Environmental DNA for the detection and monitoring of invasive species

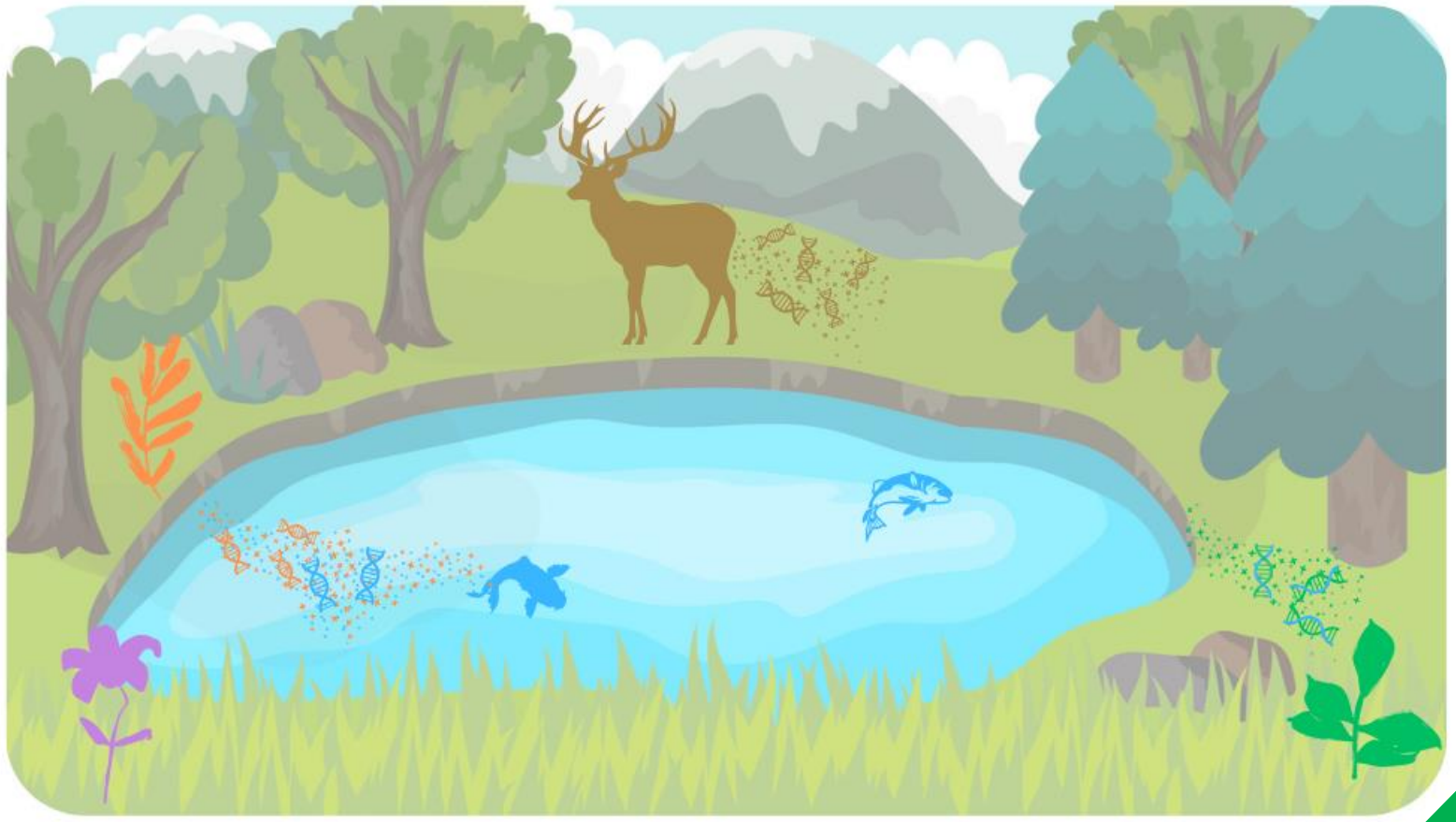
Aurélie Bonin

SOILVER webinar, June 5th 2024

What is environmental DNA?

