Environmental DNA as a basis for species conservation

Jelger Herder

Brussel, 19 april 2013

RAVON

RAVON

Reptile Amphibian Fish Conservation Netherlands

- Non Governmental Organization (NGO)
- 28 professional staff members
- 2000 volunteers

Aim: to protect and increase the number of sustainable populations of reptiles, amphibians and fish

RAVON

We collect distribution data for conservation

- Volunteers / professional / other organizations
- Coordinate national monitoring programs
- National Database Flora and Fauna
  - 60.000.000 records!

→ Data used for

→ Species protection plans

→ Habitat management

→ Red Lists

Partnership with SPYGEN

- Both organizations complement each other

SPYGEN®

- eDNA labwork
- Long experience
- Lab equipped for eDNA
- eDNA protocols
- Reference database and primers

RAVON

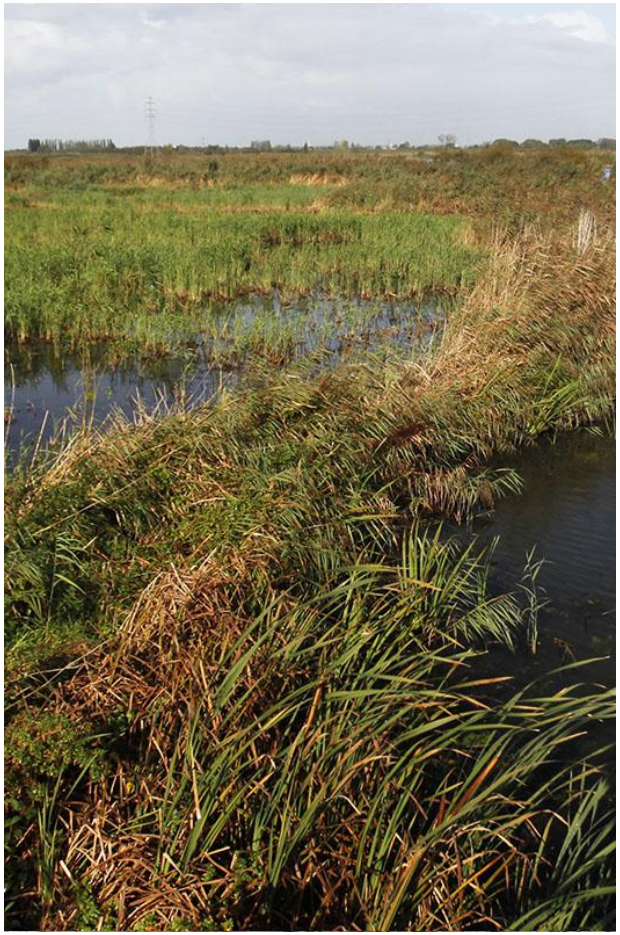
- Ecology and distribution of species
- Field methods
- Capable to organize large projects
- Collecting DNA for reference database

