eDNA as a tool for biodiversity assessments: what's next?

Eva BELLEMAIN

1) **Specific approach (eDNA barcoding)**
Experience from 3 case studies

2) **Multispecific approach (eDNA metabarcoding)**
- Markers
- Reference databases
- Case study: fish diversity assessments in streams

3) **Multigroup approach (global biodiversity screening)**
- Biodiversity inventories
- Bioindication

4) **Challenges / limits**
- Primer validation
- Laboratory requirements
- Bioinformatics

Pelobates fuscus

Emys orbicularis

Triturus cristatus

Aeshna viridis

Trichobilharzia sp.

Lithobates catesbeianus

Zingel asper

Mustela lutreola

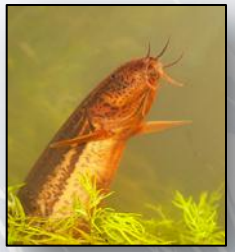
Procambarus clarkii

Neovison vison

Leucorrhinia pectoralis

Arvicola sapidus

Microtus oeconomus



Misgurnus fossilis

First study showing species detection using eDNA from water samples

Bullfrog
(*Lithobates catesbianus*)

→ Sampling of water (15 ml * 3 tubes per sampling location) over 9 ponds

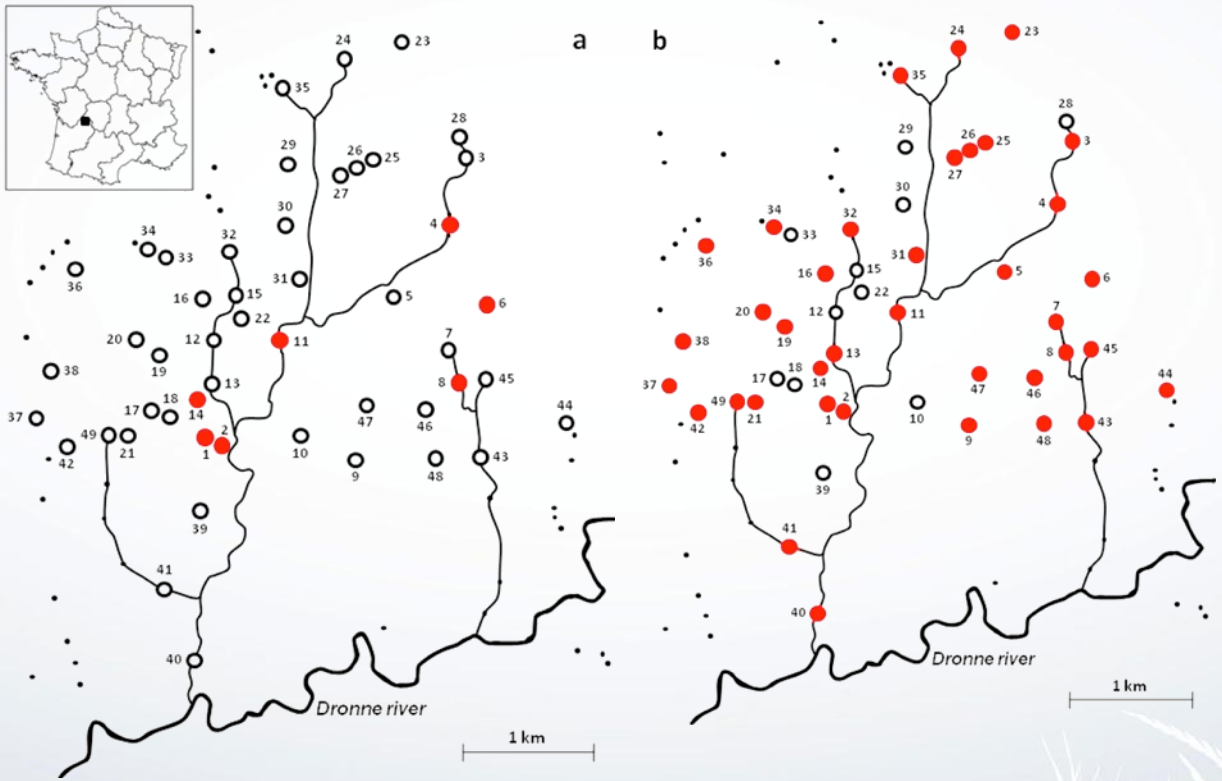
Detection

- High density 
- Low density 
- Absence 

Ficetola et al. 2008

Comparative study for the survey of the bullfrog on 49 sites (SW France)

- Classical survey: Diurnal observations & nocturnal calling surveys
- eDNA survey: 3 samples of water (15 mL) – specific primer pair for target organism



