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Environmental DNA (eDNA) as a survey tool for *Anguilla anguilla*

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What is eDNA?

Nuclear or mitochondrial DNA released from an organism into their environment

- Captured from environmental substrates e.g. water, soil, invertebrates, air or vegetation,
- Can include secreted faeces, mucus, gametes, shed skin, hair, scales and carcasses

What is eDNA?

- Taken off over the last 5/10 yrs
- Cost of molecular methods falling over last 20 yrs (Nature, 2014)
- Quick, low cost, non-invasive survey method

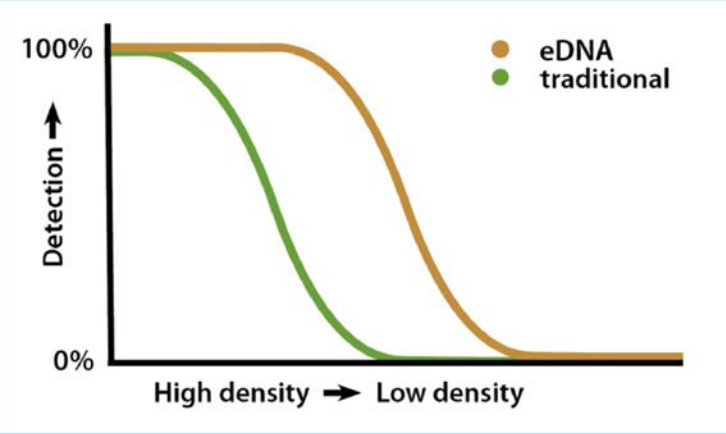


Figure - schematic advantage of the higher detection probability with the eDNA method compared to traditional methods (drawn after Darling & Mahon, 2011)

Great crested newts

Natural England approved 2013

Detection efficiency:

eDNA	99%
Bottle trapping	76%
Torch surveys	74%
Egg searches	44%

Biggs et al., 2014

What's the potential?

Presence/absence

Distribution, range

Habitat preferences

Barriers to migration

Quantification, relative population abundance

Sex, life stage…

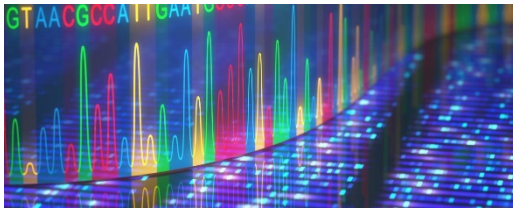
Photo: Laura Weldon

eDNA approaches

Sampling

DNA extraction

Amplification



Target species identification

Anguilla anguilla

Sequencing NGS

Species identification

Species list

How does it work?

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Sample collection

1L water samples

Filtration *in situ*

Peristaltic pump head

3 µm nitrocellulose pore filter

Storage buffer

How does it work?

Sample collection

Target amplification

Total DNA extracted

Quantitative PCR

Species specific primers & hydrolisis probe

Mitochondrial genome

Targets cytochrome *b* gene

80-150bp

Organism Presence / absence

eDNA advantages

- Non-invasive
- Time & cost effective
- Simple to collect (citizen science opportunities)
- Any time of the day
- Sample from remote, hard to reach areas
- Higher detection probabilities when compared to traditional methods – illusive or rare species, IAS, diseases
- Increased taxonomic resolution
- High sensitivity

eDNA limitations

- High sensitivity - contamination
- False negatives
- Sample storage
- Quantification
- Sex, life stages, population structure
- Detection probabilities differ with species and habitat type

Research overview:

Lab development & optimisation

Aquarium testing & optimisation

Initial field trials

Field sampling & method validation

1) Irish Loughs

2) Somerset levels

3) Corfu, Greece

Irish Loughs

Iascach Intíre Éireann
Inland Fisheries Ireland

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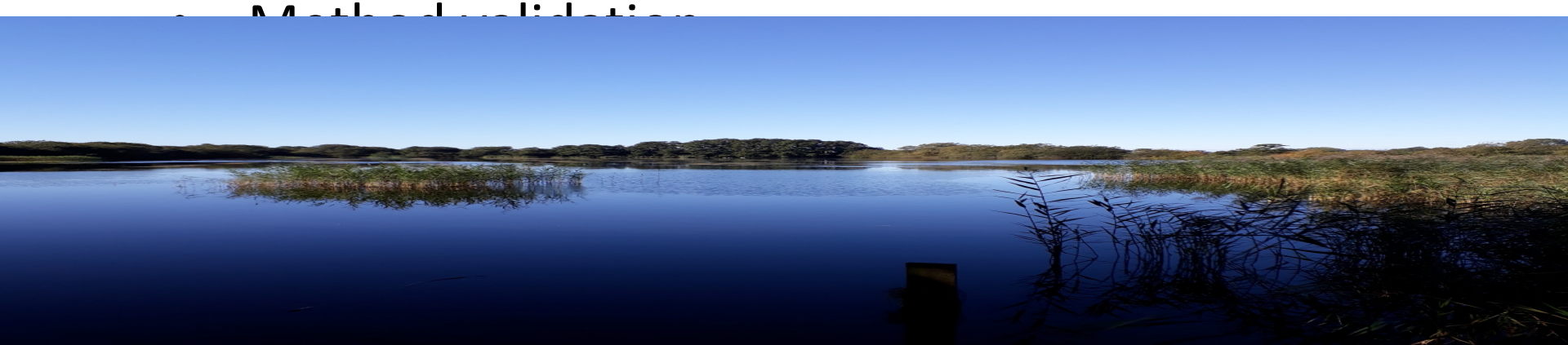
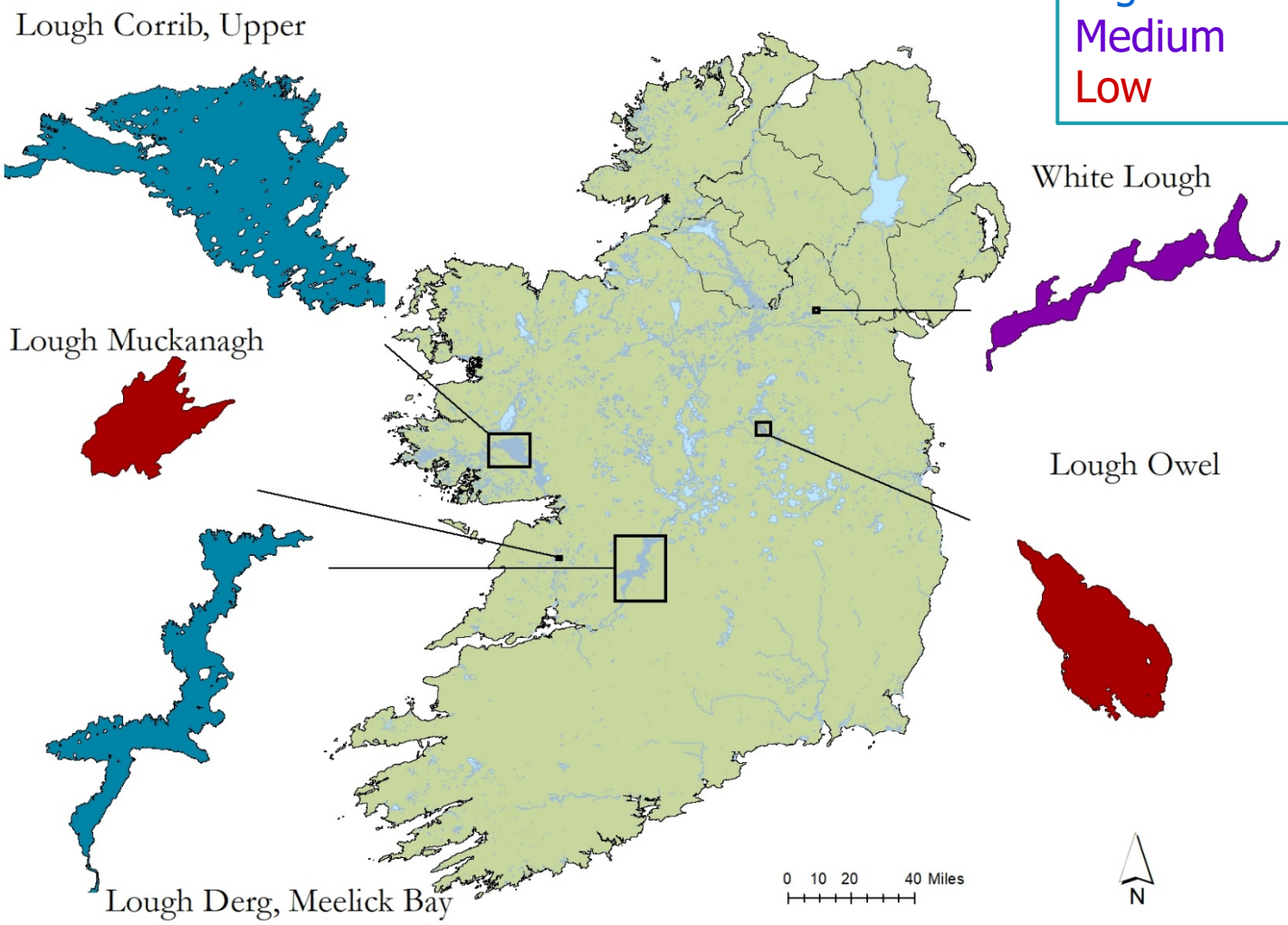
Aims:

- Can *A. anguilla* be detected in lakes?
- How does this method compare to fyke netting?
- Does eDNA recovery reflect eel populations suggested by the high, medium and low fyke net data.
- Method validation



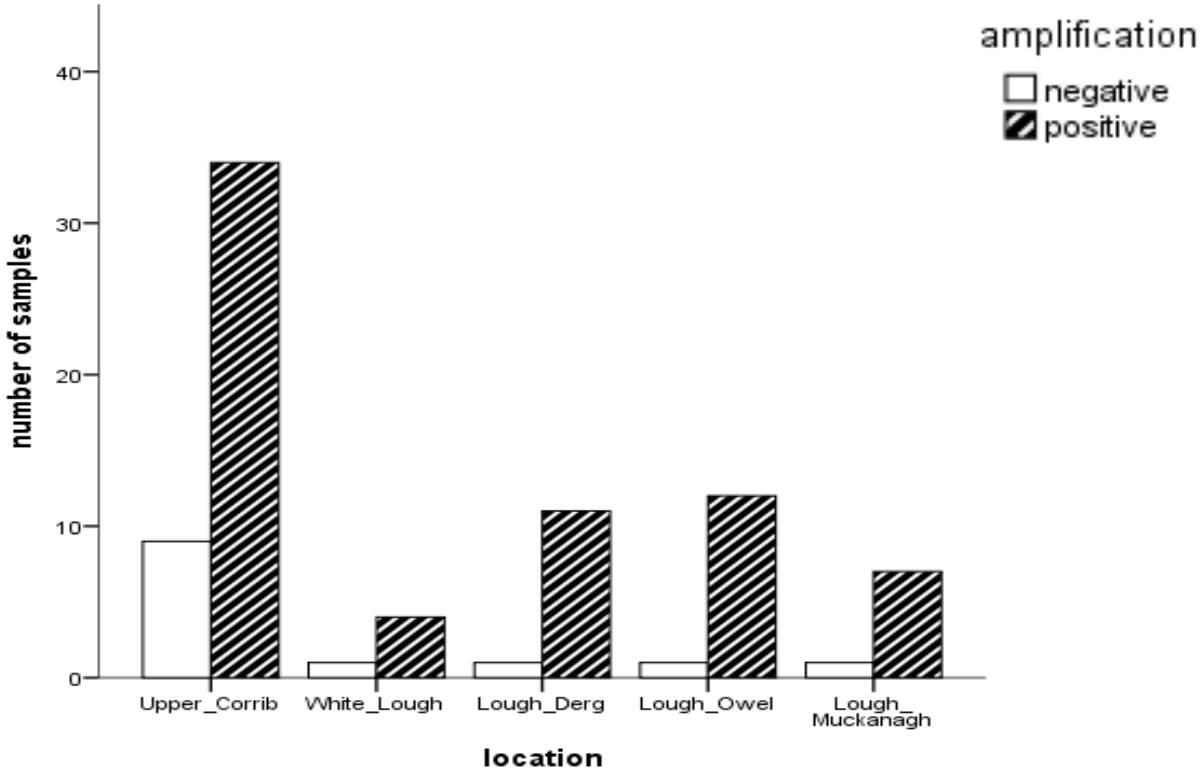
Irish Loughs

Eel population classification:
High
Medium
Low

