@N_P_Griffiths

nathan.griffiths.ic@uhi.ac.uk

A confidence framework for fish detection through environmental DNA metabarcoding:

Achieving confidence in absence

Nathan P. Griffiths

Bernd Hänfling | Rosalind M. Wright | Marco Cattaneo | James Macarthur | Sara Peixoto | Jonathan D. Bolland

UNIVERSITY OF HULL

EVO Hull

UNIVERSITY OF Hull International Fisheries Institute HIFI

Environment Agency

UHI University of the Highlands and Islands Oilthigh na Gàidhealtachd agus nan Eilean

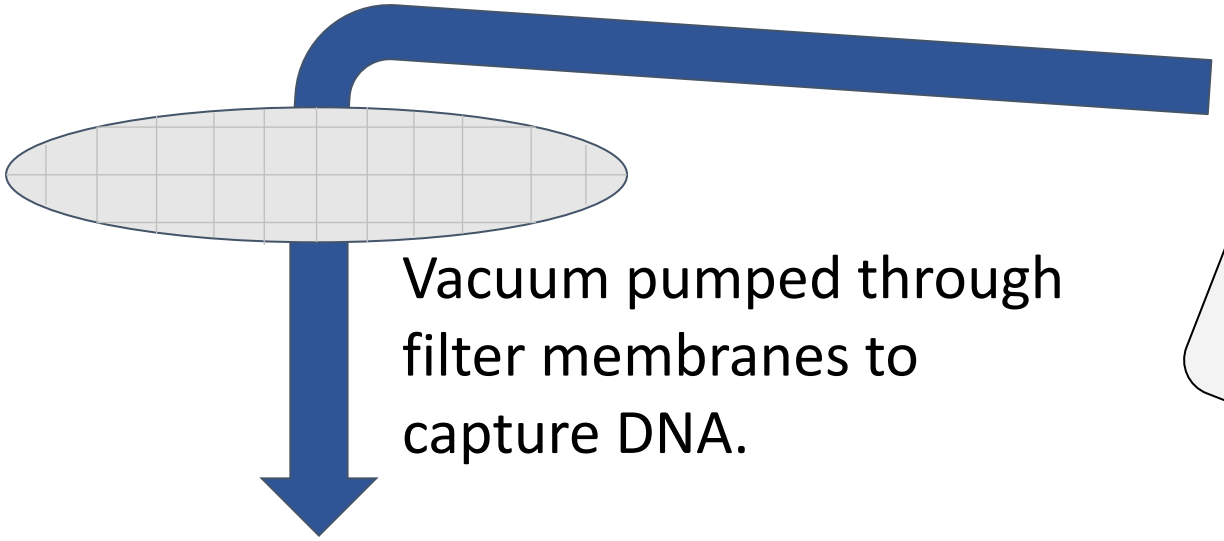
IOB Institute of Molecular and Clinical Ophthalmology Basel

University of Basel

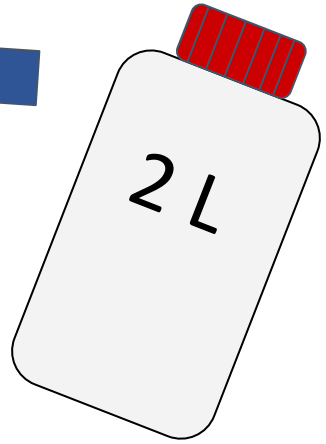
Environmental DNA

- As species interact with the environment, DNA is shed.
- This DNA is referred to as Environmental DNA (eDNA).
- eDNA can be sampled, to identify species present in the watercourse.

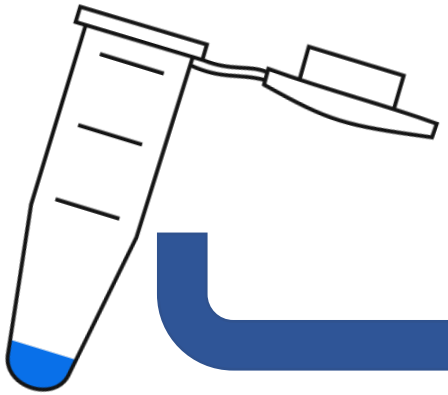
eDNA metabarcoding workflow



Vacuum pumped through filter membranes to capture DNA.



Water samples are obtained from the field.



DNA is extracted from filters.

Sellers *et al*. 2018

Extracted DNA is sequenced.