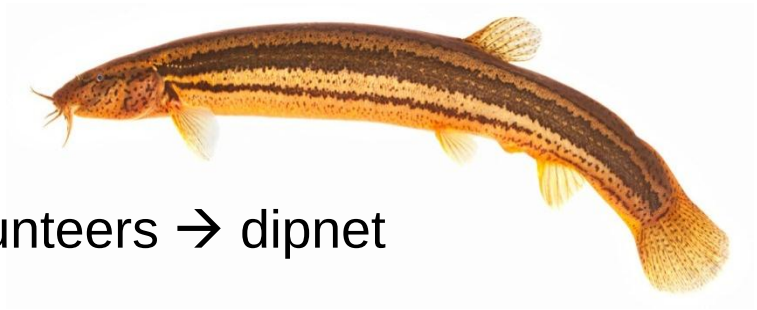
De Vlinderstichting

Zoogdier Vereniging

Some species are hard to monitor

- **Pond loach (*Misgurnus fossilis*)**





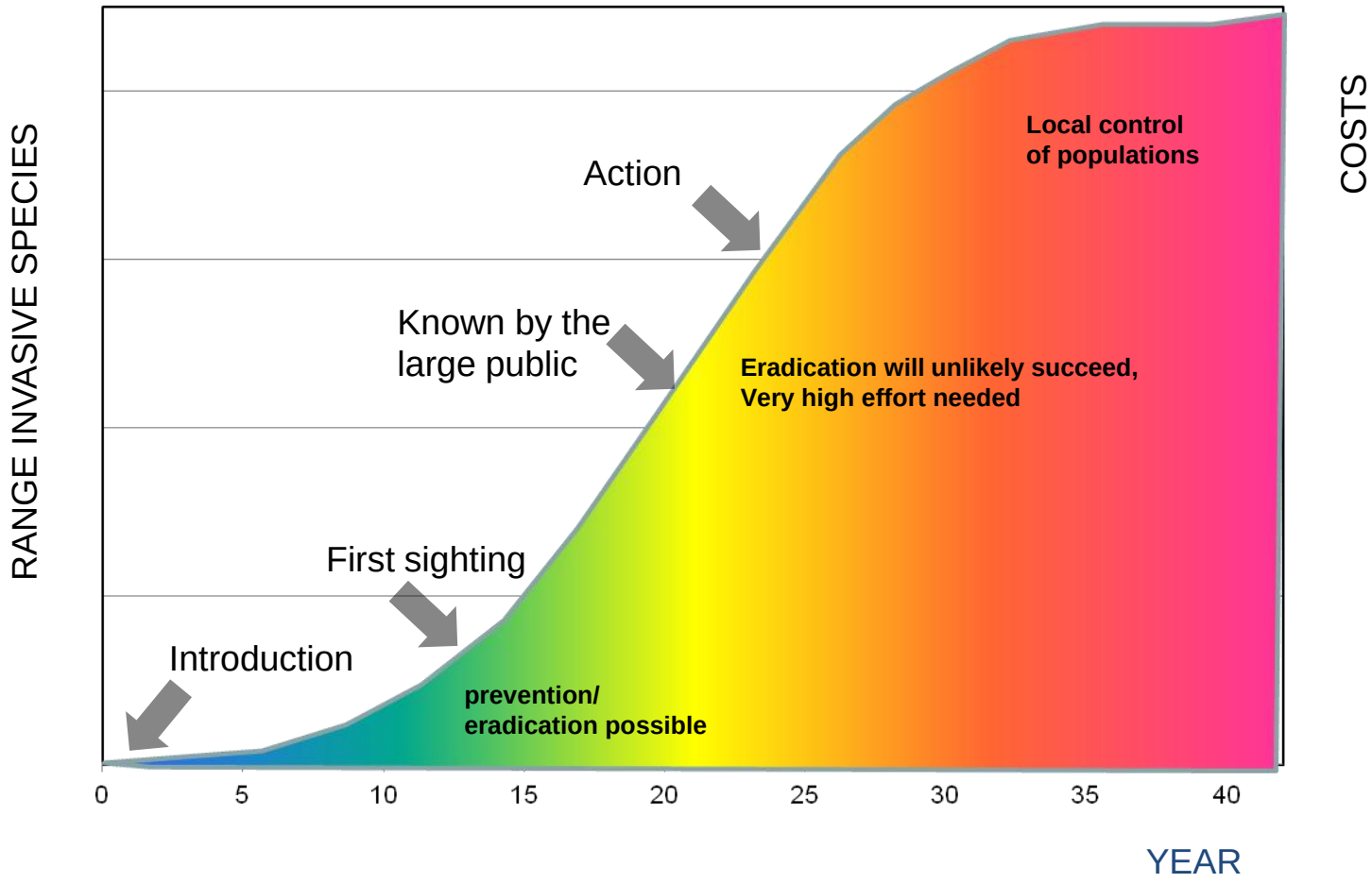
- Volunteers → dipnet
- Professionals → electro fishing
- However pit-tag research showed low detection chance
- Habitat