Every living organism lives DNA traces in its environment…

- Living cells
- Mucus
- Gametes
- Urine
- Feces
- Decaying tissue
- …



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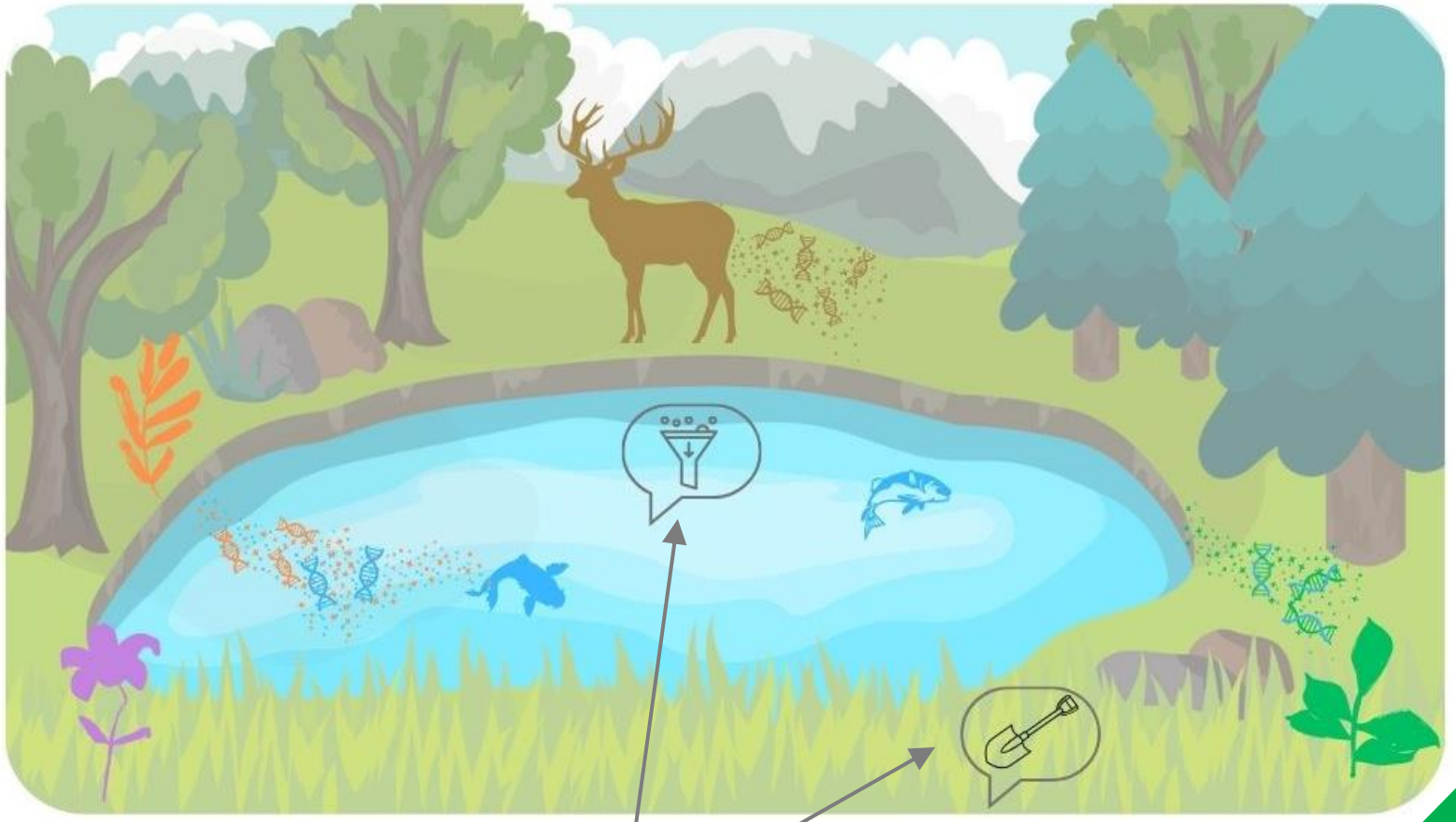


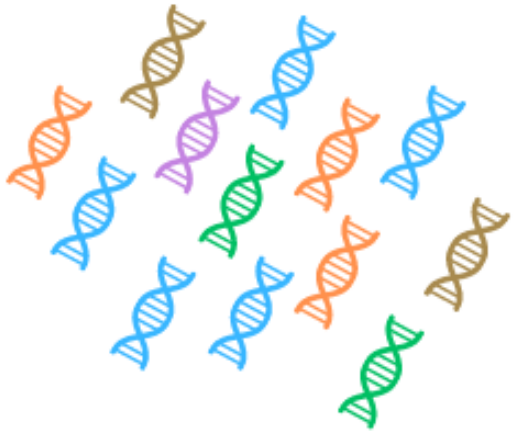
… that we can collect and use for biodiversity analyses

What is environmental DNA?

Environmental DNA
= a complex mixture of DNA fragments, more or less degraded, originating from a wide range of organisms

Environmental sample

DNA extraction



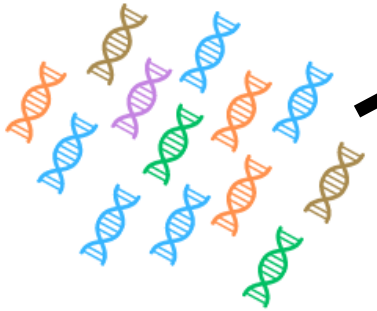
Environmental DNA

How to extract biodiversity information of this complex mixture?

➡ Two main strategies

How to extract biodiversity information from this complex mixture?