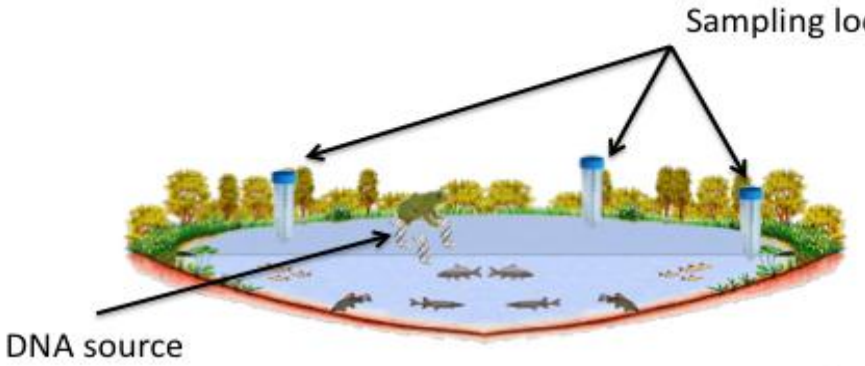
Classical survey: Detection on 7 sites

eDNA survey: Detection on 38 sites

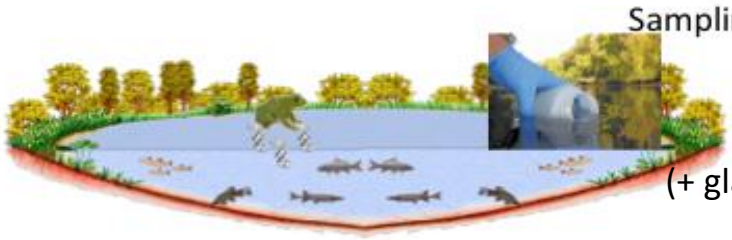
→ eDNA: 2,5 times faster in the field and 2,5 times cheaper than traditional surveys

Dejean et al. 2012

Local versus widespread sampling strategies

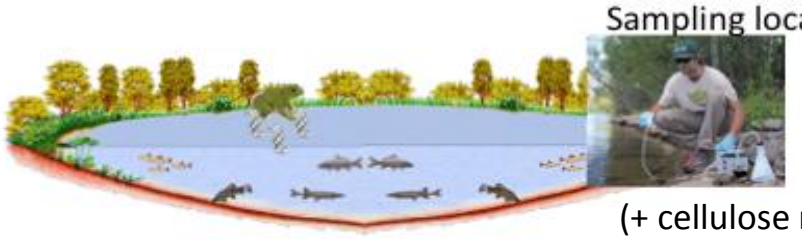


Ficetola et al., 2008



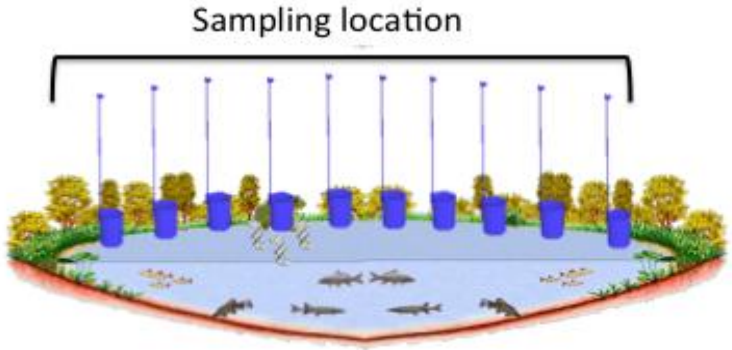
Jerde et al., 2011

(+ glass fiber filter)



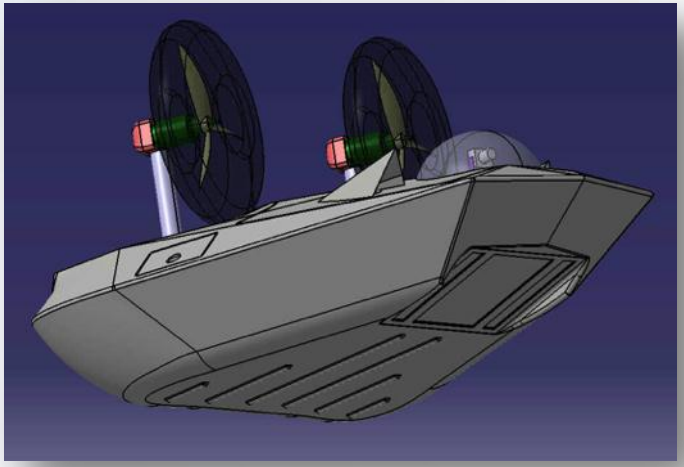
Goldberg et al., 2011

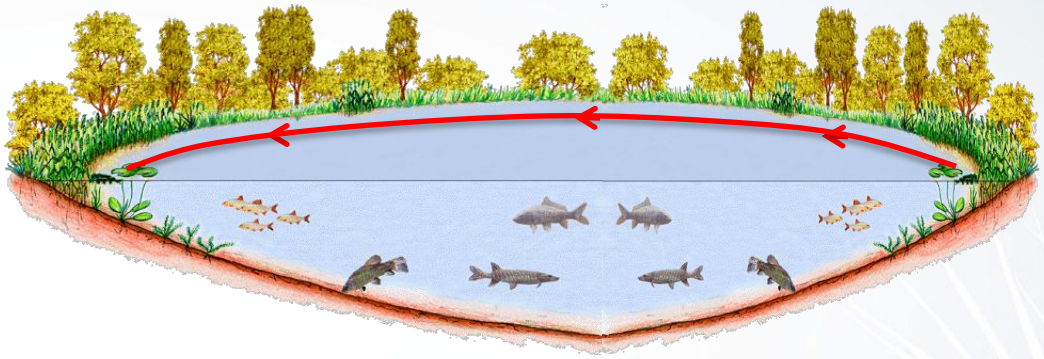
(+ cellulose nitrate filter)