Some species are hard to monitor

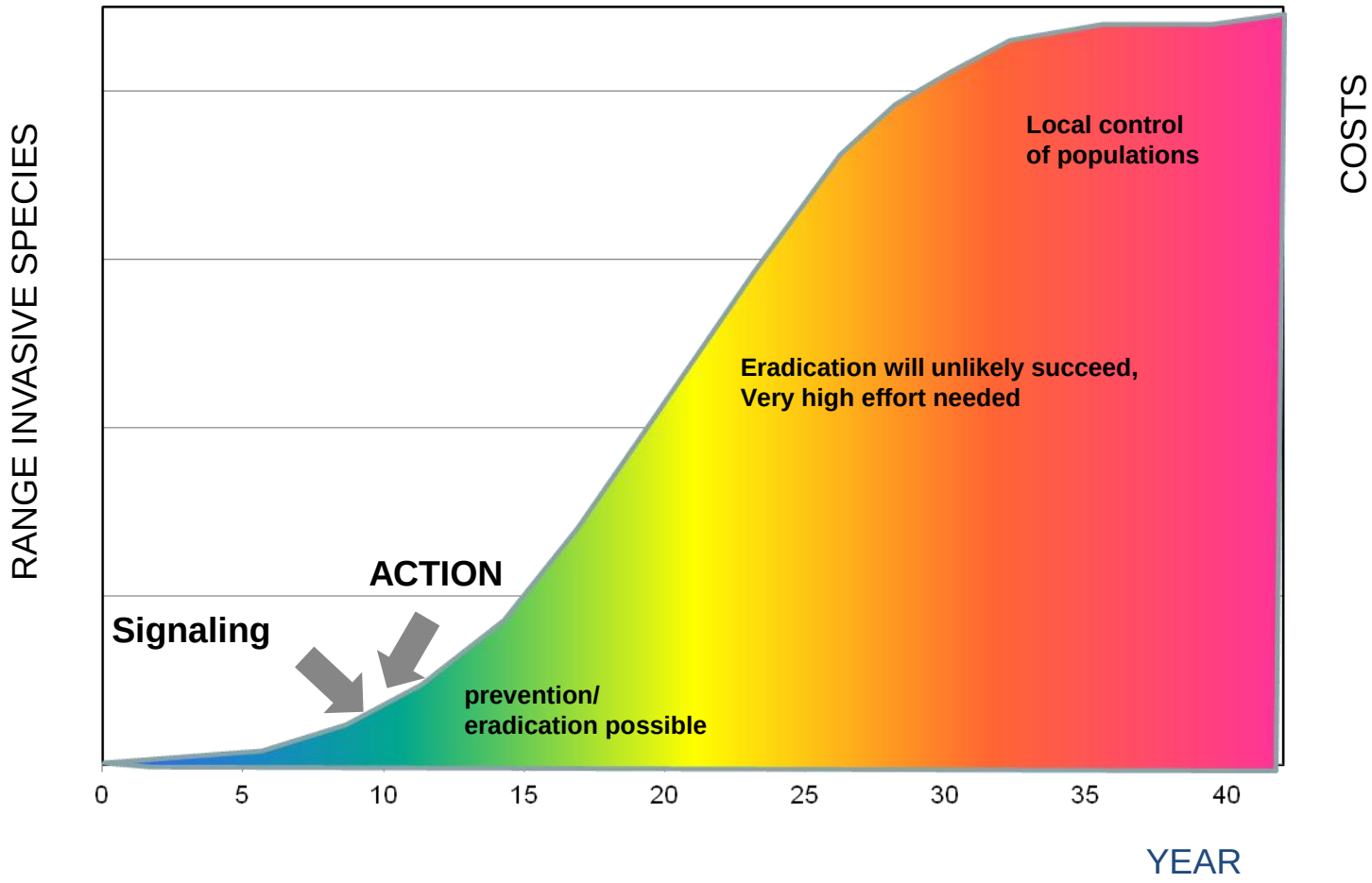
- **Common spadefoot toad (*Pelobates fuscus*)**

- Cryptic species → **nocturnal**
- Short breeding period → calls underwater
- → Endangered (74% decline since 1950)