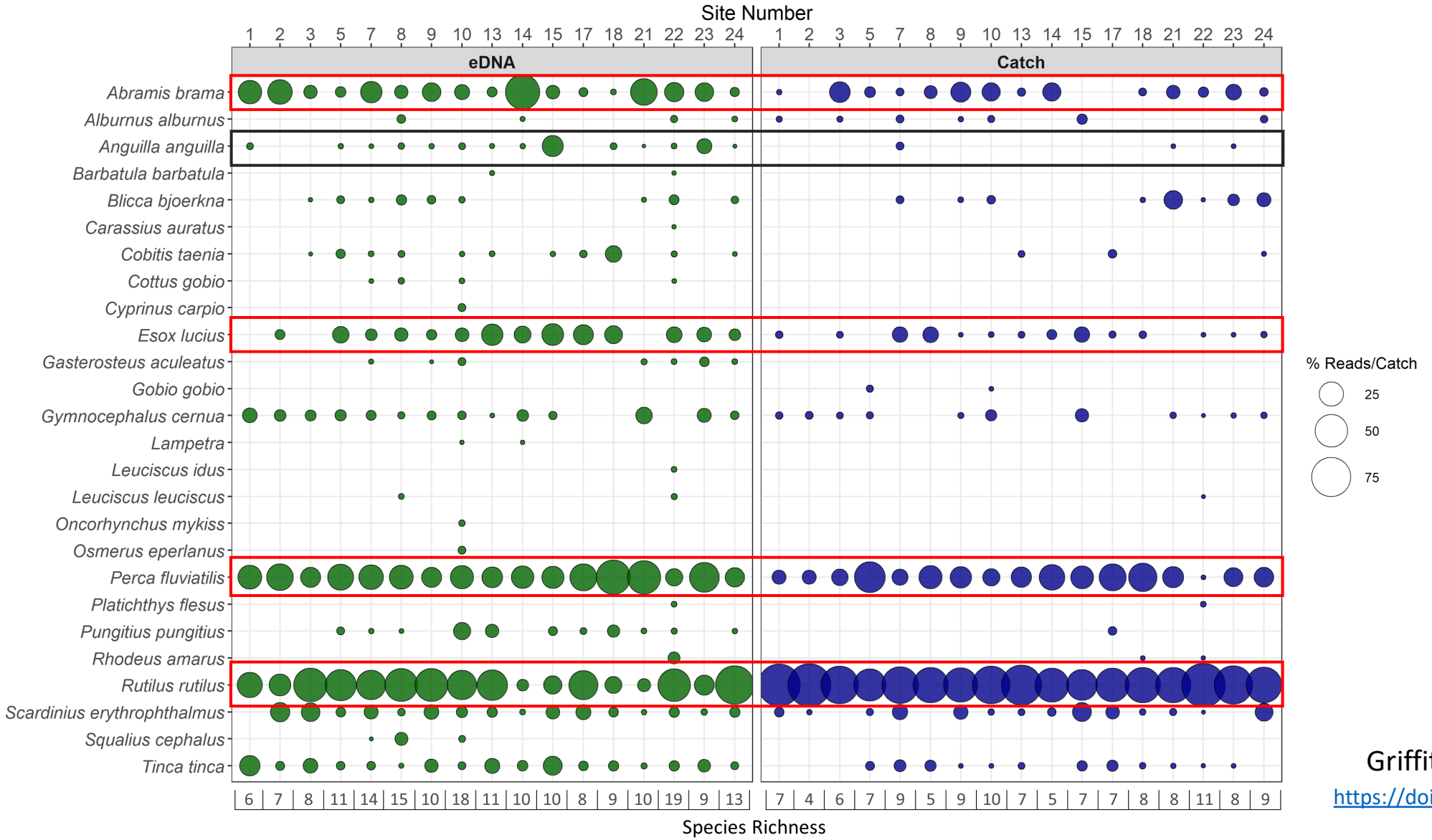
Sequenced reads are assigned to species.

Aims

- Optimise eDNA methods to determine species composition in heavily managed catchments.
- Apply this method as a tool to enable targeted management.

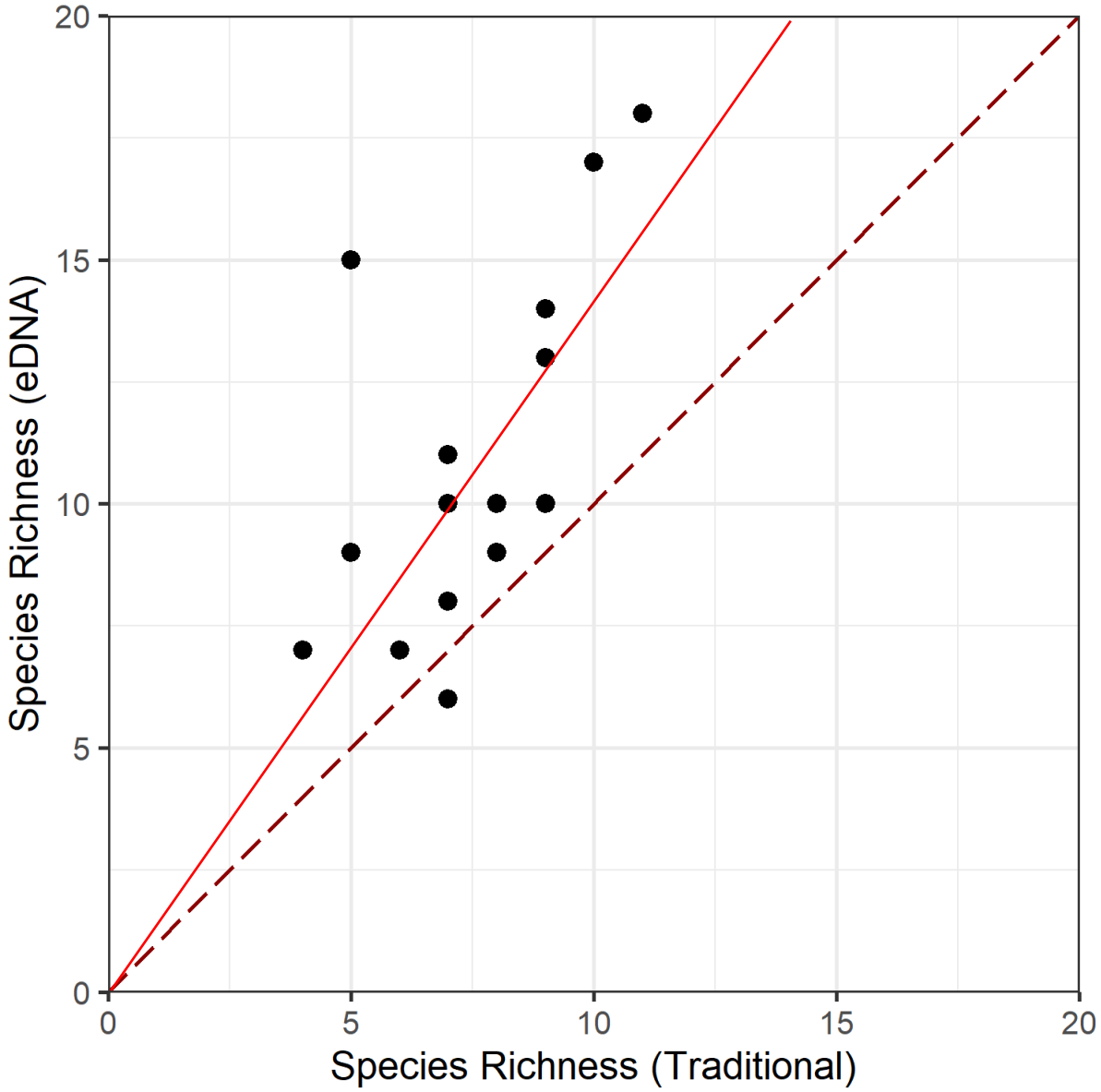
End goal → **Integrate eDNA based monitoring into prioritisation frameworks**