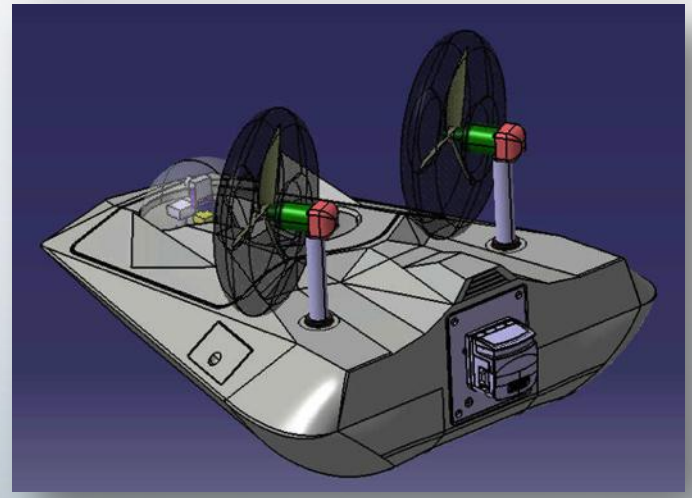


Proposed strategy

Perspectives: SPYBOAT → more exhaustive sampling
(optimised detection of rare species)



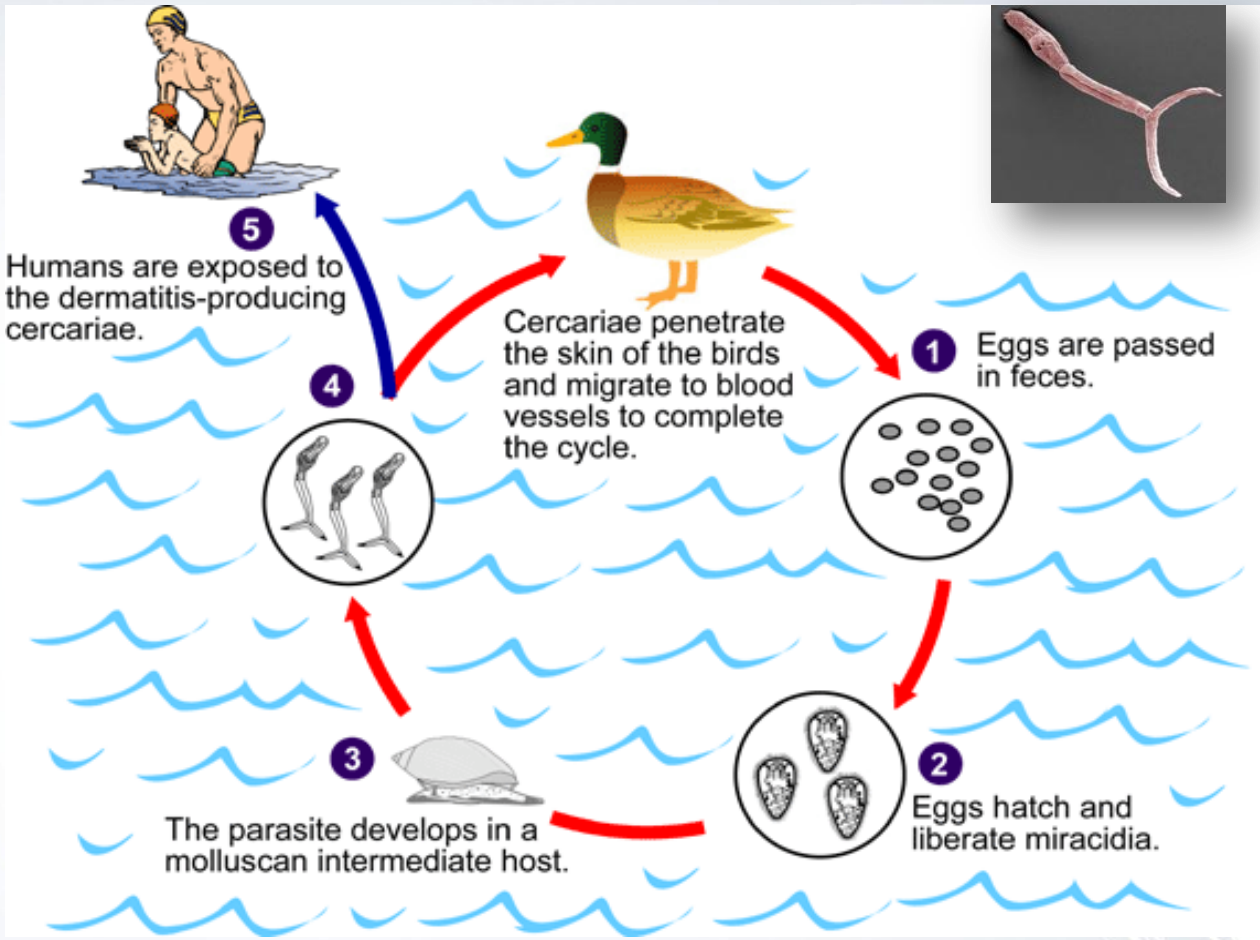




Airboat equipped with

- Peristaltic pump
- Interchangeable hull
- Remote control
- Video camera

Life cycle of *trichobilharzia* sp. causing cercarial dermatitis or swimmer 'itch



SILA l'oxygène à la source — **Annecy**

Antwerpen

Comparative study to evaluate the efficiency of the eDNA approach to detect *Trichobilharzia* sp. within natural swimming areas

Method for detecting the parasite in the field

1) Collect substrate on the bottom using quadrats

2) Isolate lymnea, count and measure them

3) Put the lymnea in the fridge then under a lamp to stimulate the release of cercariae

4) Count the number of cercariae released (usually less than 1%)

Proposed method for detecting the parasite using eDNA

1) Sample around the pool (water samples, filtration or sediments)

2) Develop short and specific primers