Early warning invasive species

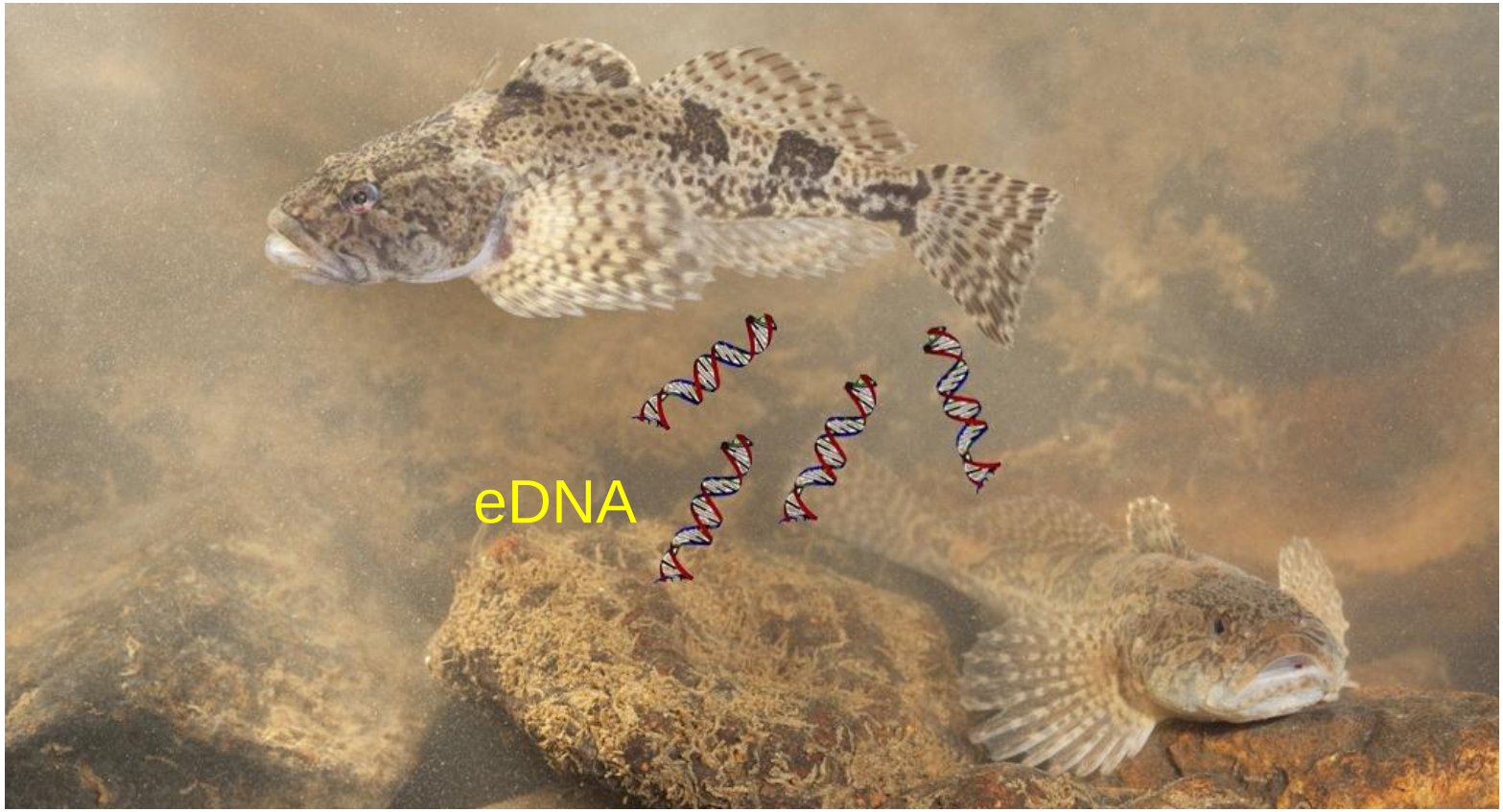


Early warning invasive species



New approach: environmental DNA

- Species that live in the water release DNA in the water via skincells, faeces and urine.



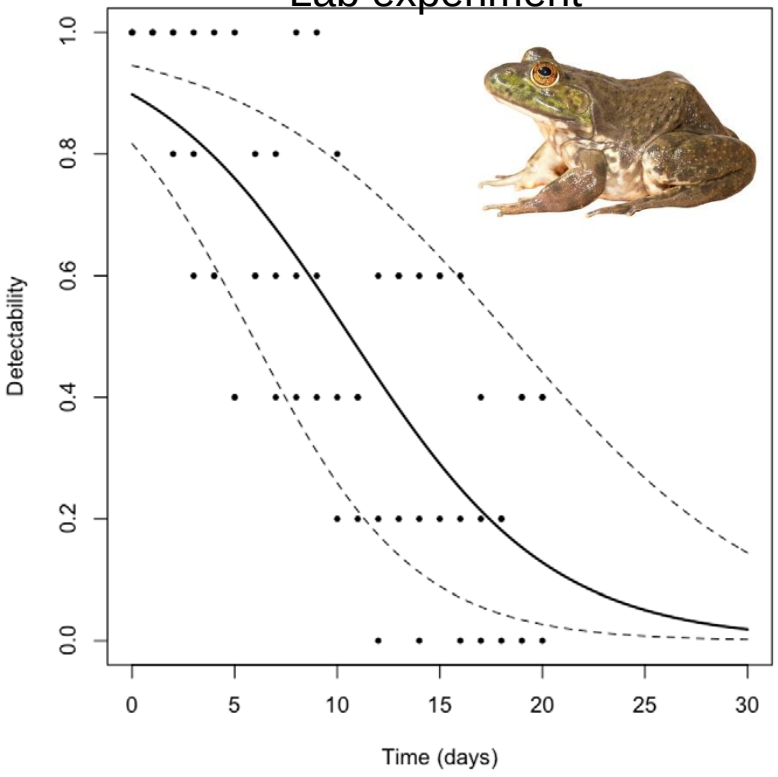
eDNA can be collected

- DNA spreads due to dissolving properties of water
- Collecting water samples = fast and efficient
- Those samples can be analysed for eDNA