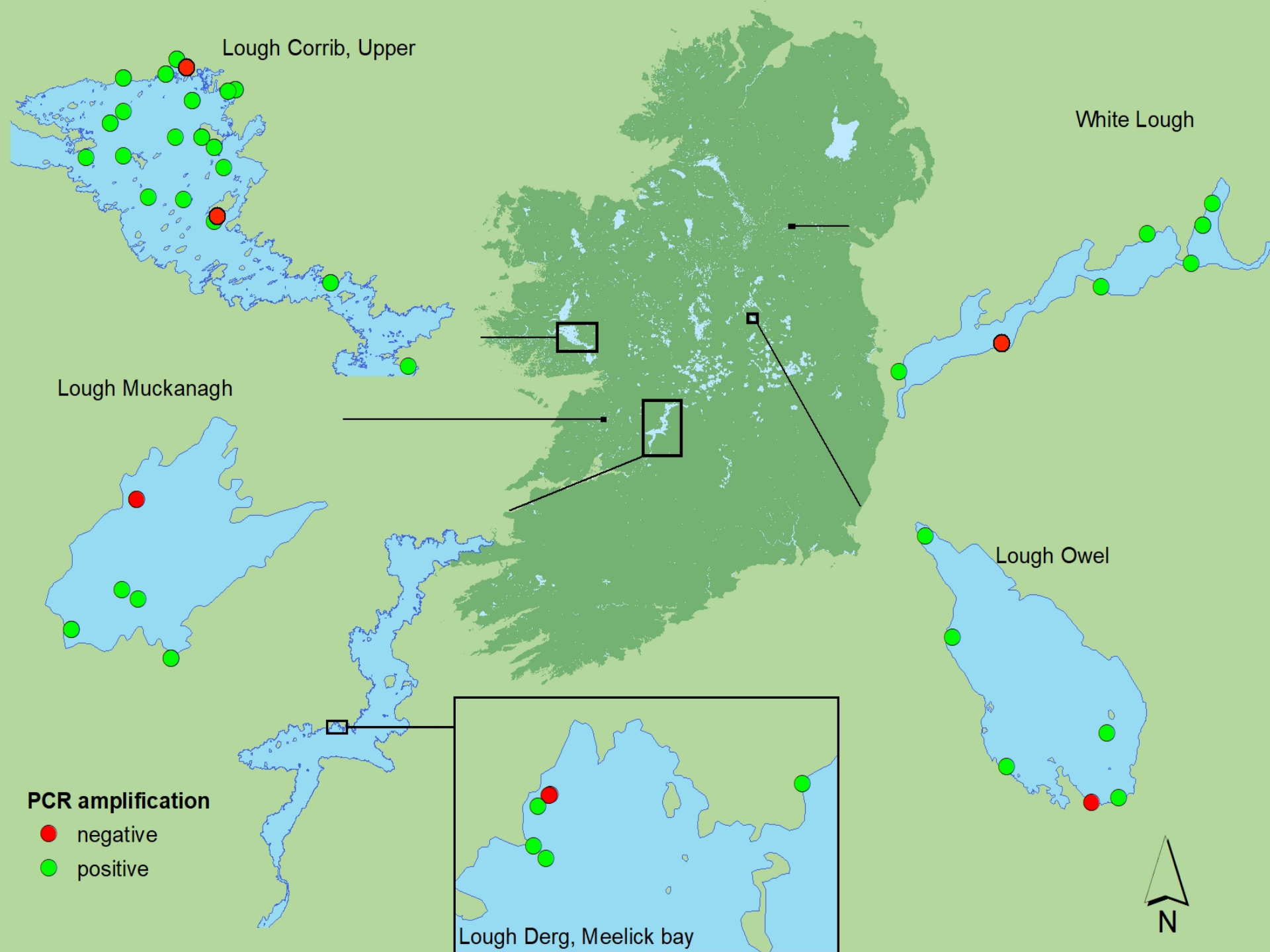


Irish Loughs

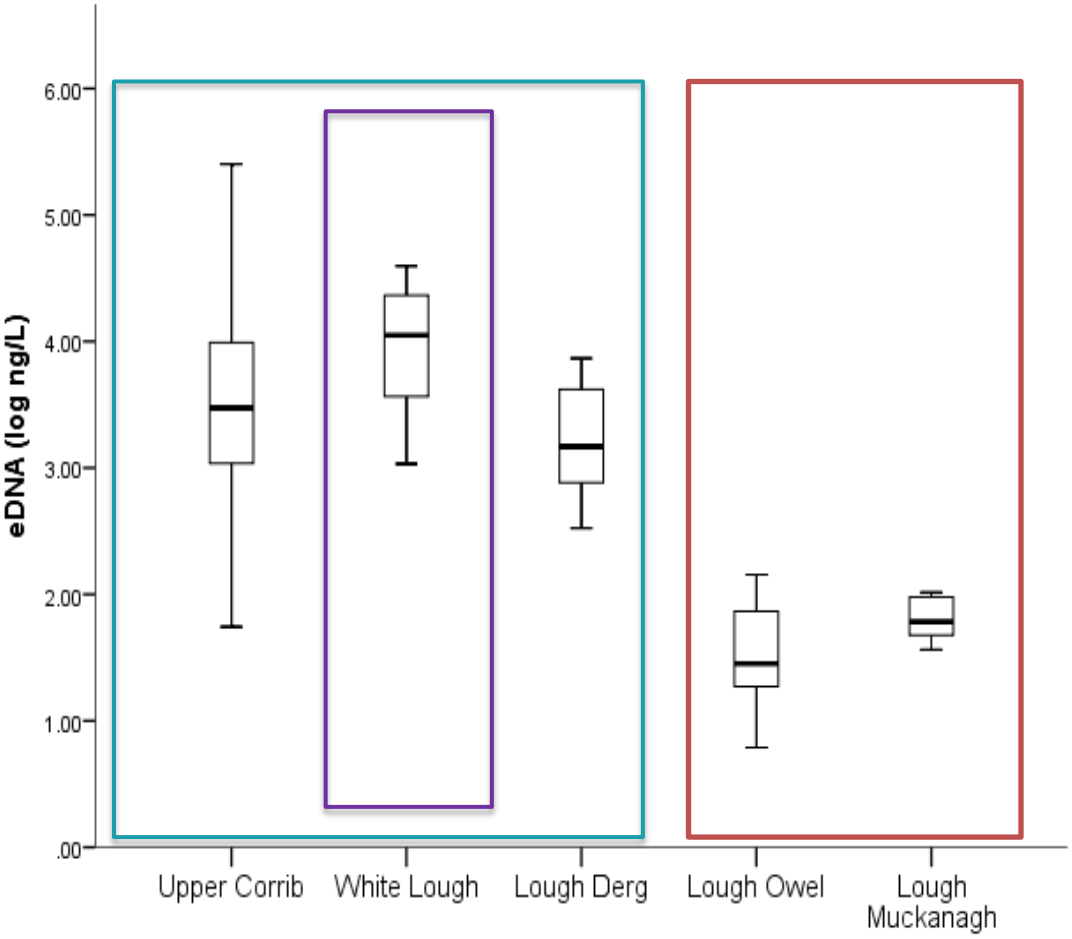
- Total of 83 water samples
- qPCR 6 replicates/sample