Strategy 1 based on sequencing = DNA metabarcoding



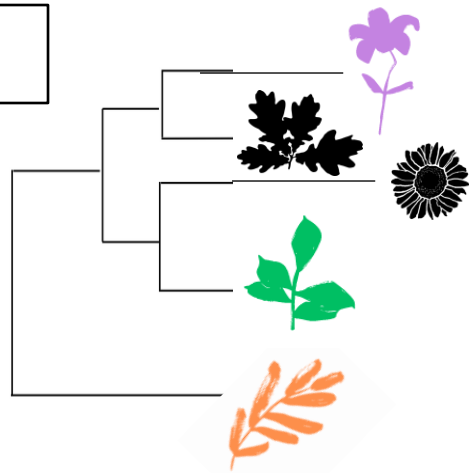
eDNA

GTARTTCGACTA

Generalist marker
(e.g. Polygonaceae)

GTAATTCGACTA....ATGCCATTAGC
GTAATTCGACTA....ATGTCATCAGC
GTAGTTCGACTA....AACCCATTAGC

Amplification and sequencing



Comparison to a sequence database

How to extract biodiversity information from this complex mixture?

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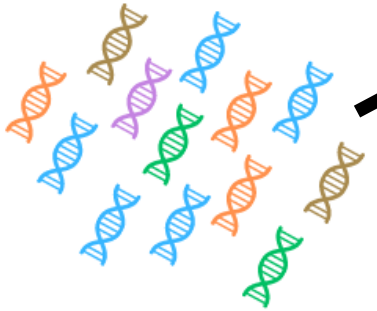

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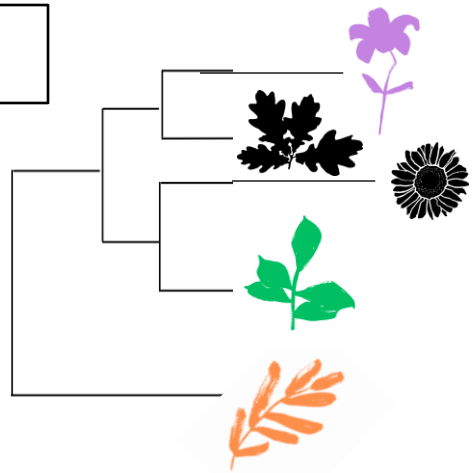


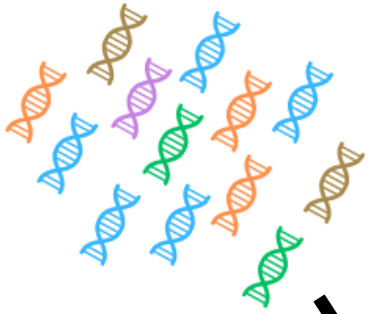
Comparison to a sequence database

Order	Family	Genus	Species	Sample 1	Sample 2	Sample 3
Caryophyllales	Polygonaceae	Rumex	*Rumex conglomeratus*	0	74101	1061
Caryophyllales	Polygonaceae	Reynoutria	*Reynoutria japonica*	63268	122	0
Caryophyllales	Polygonaceae	Persicaria	*Persicaria viscosa*	14178	0	3111

Number of reads for the taxon in the sample

How to extract biodiversity information from this complex mixture?

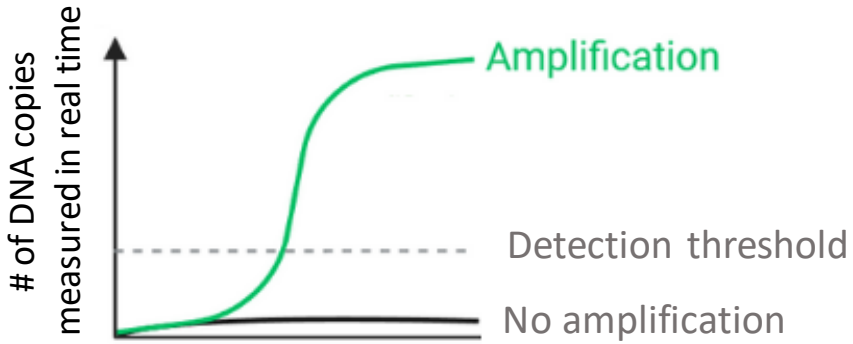
Strategy 2 based on qPCR (quantitative PCR)
Detection of a particular species (e.g. *Reynoutria japonica*)



eDNA

GTAATTCGACTA

Specific marker



qPCR amplification and detection

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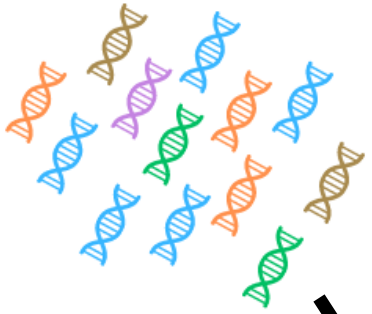

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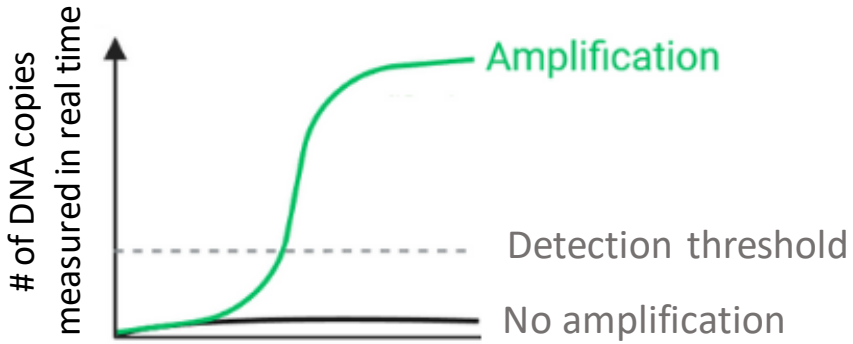


qPCR amplification and detection

	Presence	Absence
Sample 1	✓	
Sample2	✓	
Sample 3		✗

How to extract biodiversity information from this complex mixture?

