Molecular library of groundwater macrofauna

Weaver, L.,[1] Wilkinson, S.,[2] Bolton, A.,[1] Webber, J.,[1] Smith, B.,[3] Gowo, P.,[4] van der Reis, A.,[5] Hicks, A.,[6] Handley, K.,[4] Liggins, L.[4]

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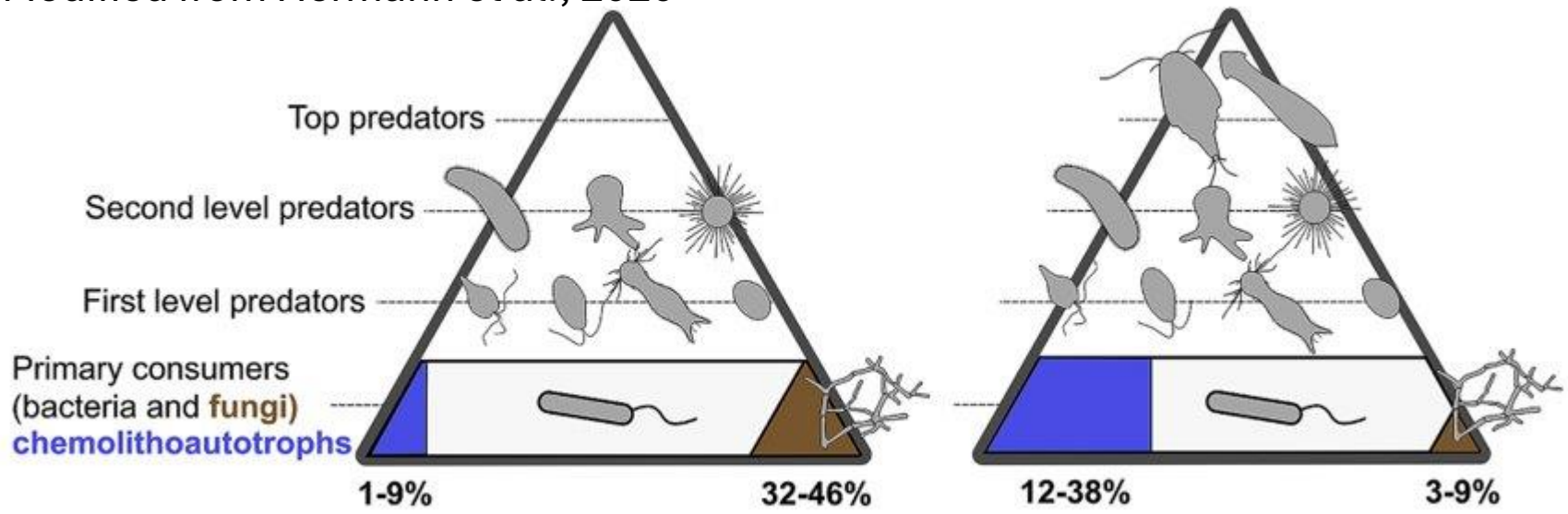
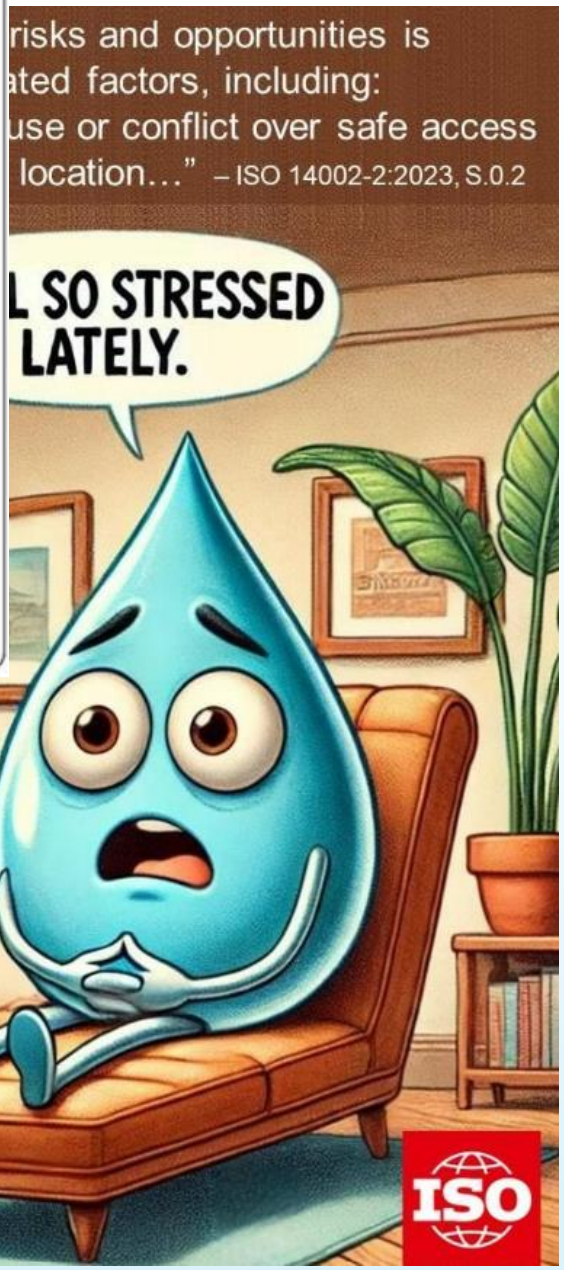
Modified from Hermann et al., 2020





