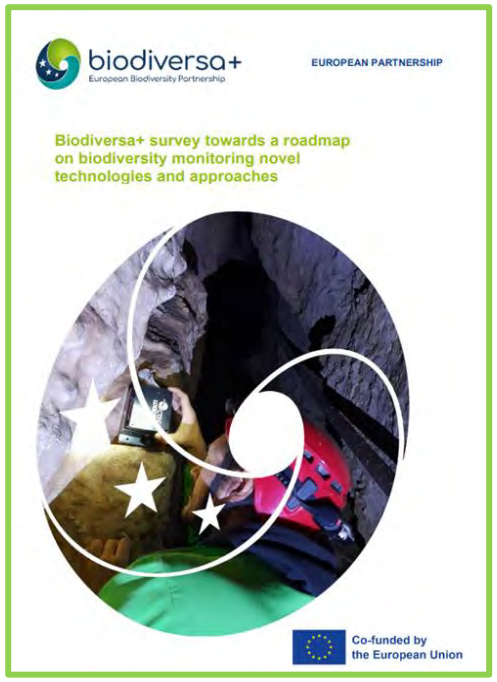
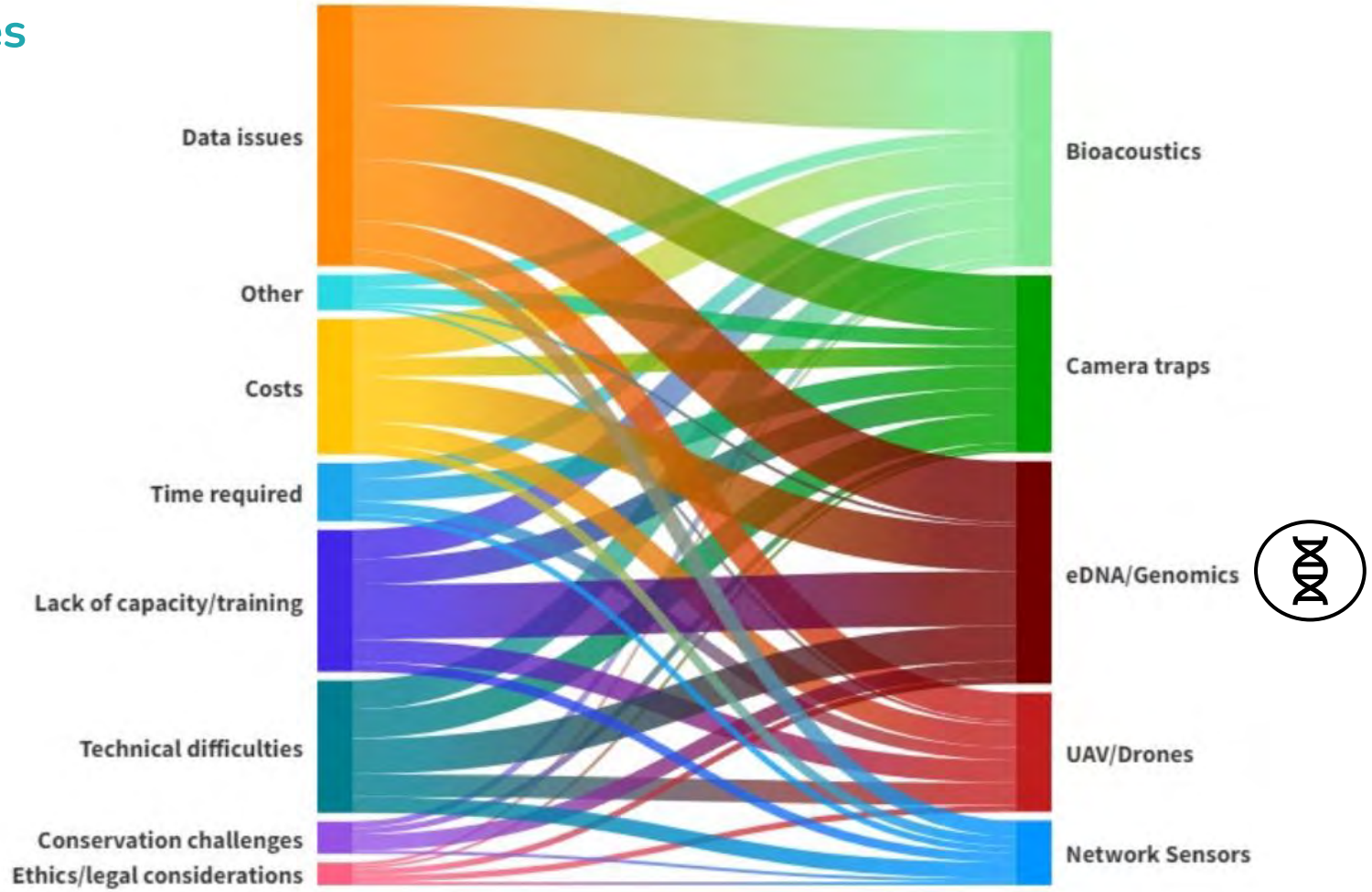
# Biodiversa+ Workpackage 2, Task 2.3.1”Novel technologies and approaches”

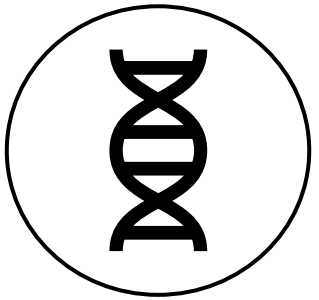
## Survey of novel technologies monitoring across partners

- Deployment state / interest
- Targeted taxa and EBVs
- Challenges and constrains



# Webinar agenda



1. Introduction of the webinar and speakers
2. Presentation by Katrine Hansen Lemming (Gov. of Denmark)
3. Presentation by Julia Seebers (EURAC Research & University of Innsbruck/ Gov. of Bolzano)
4. Presentation by Nina Prasil Delaval (PatriNat, OFB-MNHN-CNRS-IRD / France)
5. Questions / debate

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# The use of eDNA in soil biodiversity monitoring

Julia Seeber

Eurac Research, Bozen, Italy

- Aims of the pilot study
- Traditional vs. molecular methods
- The use of eDNA in soil monitoring

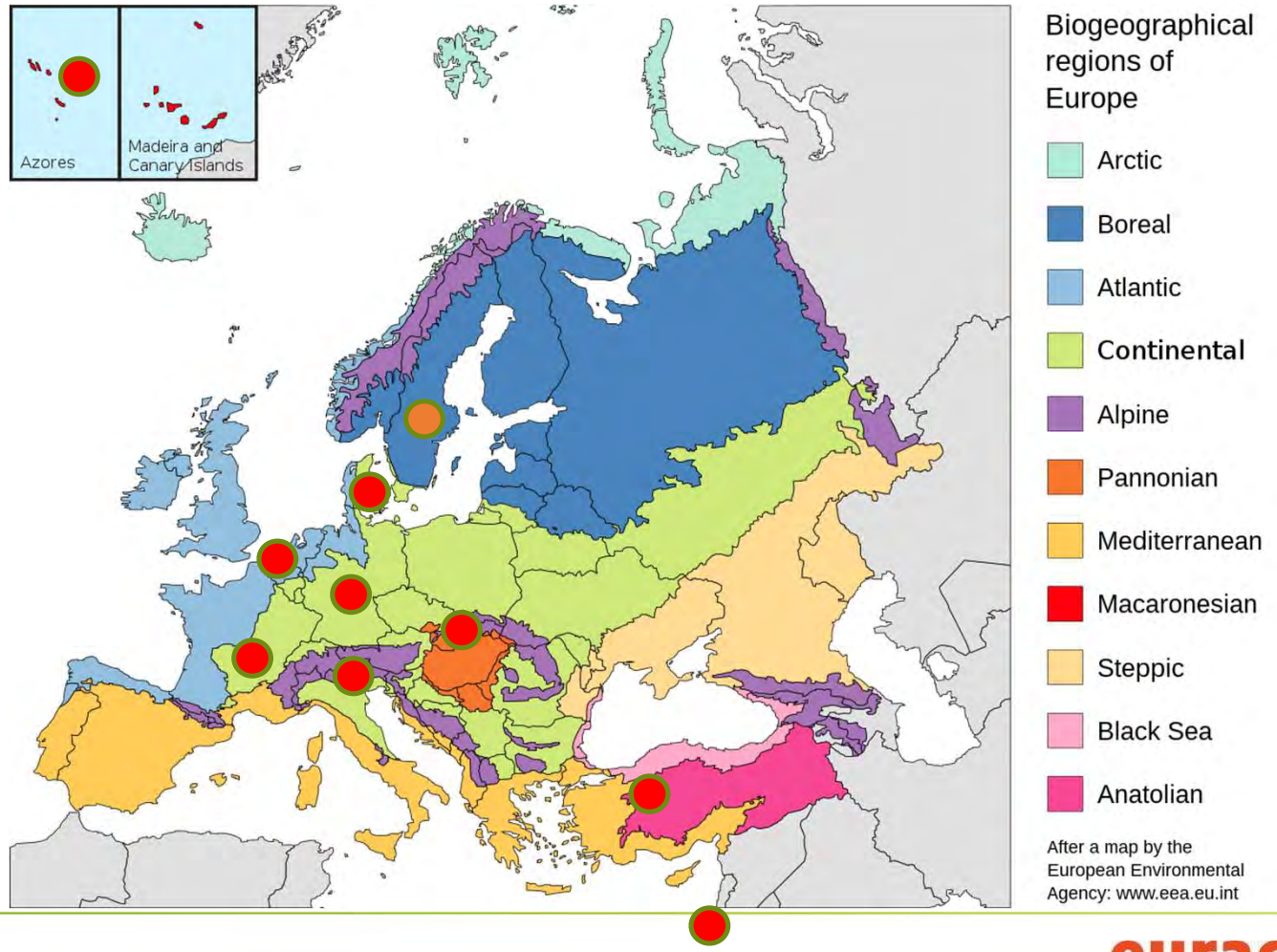
I. Collect comprehensive data on soil biodiversity in near-natural forests, preferably in Natura 2000 (or at least protected) sites, including a standardized vegetation survey and soil surveys