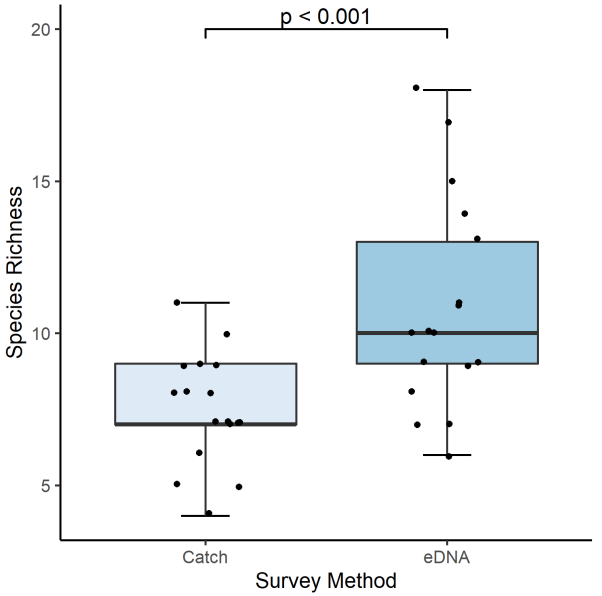
This method has already proven effective in highly managed catchments in the UK



Griffiths et al. 2020:
https://doi.org/10.1111/jfb.14497

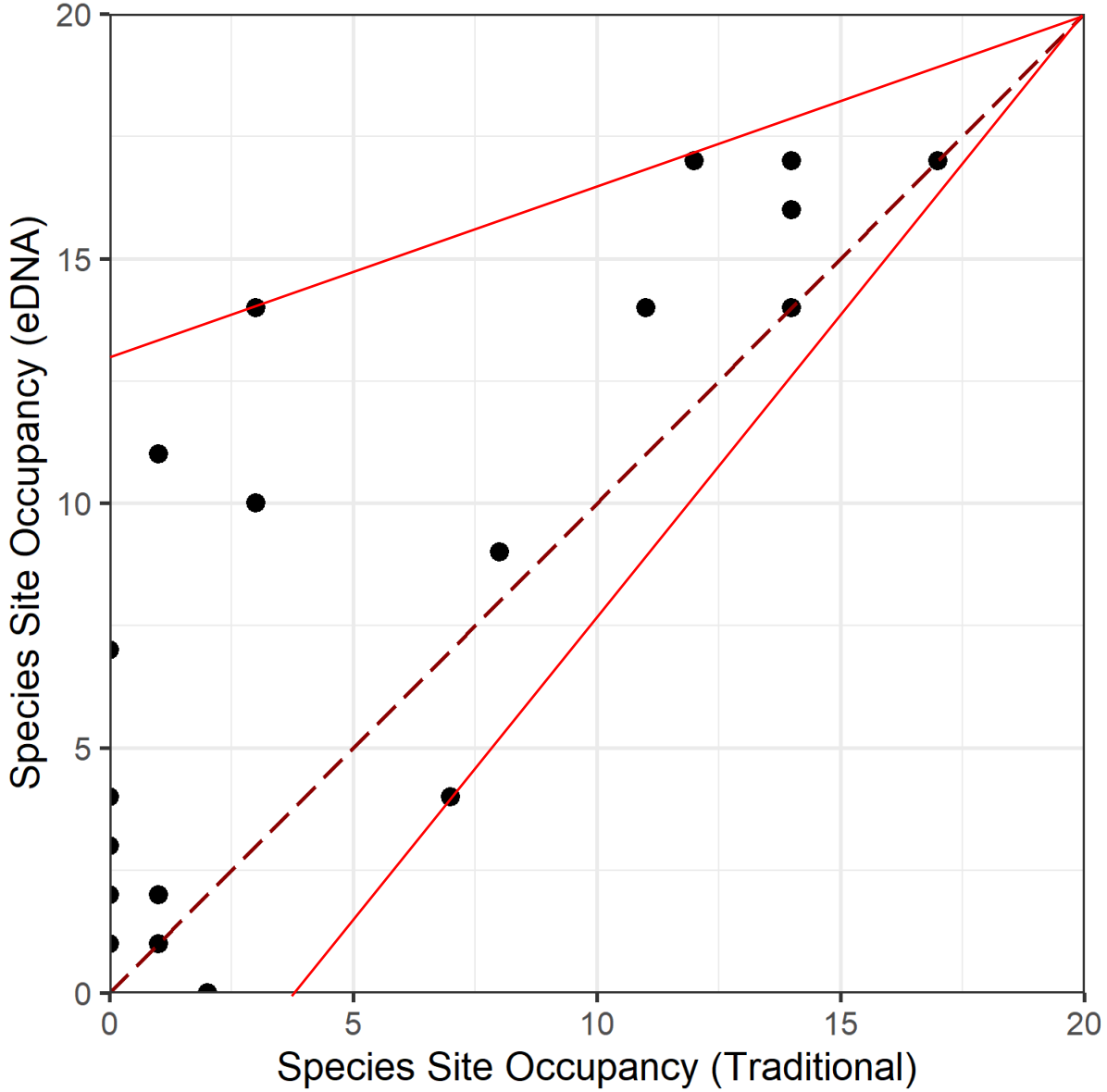
Species Richness

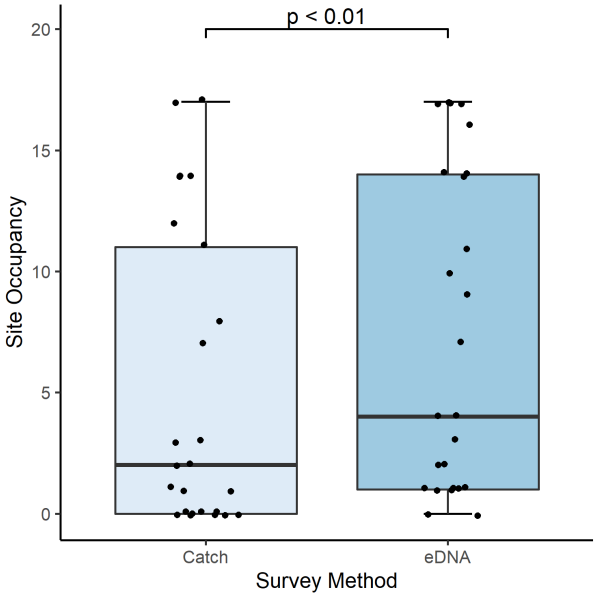




- Species richness was higher for 16/17 sites when using eDNA.
- Average species richness across the catchment was significantly higher when using eDNA methods.

Species Site Occupancy





- eDNA site occupancy was ≥ traditional methods for 22/25 fish species detected across all surveys.
- Catchment wide occupancy was significantly higher when using eDNA methods.

Prioritising fish pass solutions

