Sveriges lantbruksuniversitet
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Fish, crayfish & mussels as eDNA

in environmental monitoring?



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Why eDNA as monitoring?

Red listed or alien species ⟶ very few
= Hard to detect

Trad Methods:

- Specific to the study organisms (non generalized)
- Time consuming
- Expensive
- Some methods is counterproductive for conservation



Why eDNA as monitoring?

A new **tool** for monitoring:

- Detection: Very sensitive (DNA-fragments)
- Non destructive (water sampling)
- More effortless (cheap, less time & effort)
- A more generalized approach (for EVERY species)

BUT:

- New approaches in the field & lab (risk to contaminate)
- More skilled labor at lab analysis (bioinformation)



What's eDNA?

- Cocktail of DNA-fragments
  (**water**, air, earth, sediment, ice, fossils)
- Living or dead tissue
  (egg, larvae, mucus, scales, feces)
- Particulate or disolved material
  (different state of decomposition)

NOTE: eDNA = environmental DNA (aDNA = ancient DNA)

What's eDNA?

- Free floating/dissolved in water
- A "snapshot" in time/space
- Decomposes over time

Freshwater: DNA decompose within 7-30 dagar (Dejean *et al*, 2011; Thomsen *et al*, 2011)
Sea/coast: DNA decompose within 3-7 dagar (Thomsen *et al*, 2012)

eDNA can be sampled!

Several methods exists (but NO stand protocols!):

- Volume of water: 15ml – 60l (into one or several samples)
- How to collect: ruttner, ramberg, bottle, real-time filtering…
- Where, When to collect…

…and concentrated!

What filter/method to use?
Filter kit (0.22 – 1.2µm) **For fish:** 0.45µm

- Filtration in the field (real-time) or at lab
- Pre-filtering is possible (filtering in 2 steps)

We used:

MoBio Lab Systems Kit
0.45µm
Disposable filters (reduce contamination)
Sampled 5l water
Filter 2-5 liter (depending on algae/humic…)

A **frozen filter** is preserved!
The site can then be ”**revisited**” again… and again…

Can eDNA be used for monitoring?

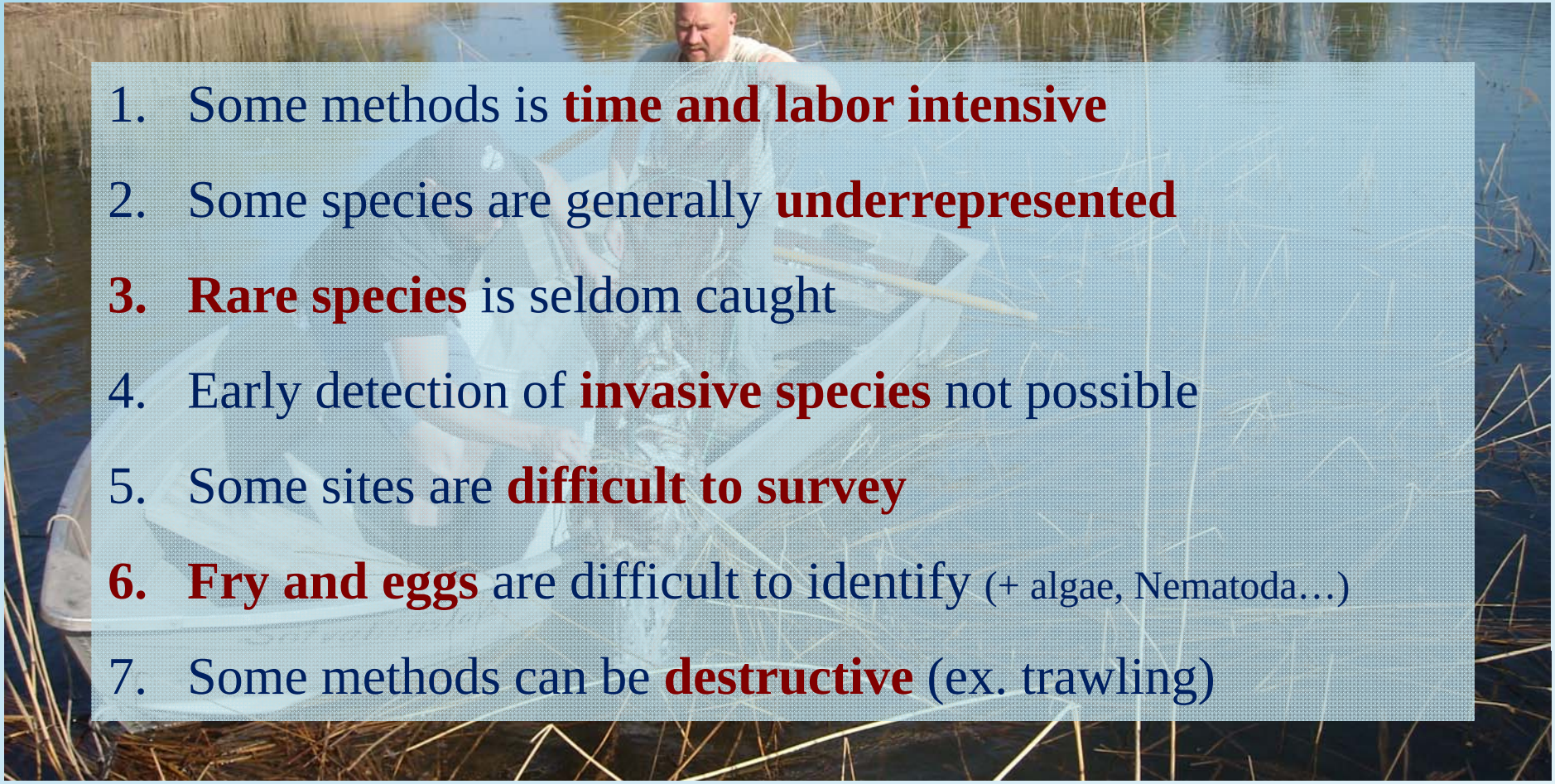
So….

- Aquatic species are hard to detect
- eDNA: Quick, sensitive and time/cost effective
- eDNA: Simple method (water sampling)

May complement traditional monitoring methods!