Comparison of the two approaches for Japanese knotweed detection

Metabarcoding and qPCR

- ⊕ Non-invasive methods
- ⊕ No need for botanical expertise in the field
- ⊕ Sampling at any season
- ⊖ Risk of contaminations

How to extract biodiversity information from this complex mixture?

Comparison of the two approaches for Japanese knotweed detection

Metabarcoding and qPCR

- (+) Non-invasive methods
- (+) No need for botanical expertise in the field
- (+) Sampling at any season
- (−) Risk of contaminations

Metabarcoding

- (+) Operational for Japanese knotweed
- (−) Semi-quantitative signal (relative abundances)
- (−) More expensive than qPCR

How to extract biodiversity information from this complex mixture?

Comparison of the two approaches for Japanese knotweed detection

Metabarcoding and qPCR

- (+) Non-invasive methods
- (+) No need for botanical expertise in the field
- (+) Sampling at any season
- (−) Risk of contaminations

Metabarcoding

- (+) Operational for Japanese knotweed
- (−) Semi-quantitative signal (relative abundances)
- (−) More expensive than qPCR

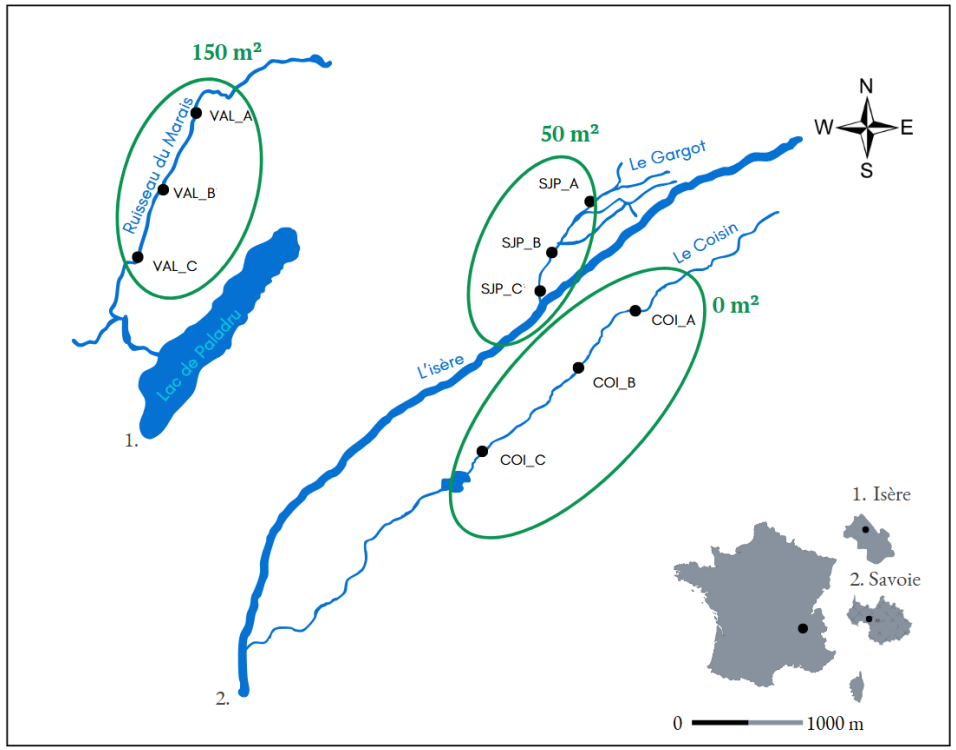
qPCR

- (+) Cheaper and quicker than metabarcoding
- (+) Quantitative signal
- (+) Higher detection rate than metabarcoding
- (−) Not completely operational for Japanese knotweed
- (−) Possibility of false positives

eDNA and Japanese knotweed: case study 1

Can we detect Japanese knotweed eDNA from rivers?

→ Japanese knotweed known to disseminate well in surface freshwaters
→ Sampling at river confluences could inform on presence upstream
→ Influence of distance to the close knotweed patch, weather conditions, size of the patch?