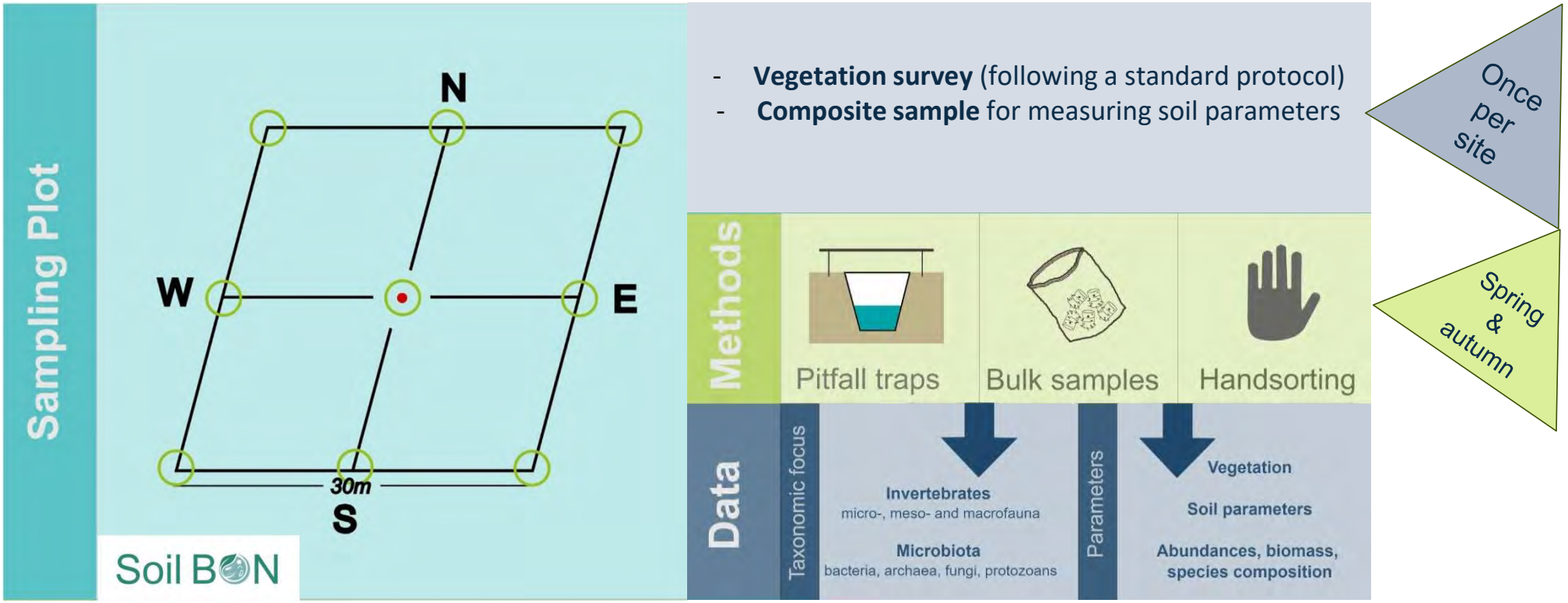
II. Compare traditional and molecular methods

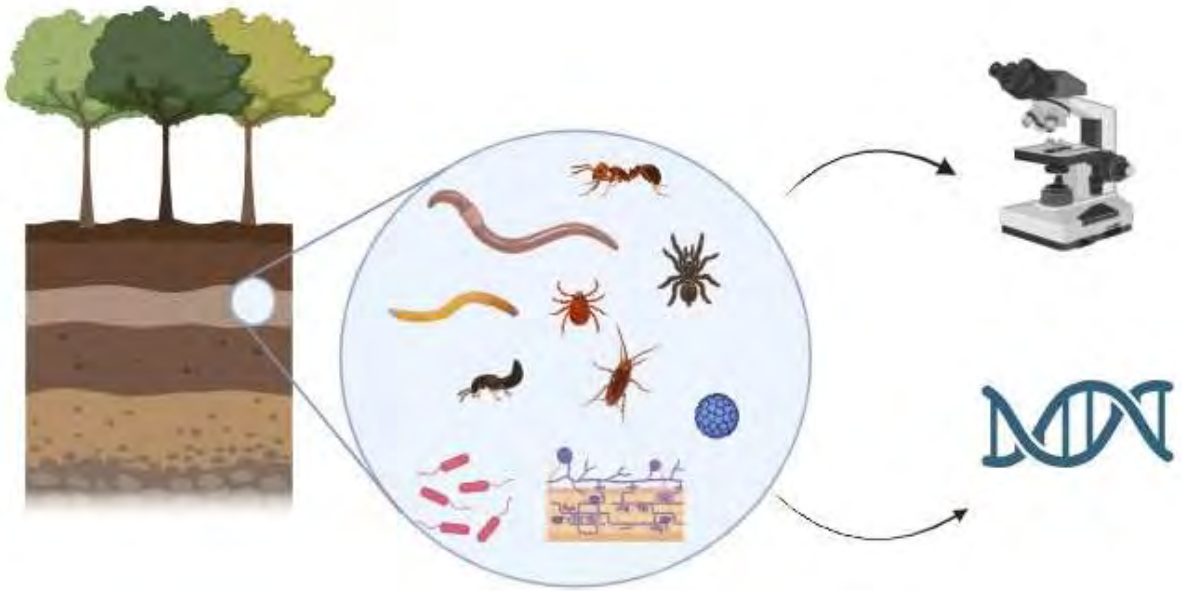
III. Define a transnational monitoring scheme, including protocols and methods, sampling design, administrative and logistic issues, identify limitations and risks and propose solutions, link to EU and international policies (e.g. Soil Monitoring Law)

## Active contributors:

Autonomous Province of Bolzano
Azores
Belgium
Denmark
Germany
France
Israel
Slovakia
Sweden
Turkey
(Catalonia)




Sampling Plot
N
W
E
S
30m
Soil BON
- Vegetation survey (following a standard protocol)
- Composite sample for measuring soil parameters
Once per site
Spring & autumn
Methods
Pitfall traps
Bulk samples
Handsorting
Data
Taxonomic focus
Invertebrates
micro-, meso- and macrofauna
Microbiota
bacteria, archaea, fungi, protozoans
Parameters
Vegetation
Soil parameters
Abundances, biomass, species composition



**Traditional methods:** sorting of pitfall and soil core samples, morphological invertebrate taxa identification

**eDNA:** archaea, bacteria, fungi, invertebrates

JOINT RESEARCH CENTRE
EUROPEAN SOIL DATA CENTRE (ESDAC)
LUCAS

**LUCAS: Land Use and Coverage Area**

## Table 1: Primers used in the pilot

| Taxon | Primers |
|---|---|
| Archaea 16S (SSU) | SSU1ArF (TCCGGTTGATCCYGCBRG) and SSU1000ArR (GGCCATGCAMYWCCTCTC) |
| Bacteria 16S (SSU) | 515F (GTGYCAGCMGCCGCGGTAA) and 926R (GGCCGYCAATTYMTTTRAGTTT) |
| Eukaryote ITS2 | ITS9mun (GTACACACCGCCCGTCG) and ITS4ngsUni (CGCCTSCSCTTANTDATATGC) |
| Eukaryote 18S (SSU) | Euk575F (ASCYGYGGTAAYWCCAGC) and Euk895R (TCHNHGNATTTCACCNCT) |