- There are over 900 water pumping stations in England alone.
- Lots of innovative solutions to fish passage in development.
- We need informed prioritisation to make use of limited resources!

Priority species

- Specific legislation means some species take high priority.

- For these, a ‘false negative’ would be in breach of policy.

COUNCIL REGULATION (EC) No 1100/2007
of 18 September 2007
establishing measures for the recovery of the stock of European eel

2009 No. 3344
FISHERIES, ENGLAND AND WALES
RIVER, ENGLAND AND WALES
The Eels (England and Wales) Regulations 2009

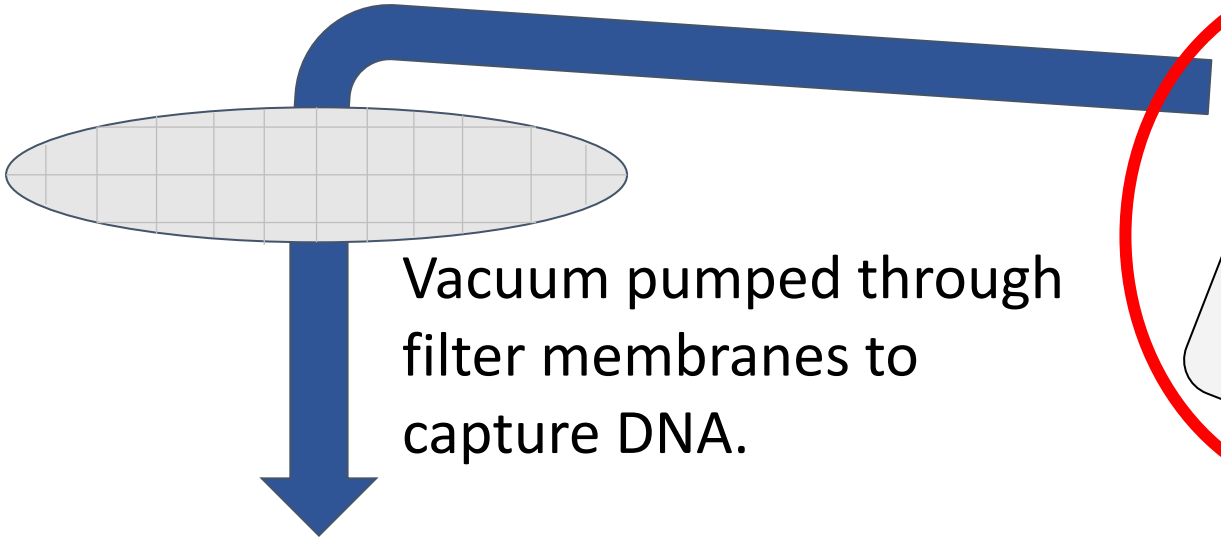
➢ To enable targeted management, we must be **confident** of where priority species are **present**, and **absent**.