Pilot study with Aquabio

- 3 rivers: one big patch of knotweed, one small patch, no plant upstream
- 3 sites along each river, 3 replicate samples
- 2 weather conditions: after at least 2 days of rain or 2 days of sunshine

eDNA and Japanese knotweed: case study 1

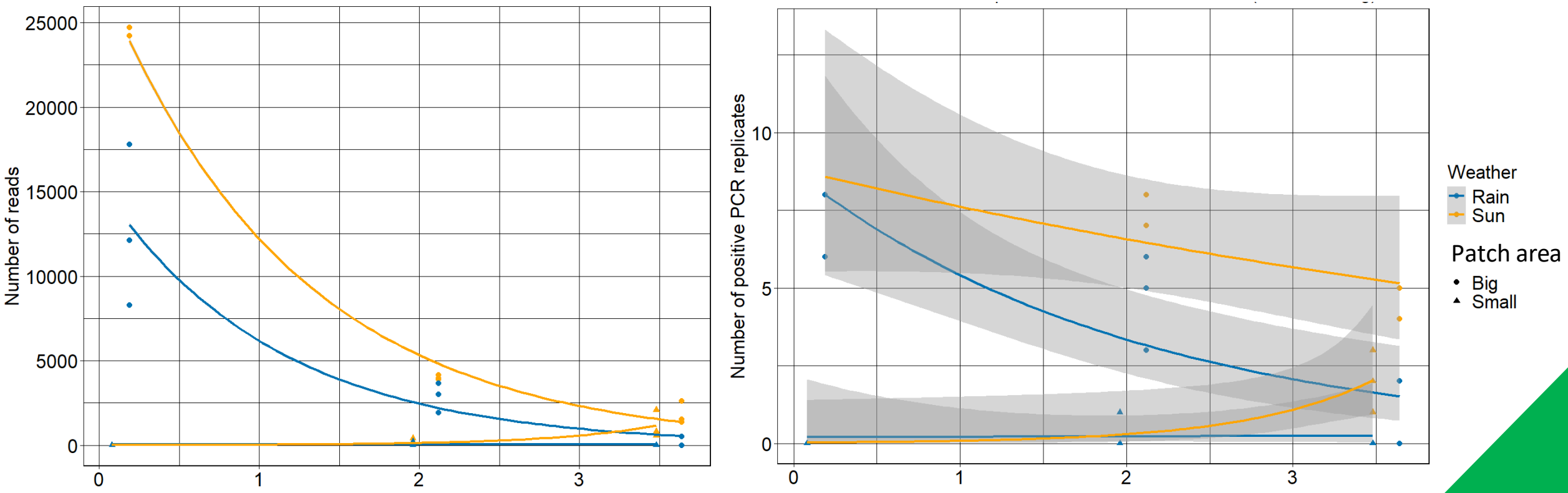
Can we detect Japanese knotweed eDNA from rivers?

Three proxies to measure detection

- Number of sequence reads (metabarcoding)
- Number of positive PCR replicates (metabarcoding)
- Number of positive qPCR replicates (qPCR)

eDNA and Japanese knotweed: case study 1

Can we detect Japanese knotweed eDNA from rivers?
Metabarcoding results



Distance in km from the closest Japanese knotweed patch upstream

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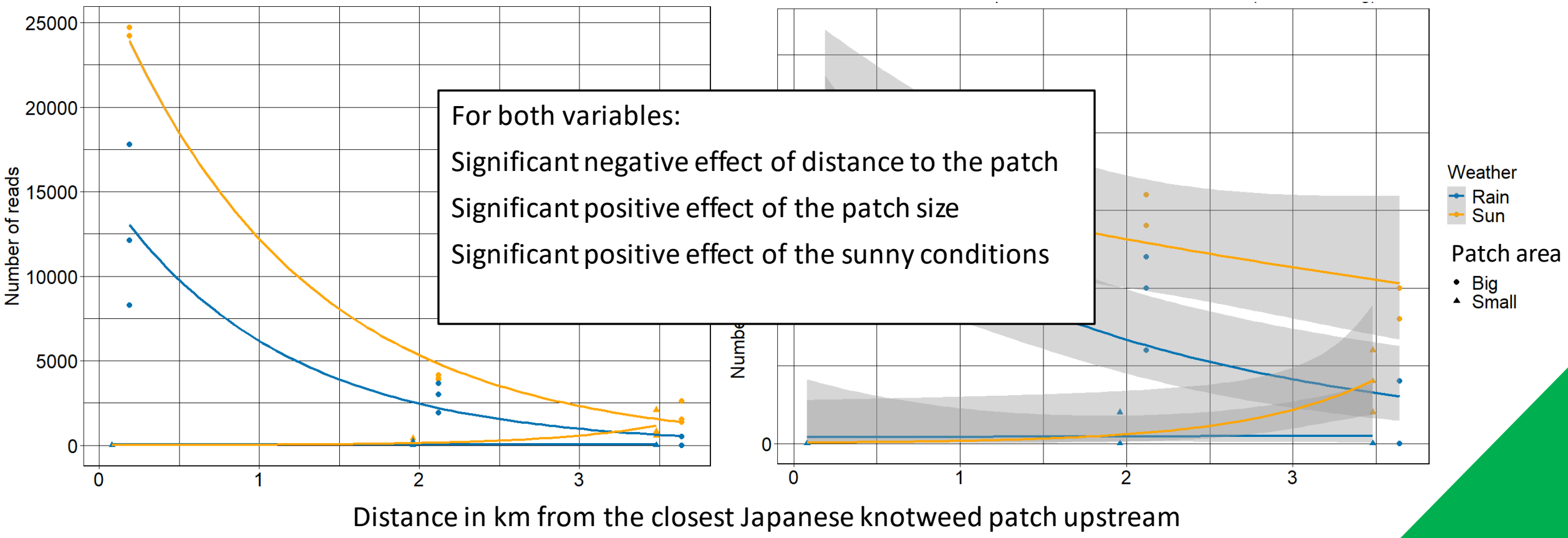
Metabarcoding results