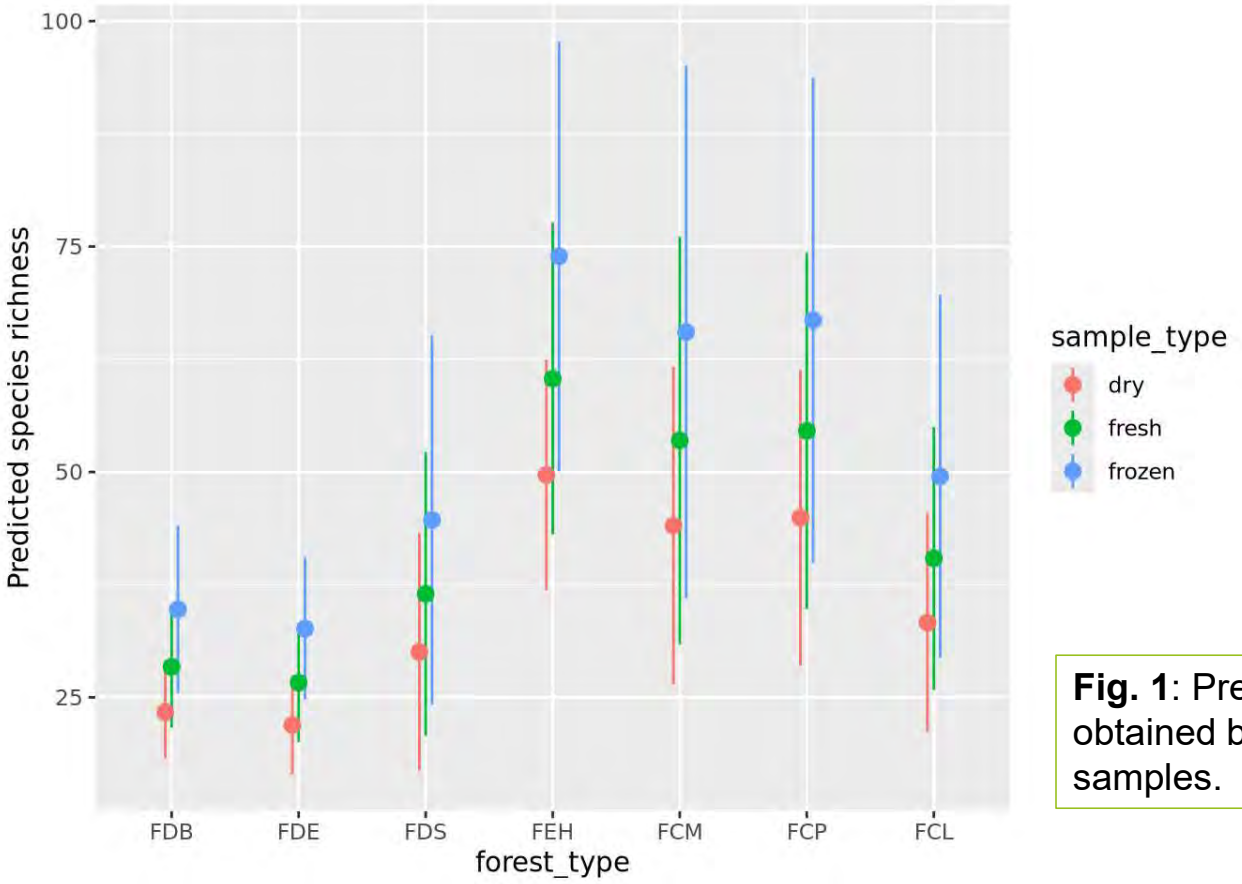



**Fig. 1**: Predicted mean species richness of Eukaryota obtained by eDNA analysis from dried, fresh and frozen samples.

- 40% Annelida
- 34% Nematoda
- 19% Arthropoda

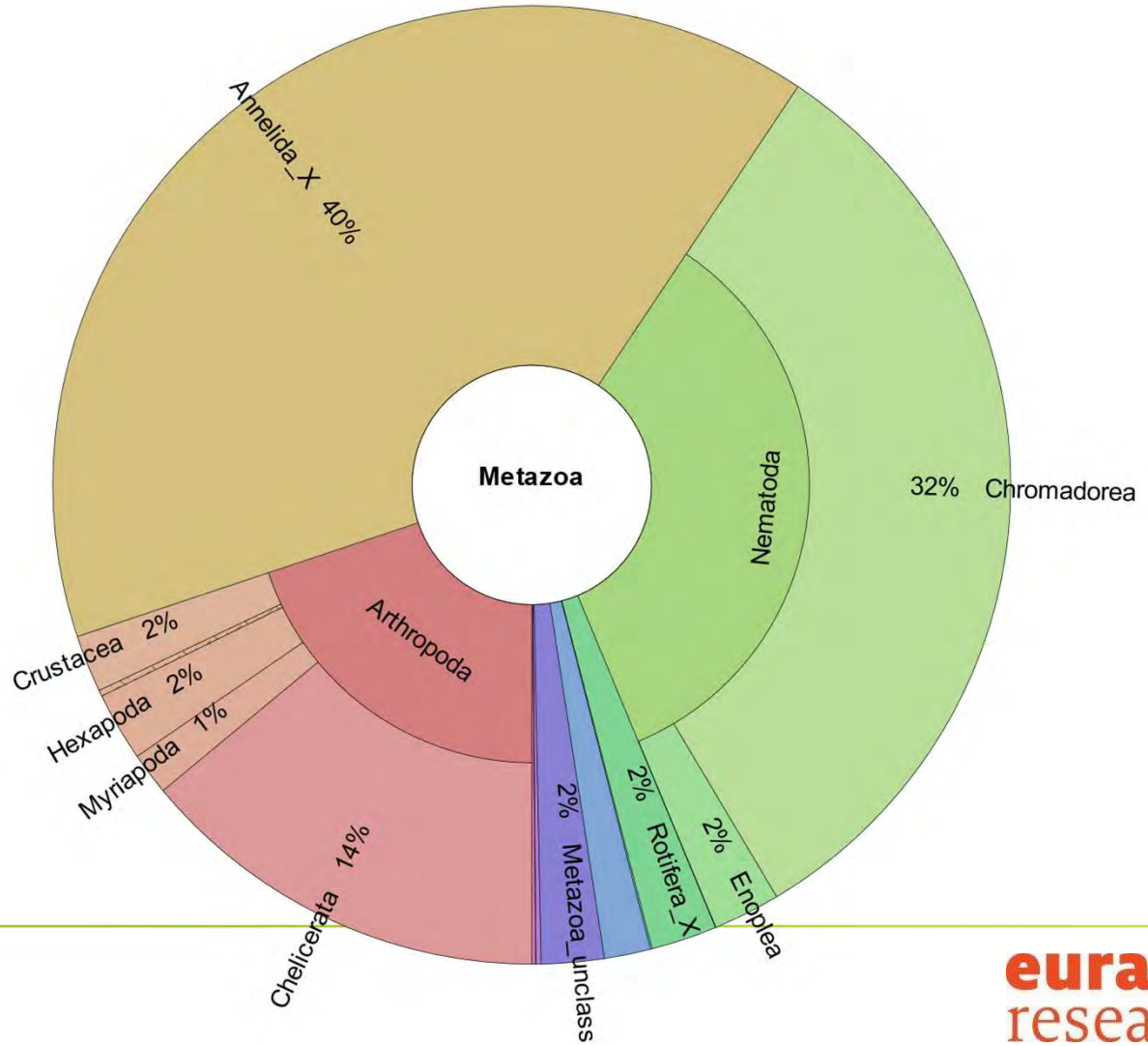



**Fig. 2**: Proportion of main Eukaryota taxa (without Fungi) identified from eDNA samples.

- 49% Annelida
- 47% Arthropoda

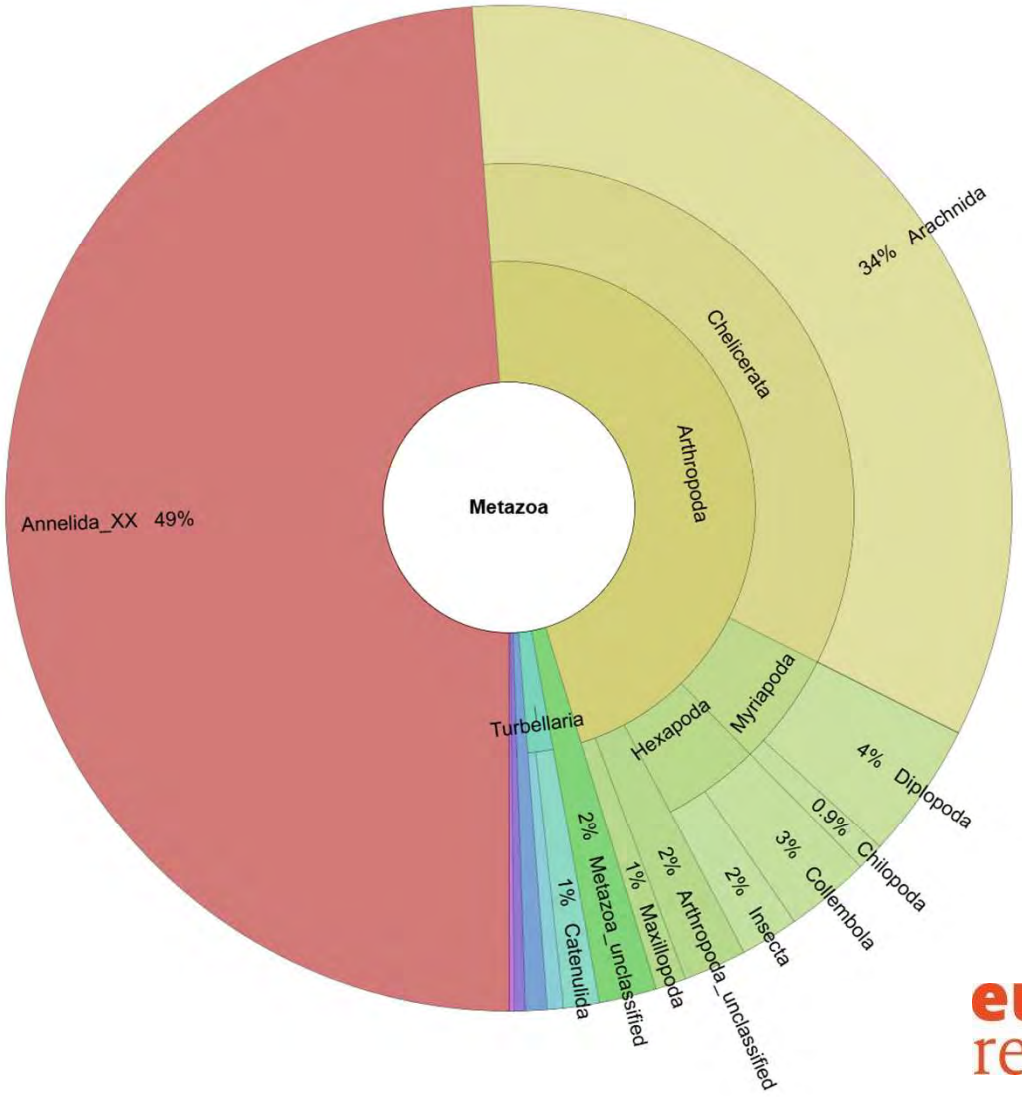



**Fig. 3**: Proportion of main macro-invertebrates identified from eDNA samples.

Arthropods obtained by traditional methods show a wider range of taxa (on order level) than those obtained by eDNA analysis