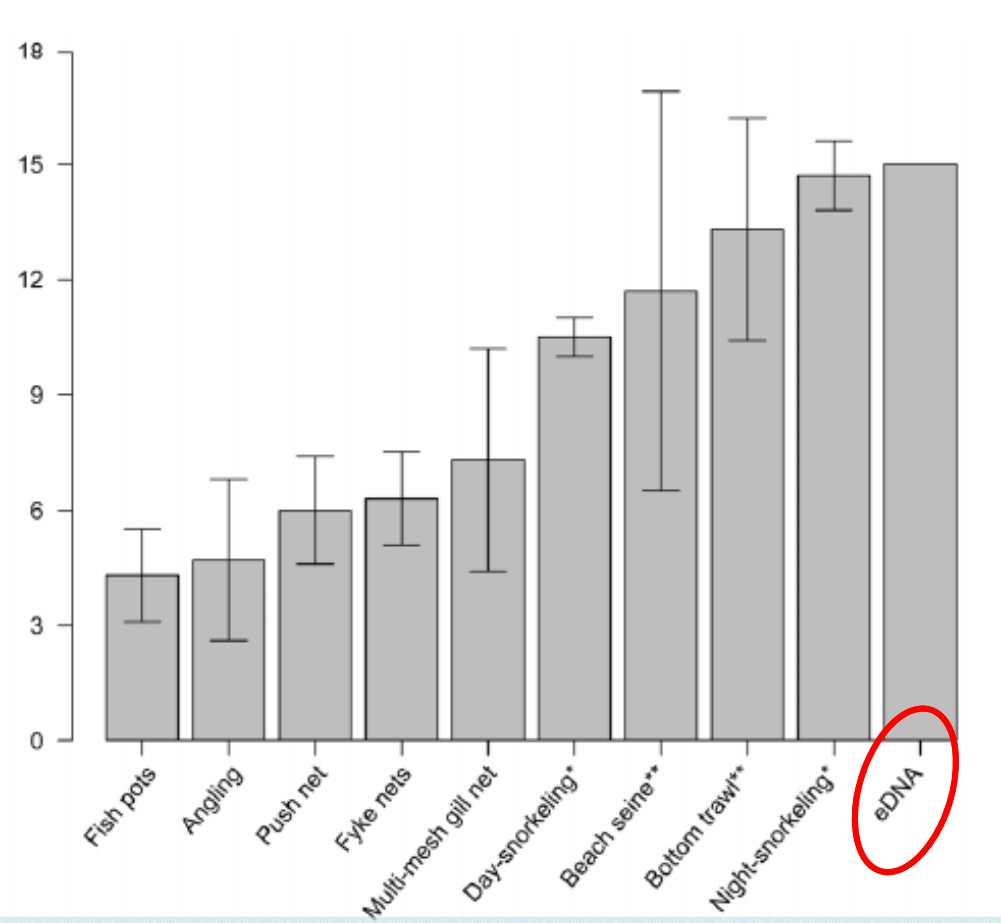
Trad methods in environmental monitoring



1. Some methods is **time and labor intensive**
2. Some species are generally **underrepresented**
3. **Rare species** is seldom caught
4. Early detection of **invasive species** not possible
5. Some sites are **difficult to survey**
6. **Fry and eggs** are difficult to identify (+ algae, Nematoda…)
7. Some methods can be **destructive** (ex. trawling)

eDNA vs trad methods

Number of fish species caught with different trad methods
(East coast of Denmark)



Källa: Thomsen *et a*l, 2012. Detection of a diverse marine fish fauna using eDNA from seawater samples

eDNA as an example in monitoring

Sample	Application	Studies of importance
Applications with potential for conservation biology and policy-making decisions		
Blood meal	Species detection	DNA of rare mammals such as the elusive Truong Son muntjac (*Muntiacus truongsonensis*) identified in leeches collected in Vietnam
Faeces Stomach contents	Population genetics	Highly fragmented and isolated populations of giant panda (*Ailuropoda melanoleuca*) were analysed and landscape genetic patterns, divergence time, and population structure identified
Honey	Species detection	Plant and insect DNA identified in just 1 ml of honey
Seawater Ballast water	Species detection	Harbour porpoise (*Phocoena phocoena*) and long-finned pilot whale (*Globicephala melas*) detected in the western Baltic
Snow	Species detection	Wolf (*Canis lupus*) DNA isolated from blood spots in the Italian Alps and Arctic fox (*Alopex lagopus*) DNA isolated from footprints
Soil	Species detection	Vertebrate mitochondrial DNA (mtDNA) identified in soil samples collected in a zoological garden and a safari park matched to the elephant and tiger inhabitants, respectively
Applications with potential for ecology (including palaeo- and macroecology)		
Cave sediments	Reconstructing past flora and fauna	Extinct biota identified from cave sediment in New Zealand, revealing two species of ratite moa and 29 species of plants from the prehuman era
Fresh water	Species detection and biomass estimation	Diversity of rare and threatened freshwater fish, amphibians, mammals, insects, and crustaceans was quantified in eDNA from small water samples collected in lakes, ponds, and streams
Ice cores	Reconstructing past flora and fauna	Plant and insect diversity from the past million years was catalogued from deep ice cores in Greenland
Nunatak sediments	Reconstructing past flora and fauna	Reconstruction of vegetation from the end of the Holocene Thermal Maximum [5528 ± 75 calibrated years before present (BP)] from bedrock protruding through ice sheets (nunatak sediments)
Permafrost	Reconstructing past flora and fauna, habitat conservation	Fungal, bryophyte, enchytraeid, beetle, and bird DNA identified in frozen sediment of late-Pleistocene age (circa 16 000–50 000 years BP)
Saliva/twigs	Species detection	DNA in saliva on browsed twigs identified browsing moose (*Alces alces*), red deer (*Cervus elaphus*), and roe deer (*Capreolus capreolus*), amplifying in some samples up to 24 weeks after the browsing event
Applications with potential for the understanding of ecosystems		
Air	Invasive-species detection	The presence of genetically modified organisms was detected from samples of air containing low levels of pollen
Fresh water	Wildlife-disease detection	Detecting the chytrid fungus *Batrachochytrium dendrobatidis*, which is likely to be a primary cause of amphibian population declines, in water samples
Fresh water	Invasive-species detection	The American Bullfrog (*Lithobates catesbeianus*) was successfully identified, showing that early detection of invasive species at low densities is possible and has implications for management

Source: Bohmann *et a*l, 2014. eDNA for wildlife biology and biodiversity monitoring (review)

Pilot project: Fish, crayfish and mussles as eDNA: testing methodology and usability

Question at issue:

1. Can we detect known/unknown species qualitatively (fish, crayfish & mussels) in a lake or stream (YES/NO)?

2. Can we develop a fast, simple, cost-effective and general methodology?

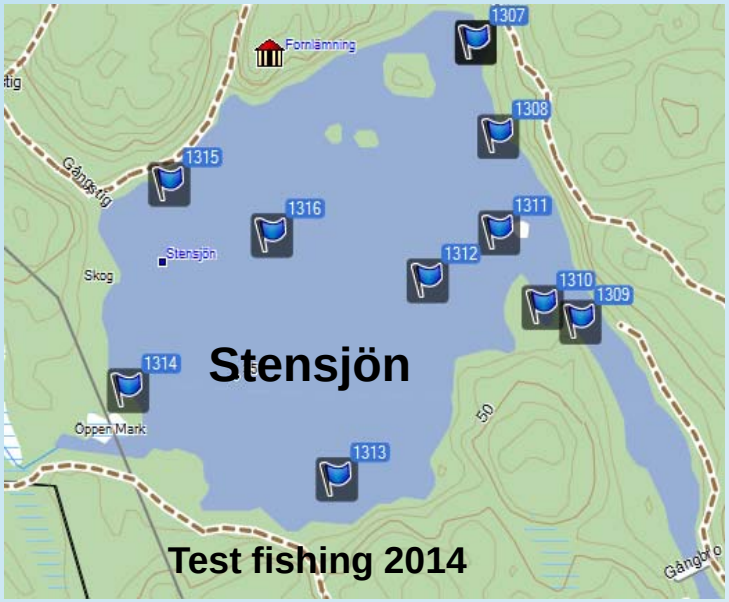
Vatten	Art (sve)	Art (latin)	Kommentar	Källa
Mälaren, Stångholmen	Siklöja	Coregonus albula	Kallvattenart	NORS
Mälaren, Stångholmen	Spetsig målarmussla	Unio tumidus		Musslor i Mälaren (NRM)
Mälaren, Stångholmen	Större dammussla	Anodonta cygnea		Musslor i Mälaren (NRM)
Mälaren, Stångholmen	Sutare	Tinca tinca	Underrepresenterad i p-fiske	NORS
Mälaren, Stångholmen	Vandrarmussla	Dreissena polymorpha		Musslor i Mälaren (NRM)
Mälaren, Stångholmen	Vimma	Abramis vimba	Underrepresenterad i p-fiske (sällsynt)	NORS
Mälaren, Stångholmen	Ål	Anguilla anguilla	Underrepresenterad i p-fiske	NORS
Mälaren, Stångholmen	Äkta målarmussla	Unio pictorum		Musslor i Mälaren (NRM)
Norasjön	Abborre	Perca fluviatilis		Anders Jonsson, fiskare (073-9825418)
Norasjön	Allmän dammussla	Anodonta anatina		NRM, Nationell övervakning av stormusslor i Norasjön, 2013
Norasjön	Braxen	Abramis brama		Anders Jonsson, fiskare (073-9825418)
Norasjön	Flat dammussla	Pseudanodonta complanata		NRM, Nationell övervakning av stormusslor i Norasjön, 2011
Norasjön	Gädda	Esox lucius	Underrepresenterad i p-fiske	Anders Jonsson, fiskare (073-9825418)
Norasjön	Gös	Stizostedion lucioperca		Anders Jonsson, fiskare (073-9825418)
Norasjön	Mört	Rutilus rutilus		Anders Jonsson, fiskare (073-9825418)
Norasjön	Spetsig målarmussla	Unio tumidus		NRM, Nationell övervakning av stormusslor i Norasjön, 2012
Norasjön	Större dammussla	Anodonta cygnea		NRM, Nationell övervakning av stormusslor i Norasjön, 2014
Norasjön	Sutare	Tinca tinca	Underrepresenterad i p-fiske	Anders Jonsson, fiskare (073-9825418)
Norasjön	Ål	Anguilla anguilla	Underrepresenterad i p-fiske	Anders Jonsson, fiskare (073-9825418)

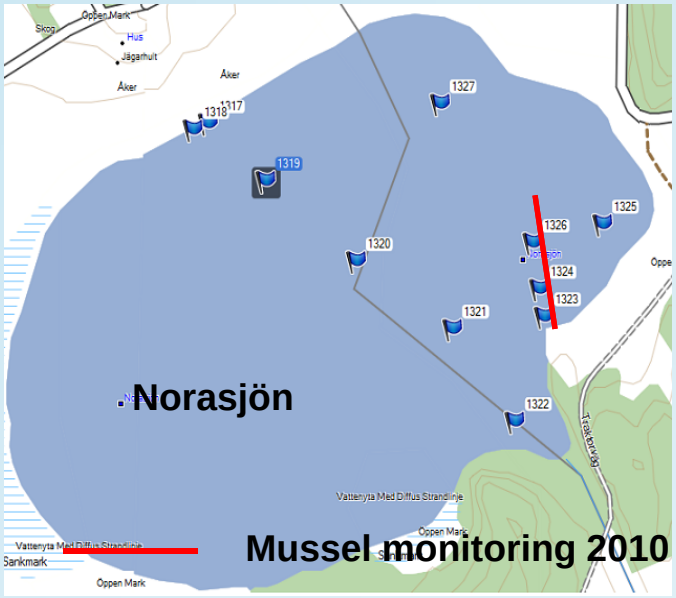
Pilot project: Fish, crayfish and mussles as eDNA: testing methodology and usability

Field work :

4 lakes och **2 streams** were chosen with known species (monitoring)

- **Stensjön** (Stockholm): signal crayfish & fish
- **Mälaren** (Stockholm): zebra mussel, signal crayfish & fish
- **Norasjön** (Trosa): stormusslor & fish
- **Öresjö** (Trollhättan): noble crayfish & fish





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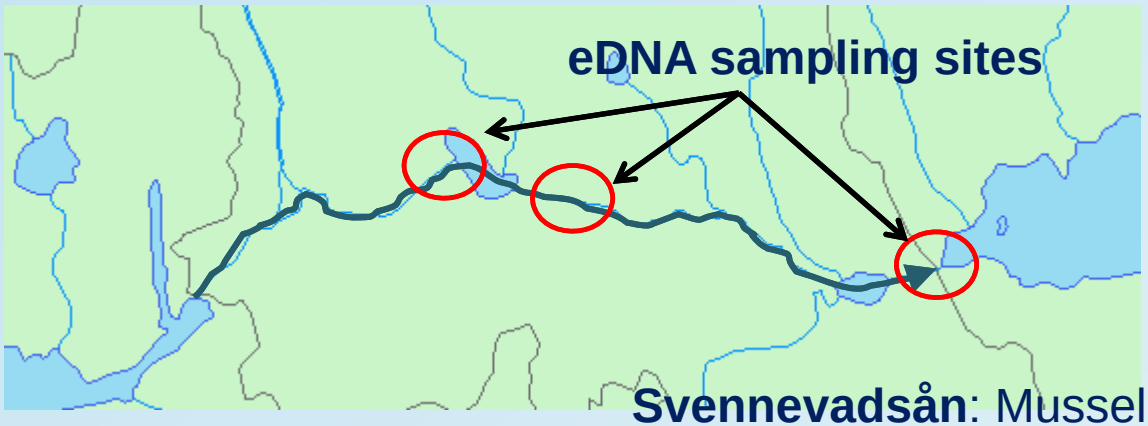
- **Svartån** (Karlskoga): Freshwater pearl mussel, signal crayfish & fish
- **Svennevadsån** (Hallsberg): stormusslor, signal crayfish & fish



Svennevadsån: Mussel monitoring 2008,
Test fishing: Tisaren (1997), Sottern (1982)

Pilot project: Fish, crayfish and mussles as eDNA: testing methodology and usability