3) Extract DNA + qPCR

4) Sequence to identify the parasite

Positive control: Annecy site where the parasite was known to be present (0,8% in 2012)

→ *Trichobilharzia frankii* detected and identified in different samples

→ eDNA method efficient, less time consuming, easier to implement

→ Perspectives for the detection of other parasites and pathogens and the prevention of health risks for humans and animals

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eDNA metabarcodes

- Must amplify short DNA fragments
- Must be adapted for the different taxonomic groups
- Must be highly versatile (to equally amplify the different target DNAs)
- Must have a good taxonomic resolution (ideally to the species level)

Identified markers based on those criteria

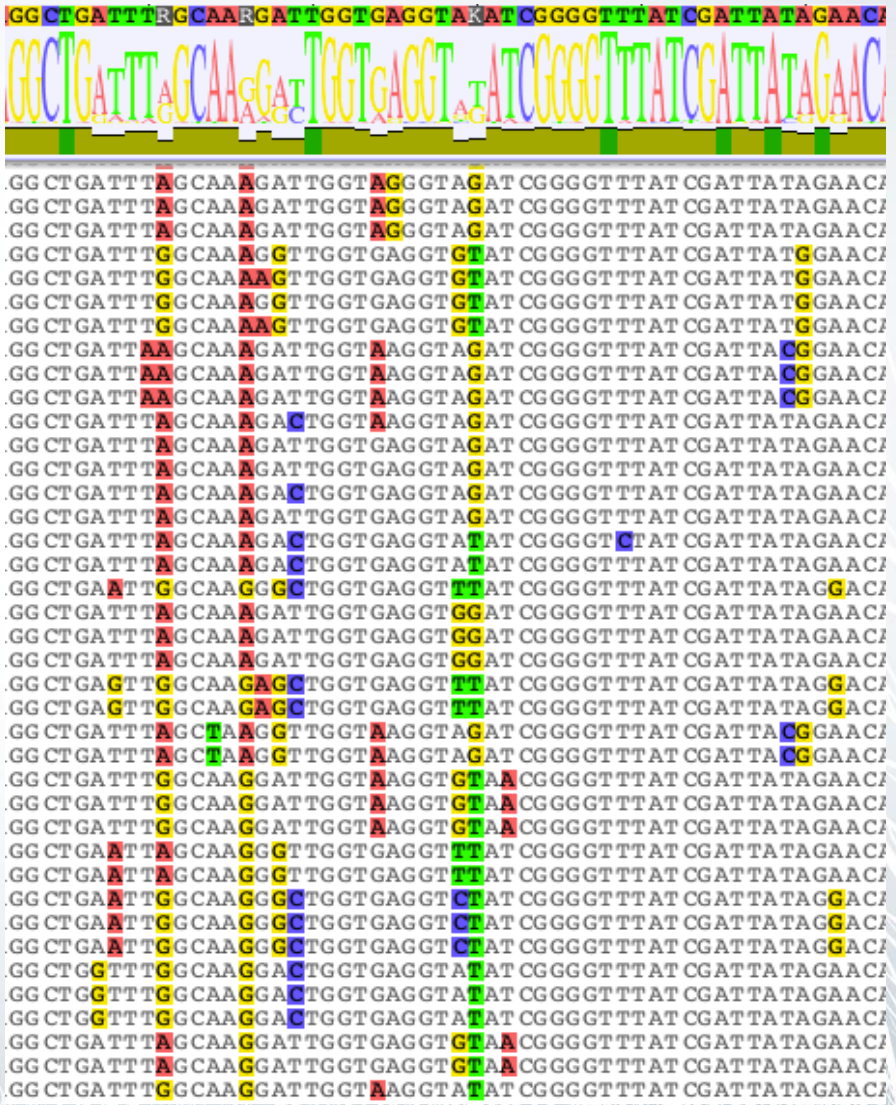
Group	Region	Amplified lenght
Amphibians	12S	23-59 bp
Teleostean fishes	12S	60-80 bp
Mammals	12S	71-87 bp
Chiroptera	12S	71-87 bp
Molluscs / Arthropods	16S	35-40 bp
Odonates	Under development	
Crayfishes	Under development	