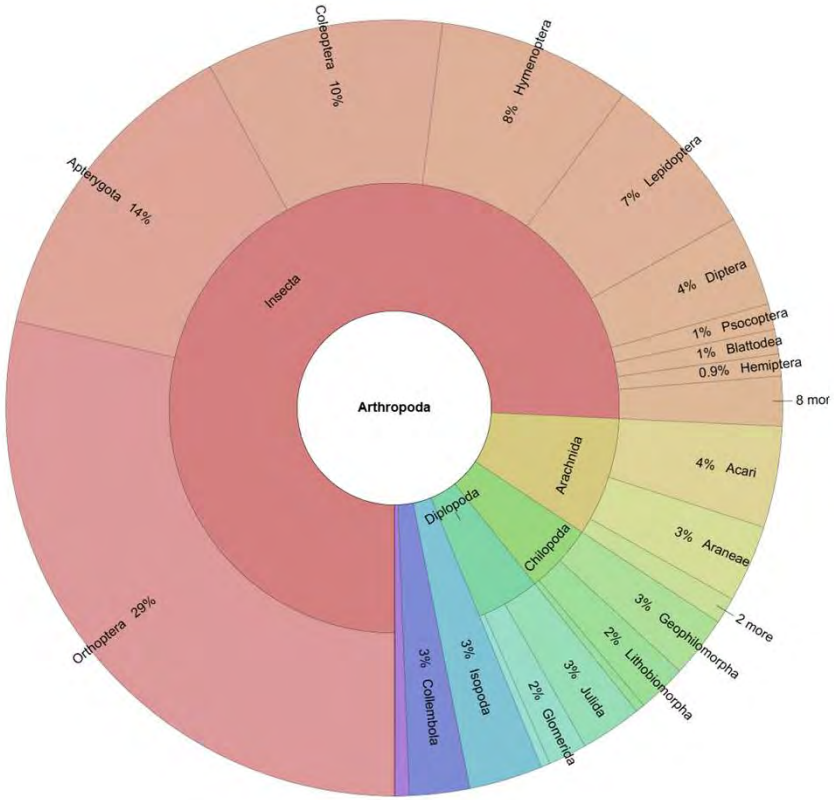



**Fig. 4**: Arthropod taxa (order level) identified in soil core samples (handsorting)

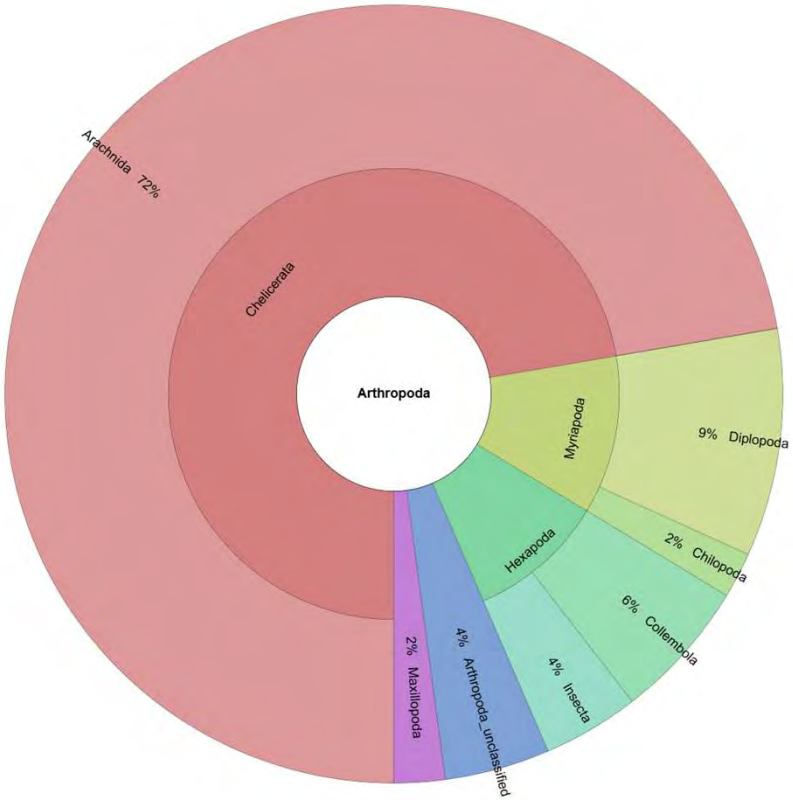



**Fig. 5**: Arthropod taxa (partly order level) identified in eDNA samples.

# Suitable methods to obtain a complete overview of soil biodiversity

## Current situation

**Traditional methods**

- captures taxonomic resolution (family level)
- time consuming

**Molecular methods**

- time efficient
- non-invasive
- captures alpha diversity
- not yet suited for all taxa

**Needs**

- optimization of protocols and workflows
- group-specific primers
- reference database (Animalia/Arthropoda)

## Future perspective

**Molecular methods**

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Co-funded by the European Union

# Thank you!


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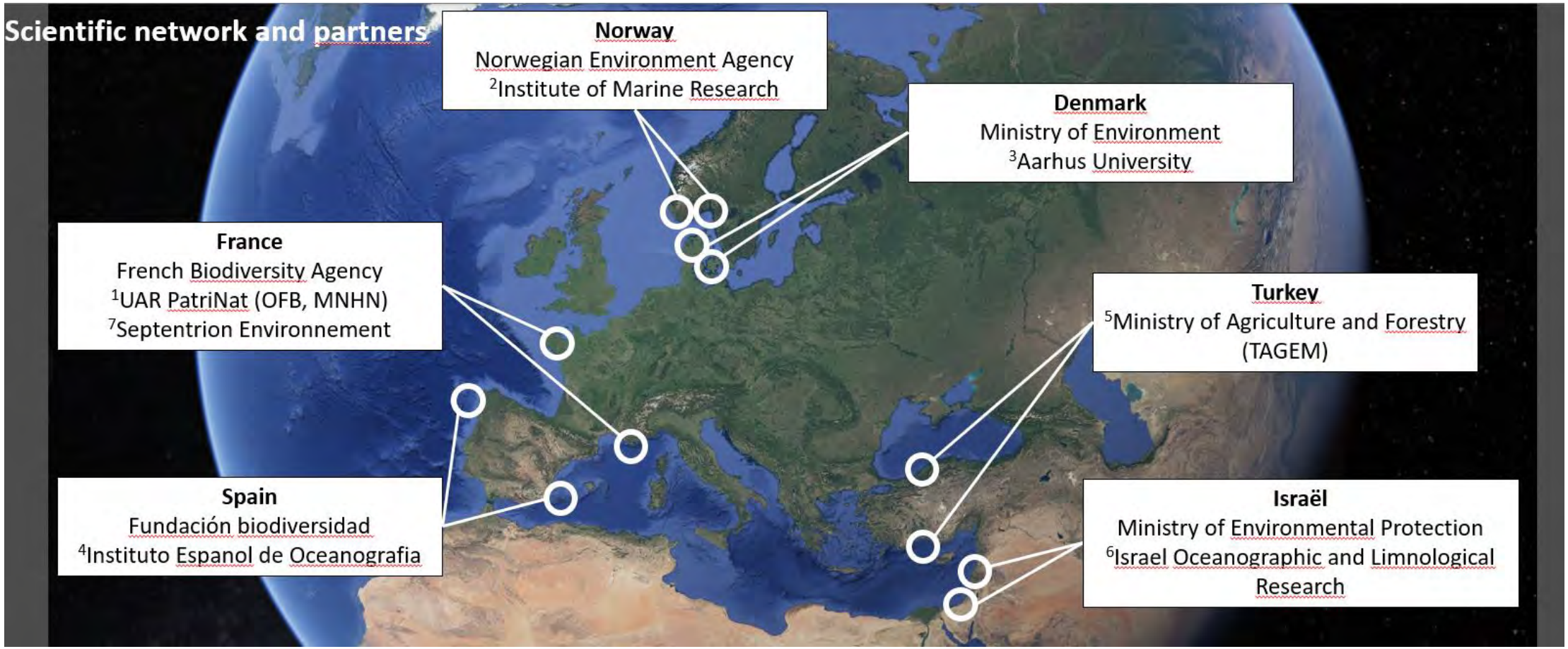
# EuRockFish and the use of eDNA for reef fish monitoring

Prasil-Delaval N.[1], Legras G.[1], Delesalle M.[1], Ring Kleiven A.[2], Martinez-Swatson K.[2], Kleiven P.[2], Staehr P.[3], Robinson B.[3], Ternon Q.[1], Guérin L.[1], Danet V.[1], Cuadros A.[4], Díez González S.[4], Cebeci, A.[5], Öztürk, R.Ç.[5], Alemdağ, M.[5], Firidin, Ş.[5], Rilov G.[6], Stern N.[6], Zilberman S. [6], Morov A.[6], Raanan G.[6], Sonego Y.[6], Haim M.[6], Nazarova S.[6], Bianchimani O.[7], Estaque T.[7] & Thiriet P.[1]