Aims

- Determine the sensitivity of our eDNA metabarcoding workflow.
- Optimise the number of sample and lab replicates required.

End goal → **Sampling designs tailor-made to suit end-user requirements.**

eDNA metabarcoding workflow



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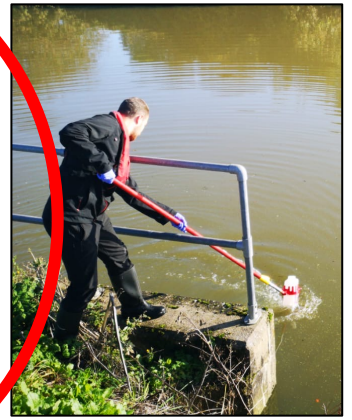
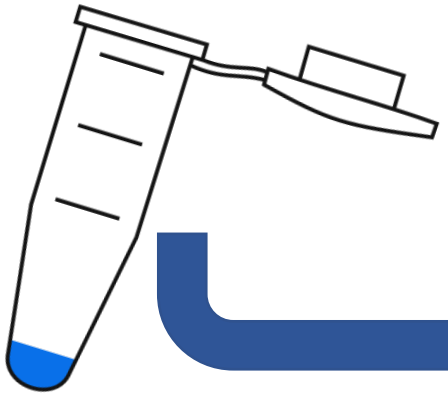


Field Replication

Water samples are obtained from the field.



DNA is extracted from filters.

Sellers *et al*. 2018

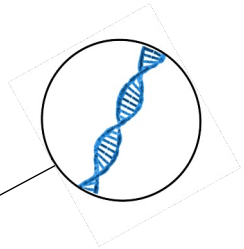
Lab replication

Extracted DNA is sequenced.

Sequenced reads are assigned to species.

Levels of replication

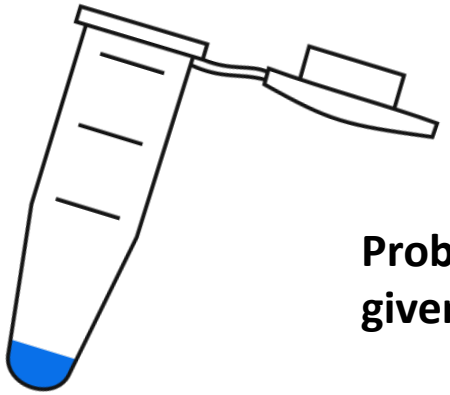
Site



Field replicate:

Probability of DNA in sample, given presence at site?

Lab replicates:



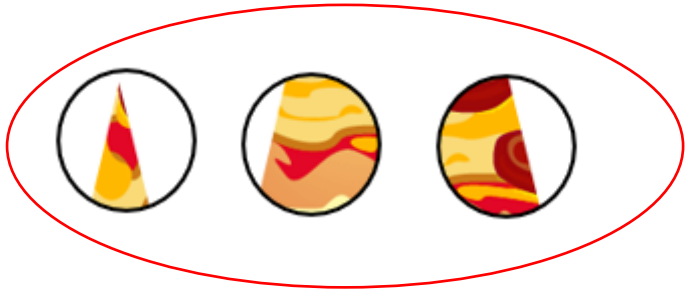
Probability DNA detection, given presence in sample?

Pizza analogy

(a) Site:

(b) Samples:

No olives?
(false negative)

1.  = 

2.  = 

3.  = 

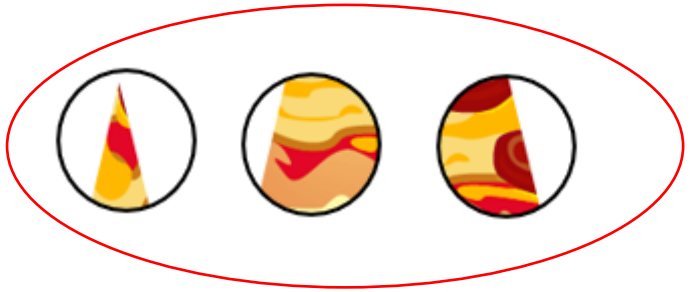
(c) Lab replicates:

Pizza analogy

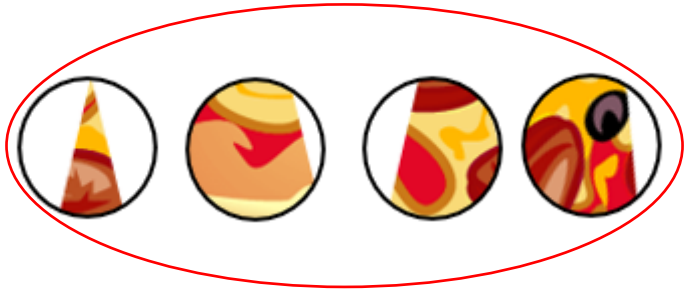
(a) Site:

olives ✓

(b) Samples:

1.  = 

2.  = 

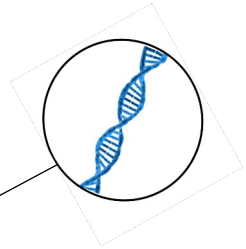
3.  = 

(c) Lab replicates:

Our Study

- **Field replication** – 44 sites each with 10x samples processed

- **Lab replication** – 10x samples obtained at a site each underwent 10x PCR replicates



Results

- We confirmed eel presence at 17 / 44 of our study sites.