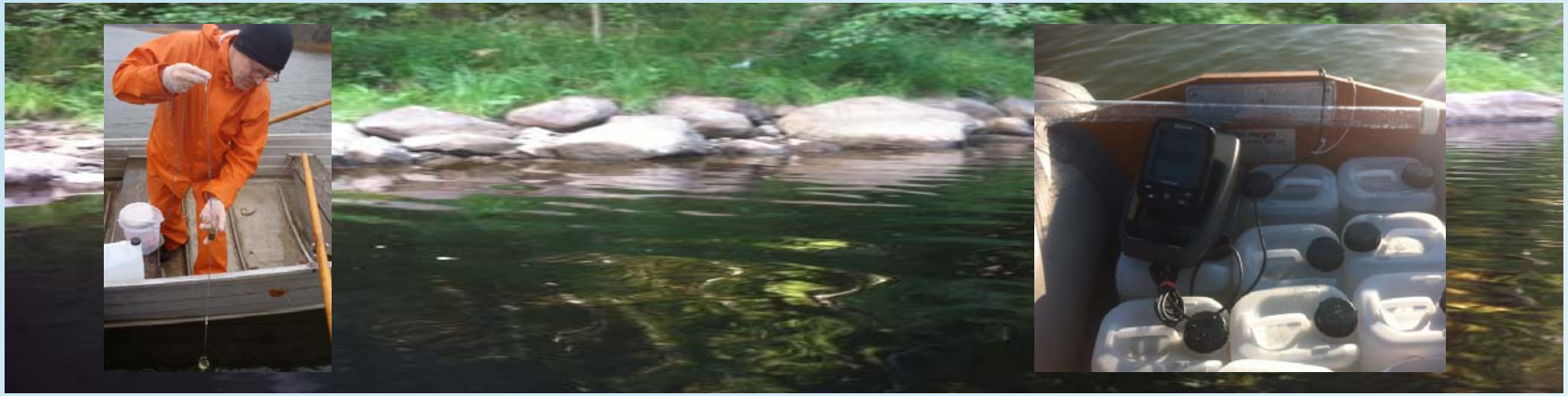
Field work (methodology):

1. Water samples: 5l from 10 sites with different depths (0,3-12m)
2. Sampling: Ramberg tube (1l) 5-6 samples on each site.
3. Neutral samples: directly in bottle (contamination control)
4. Depth, temperature, bottom substrate etc for each site
5. Filtering: The samples were filtered & frozen within 24 hours (on lab)
6. **Lab analyses & some results will be presented later…!**

Pilot project: Fish, crayfish and mussles as eDNA: testing methodology and usability

Improve field methodology:

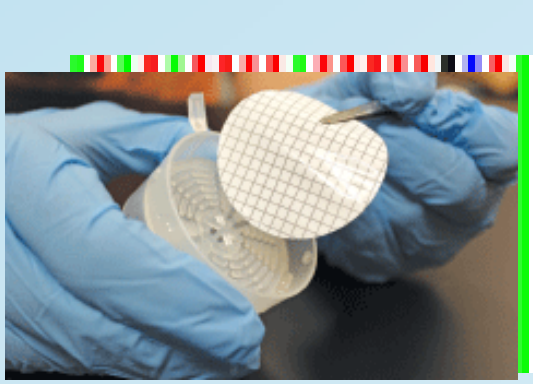
1. How to sample
   - How many liters?
   - How to filter & preserve the residue?
2. Develop a generalized protocol (if possible)
3. Identify sources of error (false positives/negatives)

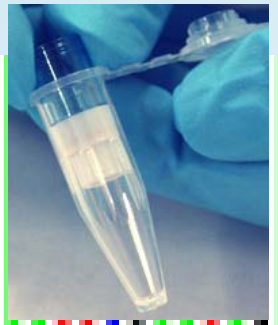


Pilot project: Fish, crayfish and mussles as eDNA: testing methodology and usability

Improve lab methodology :

1. Identify DNA sequences unique for target species
   - Sequence DB (barcoding-library) ex GenBank, BOLD, DNA-nyckeln
   - Create sequences from live tissue
2. Develop primers to amplify the sequence (PCR)
3. Ensure specificity (test it!)





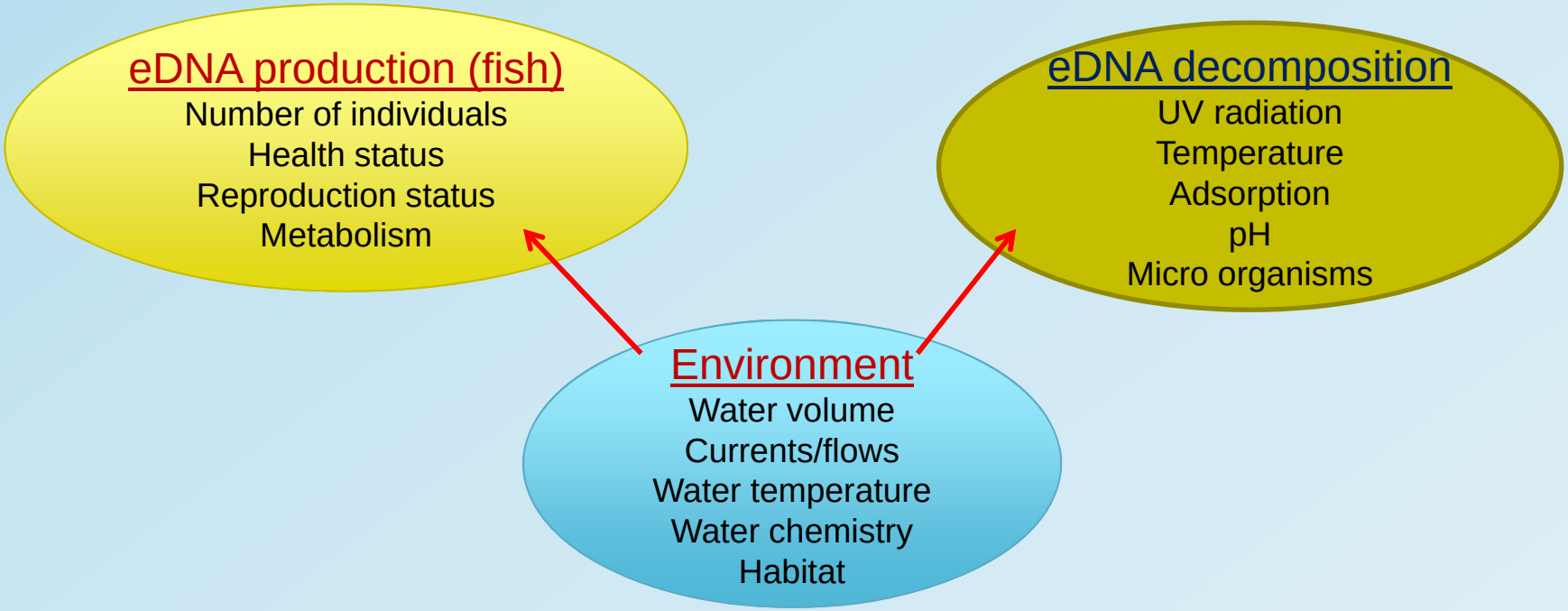
Info: Barcode = DNA-sequence unique for a single species

Challenges…

- Unanswered questions (age, sex, size, numbers…)
- Positive results **do not** answer:
  1. **How much/many** (quantitative density)
  2. **How close** (lake, stream, coast, sea…)
  3. **How fresh** (days – weeks)?
- Many environmental factors affect eDNA (several unknown)

[eDNA] = biomass/number of individuals etc?