Reference databases developed at SPYGEN (soon public)

- Chiroptera: 42 species in Europe

- Fishes: 83 species in Europe

- Amphibians: 47 species in Europe



Case study: biodiversity in streams

Electric fishing

ONEMA
Office national de l'eau et des milieux aquatiques

eDNA metabarcoding

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SPYGEN®

Multigroup approach

eDNA for biodiversity inventories and environmental watch

- Find out what organisms exist in a given area:
  - Optimise the detection and monitor rare, endangered or cryptic species
  - Evaluate conservation priorities of an area
  - Bioprospecting

- Allows an early detection of alien species:
  - ↗ chances of eradication
  - ↘ impact of the alien species on the ecosystem
  - ↘ cost of eradication action

- Allows to adapt conventional methods to the species present on the sites to gather additional field data (e.g. age classes, quantitative data, etc…)

eDNA for bioindication

→ Detect species that can be used to monitor the health of an environment or ecosystem (i.e. species whose function, population, or status can reveal the degree of integrity of an ecosystem)

e.g: Macroinvertebrates, diatoms

→ Produce aquatic biodiversity indices and follow its evolution through time

Which target groups would be interesting to survey?

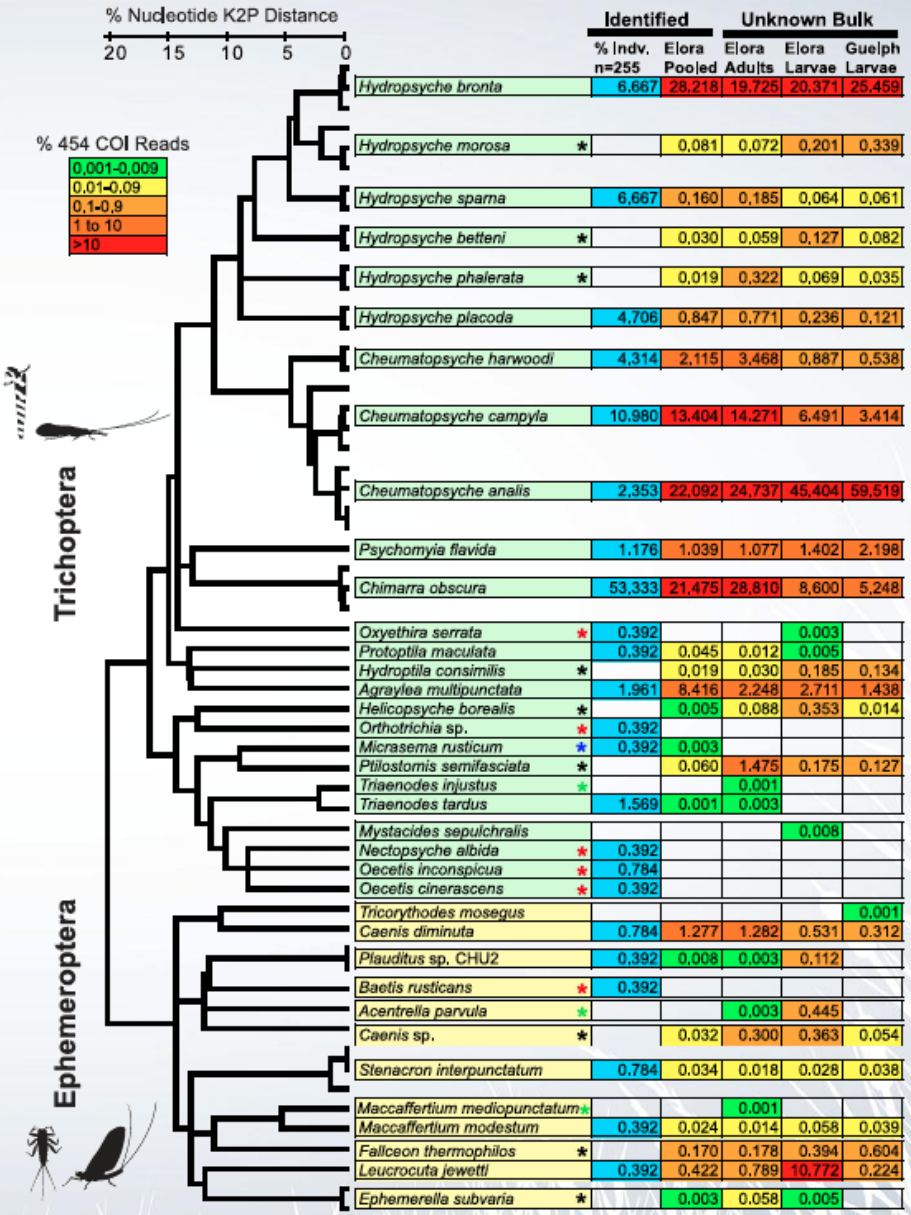
(Insects, molluscs, parasites, plants … ?)