- Who is in the groundwater ecosystem?
- The problem taxa - Stygofauna or macrofauna
- Current methods
- Pitfalls…….

Sampling methods

1974: Williams and Hynes first used the sediment core method

since 1970: first population genetic studies using gel electrophoresis techniques

003 & 2004: ebert et al. & rton et al. first lied molecular nniques (DNA oding method) and GIS

2005: Hahn resented the baited phreatic trap system

1970 - 1990: Coleman and Hynes (1970); Hynes (1974); Mathieu et al. (1984/1991) & Tabacchi (1990) invented the artficial substrates method

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Current field Sampling methods

Netting

Pumping

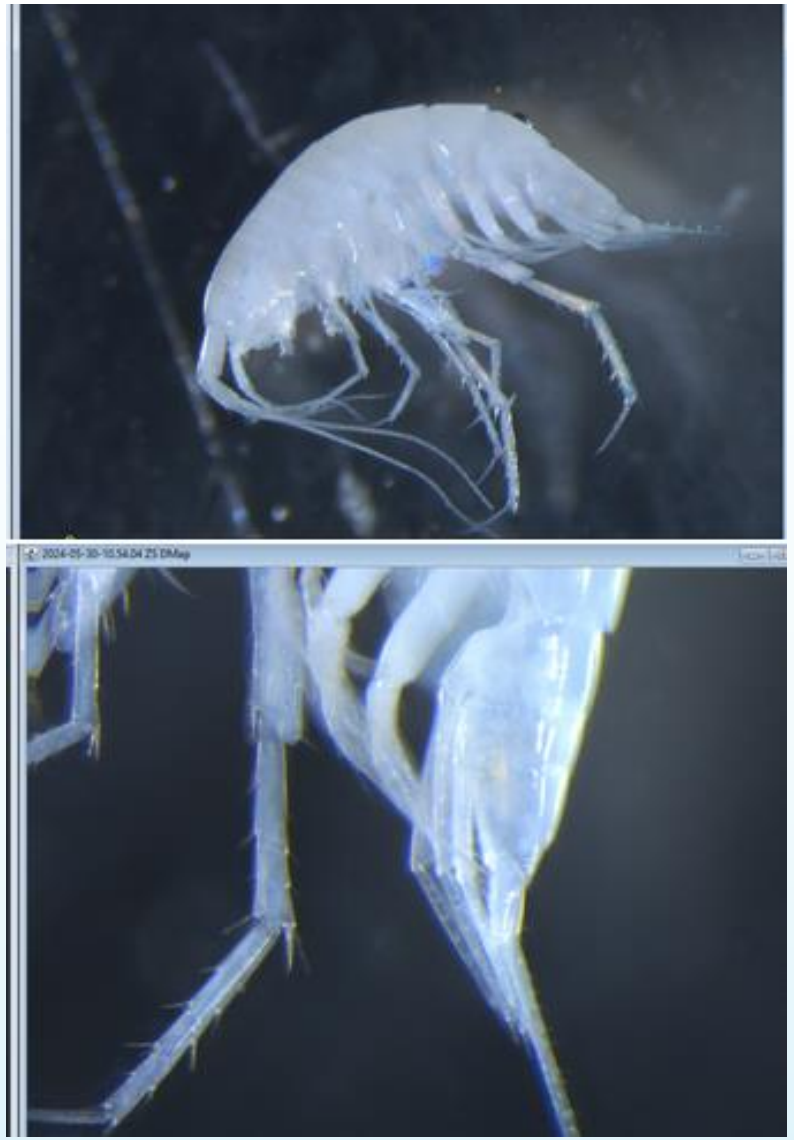
Biobag

Netting

https://www.envirolink.govt.nz/assets/Envirolink/Reports/1861-TSDC143-Sampling-considerations-and-protocols-for-assessing-groundwater-ecosystems.pdf

Who are you?

- More problems.....
- Stygofauna or macrofauna identification



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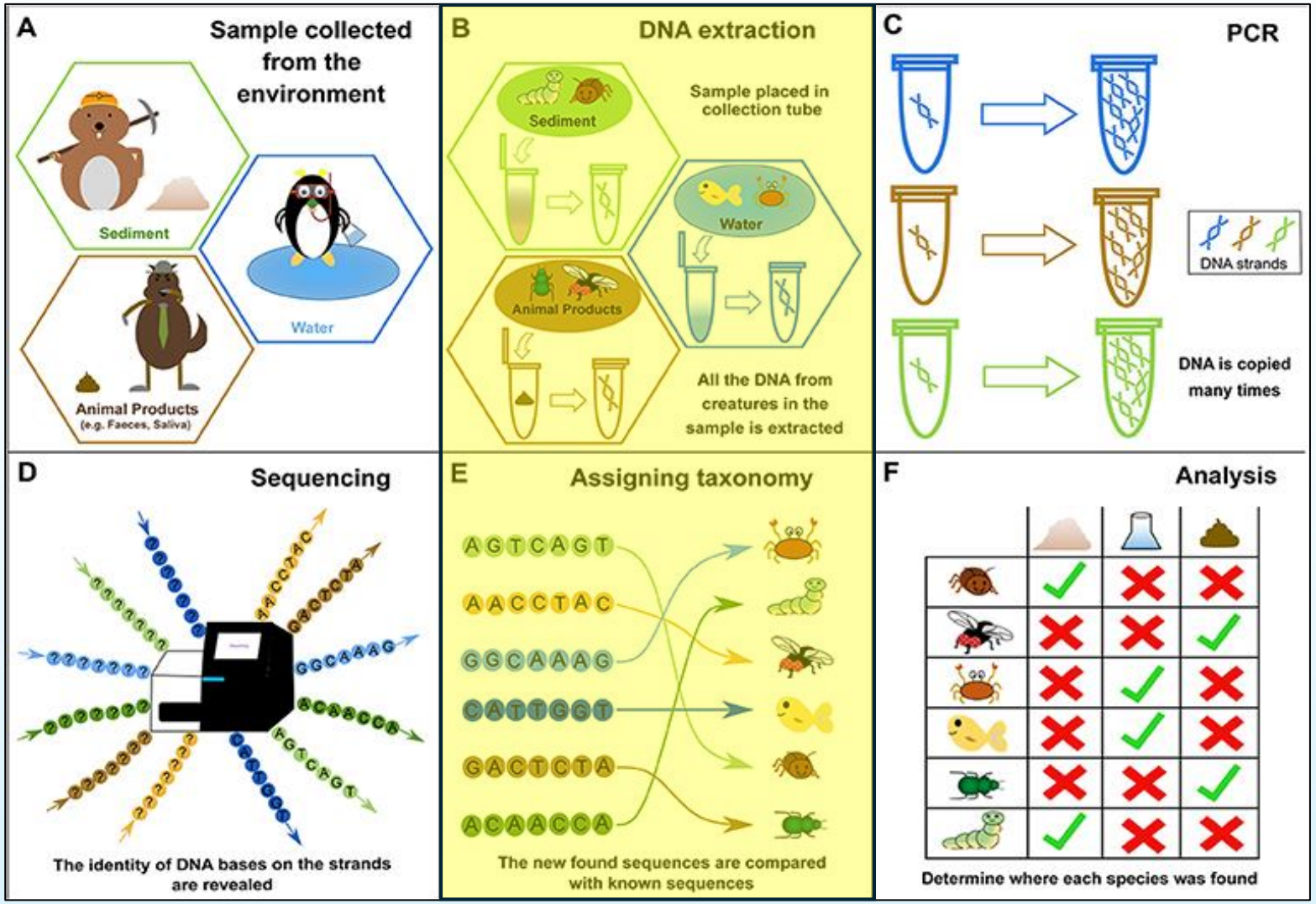
Environmental DNA