[eDNA] = Production – decomposition



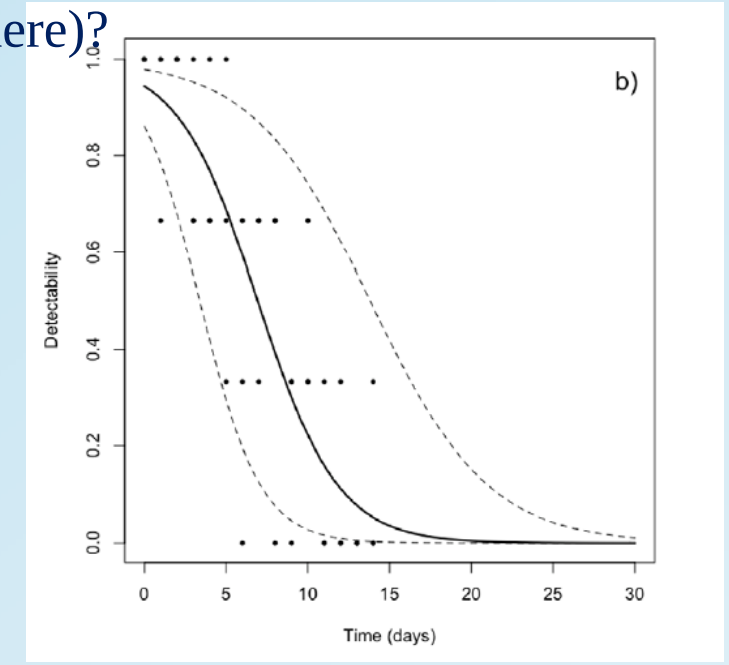
Source: M. Laramie (2014) US Geological Survey. eDNA - A new tool for monitoring imperiled species

Challenges…

Standardized protocols (sampling – final analysis)

1. **DNA movements** in water (from where)?
2. **DNA persistence** in water (time)?

Where
When
How
} to sample?