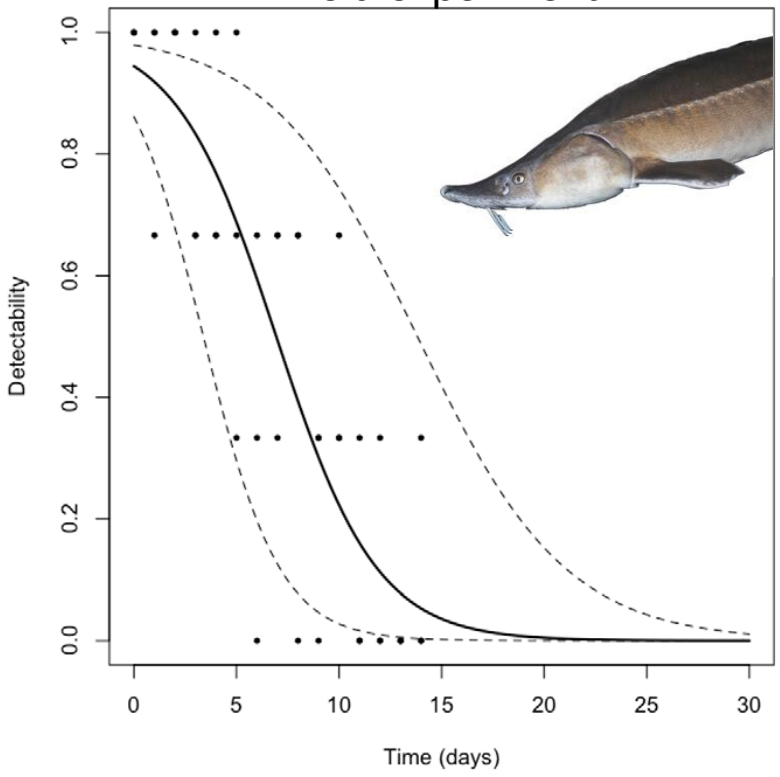
eDNA shows recent presence

- Experiments showed that eDNA in the water breaks down within three weeks (Dejean *et al.*, 2011).

Lab experiment



Field experiment



eDNA American bullfrog

- First studies with eDNA were performed on the American bull frog (*Lithobates catesbeianus*) in France (Ficetola *et al.,* 2008)
- On the IUCN list of 100 worst invasive species in the world!
  - Disease transmission
  - Predation
  - Competition

First study in the Netherlands

- In 2011 RAVON and SPYGEN → pilot study on the use of eDNA to find pond loaches (*Misgurnus fossilis*) (Herder *et al.,* 2012)
  - Detection chance of 87,5% (7 out of 8 locations)
  - Control locations (4) negative
- In 2012 large inventory projects in the Netherlands
  - Found new locations, but also missed some controls!

eDNA common spadefoot

- Endangered in the Netherlands (Red List)
  - Only 35 populations left
  - 74% decline since 1950 and still declining!
- Very difficult to monitor
- Reintroduction program → info needed for conservation!

- **Seemed a perfect species for eDNA!**

eDNA common spadefoot

- eDNA within Network Ecological Monitoring (NEM)
  - 23 historic populations (extinct/unknown)
  - 4 control locations
- Results
  - **eDNA positive for common spadefoot on 6 locations!**
  - 3 out of 4 control samples positive (75% detection)
  - 17% increase in known populations!

NETWERK NEM Ecologische Monitoring

Pilot studies dragonflies

- Green hawker

  (*Aeshna viridis*)

- 7/9 waters (detection 78%)
- Missed locations →sampling later in the year

- Large white-faced darter

  (*Leucorrhinia pectoralis)*

- 6/8 waters (detection 75%)
- Missed location → also missed with traditional methods

- Quick screening + monitoring in longer period

Pilot study watershrew

- Water shrew (*Neomys fodiens*) perfect for eDNA?

- But…. no detection with eDNA on 10 pilot locations
- Possible explanations:
  - Not present at exact location during sampling
  - Small animal, low densities
  - Ecology → lives primarily on land!