eDNA method captures and identifies *A. anguilla* DNA


Lough Corrib, Upper
White Lough
Lough Muckanagh
Lough Owel
Lough Derg, Meelick bay
PCR amplification
negative
positive
N

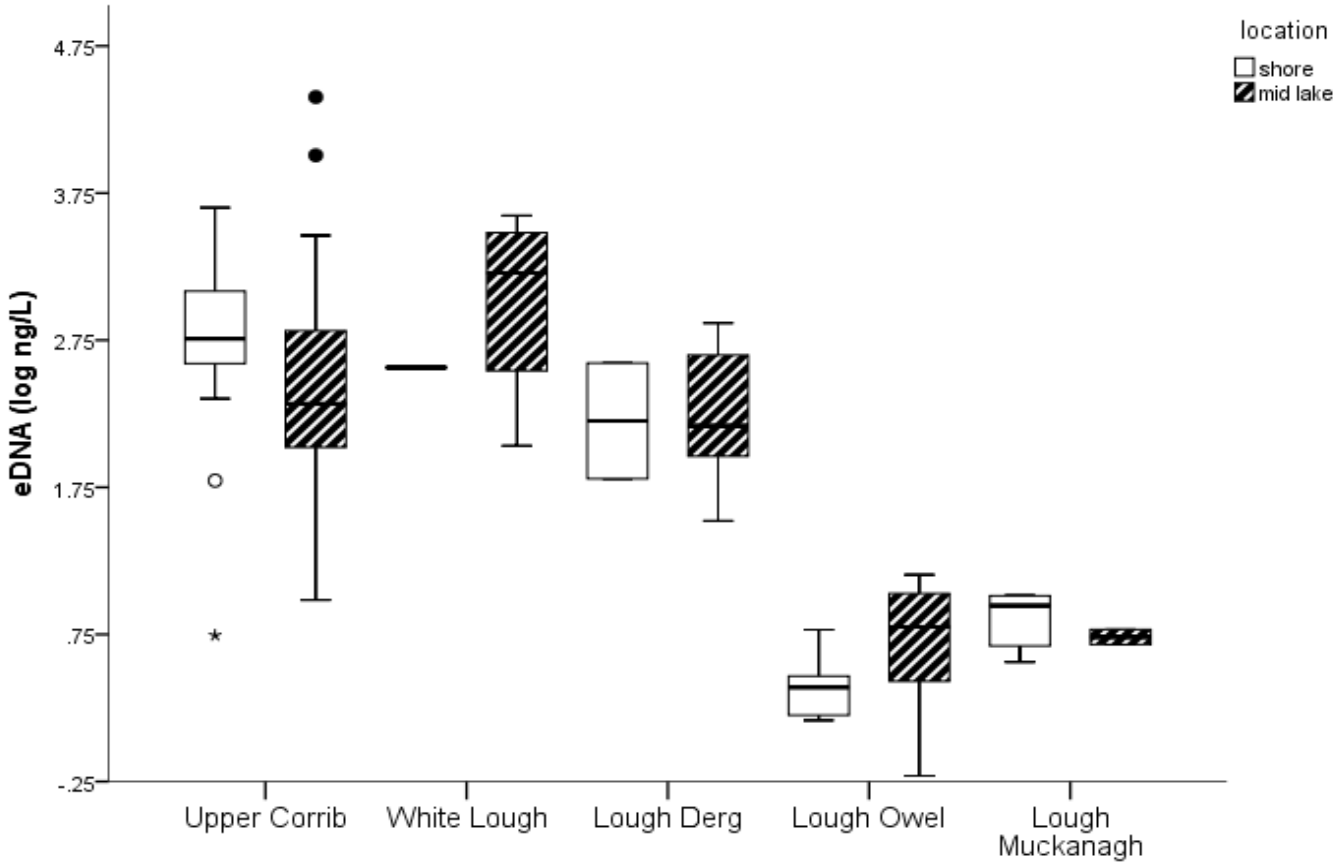
Irish Loughs



Eel population classification:
High
Medium
Low

eDNA recovery reflects relative population numbers

Irish Loughs

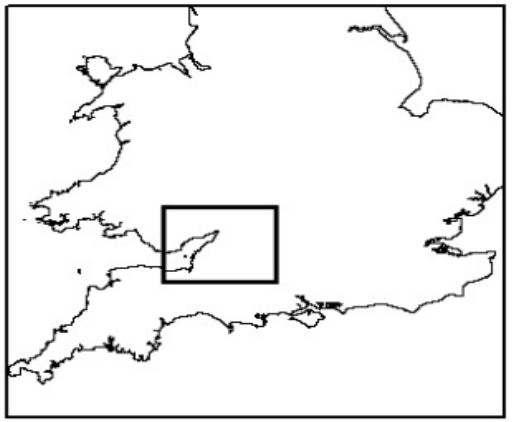


No significant difference in eDNA recovery from shore vs. mid-lake samples

Somerset

- Avalon Marshes, Somerset

- 3 freshwater ponds:
  - Westhay Moor
  - Westhay Heath
  - Catcott



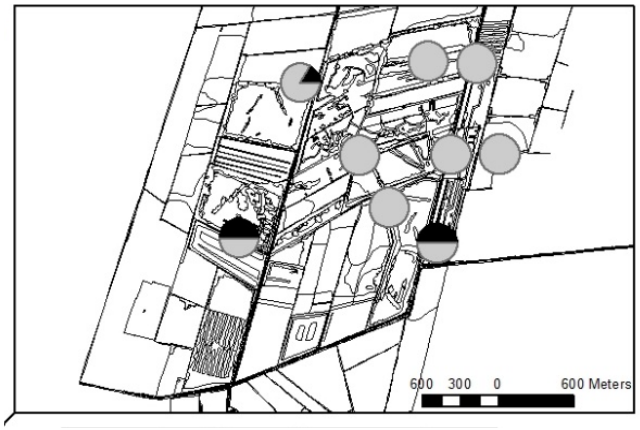
Aims:

- Explore method function in smaller water bodies
- Fine scale habitat mapping
- Depth sampling

THE wildlife TRUSTS SOMERSET

Westcountry Rivers Trust

Somerset



Westhay Moor

Westhay Heath

Catcott

Outcome of 6 qPCR replicates:

Negative
Positive

Preliminary findings: indicate habitat use and depth variation – more work to be done!

Summary

Effective non-invasive method for detection of *A. anguilla* from water samples

eDNA recovery reflects known relative populations

High sensitivity

Works well independently or in combination with traditional methods

eDNA: future potential

Lots of potential!

- Pre-survey tool
- Inform future monitoring plans
- Potential to be used for citizen science projects
- Can be used to answer more ecological questions

  e.g. habitat use, barriers to migration
- Other species
- Particularly useful for rare, elusive, invasive species or pathogens

Professor Katsumi Tsukamoto, Nihon University, Japan

Thank you for listening

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Acknowledgements

Laura Weldon, Dr Mark Steer, Dr Heather Macdonald, Dr Lyn Newton