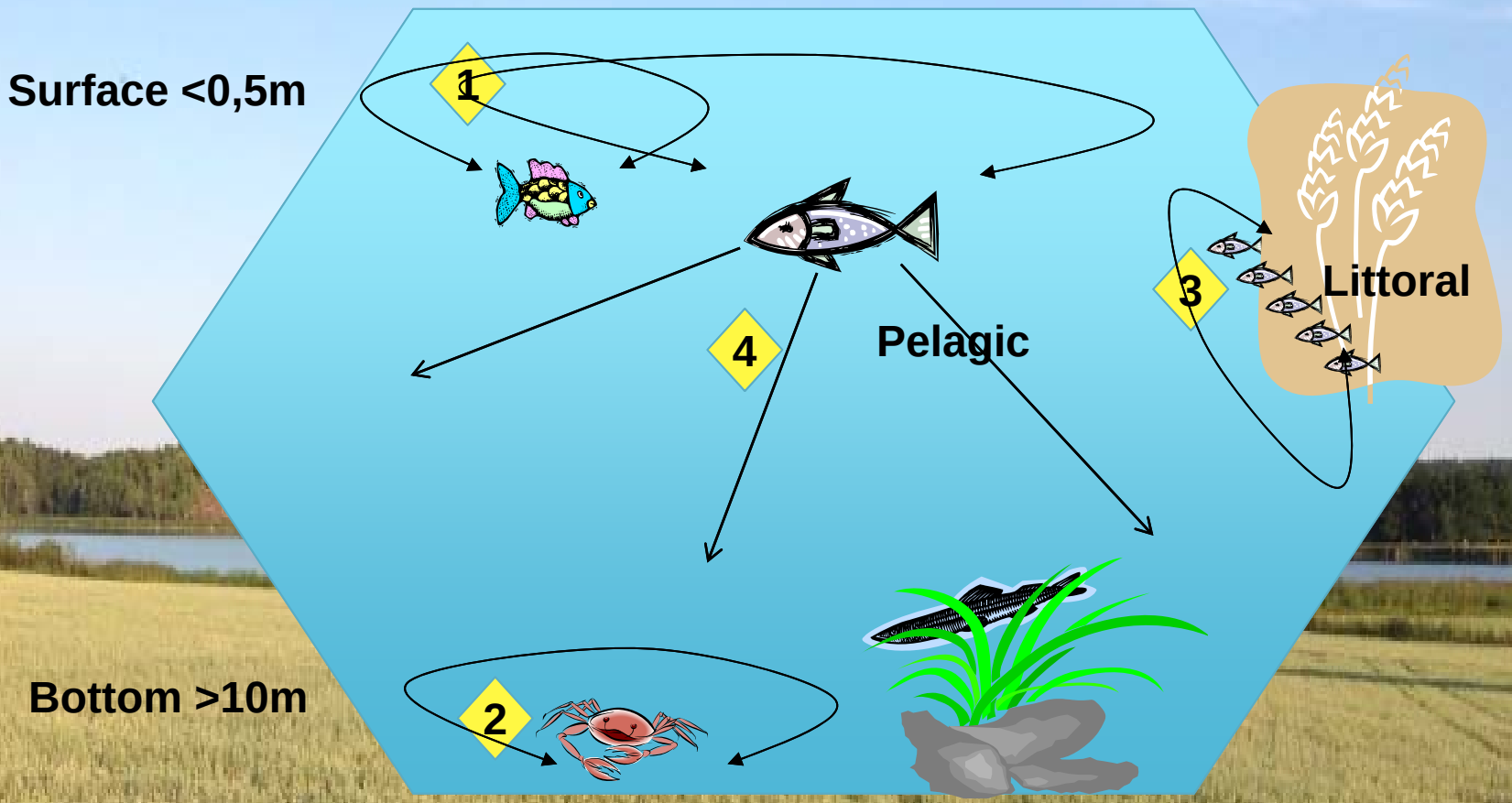


Source: Dejean et al. 2012
Case study: stagnant water

Foto: U.S. Geological Survey

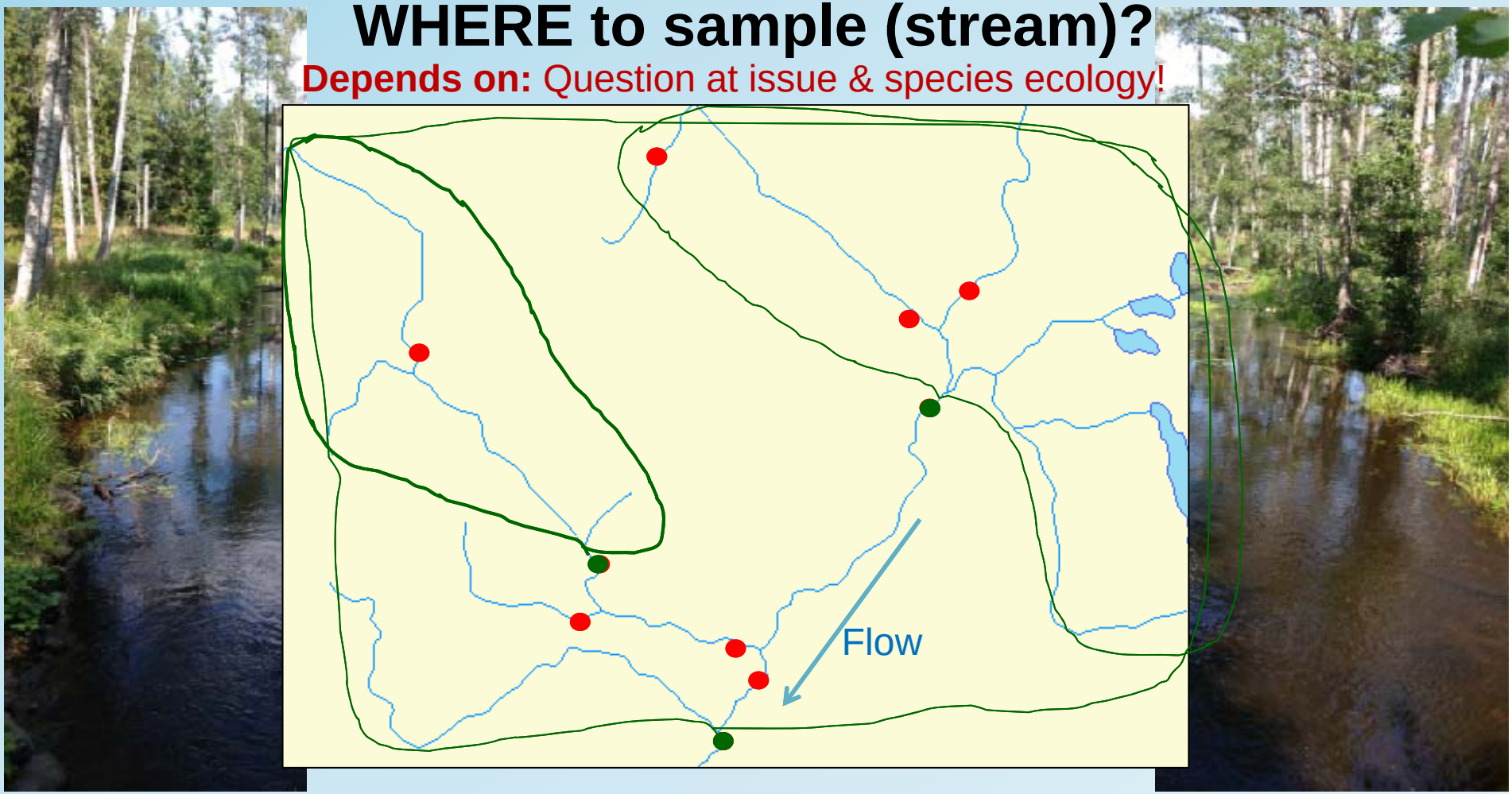
WHERE to sample (lake)?

Depends on: Question at issue & species ecology!



WHERE to sample (stream)?

Depends on: Question at issue & species ecology!



Further downstream: larger area upstream

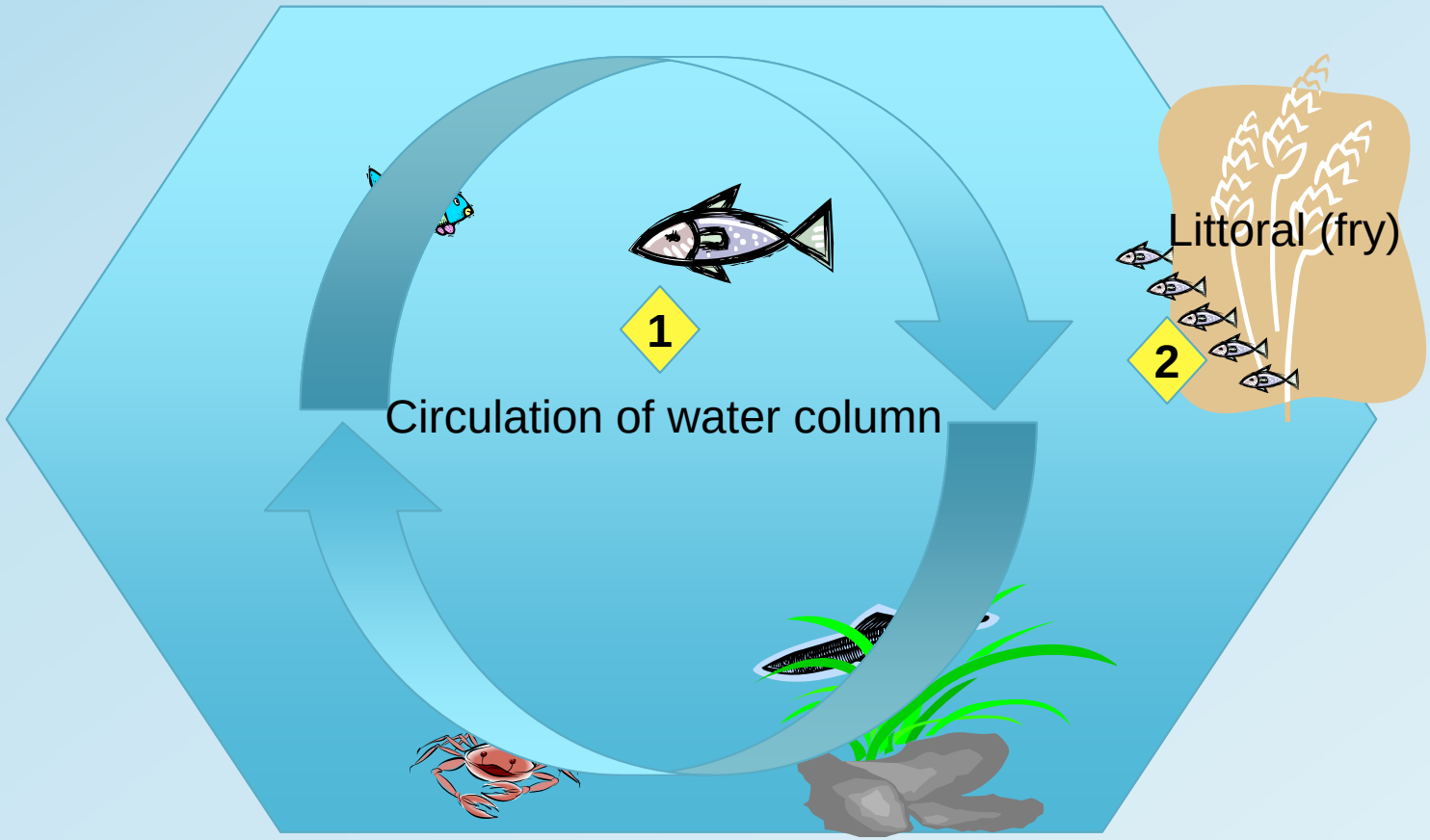
Estimate biodiversitet within a catchment area

Streams: eDNA transported >9km downstream & still detectable (Deiner. 2014. Transport distance of eDNA in a river)

WHEN to sample?

