Taking eDNA underground: detecting subterranean fauna in groundwater using eDNA

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DATE 27/02/2024

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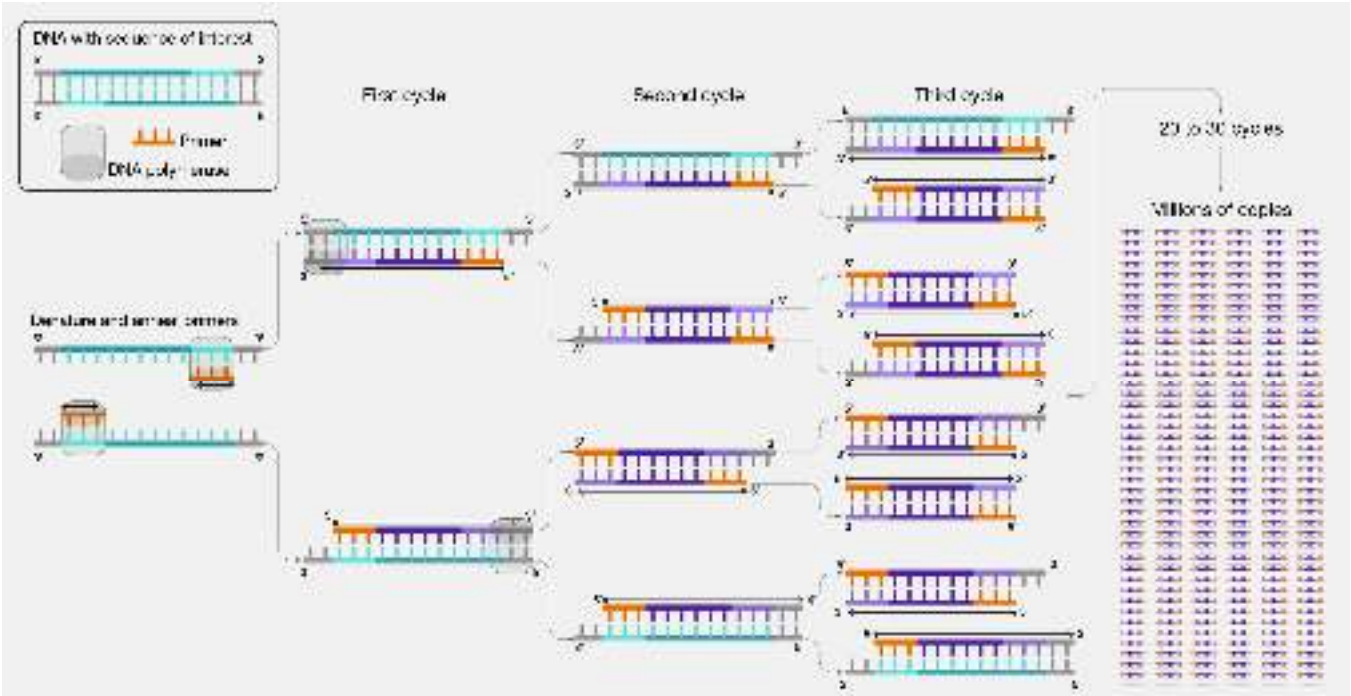
Aim 2:Develop eDNA metabarcoding assays for subterranean communities

What is a metabarcoding assay?

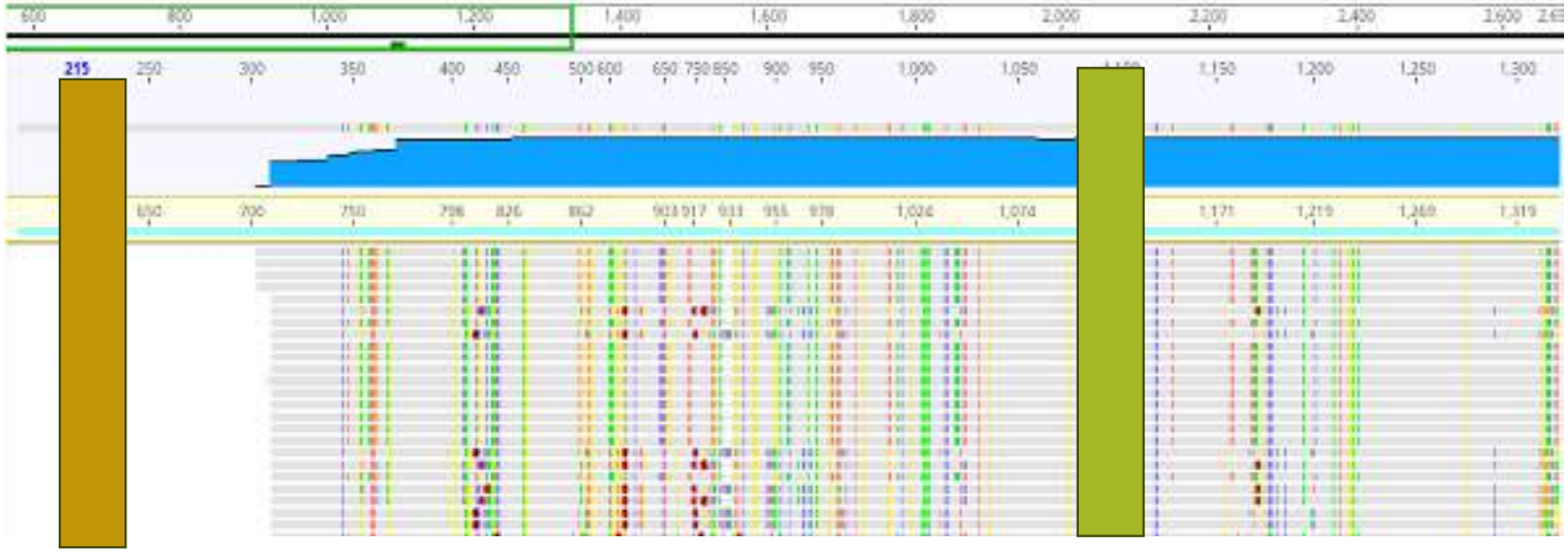
- PCR Amplification
- Combination of forward and reverse primer