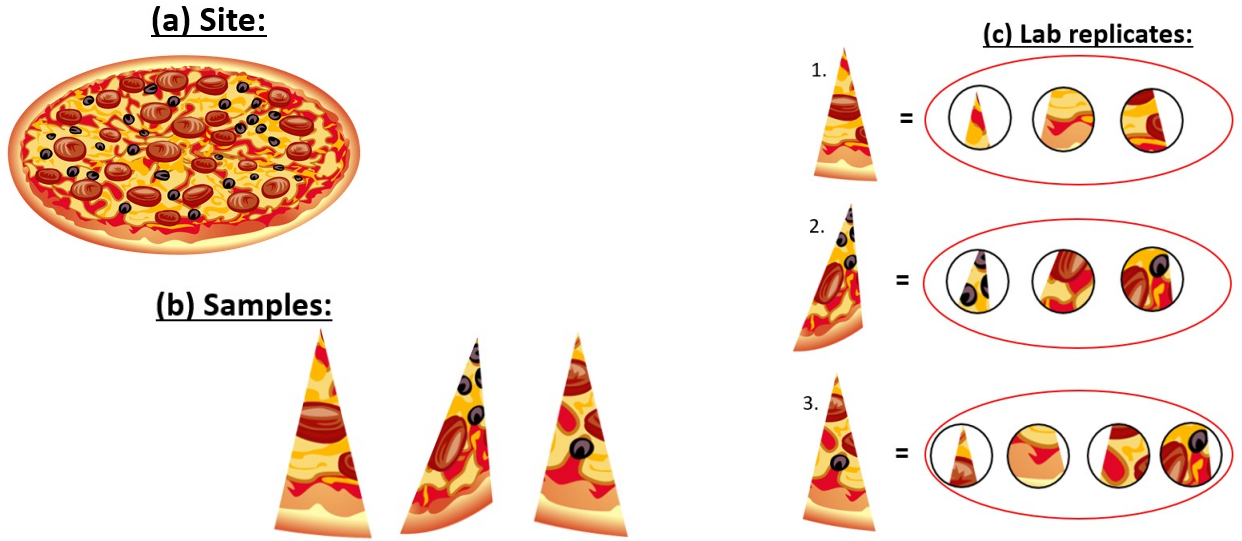
- But… does this mean we can be confident the remaining 27 should be classified as **absent** for eels?

Our model

a = probability of occupancy at a site
b = conditional probability of DNA presence in a sample given occupancy at the site
c = conditional probability of DNA detection in a replicate given presence in the sample

If **n** samples are taken, with **m** PCR replicates, and no eels are detected, then the probability of absence is:
(1-a)/(1-a+a*(1-b+b*(1-c)^m)^n)

When we apply this to eels:
a = 38.7%
b = 86.8%
c = 25.7%



Confidence of absence for Eels

	1 sample	2 samples	3 samples	4 samples	5 samples	6 samples	7 samples	8 samples	9 samples	10 samples
1 replicate	0.67	0.72	0.77	0.81	0.85	0.88	0.90	0.92	0.94	0.95
2 replicates	0.72	0.81	0.87	0.92	0.95	0.97	0.98	0.99	0.99	1.00
3 replicates	0.76	0.87	0.93	0.97	0.98	0.99	1.00	1.00	1.00	1.00
4 replicates	0.80	0.91	0.96	0.98	0.99	1.00	1.00	1.00	1.00	1.00
5 replicates	0.83	0.94	0.98	0.99	1.00	1.00	1.00	1.00	1.00	1.00
6 replicates	0.85	0.95	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
7 replicates	0.87	0.96	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
8 replicates	0.88	0.97	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
9 replicates	0.89	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
10 replicates	0.90	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
11 replicates	0.91	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
12 replicates	0.91	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
13 replicates	0.91	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
14 replicates	0.92	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
15 replicates	0.92	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
16 replicates	0.92	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
17 replicates	0.92	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
18 replicates	0.92	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
19 replicates	0.92	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
20 replicates	0.92	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Optimal strategy

Wasted effort

a = 38.7%
b = 86.8%
c = 25.7%

<95% confidence **>95% confidence** **>99% confidence**

****Numbers are rounded, 100% probability of absence does not exist!**

Cobitis taenia (Spined loach)

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
R1	0.80	0.82	0.85	0.87	0.89	0.90	0.92	0.93	0.94	0.95
R2	0.82	0.87	0.90	0.93	0.95	0.96	0.97	0.98	0.99	0.99
R3	0.85	0.90	0.94	0.96	0.98	0.99	0.99	0.99	1.00	1.00
R4	0.87	0.93	0.96	0.98	0.99	0.99	1.00	1.00	1.00	1.00
R5	0.89	0.95	0.98	0.99	1.00	1.00	1.00	1.00	1.00	1.00
R6	0.90	0.96	0.99	0.99	1.00	1.00	1.00	1.00	1.00	1.00
R7	0.92	0.97	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R8	0.93	0.98	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R9	0.94	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R10	0.95	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R11	0.95	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R12	0.96	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R13	0.97	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R14	0.97	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R15	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R16	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R17	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R18	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R19	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R20	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Rutilus rutilus (Roach)