Spring:
April-June

Depends on: Question at issue & species ecology!

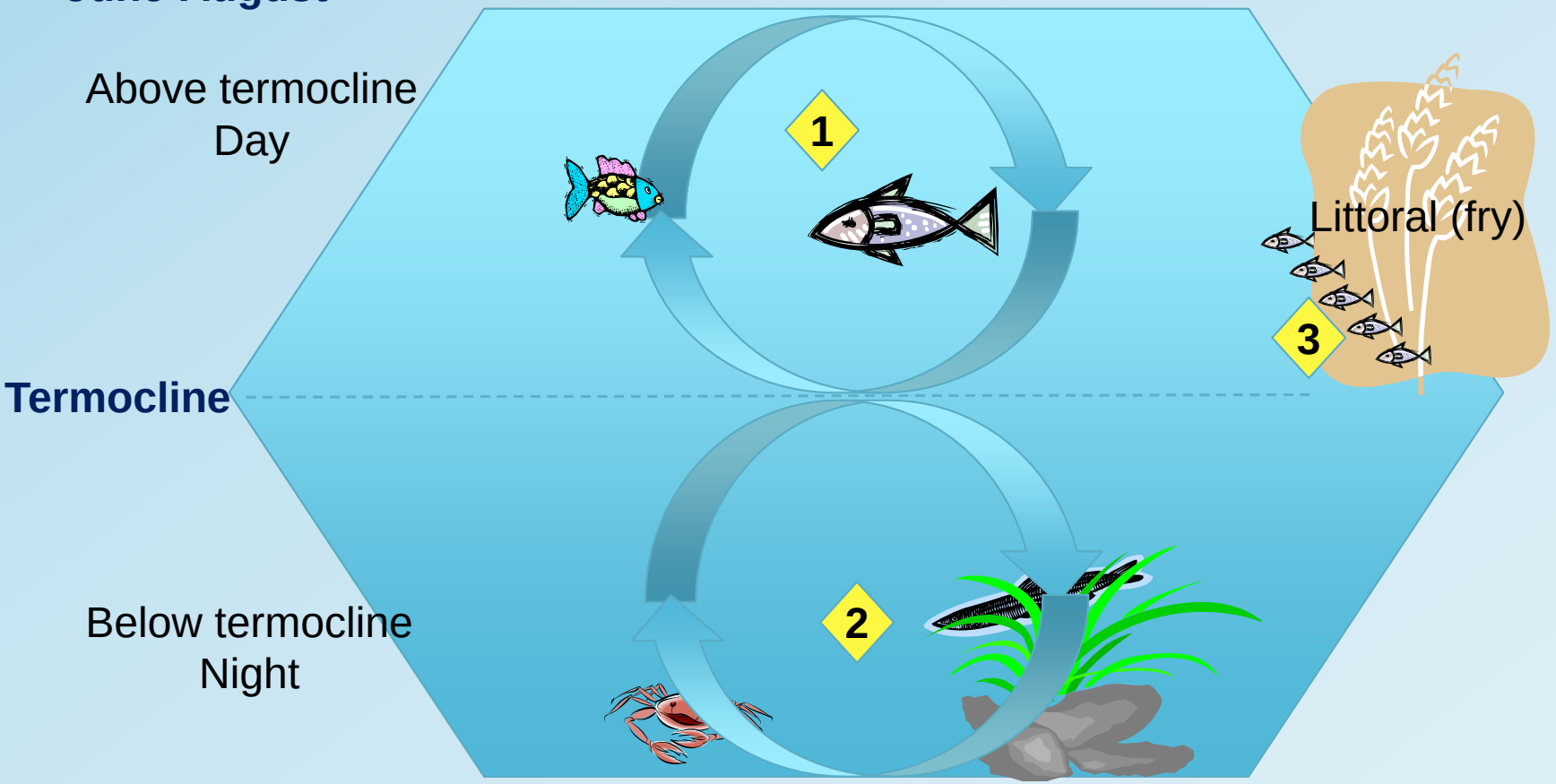


Streams: Low tide/flow best time for eDNA sampling (less dilution)

WHEN to sample?

Summer: June-August

Depends on: Question at issue & species ecology!



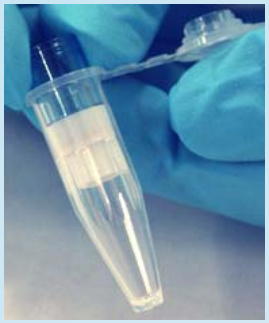
Streams: Low tide/flow best time for eDNA sampling (less dilution)

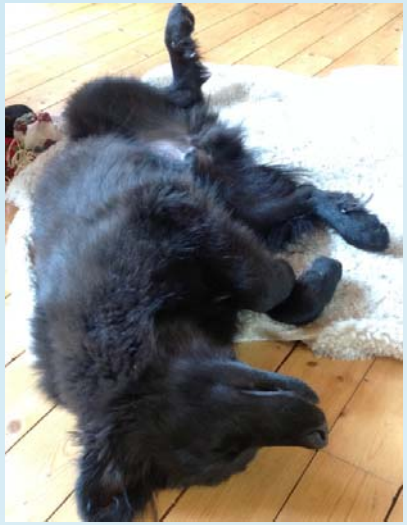
Challenges…

1. **False positives** = match **BUT** the species do **NOT** exist on the site!
2. **False negatives** = **NO** match **BUT** the species exist on the site!