https://www.google.com/url?sa=i&url=https%3A%2F%2Fwww.genome.gov%2Fgenetics-glossary%2FPolymerase-Chain-Reaction&psig=AOvVaw1c-y5oPRGsKpWQer8_18Zq&ust=1709006888752000&source=images&cd=vfe&opi=89978449&ved=0CBMQjRxqFwoTCLiy_PWQyIQDFQAAAAAdAAAAABAE

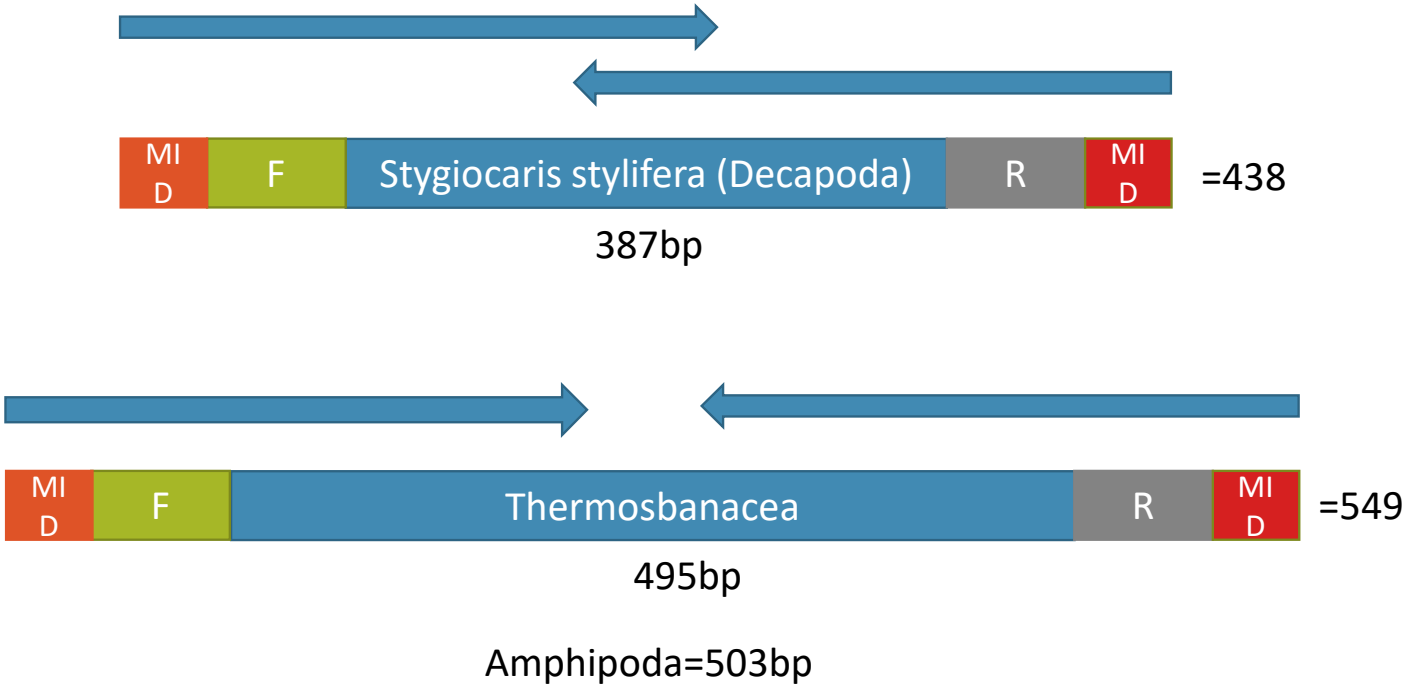
18S rRNA

- ~1800bp but variable in length
- Common Eukaryote assay
- Good first pass
- Need to trim first 50 bp