ZOOGDIER VERENIGING

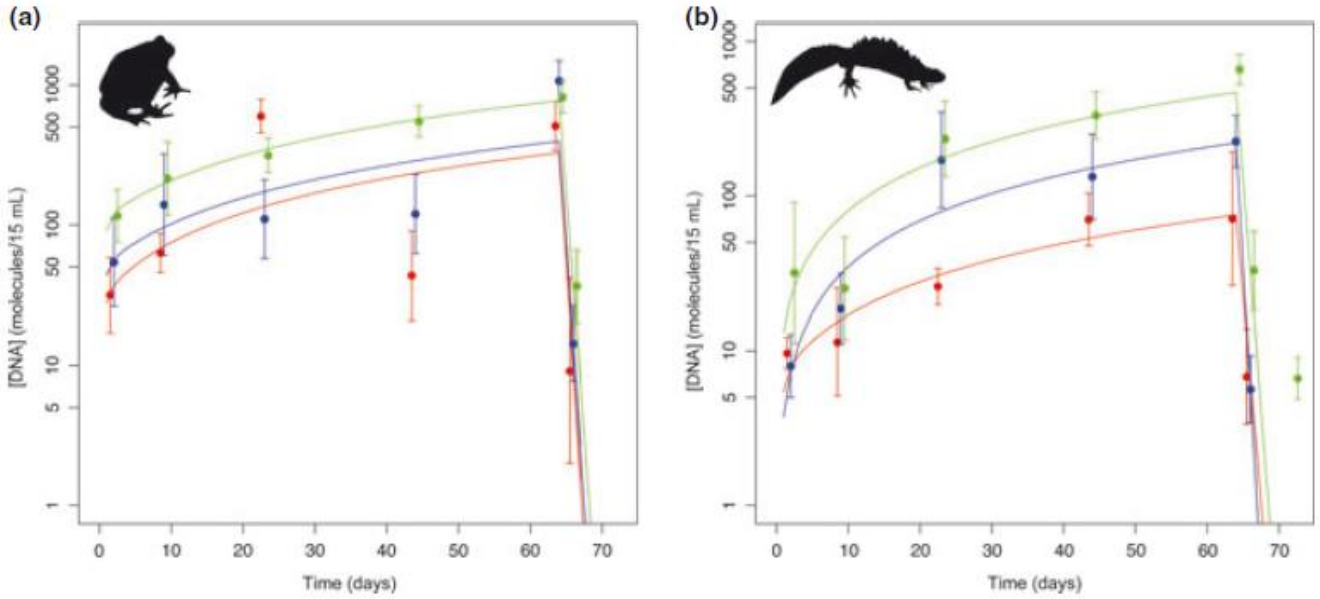
Pilotstudy Root vole

- Root vole (*Microtus oeconomus*) → habitat directive?
  - Higher densities, lives close to the water and swims.

- Yes…. detection of root vole on 5 out of 10 pilot locations
- Uncertain if eDNA “missed” the root vole on the other 5 locations or if the species was not present → follow up in 2013

Estimating densities via eDNA

- In the lab
  - A significant relation between the number of larvae of the Northern crested newt and common spadefoot and the amount of eDNA in the water was found.



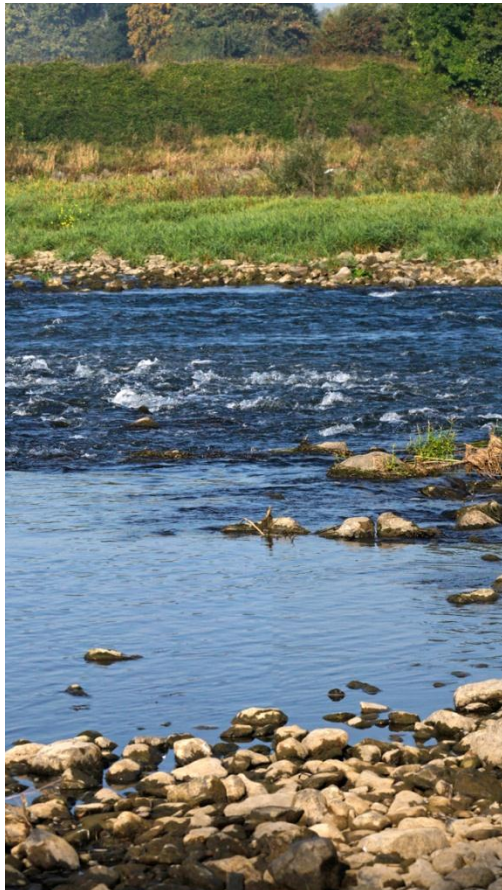
Thomsen *et al*., 2012

Estimating densities in the field

- Study on Northern crested newts near Tilburg
  - 9 waters checked with eDNA and with traditional methods (dipnet + amphibian traps)
- Results traditional methods
  - 2 ponds with crested newts
- Results eDNA
  - 5 ponds with crested newts
- **The eDNA signal was clearly stronger in the ponds with many larvae!**

Estimating densities in the field

- But results are variable
  - For the pond loach we sampled on the same location with variable results.
- Many factors might influence amount of eDNA
  - Species / activity
  - Microbial activity
  - Temperature
  - pH
  - Conductivity
  - Organic material
  - Watertype (flow rate, size → dillution)
- **Research needed per species, per habitat and per period.**



eDNA for more species?

- **Not for species that are easy to monitor!**

Bitterling
Rhodeus amarus

Spined loach
Cobitis taenia

Dipnet

eDNA for more species?