For both variables:

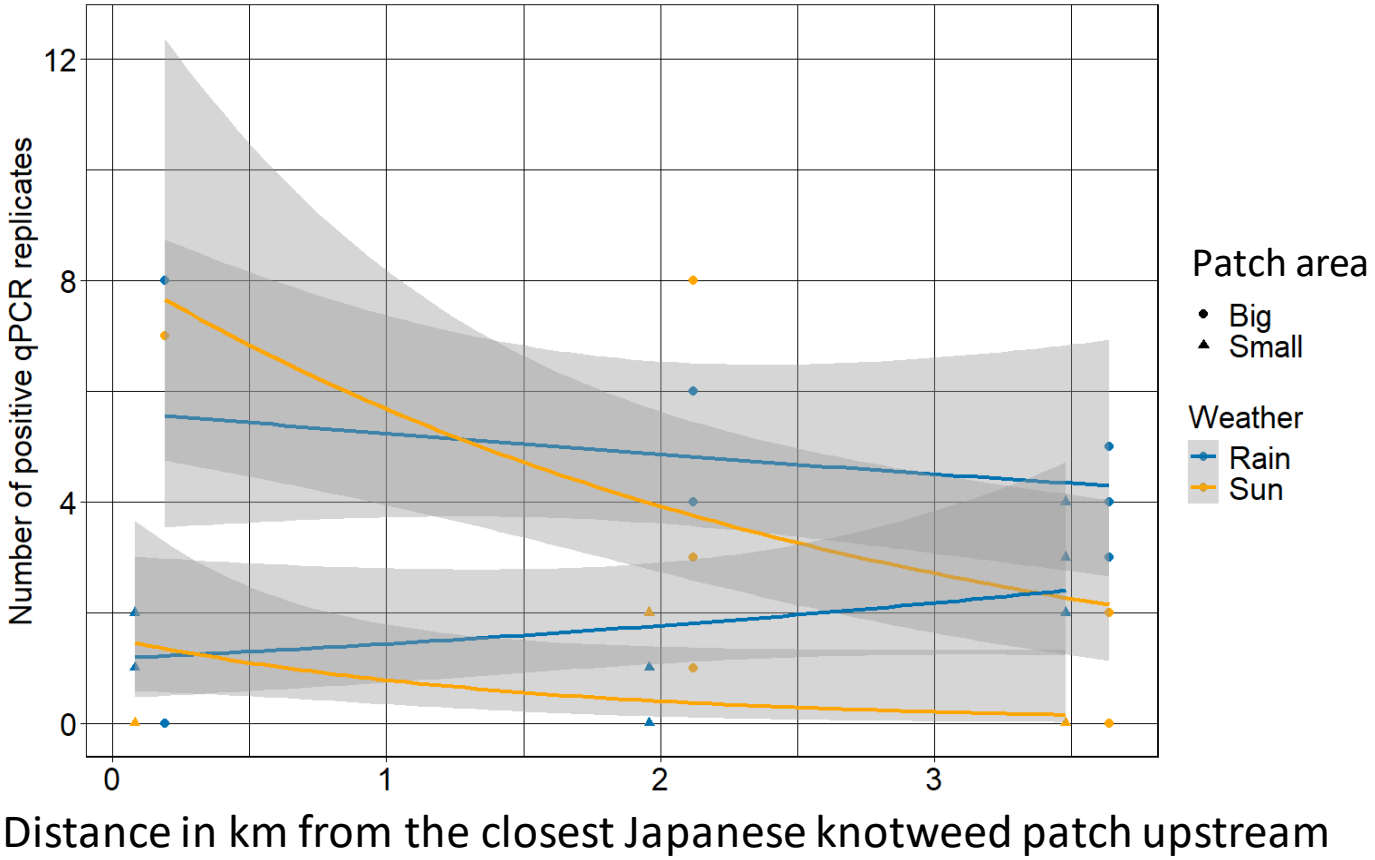
Significant negative effect of distance to the patch