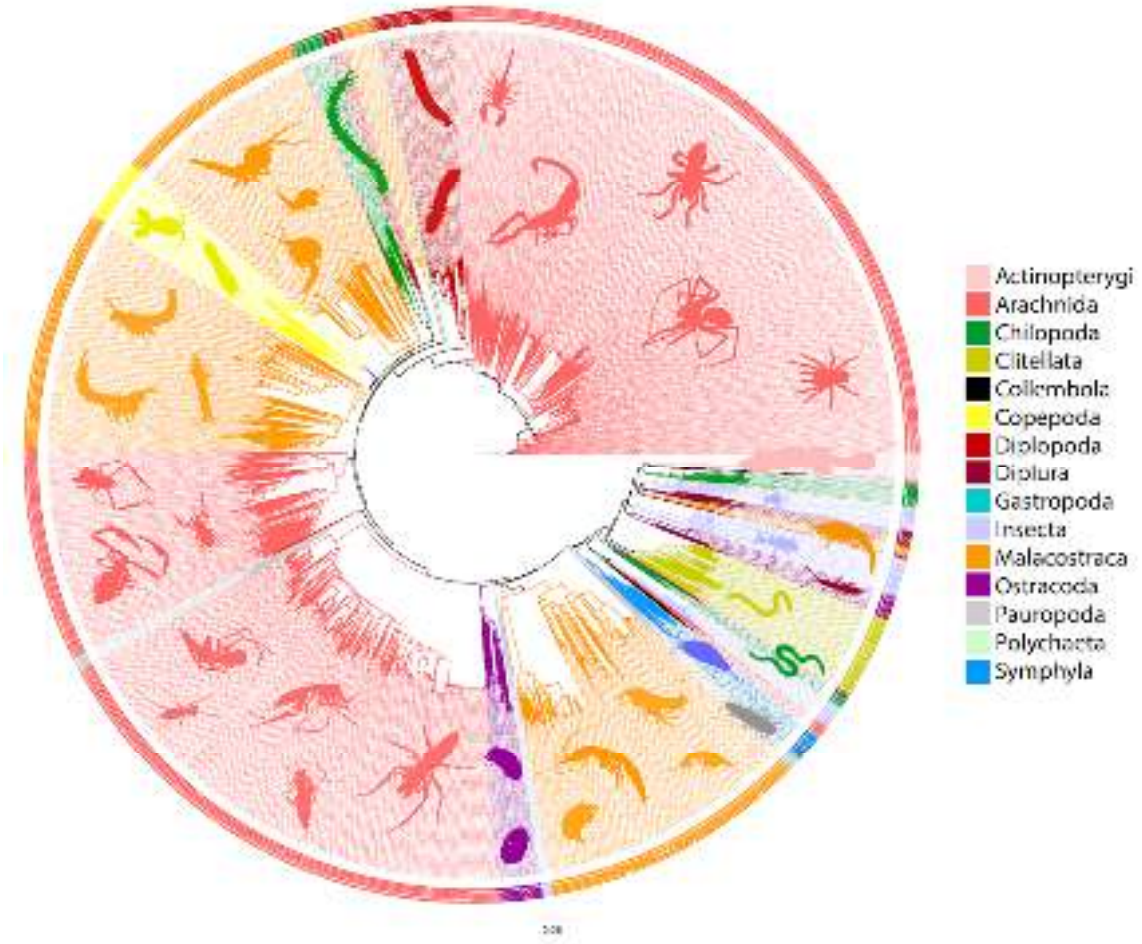
18S rRNA

- Variable length poses challenge for some taxa
  - Ispopoda, Thermosbanacea, Amphipoda
- Enough variability for Family genus identification of most taxa, species for some
  - Minimum ~98-99% id



COI

- Cytochrome c oxidase subunit I
- Super variable, up to 10% variability within a species
- Most commonly used barcode for animals
  - 4488 COI sequences in Subfauna reference database