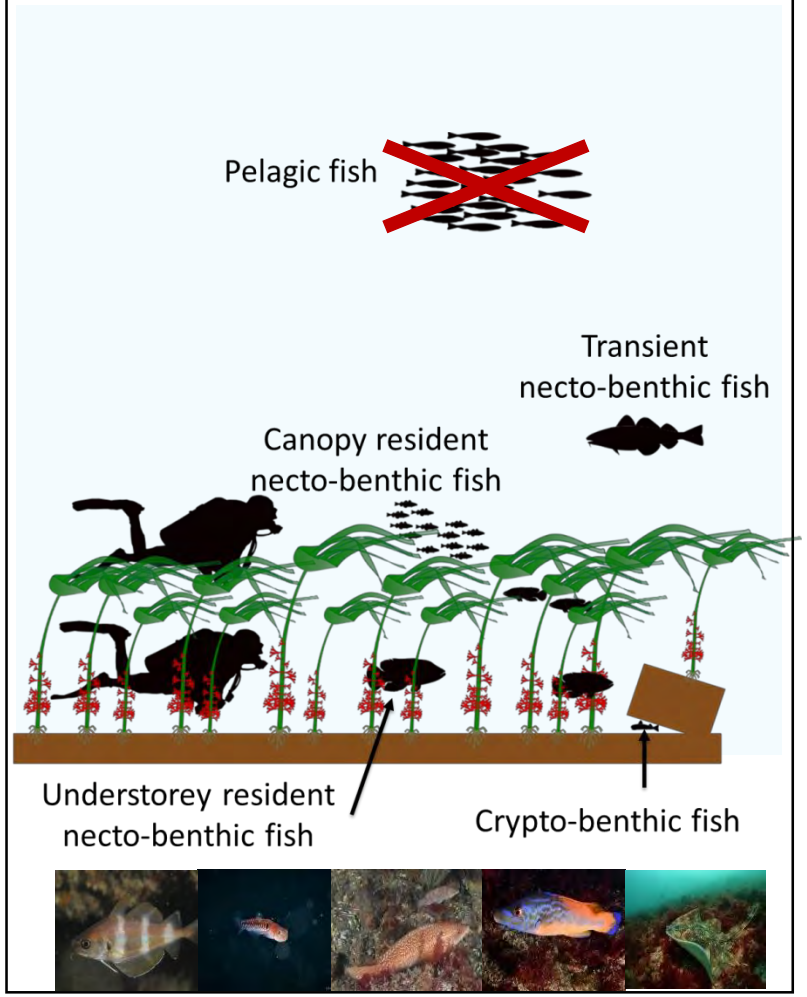
18th of December, 2024

# Our wonderful partners

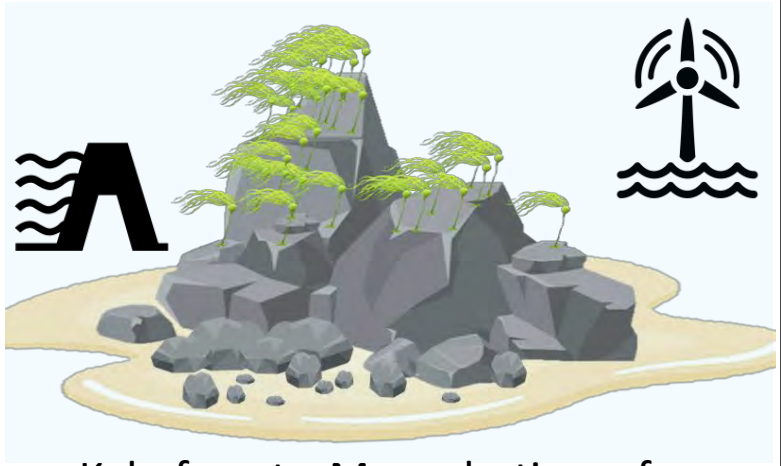


# Object of Study: Reef fish in European coastal waters

## Demersal fish



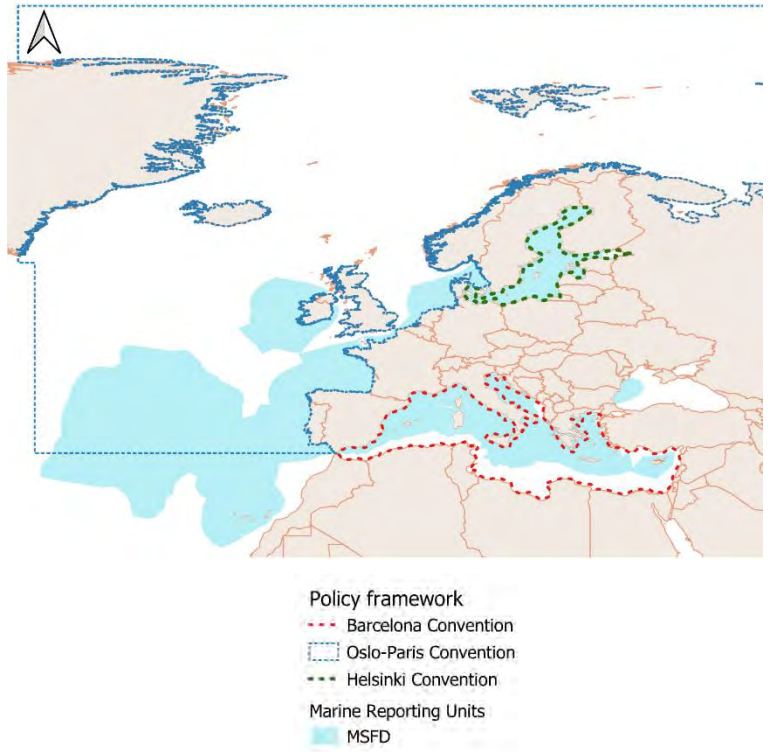

## Temperate rocky reefs



Kelp forests, Mesophotic reefs,…
and marine infrastructures

**Sensitive to human threats**
Fisheries, Habitat degradation
Climate change, Invasive species

## Geographical Europe
x
UE Directives
and Regional Sea conventions



# Problematic and aim of the project

## Problematic

- Long term monitoring is **missing** in several European countries
- Observational methods used throughout Europe are **heterogeneous**
- European reef fish scientific experts are **not well organized** in network

## General Aim of the project

- Develop and test **homogenized protocols** for two traditional methods (scuba diving and video) and a new complementary method (eDNA)
- **Validate the methodological framework** that combines the 3 sampling methods to more accurately assess infralittoral and circalittoral reef fish
- Test the potential of this combination of methods to detect the arrival of NIS
- Produce a **methodological guide** for monitoring European reef fish under MSFD, regional sea conventions and MPAs, and provide guidance for OWF monitoring

Fish population and assemblage structure (conspicuous species only)
Indices related to abundances, biomasses and body-size distributions

Diversity indices (all species)
computed on P/A of taxa and MOTU

## Underwater visual census by scuba (UVC)

## Baited Remote Underwater Stereo-Video (BRUV)

## Metabarcoding on environmental DNA (eDNA)

Both methods allow counting and measuring individuals of conspicuous fish species
They have distinct Pro/Cons as regards **environmental constraints** and sampling biases

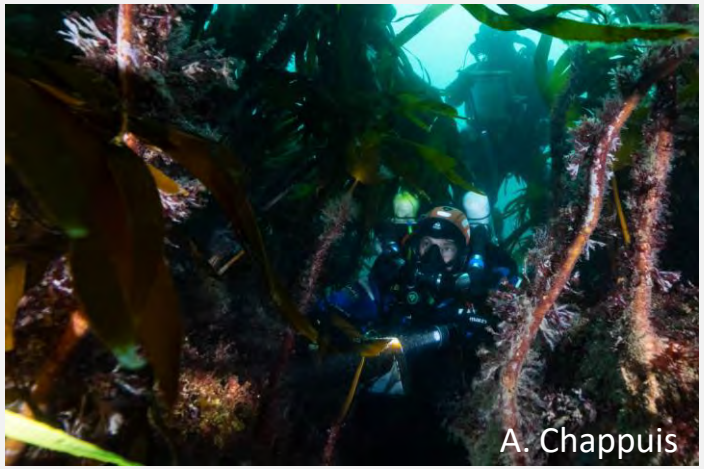
UVC is the best choice when:
Underwater Visibility > 4m
Bathymetry < 30 m
Presence of Kelp canopy

Optimum choice in intermediate conditions have to be discussed

BRUV is the best choice when:
Underwater Visibility < 2m
Bathymetry > 30 m
Absence of Kelp canopy

Detect Presence of all fish species including small hiden fish, as well large, vagrant and/or shy fish species

Molecular markers and primers have to be discussed, as well water filtration protocols (volume and position, surface vs bottom)


A. Chappuis


Langlois et al. 2018

P. Thiriet

Fish population and assemblage structure (conspicuous species only)
Indices related to abundances, biomasses and body-size distributions

**Underwater visual census by scuba (UVC)**

**Baited Remote Underwater Stereo-Video (BRUV)**

Both methods [illegible] fish species
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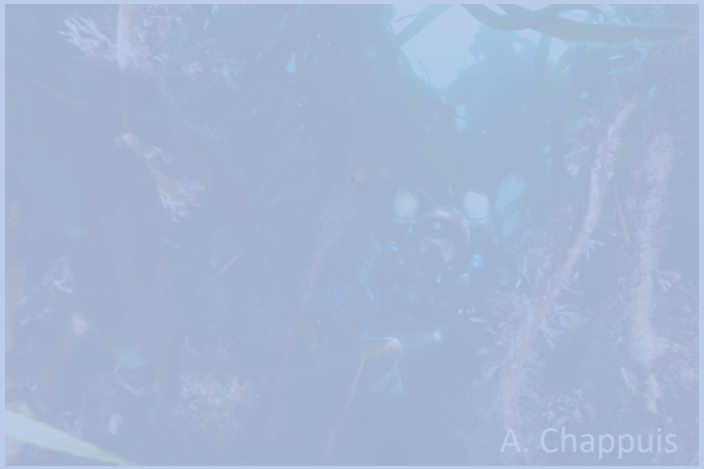

A. Chappuis


Langlois et al. 2018

# Why use eDNA to monitor european reef fish ?

Diversity indices (all species) computed on P/A of taxa and MOTU

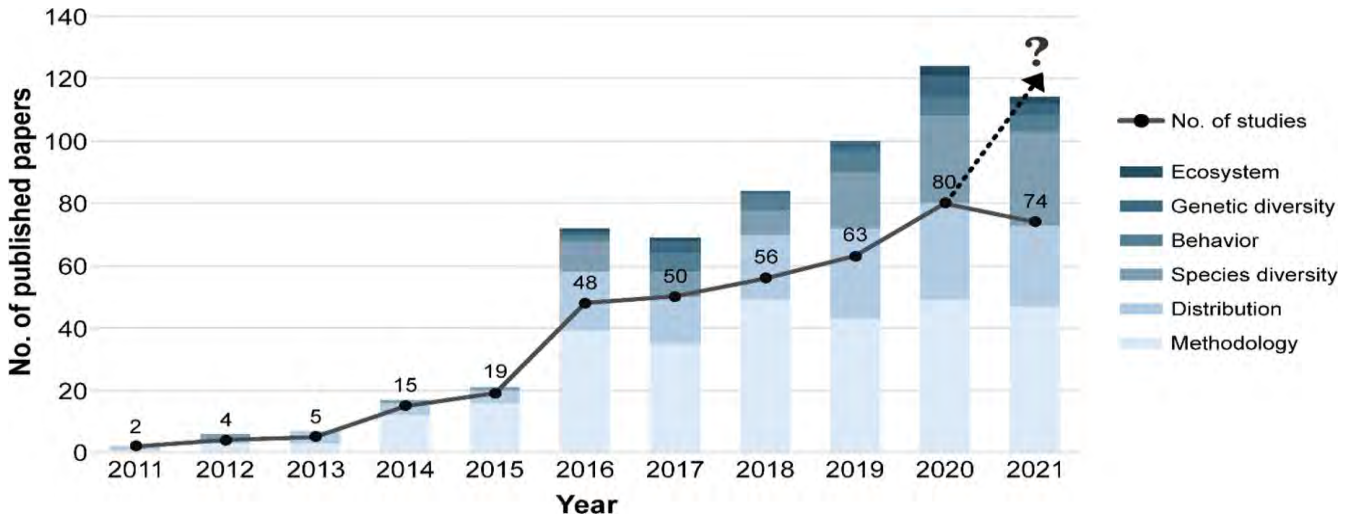
**Metabarcoding on environmental DNA (eDNA)**

Detect Presence of all fish species including small hiden fish, as well large, vagrant and/or shy fish species

Molecular markers and primers have to be discussed, as well water filtration protocols (volume and position, surface vs bottom)

P. Thiriet

# The rise of eDNA




Molecular Ecology, Volume: 31, Issue: 20, Pages: 5132-5164, First published: 16 August 2022, DOI: (10.1111/mec.16659)

Variety of protocols used:

- Volume (1l, 2l, 30l)
- Porosity of the filter
- Type of sampling
- Different primers
- Molecular analyses

**Issue to analyse data and compare results!**

# How do we use eDNA collectively, in a project involving several institutions and countries?

Additional constraints:

- Limited time (3 methods ; no time to filter water in the lab after the day is over)
- Limited space on the boat
- Must be compatible with upscaling at European level