Significant positive effect of the patch size

Significant positive effect of the sunny conditions

eDNA and Japanese knotweed: case study 1

Can we detect Japanese knotweed eDNA from rivers?
qPCR results



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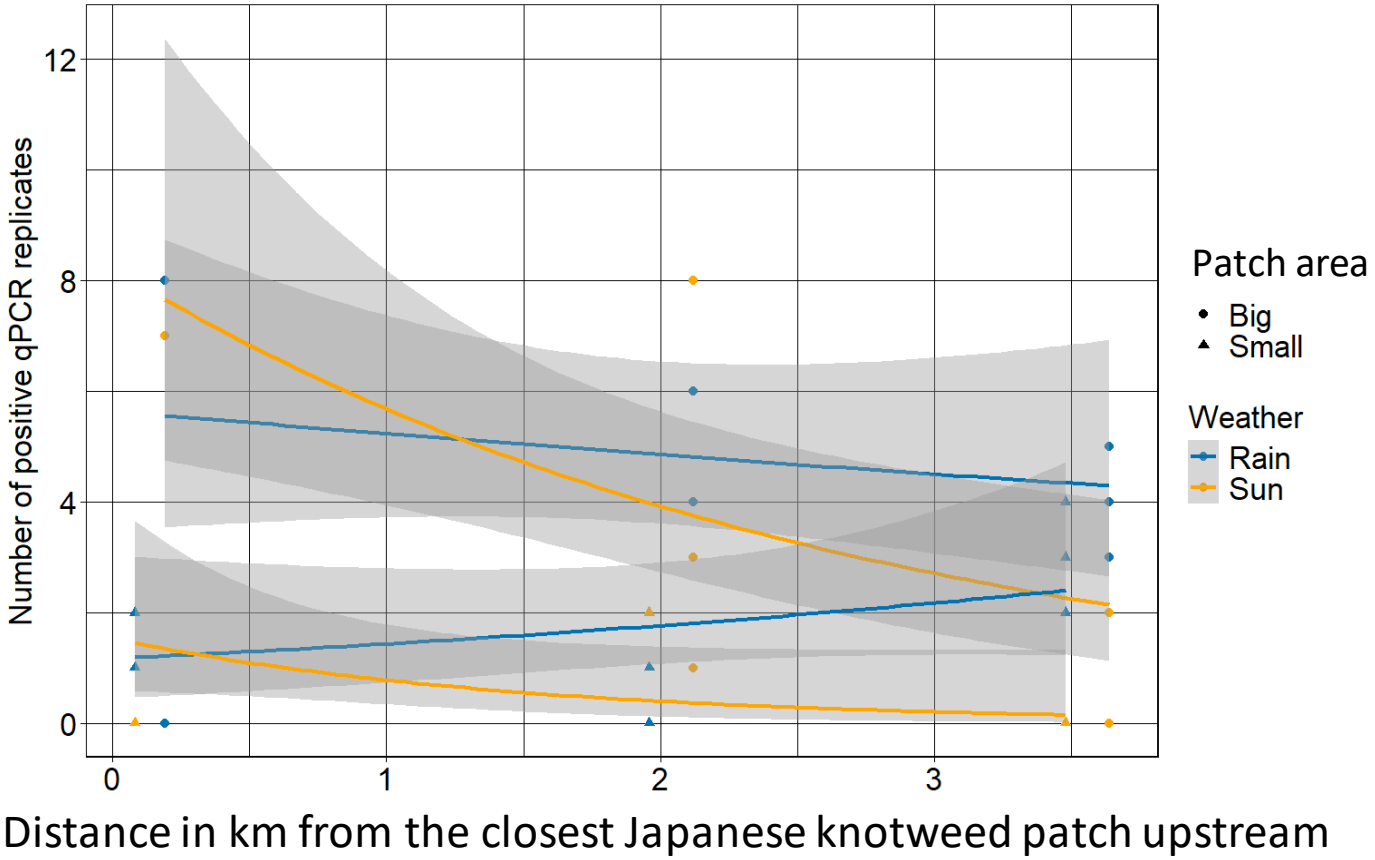


Significant negative effect of distance to the patch

Significant positive effect of the patch size

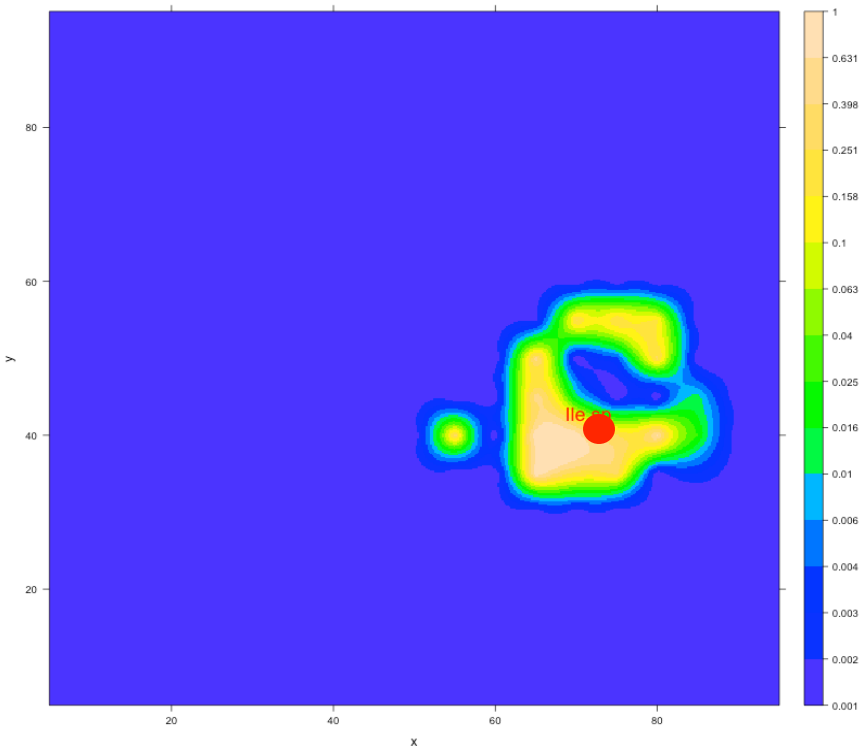
eDNA and Japanese knotweed: case study 1

Can we detect Japanese knotweed eDNA from rivers?
Conclusions

- Promising results with both methods!
- Need to repeat the study during the growing season
- Need to evaluate the sensitivity for both approaches (more technical replicates)

eDNA and Japanese knotweed: case study 2

Can we detect Japanese knotweed eDNA from soil and what does it mean?