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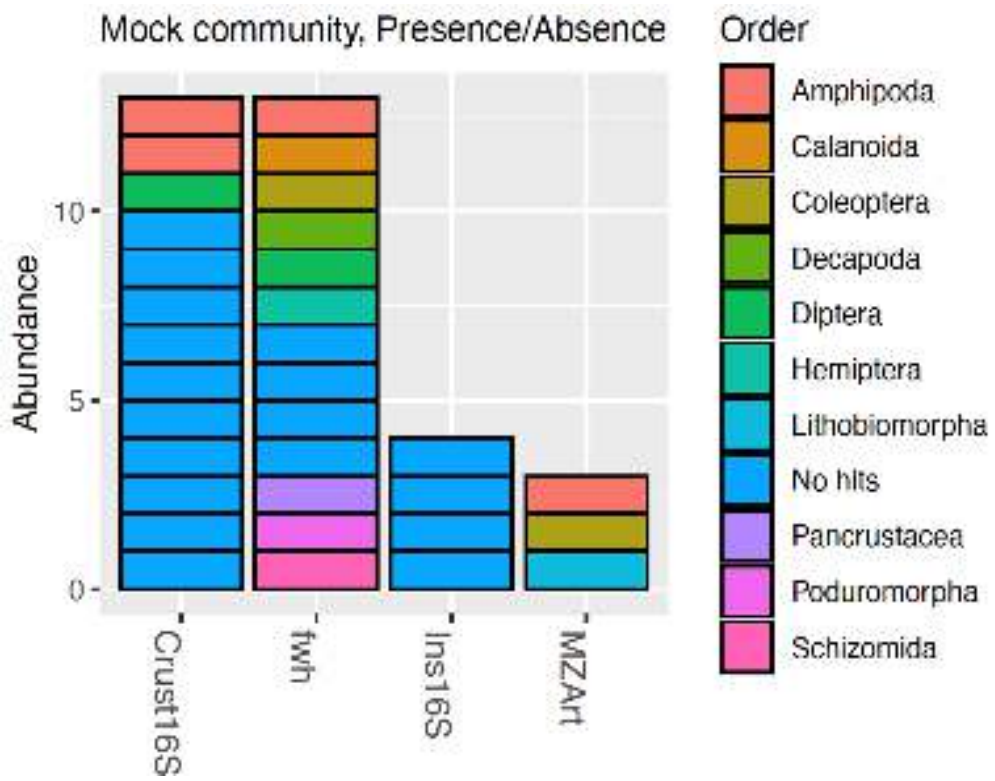
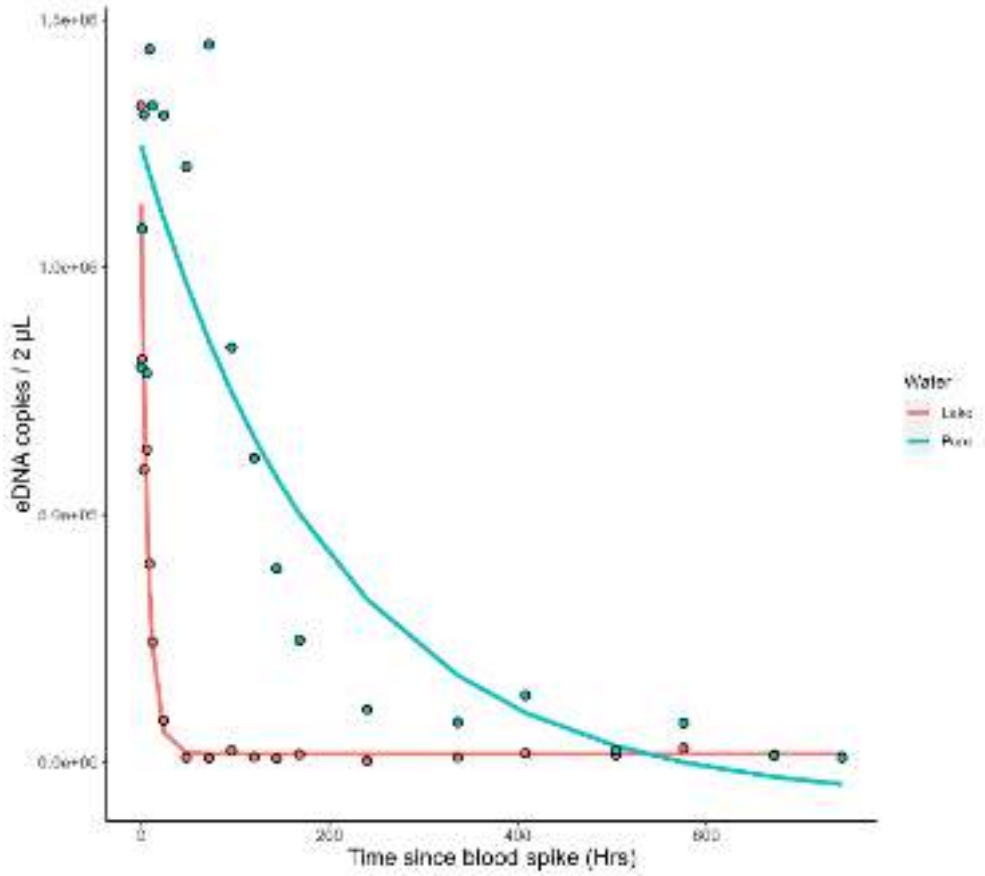
16S assays

- Lack of reference material
  - No hits
- Difficulty barcoding
- Mitogenomes
- Next steps
  - Assays targeting Bathynellacea, Isopoda



eDNA longevity

- Covid delays PhD starting
- According to the literature
  - Up one month (Dejean et al. 2011)
  - As little as one day (Barnes et al. 2014)
  - Microbial action
  - Longer in sediment (Mao et al. 2014)



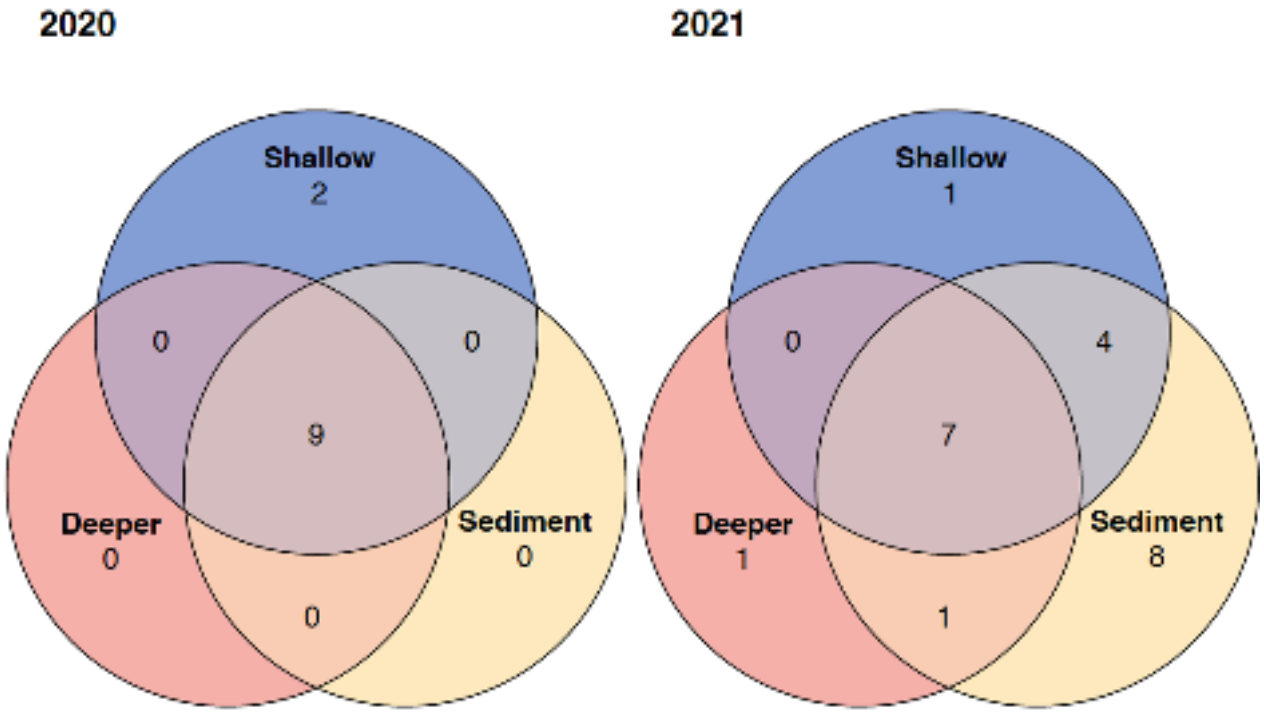
Aim 3: Develop a replicable sampling protocol