➡ Protocol must be **easy**, **user friendly and reproducible**

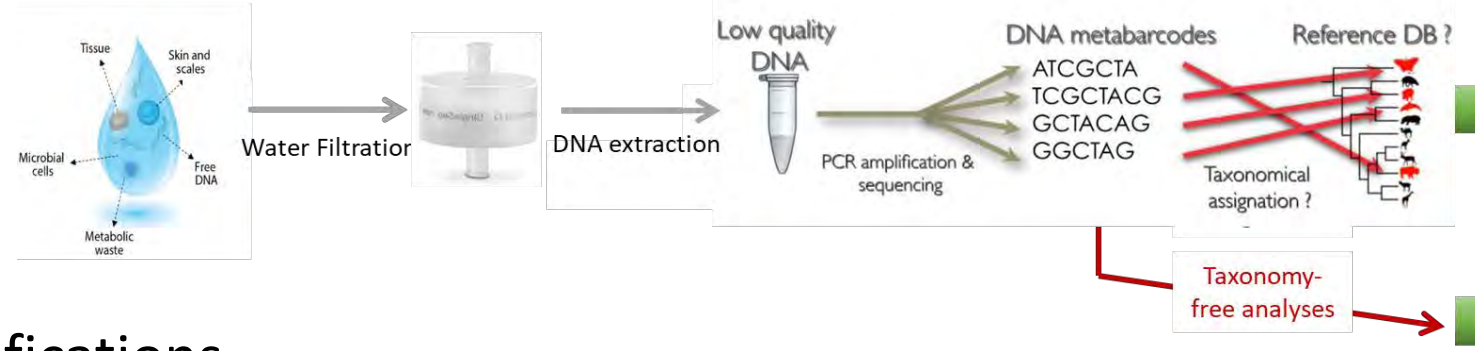
Selected method:

- Filtration directly in the field
- Using filtration capsules of 30l
- Only one private lab will be in charge of supplying the 432 kits and performing the analyses

➡ More expensive this way, but only viable solution

**Conclusion: Launch of a call for tender**

# Use of eDNA : technical requirements




## 1/ Selection of key technical specifications

**Sampling kits**

- ❑ Important filtration area that guarantees the filtration of at least 30L of water
- ❑ Buffer allowing the storage of the samples at room temperature for up to one month before sending the samples to the contractor for DNA extraction. Not dangerous (international shipping)
- ❑ Sampling kits must be sterile and easy to use
- ❑ Must allow sampling up to 200m depth

**Biomolecular and bioinformatic analyses**

- ❑ Chosing the primers for eDNA amplification:
  - Persistence of intact DNA strands in the water
  - Good taxonomic resolution
  - Completeness of reference bases
  - Number of PCR replicates, depth of sequencing
  - Pipeline used for bioinfo should apply to FAIR principles

## 2/ Selection of a unique service provider to supply the sampling kits and perform the molecular and bioinformatic analyses

Exhaustive list of technical requirements → European call for tender (5 respondents) → Selection of the best suited

# The system in itself

- Peristaltic pump with 2 heads
- One/Two filtration capsules and tubing
- Possibility to filter two replicates at the same time


Underwater pump and its hardware modifications (rope with loops and floating panel)

# And on the field, description of the methods and protocols used

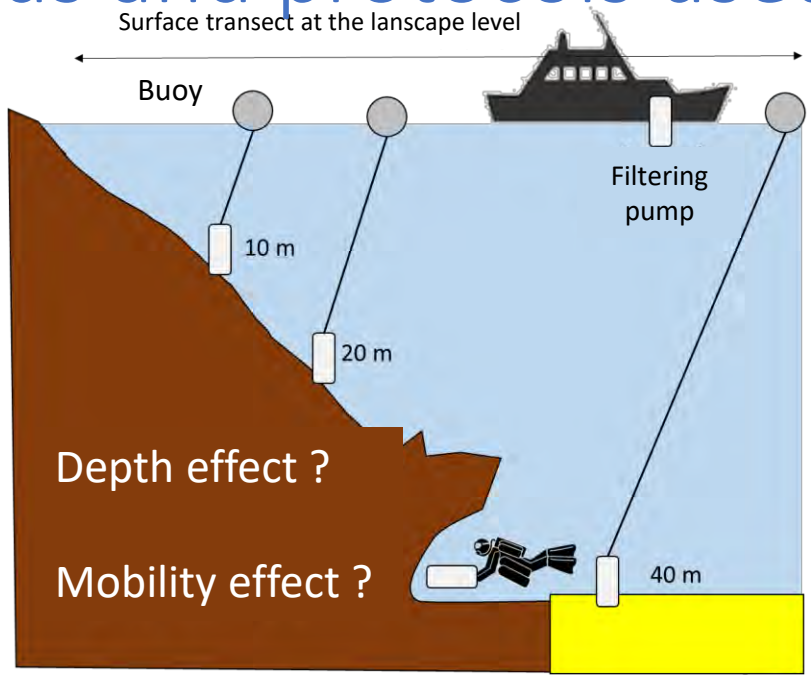
Nb of samples/partner = 72

Where and how should we sample ?

- ❑ Sampling depth(s)
- ❑ Stationnary or mobile?

Should the sampling protocol depend on environmental variables (wich are heterogous within and between countries)

- ❑ Water body movement (e.g. estuaries, tides)
- ❑ Water stratification(e.g. thermocline, halocline)
- ❑ 3D complexity of habitat (e.g. canopy, underwater cliff)
- ❑ Impossibility to dive (skills, security, law)




→ Conclusion to come at the end of the project, in our methodological guide!

Two possibilities at this stage: Standardisation, or Harmonisation

Harmonisation through a decision tree, allowing the user to select the sampling strategy that best suit his needs depending on the environmental context

# First feedback from partners after fieldwork

Easy to deploy

Main issues (but rare):

- The pump did not start
- Algae got stuck and impacted the volume filtered

Overall, good feedback on the method

However, some issues with customs for countries outside UE

# Next steps

Samples arrived at the lab for most partners

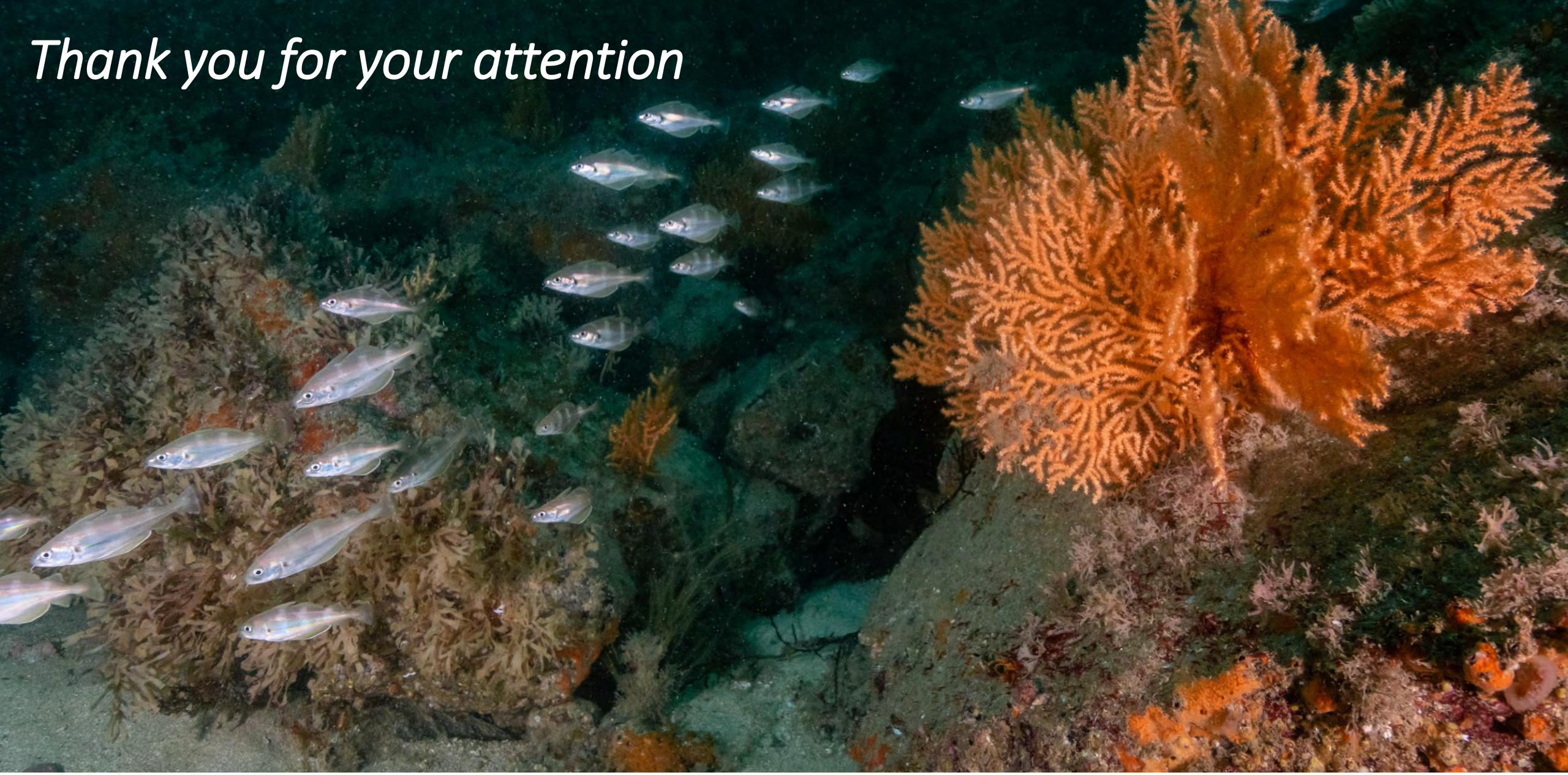
⟶ Still facing some issues for countries that are outside of the European Union

Results should come back from the lab in March, 2025

⟶ Start of the analyses

Remaining (open) question : on which online, global biodiversity repository should we share these data?

# *Thank you for your attention*



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# Additional content

| point | drop (fixe) | dive (mobile) | comment |
|---|---|---|---|
| need **diver** | no | yes | Diver competences are needed to bring the pump underwater with mobile methodology |
| go to **other site** during sampling | yes | no | During the drop sampling something else can be done in other sites while with dive you can only do UVC at the same time in the same station (site and depth) |
| **control** well functionning underwater | no | yes | With dive you can control any misfunctioning of the pump and react directly to solve the problem |
| **target** microhabitat (cave, crevices, holes…) | no | yes | With dive you can bring the pump in many nested microhabitat with some kind of low mixed water mass (with specific DNA signature) |
| **describe** local habitat and present fish | no | yes | With dive you can add covariate of the habitat explored during sampling (nearby seagrass, kelp, sand basin…) as well as the fish present (that must be detected and used as control) |
| **environmental constraint** (current, swell…) | low | high | With dive you are mostly constrained by environmental condition, more precisely current and swell, while the drop allows to sample in most of the acceptable condition (limited by weather condition that do not allowed to go at sea) |

# Project: eDNA in water holes

**Agency for Green Transition and Aquatic Environment, Denmark**

*Triturus cristatus* (Great crested newt)
*Graphoderus bilineatus* (Water beetle sp.)
*Dytiscus latissimus* (Diver beetle sp.)