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
R1	0.50	0.68	0.82	0.91	0.95	0.98	0.99	1.00	1.00	1.00
R2	0.63	0.86	0.96	0.99	1.00	1.00	1.00	1.00	1.00	1.00
R3	0.70	0.92	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R4	0.73	0.94	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R5	0.75	0.95	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R6	0.75	0.95	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R7	0.75	0.95	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R8	0.75	0.95	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R9	0.75	0.95	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R10	0.75	0.95	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R11	0.75	0.95	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R12	0.75	0.95	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R13	0.75	0.95	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R14	0.75	0.95	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R15	0.75	0.95	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R16	0.75	0.95	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R17	0.75	0.95	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R18	0.75	0.95	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R19	0.75	0.95	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R20	0.75	0.95	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Scardinius erythrophthalmus (Rudd)

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
R1	0.87	0.95	0.98	0.99	1.00	1.00	1.00	1.00	1.00	1.00
R2	0.90	0.97	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R3	0.90	0.97	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R4	0.90	0.97	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R5	0.90	0.97	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R6	0.90	0.97	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R7	0.90	0.97	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R8	0.90	0.97	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R9	0.90	0.97	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R10	0.90	0.97	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R11	0.90	0.97	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R12	0.90	0.97	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R13	0.90	0.97	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R14	0.90	0.97	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R15	0.90	0.97	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R16	0.90	0.97	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R17	0.90	0.97	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R18	0.90	0.97	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R19	0.90	0.97	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R20	0.90	0.97	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Esox lucius (Pike)

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
R1	0.50	0.71	0.85	0.93	0.97	0.99	0.99	1.00	1.00	1.00
R2	0.71	0.93	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R3	0.85	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R4	0.93	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R5	0.97	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R6	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R7	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R8	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R9	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R11	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R12	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R13	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R14	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R15	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R16	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R17	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R18	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R19	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R20	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

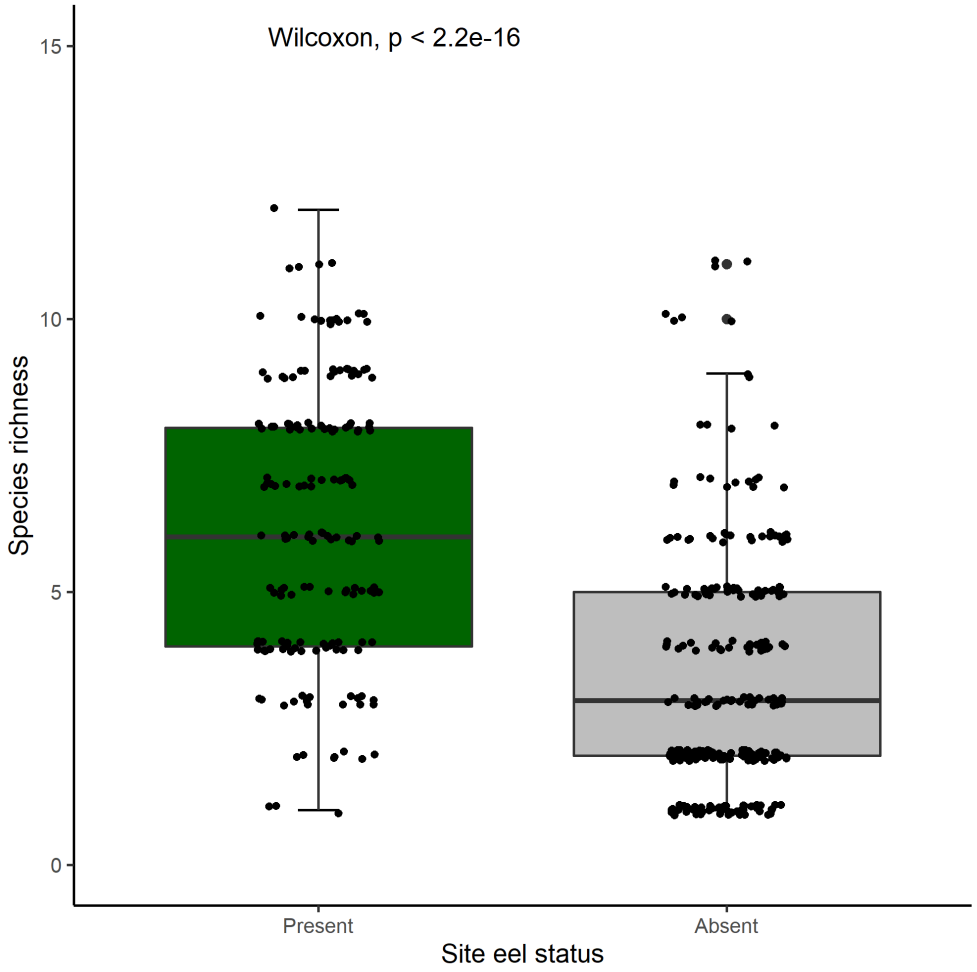
Gasterosteus aculeatus (3-Spine stickleback)