Aim 3

- Aim 3. Develop a replicable sampling protocol suitable for adoption into EIA
  - Test three different sampling approaches (water, sediment and bore scrapes).
  - Test the consistency of sampling eDNA over multiple years.

Sampling protocol

- Shallow water and sediment contain the most stygofauna diversity

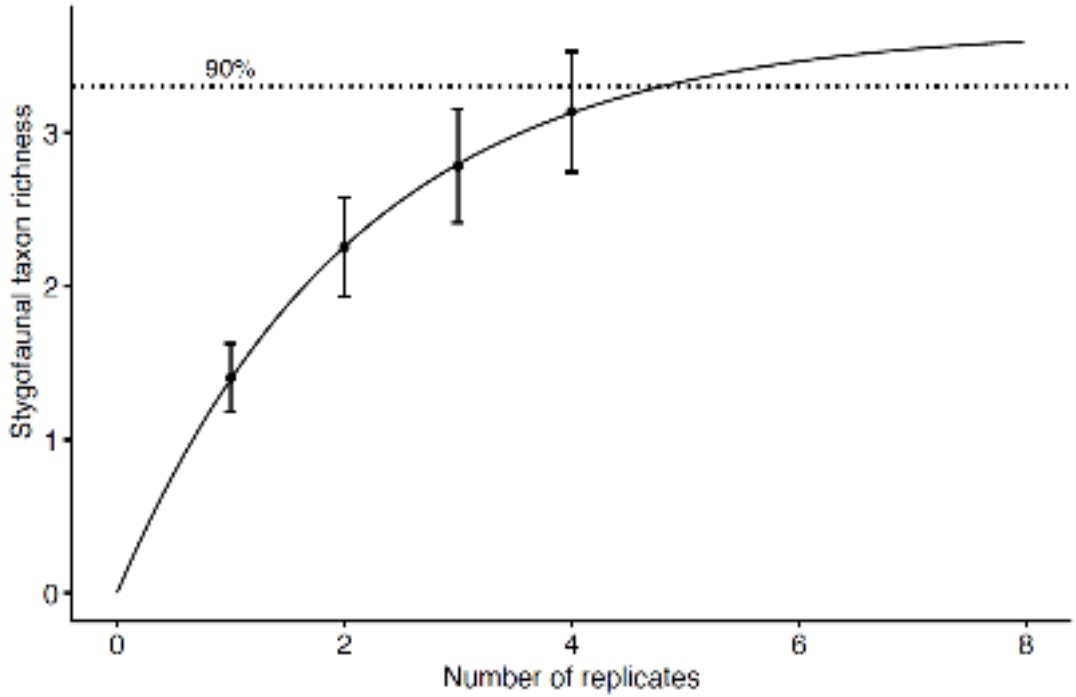


Sampling protocol

- Shallow water and sediment contain the most stygofauna diversity
- Order of sampling (nets or eDNA) makes little difference to stygofauna detection using eDNA