**Katrine Hansen Lemming**
December 18th 2024

# Introduction

Katrine Hansen Lemming

*Master of science in Biology*, Aarhus University 2016

*Project manager* at the Agency for Green Transition and Aquatic Environment, Denmark, Since 2016

- Water courses
- Natura 2000
- eDNA in aquatic environments

# Agenda

1. Natura 2000 monitoring program
2. Short introduction to eDNA
3. Aim and objectives of the eDNA project
4. Experiences and results up till now

# 1. Natura 2000 monitoring program

## What and why do we monitor?

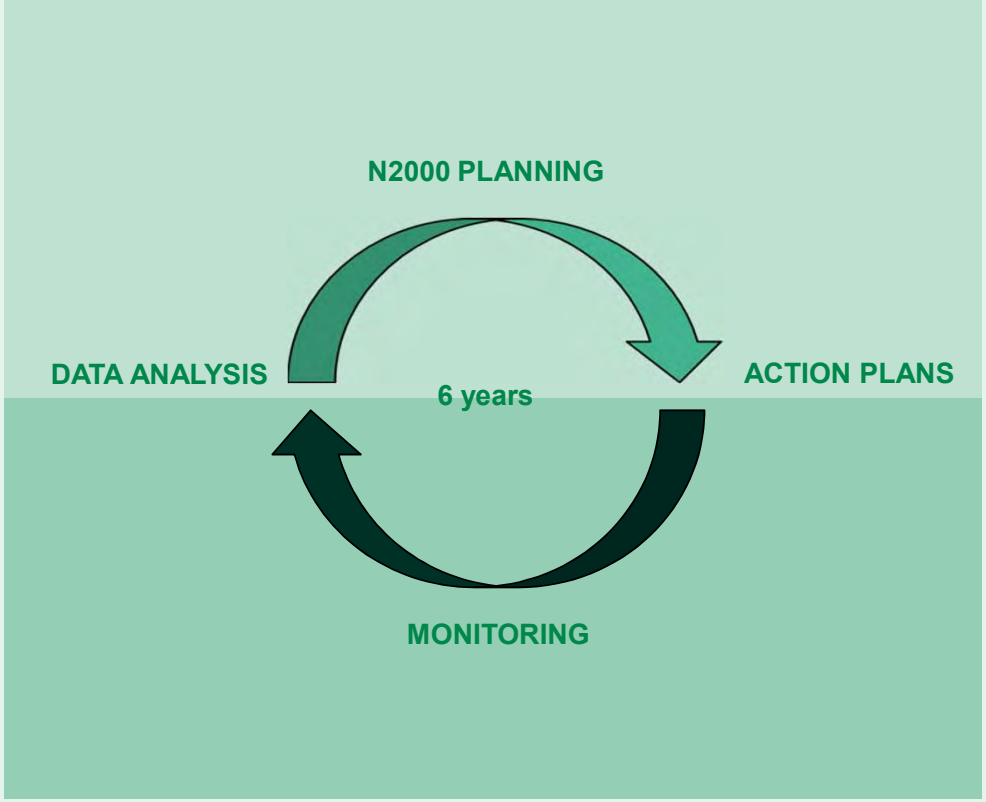
# Natura 2000 monitoring program

## EU obligations

Monitoring reports[1,2]

## Natura 2000 in Denmark

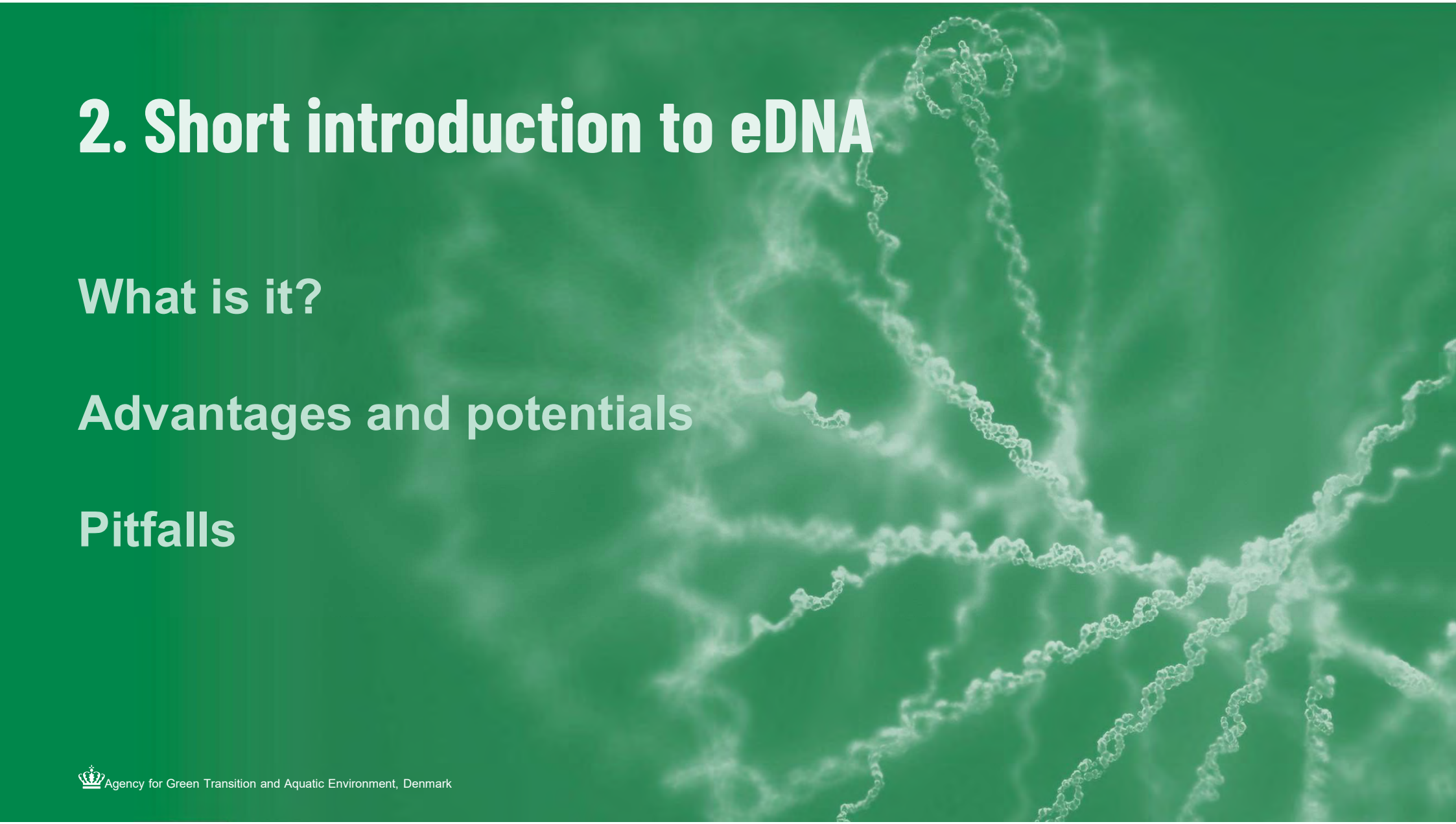
- 250 Natura 2000-sites






1) The Birds Directive (Directive 79/409/EEC)
2) The Habitats Directive (Council Directive 92/43/EEC)

# 2. Short introduction to eDNA

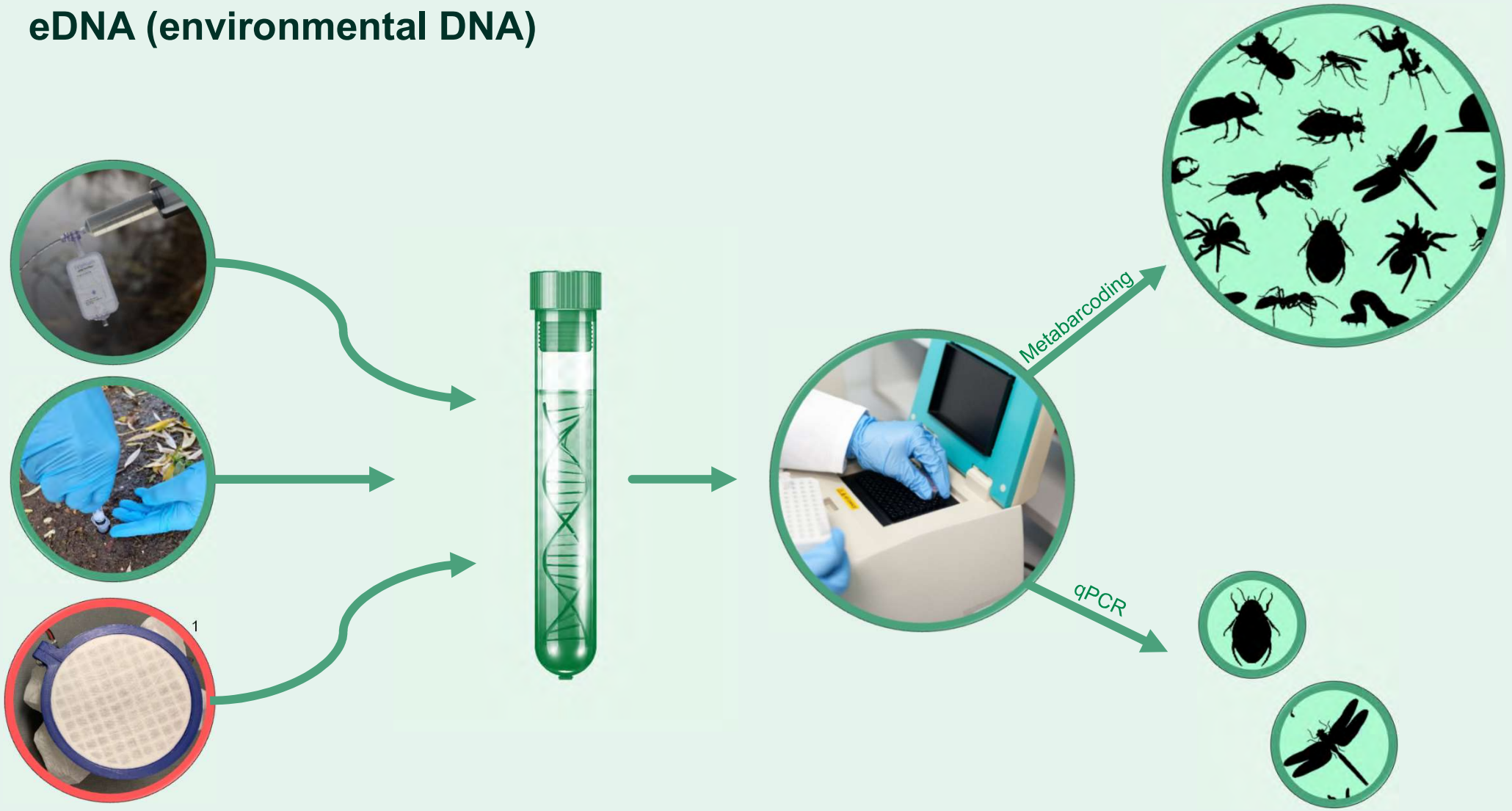


## What is it?

## Advantages and potentials

## Pitfalls

# eDNA (environmental DNA)




Agency for Green Transition and Aquatic Environment, Denmark

1) Garret, N.R et al., 2022, *Airborne eDNA documents a diverse and ecologically complex tropical bat and other mammal community.*