Multigroup approach

Species composition of mayflies and caddisflies from bulk samples

(454 pyrosequencing using a 130 base COI mini-barcode)

→ Need for optimised metabarcodes



Hajibabaei et al. 2011

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Risk of errors:

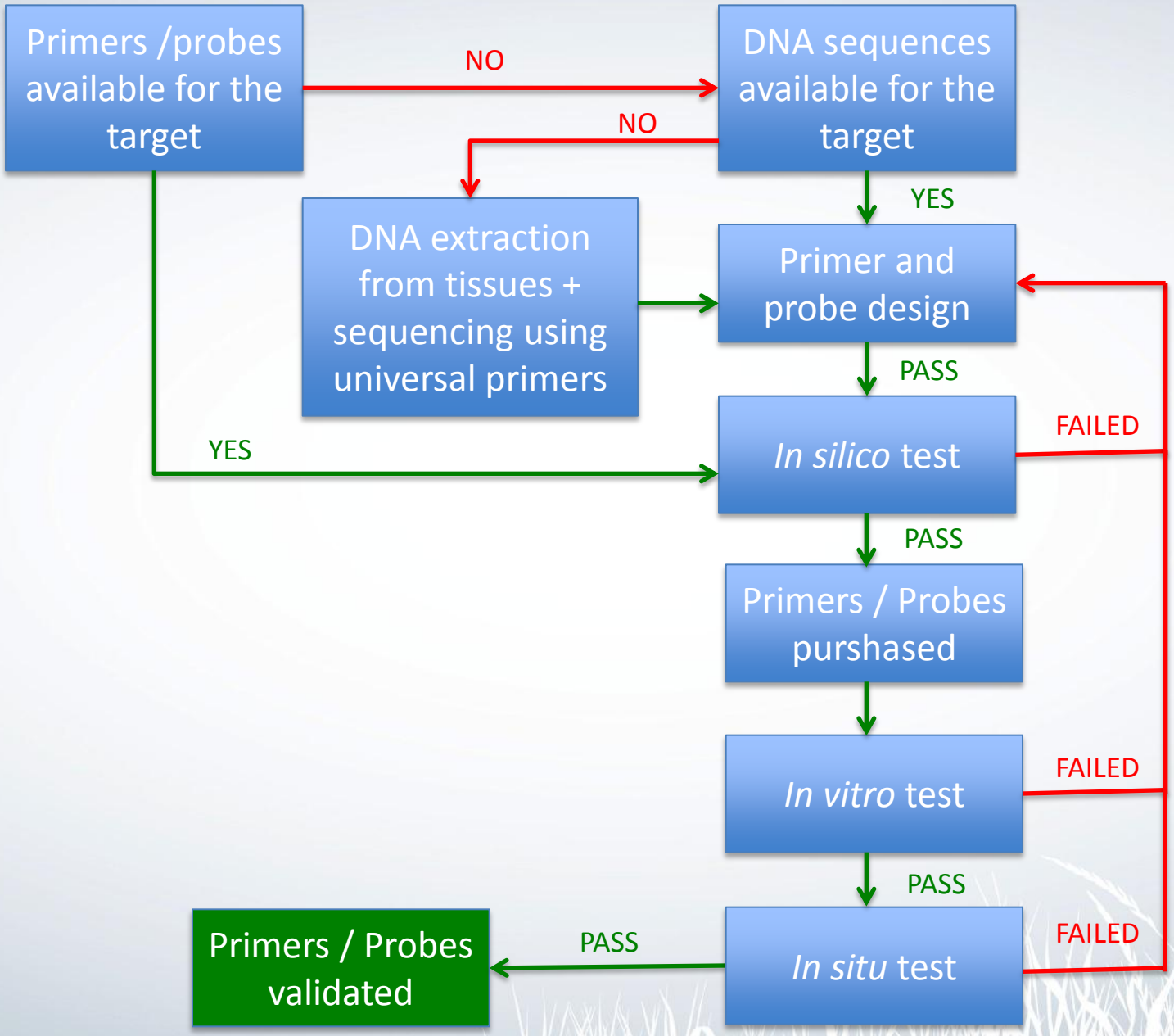
- False positives: species detected while it is not present