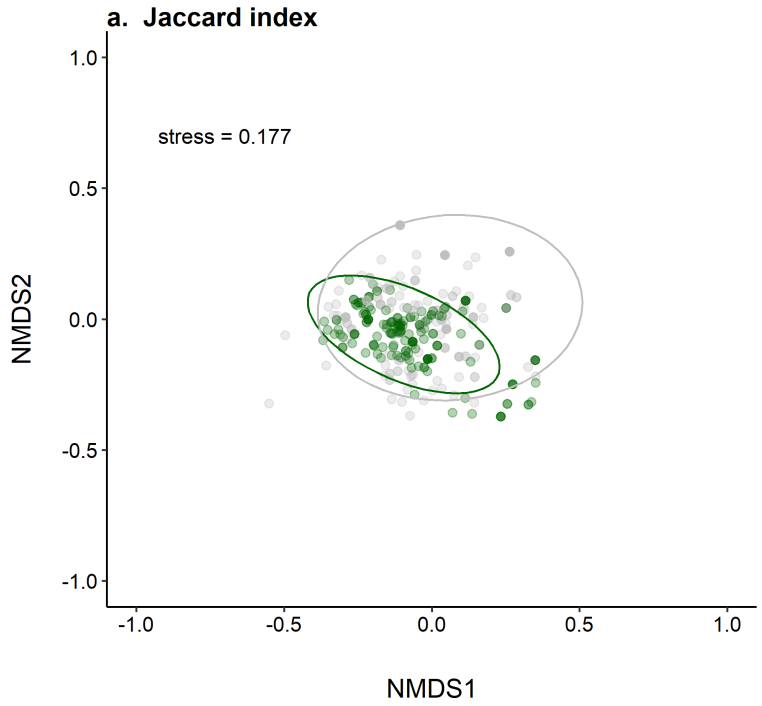
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
R1	0.33	0.46	0.59	0.71	0.81	0.88	0.92	0.95	0.97	0.98
R2	0.46	0.71	0.88	0.95	0.98	0.99	1.00	1.00	1.00	1.00
R3	0.59	0.88	0.97	0.99	1.00	1.00	1.00	1.00	1.00	1.00
R4	0.71	0.95	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R5	0.81	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R6	0.88	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R7	0.92	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R8	0.95	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R9	0.97	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R10	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R11	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R12	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R13	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R14	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R15	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R16	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R17	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R18	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R19	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R20	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

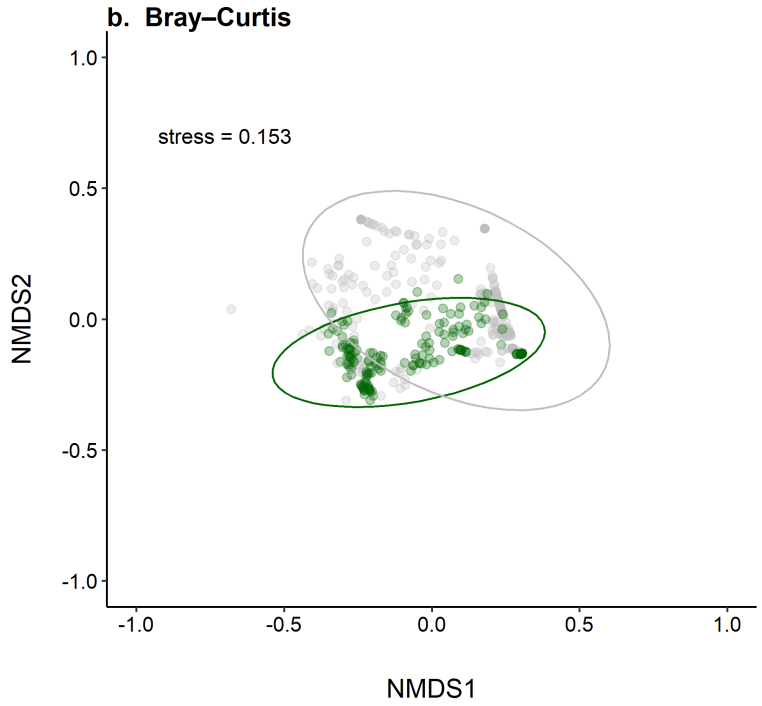
Pungitius pungitius (9-Spine stickleback)

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
R1	0.30	0.45	0.61	0.76	0.86	0.92	0.96	0.98	0.99	0.99
R2	0.41	0.69	0.88	0.96	0.99	1.00	1.00	1.00	1.00	1.00
R3	0.49	0.81	0.95	0.99	1.00	1.00	1.00	1.00	1.00	1.00
R4	0.54	0.86	0.97	0.99	1.00	1.00	1.00	1.00	1.00	1.00
R5	0.56	0.88	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R6	0.57	0.89	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R7	0.57	0.89	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R8	0.57	0.89	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R9	0.57	0.89	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R10	0.57	0.89	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R11	0.57	0.89	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R12	0.57	0.89	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R13	0.57	0.89	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R14	0.57	0.89	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R15	0.57	0.89	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R16	0.57	0.89	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R17	0.57	0.89	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R18	0.57	0.89	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R19	0.57	0.89	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00
R20	0.57	0.89	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Site categorization







Jaccard index (PERMANOVA, R2 = 0.08, DF = 1, P = 0.001)

Bray-Curtis (PERMANOVA, R2 = 0.11, DF = 1, P = 0.001)

Conclusions

@N_P_Griffiths

nathan.griffiths.ic@uhi.ac.uk

- We developed a model which allows assessment of the ‘confidence in absence’ of priority species.
- A >99% certainty that 27 of our sites were eel negative.
- **Can be applied to inform cost-benefit analysis and survey designs of future work (Confidence, Lab & Field resources).**