# Advantages and potentials

**… eDNA can increase the quality of data**

**... eDNA can simplify monitoring processes**

**... eDNA can ease the pressure of a busy field program**

**... eDNA can be resource-saving**

# Pitfalls with eDNA

Transferred DNA (false positive)

- Hygiene around sampling
- Negative control samples

eDNA analysis (false positive/negative)

- Not enough DNA (false negative)
- Error in the analysis (false positive/negative)
- Imprecise analysis (false positive/negative)
- qPCR: No threshold value/limit of detection (false positive)

Passing/migrating individuals (”false” positive)

- Often little concentration of DNA
- Change of perspective: area dispersal instead of specific area?

Dead individuals (false positive)

- Often little concentration of DNA

**False negatives in eDNA as well as the conventional monitoring**

# 3. Aim and objectives of the project

## What and how?

# What: Aim and selected objectives of the project

## Overall aim

**Is it possible to apply eDNA to the national monitoring program?**


Great crested newt

## Selected objectives

### Inspiration seeking and networking

- Harmonized protocols yet?
- England and Germany


Water beetle sp.

### Examine different laboratories

- Accreditation, standards and methods
- Parallel laboratory testing – do they find the same?

### eDNA and the conventional monitoring program

- Compare laboratory results to results from the conventional method
- eDNA works on *Triturus cristatus* (Great crested newt). Does it work on a large scale?
- Is eDNA ready to apply to diver and water beetles?
  - *Graphoderus bilineatus* (Water beetle sp.)
  - *Dytiscus latissimus* (Diver beetle sp.)


Diving beetle sp.

# How

## Networking

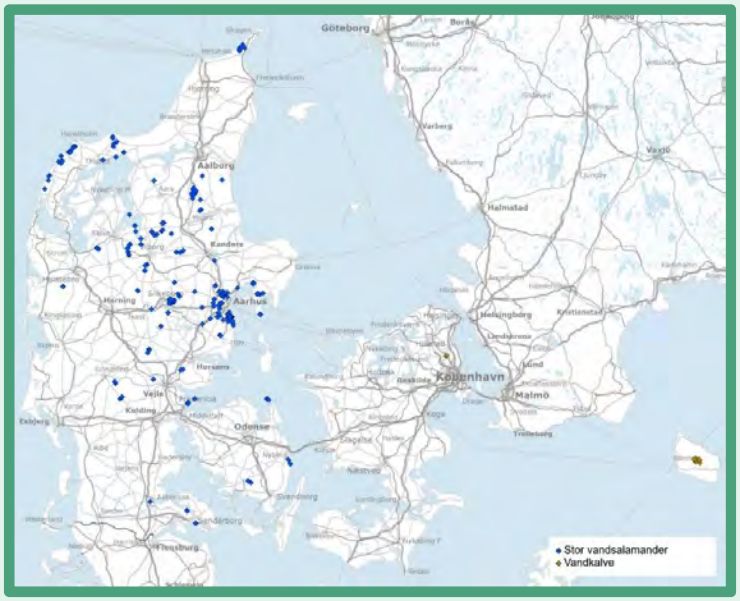
- Visited England in 2023
- Online conversations

## Finding and testing laboratories

- English lab – accredited
- Danish lab – not accredited

## Parallel testing

- Between the laboratories
- Between the lab-results and the results from the conventional monitoring program




## Testing on large scale

Great crested newt

- 230 locations

## Testing eDNA as a method for:

Water beetle and diving beetle

- 12 locations (few locations in DK)

# 4. Experiences and results up till now

Networking

Results

Potential challenges

# Networking

Department
for Environment
Food & Rural Affairs

# Networking

**Joanne Littlefair**

Queen university of London[1], England

- eDNA in other substrates
- Airborne eDNA

**Florian Leese**

Duisburg-Essen University, Germany

- Advantages >< disadvantages
- eDNA as a monitoring method
- Standardization methods
- Abundance

**Fraser Morgan og Joe Huddart**

NatureMetrics, England

- Great crested newt
- eDNA in water and soil

Department for Environment Food & Rural Affairs

**DEFRA**

England

- eDNA as a tool
- eDNA can't replace the current monitoring tools
- Early warning
- Collaboration across countries
- Participate in networks



1) Now working at University College London

# Results - qPCR


Diving beetle sp.


Water beetle sp.

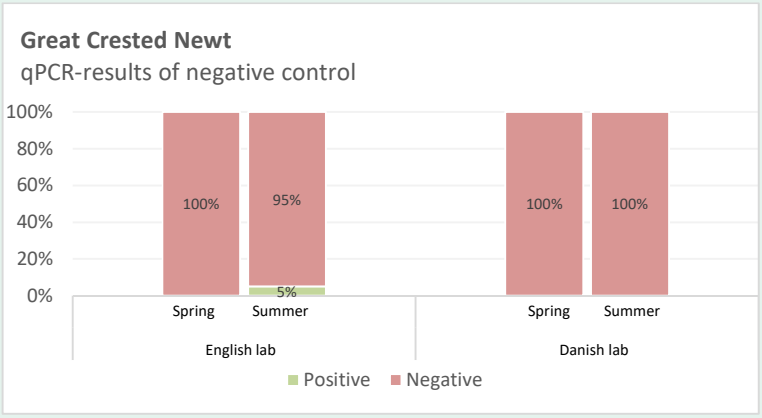

Great crested newt

# Results – Great crested newt *(Triturus cristatus)*

## Comparison of results

Comparison of qPCR results from the two labs and from the conventional method – results based on *positive* finds with the conventional method.



# Results – beetles *(Graphoderus bilineatus* and *Dytiscus latissimus)*

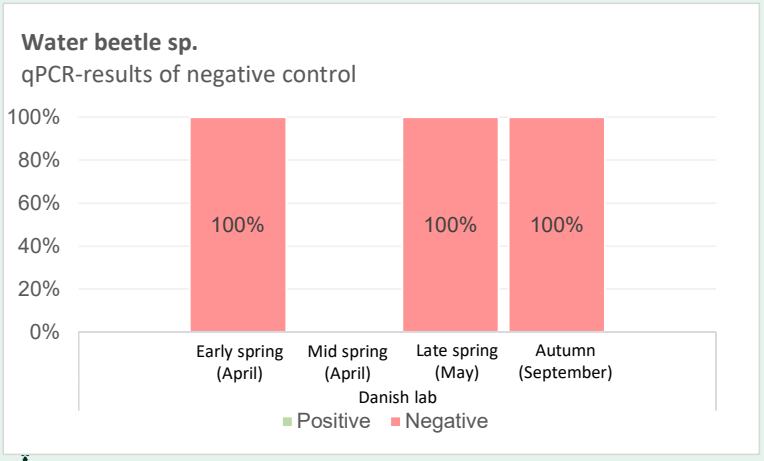
## Comparison of results

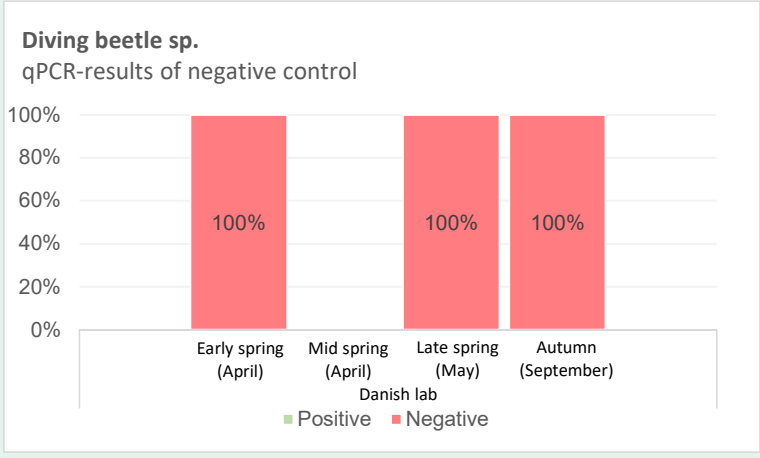
Comparison of qPCR results from the two labs and from the conventional method – results based on *positive* finds with the conventional method.









# Potential challenges


Diving beetle sp.


Water beetle sp.


Great crested newt

# Potential challenges – what have we thought of?

False positives (eDNA)

- False positives may be handled with a threshold/limit of detection
- Good hygiene
- No monitoring method is flawless – which is better?

How to convince others that eDNA works?

- Parallel testing (conventional >< eDNA) before and during
- Expert check ups at least once during a period (conventional)

How to argue for changing a monitoring method?

- Not changing WITHIN a period of monitoring, but from a new period
- Improving existing method
- Working towards harmonisation
- At least as good results, but cheaper in the long run
- Less subjective

Laboratories

- Blind tests
- Accreditation
- Iso-standards

# THANK YOU


Diving beetle sp.


Water beetle sp.


Great crested newt

# Possible subjects for discussion in plenum

Data reporting

- How to assure uniform data from all EU-countries?

Performance criteria instead of iso-standards?

- Avoiding specific primers that do not fit across countries.
- Avoiding being restricted to one method in the legislation and thus not being able to improve the method.

How to ensure harmonization across EU-countries?