So… false positive results

do NOT reflect reality …





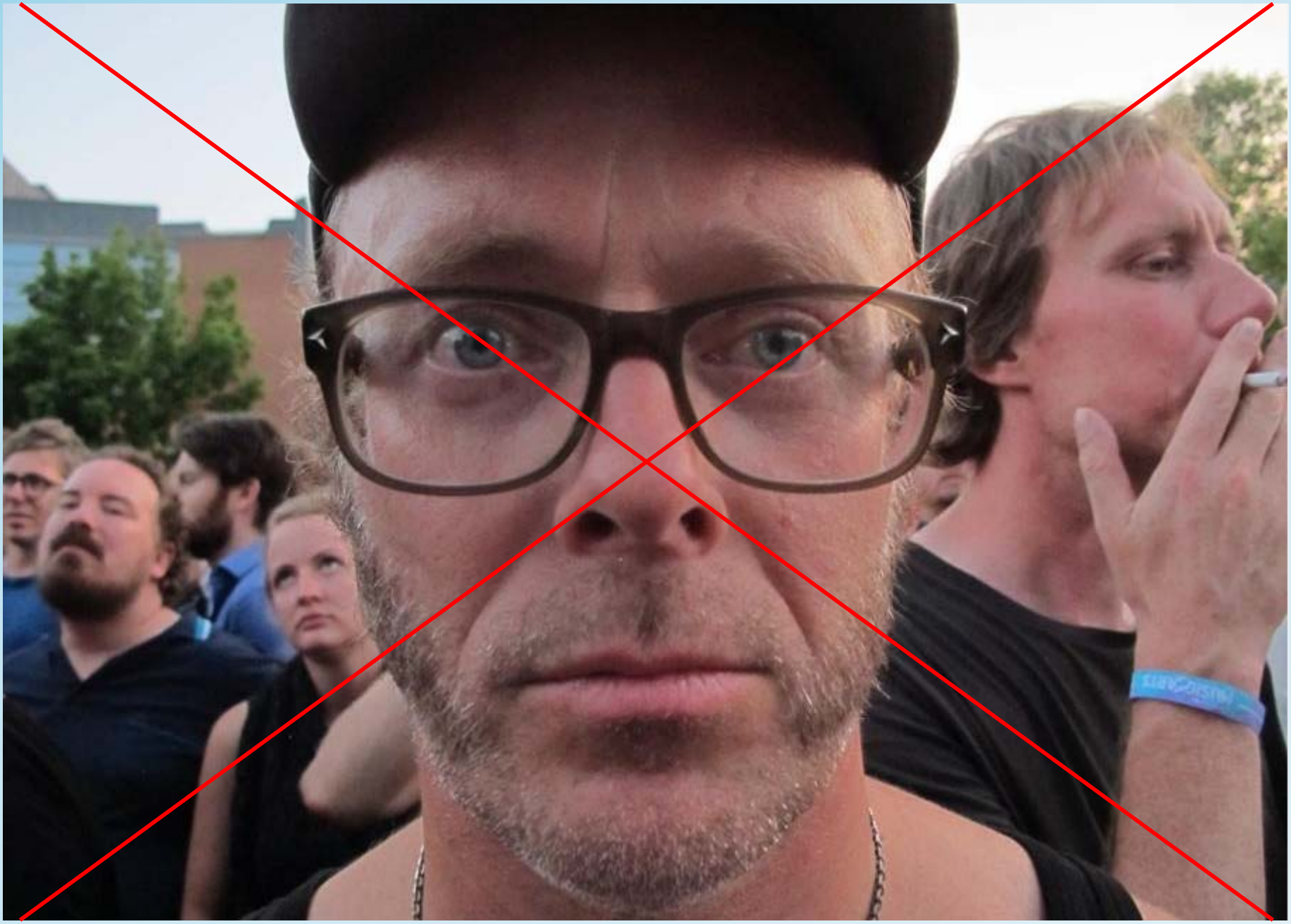
Challenges…

False positive results (species NOT on site)

Contamination (many sources from sampling to analysis)

1. At sample site (natural or human). Sampling over time!
2. Field equipment - sterilization and negative controls (increase cost)
3. Lab equipment / reagents - sterilization, separate labs, negative controls (increase cost)

Source: Darling & Mahon, 2011. From molecules to management: Adopting DNA-based methods for monitoring biological invasions in aquatic environments.



Challenges…

False positive results (species NOT on site)

Sequenses & PCR-primers

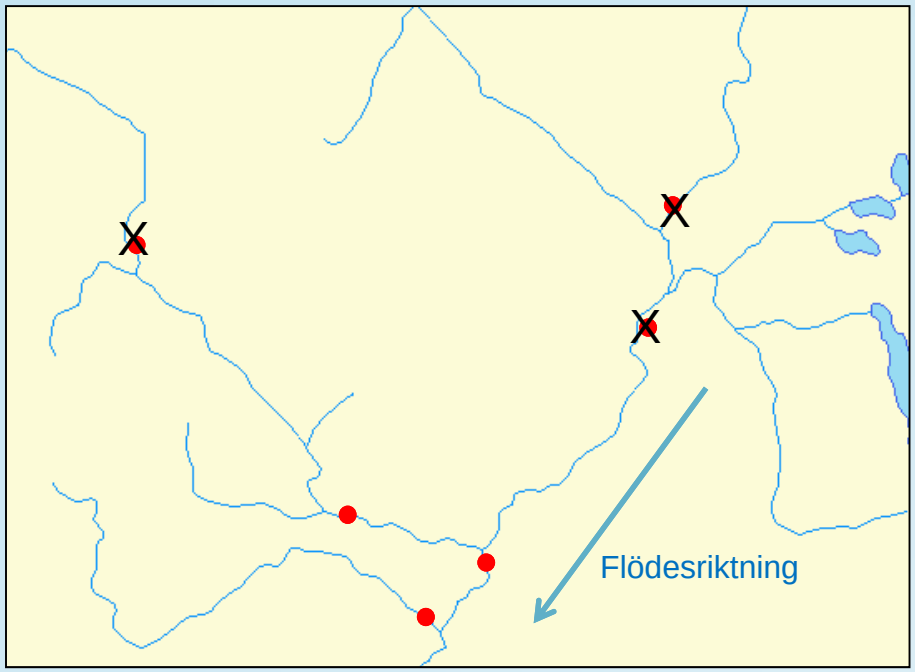
1. DNA sequences not specific enough
2. PCR primers / probes not specific enough

To be continued….

Challenges…

False negative results (species on site BUT no match)

1. Poor sensitivity or incorrect methodology
2. Species ecology etc…



Future uses of eDNA

New & powerful tool for monitoring!

- **Fast surveys** provide room for different prioritization (more focus on other areas)
- **Quantitative improvements** (PCR – qPCR – ddPCR…)
- **Assessment of biodiversity** in completely new areas
  - Calculate biodiversity in catchment areas
  - Foodweb analysis within ecosystems
- **Functional genes**

New technologies and advances improve barcoding libraries

- **More species** and better **geographical coverage**
- More robust **DNA sequences**
- Test for more **DNA subunits** (CO1, 16S, ITS…)

Etc etc…

Future needs…

1. Interpret "the wave of data" coming from sequencing (bioinformation). Shotgun sequencing = *a lot* of data
2. Develop methodology/protocols (how to…)
3. Manage sources of error better (site occupancy models*)
4. Reduce cost by using joint infrastructure more efficiently
5. Develop strategies to quantify [DNA]
6. Basic research on DNA transport and degradation in water
7. Develop skills to move more freely between green & white biology (cost effective)

Etc etc…

*: B Schmidt et al (2013) Site occupancy models in the analysis of eDNA presence/absence surveys


THANK YOU!
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