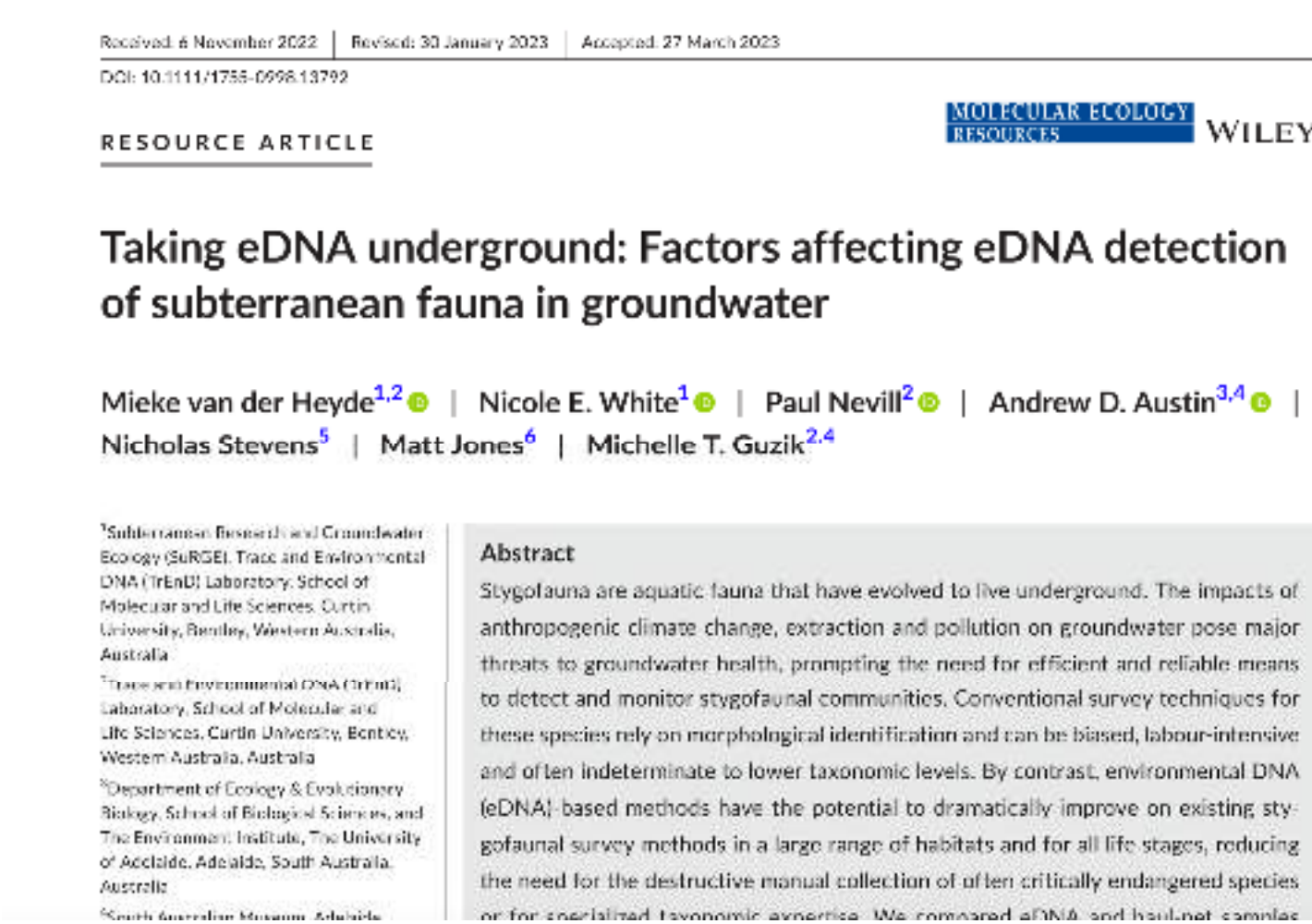
Sampling protocol

- Shallow water and sediment contain the most stygofauna diversity
- Order of sampling (nets or eDNA) makes little difference to stygofauna detection using eDNA
- Minimum 5 samples, preferably 6+
- Field controls



Consistency

- Bores tended to cluster together across sample years
- There are exceptions
  - e.g. MW34
- To come: three years of data using COI assay



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RESOURCE ARTICLE

MOLECULAR ECOLOGY RESOURCES WILEY

Taking eDNA underground: Factors affecting eDNA detection of subterranean fauna in groundwater

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Abstract

Stygofauna are aquatic fauna that have evolved to live underground. The impacts of anthropogenic climate change, extraction and pollution on groundwater pose major threats to groundwater health, prompting the need for efficient and reliable means to detect and monitor stygofaunal communities. Conventional survey techniques for these species rely on morphological identification and can be biased, labour-intensive and often indeterminate to lower taxonomic levels. By contrast, environmental DNA (eDNA)-based methods have the potential to dramatically improve on existing stygofaunal survey methods in a large range of habitats and for all life stages, reducing the need for the destructive manual collection of often critically endangered species or for specialized taxonomic expertise. We compared eDNA and haul-net samples