→eDNA can persist longer in soil and doesn't diffuse

→Implications for the sampling design and sampling effort

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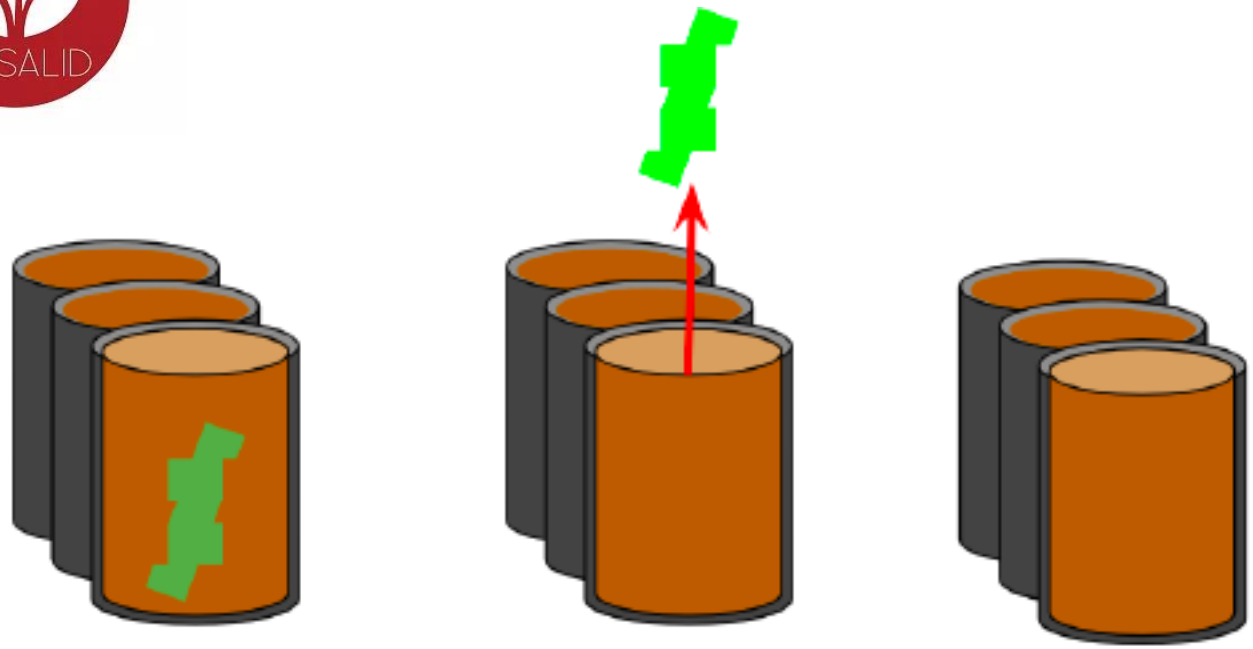


Rhizome remains during the sampling period

Rhizome removed prior to the sampling period (T=7)

No rhizome

Experimental study – Part A:

- Calibrated rhizomes (same biomass)
- Controlled conditions
- Sampling after 7 and 14 days

Detection possible after a few days?
How long does the signal persist?

eDNA and Japanese knotweed: case study 2

Can we detect Japanese knotweed eDNA from soil and what does it mean?

Big rhizome | Medium rhizome | Small rhizome

Experimental study – Part B:

- Different biomasses
- Controlled conditions
- Sampling after the optimal number of days determined in Part A

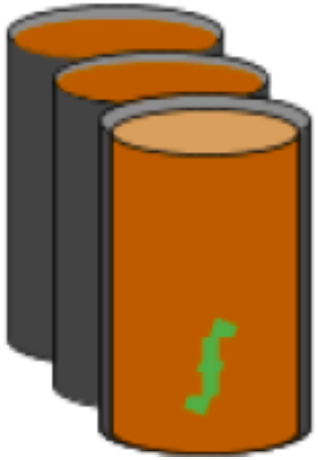
➡ What is the sensibility of the system?

eDNA and Japanese knotweed: case study 2

Can we detect Japanese knotweed eDNA from soil and what does it mean?

Big rhizome

Medium rhizome



Small rhizome

Perspectives

Detection

- Promising results for aquatic eDNA
- Remaining challenges for soil eDNA
  → Designing efficient sampling designs for soil
- qPCR assays still to be improved
  → Use of qPCR as a quick and cheap test, metabarcoding in a second time?
- Development of MinIon sequencing
  → To help decrease turnaround time

Monitoring the efficiency of control measures

- Explore the quantitative nature of the eDNA signal

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