- **Promising for …**

Rare / cryptic species

Invasive species

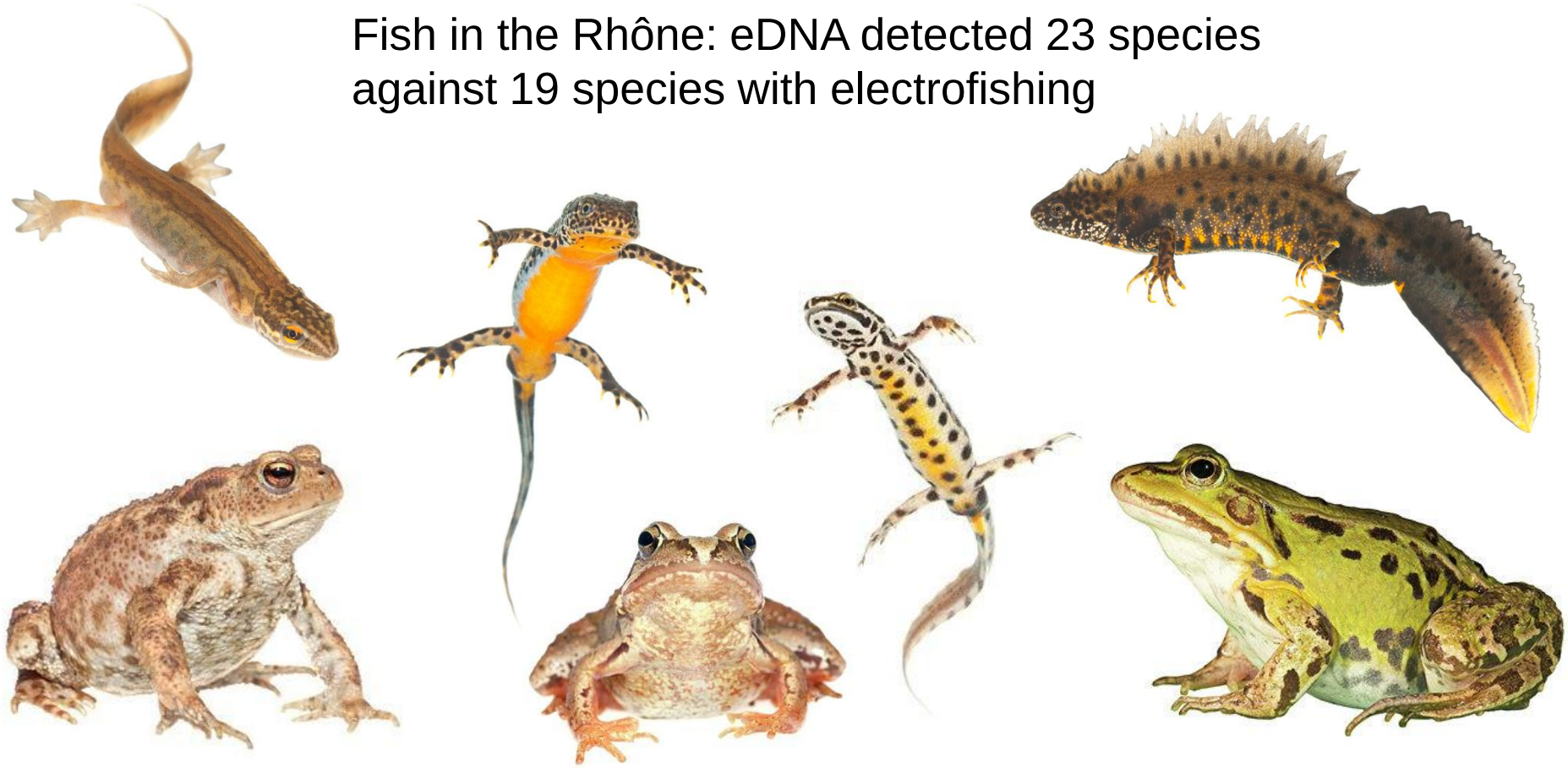
The next step: multispecific approach

- Universal primer for group of species
- All DNA of these group is amplified in the PCR
- Al amplified DNA is sequenced using Next Generation Sequencing (NGS)
- Matching the DNA to a reference database on a computer

The next step: multispecific approach

- Succesfully tested by SPYGEN in France
  - Amphibians in ponds: eDNA gave a similar amount or more species than traditional

    Fish in the Rhône: eDNA detected 23 species against 19 species with electrofishing



The next step: multispecific approach

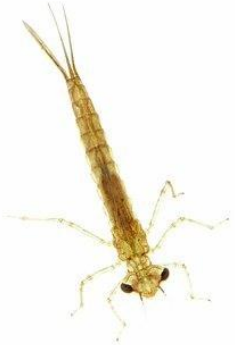
- RAVON carried out research in the Netherlands
  - Comparisson between Water Framework Directive sampling and eDNA
  - Preliminary results (eDNA samples have not been fully analyesed yet)
    - eDNA 19 species, electrofishing 20 species.