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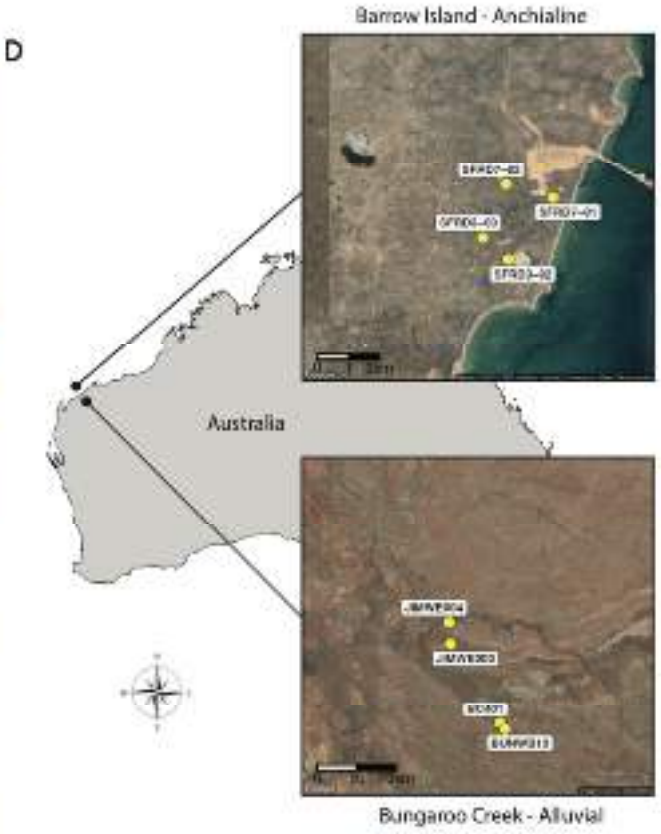
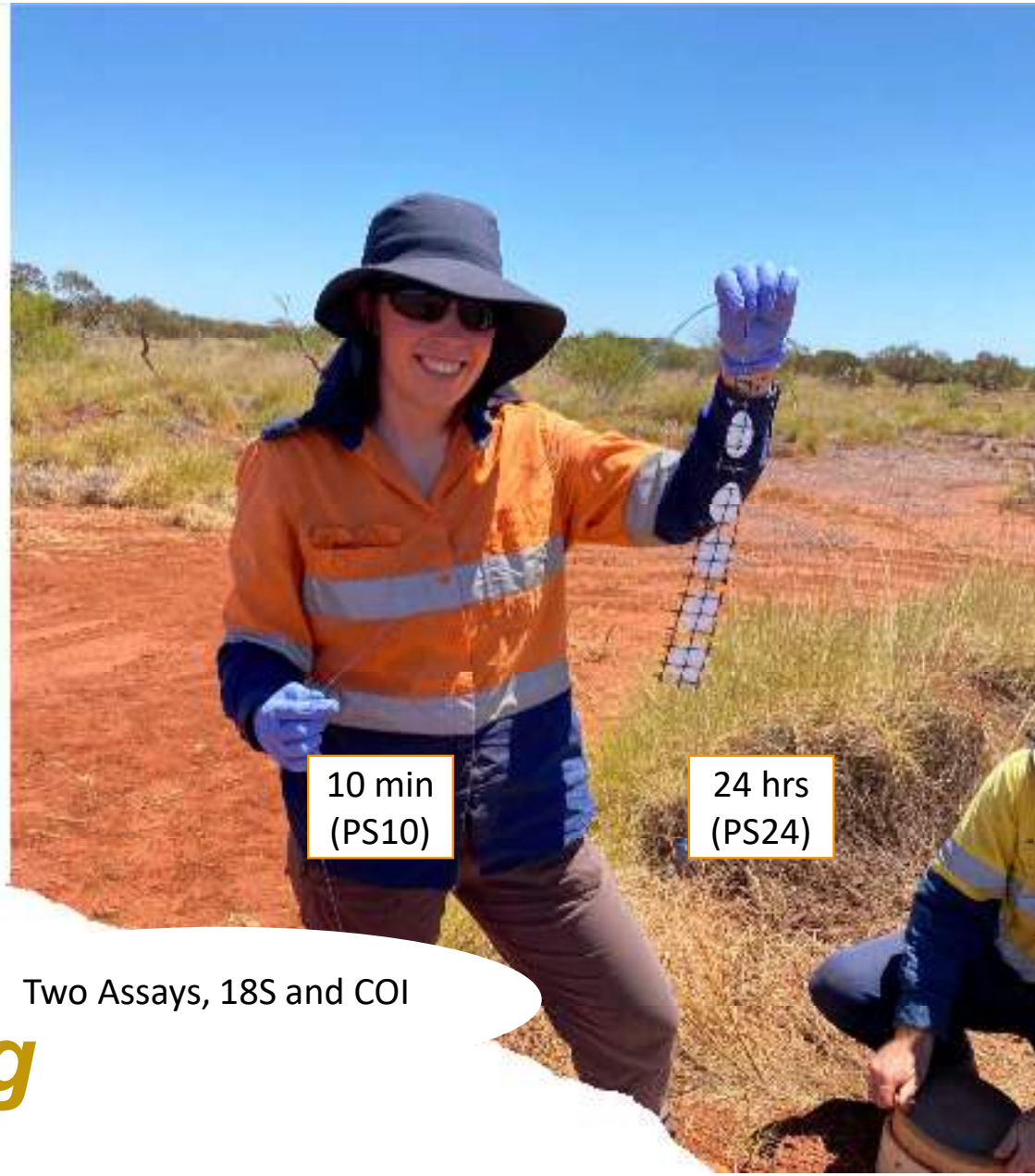
Filtering water is time consuming