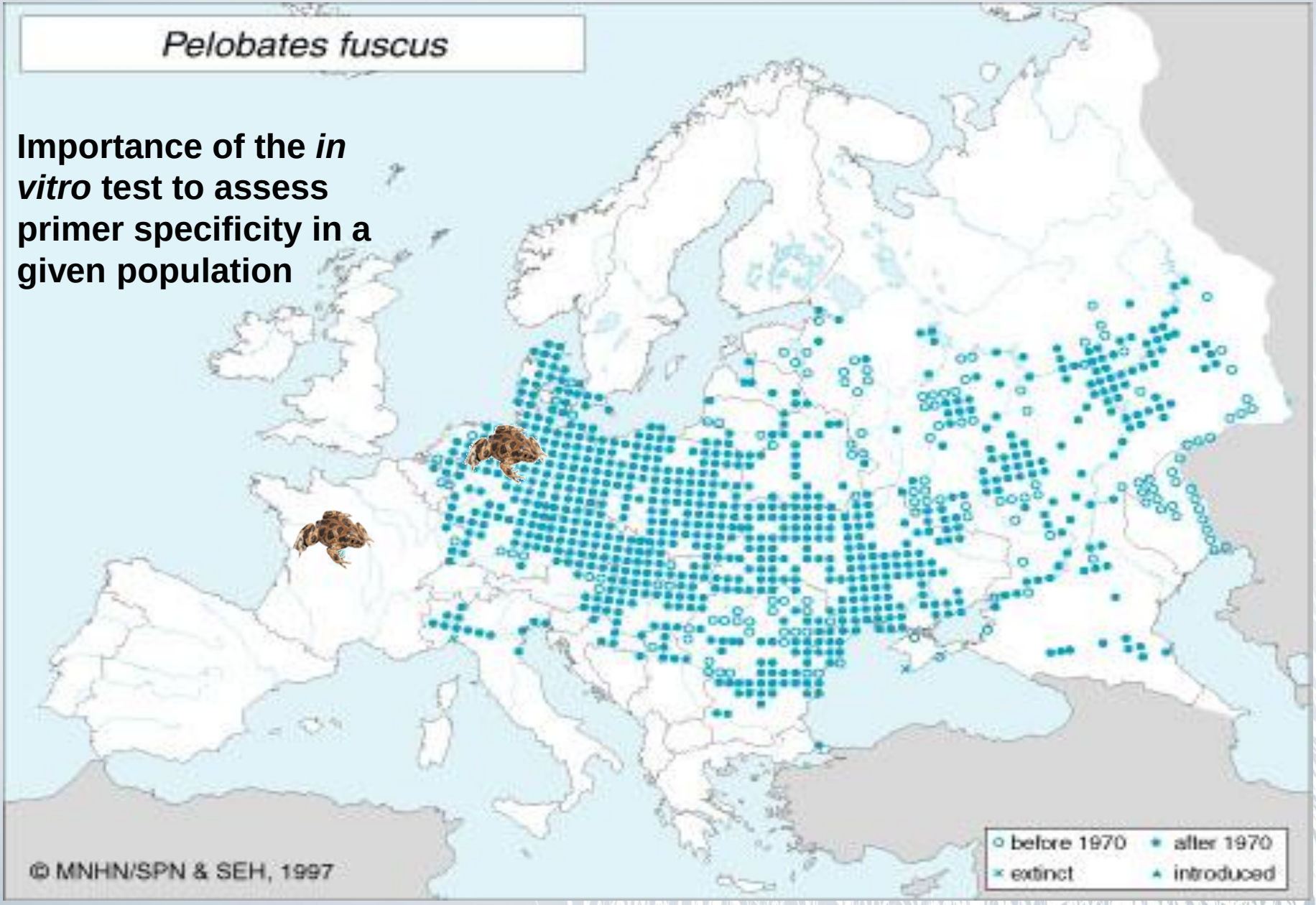
  → non-specificity of the primers used for DNA amplification
  → contaminations (in the field and/or in the laboratory)
  → protracted DNA persistence after the death of the organism
  → Poor reference database

- False negatives: species not detected while it is present

  → non-adapted primers
  → poor sampling
  → poor extraction protocol efficiency
  → presence of PCR inhibitors in the samples
  → Insufficient amount of DNA of the focus species/group in the ecosystem
  → Poor reference database

Primer validation





Importance of the *in vitro* test to assess primer specificity in a given population