Challenges

- Data management
  - 1 run → 6 billion codes
  - Pile of paper of 48 km!
- Reference databases
  - Genbank contains many errors
  - DNA codes unknown for many species
  - Building own reference database!

Pitfalls in the field

- **False positives – Species not present → positive eDNA score!**



- Contamination
  - Fieldwork protocols
  - Working sterile

- Theory of excrements and movement of DNA by herons/ducks
  - Chance thought to be very small!

Pitfalls in the field

- **False negatives – Species is present, eDNA scores negative!**

- Sampling
  - Method — Fieldwork protocols
  - Location — Species experts for sampling
  - Period — Tests + Ecological Knowledge

Pilot studies!

Implications false negatives

- Endangered & protected species
  - No species protection measures (for example on construction sites)
  - Locations not integrated in species protection plans

- Invasive species
  - Later signaling →

    higher costs

    + ineffective eradication

Implications false positives

- Endangered & protected species
  - Waste of means for habitat improvements (for example the construction of breeding ponds)
- Invasive species
  - Unnecessary actions taken for eradication and control

Traditional methods are neither perfect!

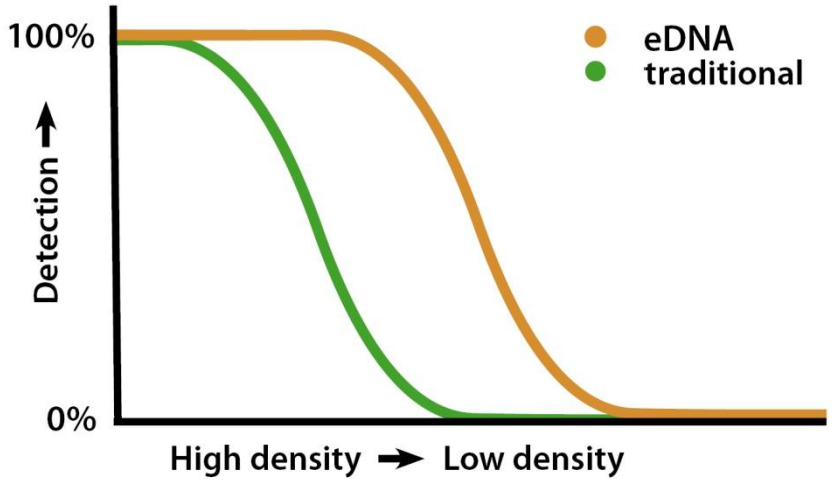
- False negatives
  - The efficiency of each method differs per species!

- False positives
  - Misidentification of species

  - Movement by predators

Advantages of eDNA

- Higher detection chance
- Lower costs
- Species specific
- No stress
- No spread invasive species/diseases
- More reliable negatives



Benefits traditionel methods

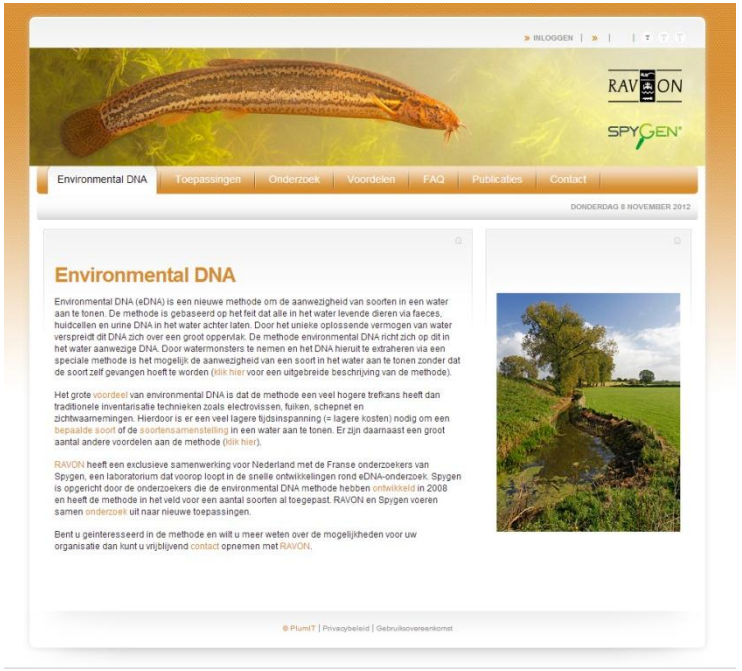
- Collecting info on length, age, condition
- Feeling with the species → support for conservation
- Invaluable work of volunteers cannot be replaced

Ranavirus

Support for conservation

Volunteers

Questions?



www.environmental-dna.com