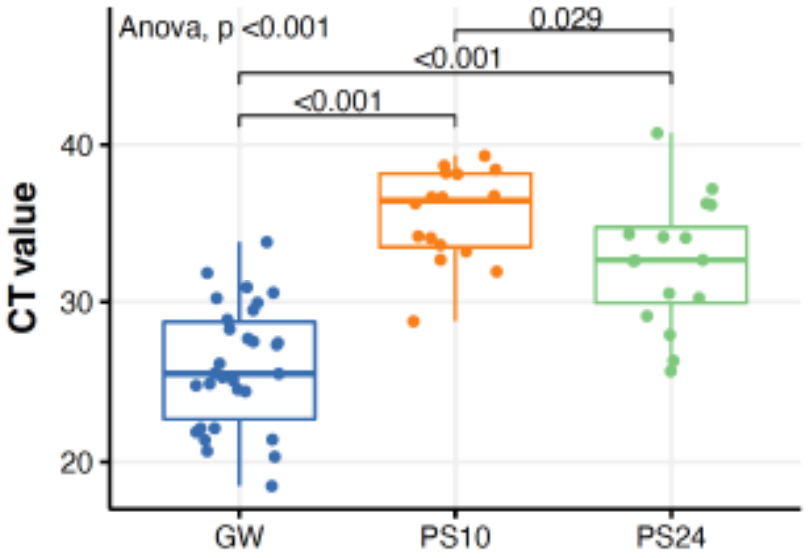




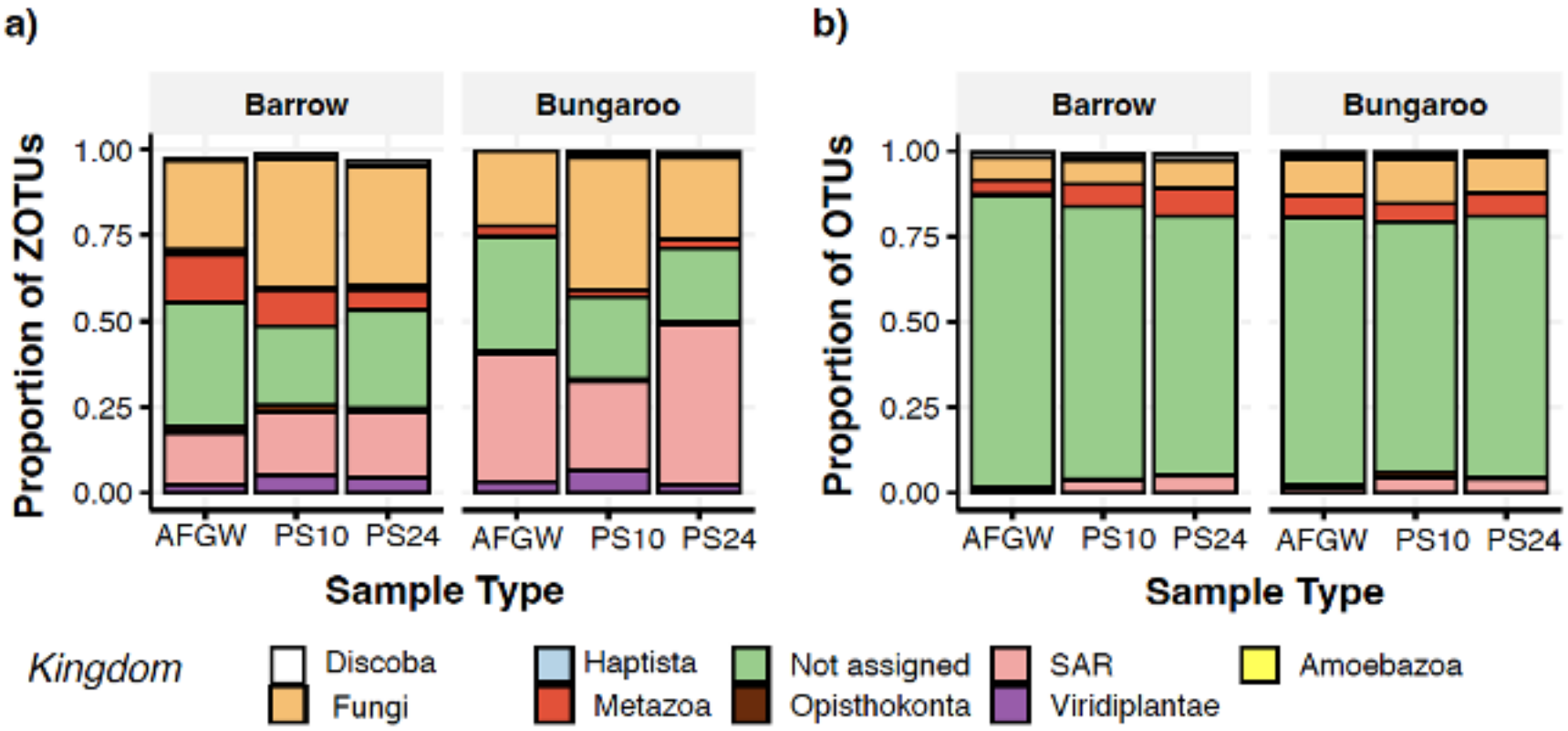
Two Assays, 18S and COI

Passive eDNA sampling

Passive samples capture less DNA



Passive samples capture similar diversity



Passive samples can detect Subterranean fauna

- But not as filtered wa
  - Passive
  - Filtered

stygofauna

troglofauna

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ORIGINAL ARTICLE

Environmental DNA
WILEY

Rapid detection of subterranean fauna from passive sampling of groundwater eDNA

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Abstract

Groundwater is an essential source of freshwater that supports surface ecosystems as well as organisms adapted to living underground. The impacts of anthropogenic climate change, extraction, and pollution pose major threats to groundwater ecosystem health, prompting a need for efficient and reliable means to detect and monitor subterranean faunal communities. Conventional survey of subterranean fauna relies on the collection of organisms for morphological identification, which can be biased, labor intensive, and often indeterminate at lower taxonomic levels. Environmental DNA (eDNA)-based methods can dramatically improve on stygofaunal surveys, but

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Conclusion

Subterranean fauna can be detected using eDNA

Multiple assays and sample types are required to detect the full breadth of biodiversity

Shallow water and sediment samples contain the most biodiversity

Passive samples can detect subterranean fauna and PUFs outperform membranes

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