Classical laboratory

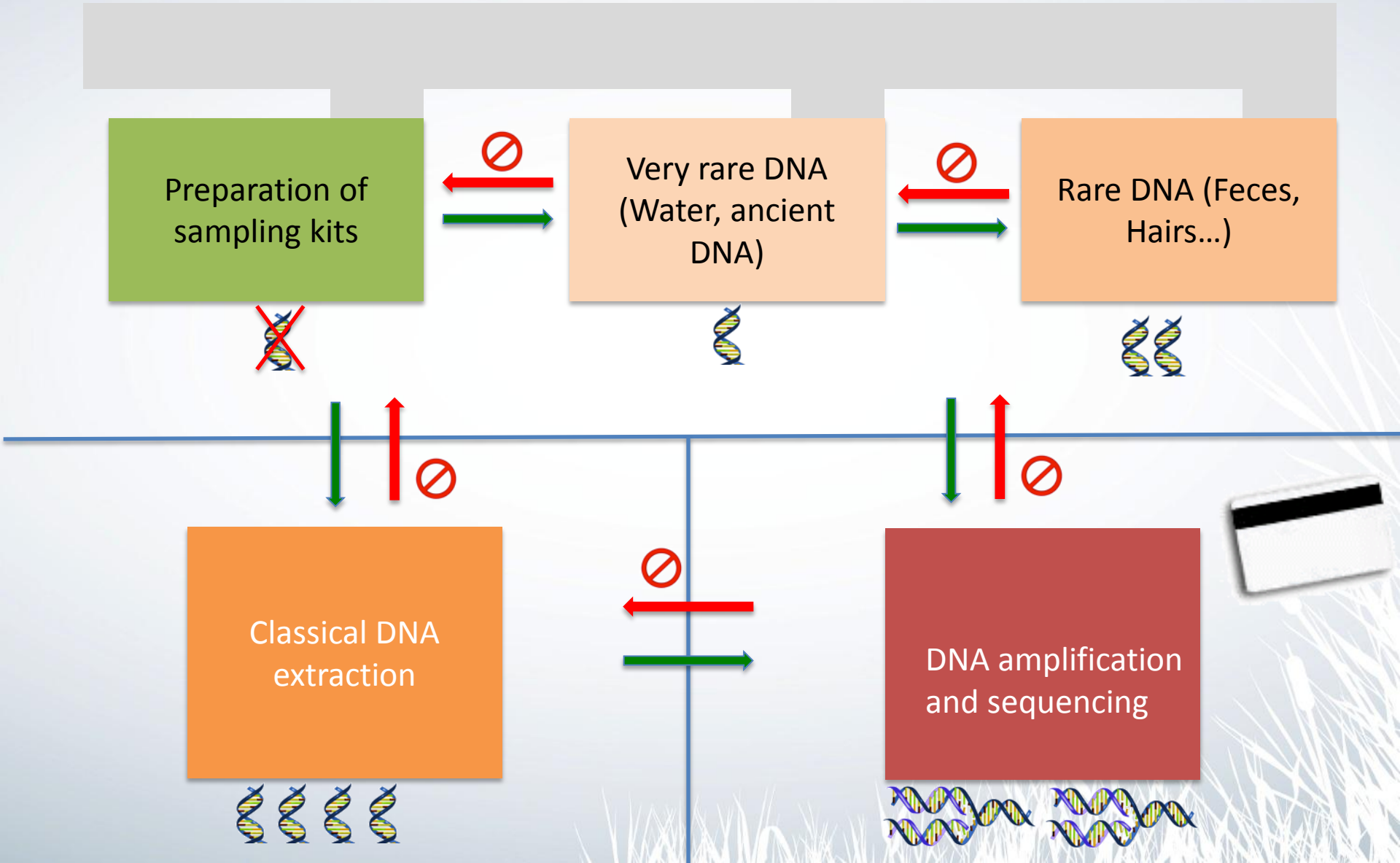
DNA extraction room

DNA amplification room

eDNA laboratory

- Physical separation between rooms
- Differential pressures between rooms
- UV treatments
- Special equipments
- Specific rules

eDNA laboratory:



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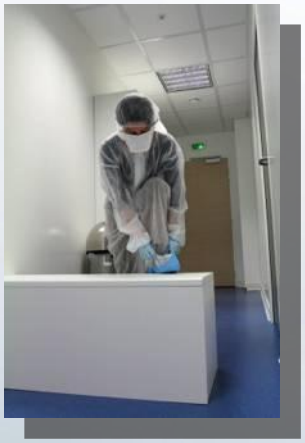

- Increasing amount of data produced
  (e.g. HiSeq 200: 6 billions of reads of 100 bases representing 3000 tons of paper if printed)

  → Need for more server storage capacity, computing, **reliable softwares**

  → Time consuming!

- Difficulties with amplification/sequencing errors (difficulties to work with rare species/MOTUs)

  → Need for improved bioinformatics softwares

- eDNA as a useful and promising tool for biodiversity assesment and conservation, complementing field methods

- Need for high quality reference databases from different countries, using defined markers → **Partnership important**

RAVON

De Vlinderstichting

ZOOGDIER VERENIGING

(fishes, amphibians, mammals, insects)

PROVINCIE ANTWERPEN

(macro-invertebrates)

muséum genève

(chiropters)

→ A consortium (similar to CBOL) would be very useful!

Thanks for your attention!

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