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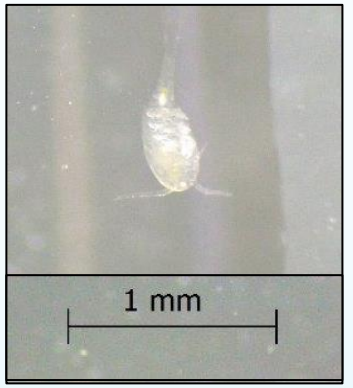
Methods

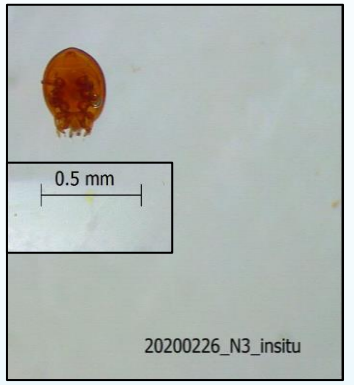
- Previously taxonomically identified specimens:
  - Niwa and ESR collections
- 66 specimens tested:
  Amphipoda, Isopoda, Copepoda, Acaria, Nematoda

- Extraction methods tested
  - magnetic bead protocol using the Genolution Nextractor instrument
  - MagMAX Microbiome Ultra Nucleic Acid Isolation Kit (Applied Biosystems),
  - DNeasy PowerSoil Pro Kit (Qiagen),
  - DNeasy Blood & Tissue Kit (Qiagen),
  - QIAamp DNA Micro extraction kit (Qiagen),
  - Phenol/Chloroform extraction method

- Sequencing tested:
  - COI barcoding (WilderLab)
  - Megabarcoding (MinION) – new primer design (thanks Shaun!)
    - Targeting
      - 16s
      - 12s
      - control region D loop
      - NAD2
      - Folmer region of COI

Photograph: Fenwick et al (2007)

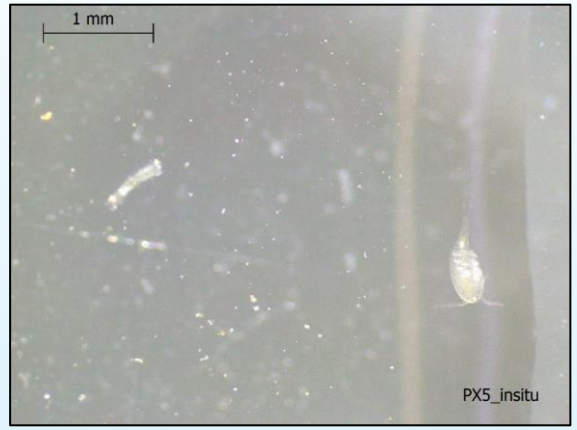




Results – Specimen DNA extraction

- 66 specimens tested:
  - 100% Success! Thank you Judith!
    - QIAamp DNA Micro extraction kit (Qiagen)
    - Overnight incubation @56˚C
    - Lysis
    - DNA bound to silica-membrane spin column
    - 5 min incubation
    - Washed and eluted
  - BUT….
  - Variation in amount DNA extracted:
    - Specimen size
    - Age
    - Preservation method
    - Type Amphipods > Isopods > Copepods > Mites

Photograph: Fenwick et al (2007)



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Results – Sequencing methods

eDNA method

- Standard Wilderlab sequencing – 313 bp Leray Region nested within 658 bp Folmer CO1 barcode region
  - 33 successful (50%)
    - Lack of ID in database!

Megabarcoding

Test of eDNA method on groundwater

Results – Sequencing methods

eDNA method

Megabarcoding

- New primerset (Designed by Shaun Wilkinson)
  - 16s, 12s, control region D loop, NAD2, Folmer region of COI
    - 2/6 successful – WHY?
    - Results confirmed the taxonomic ID of specimens

Photograph: Fenwick et al (2007

Test of eDNA method on groundwater

Results – Sequencing methods

eDNA method

Megabarcoding

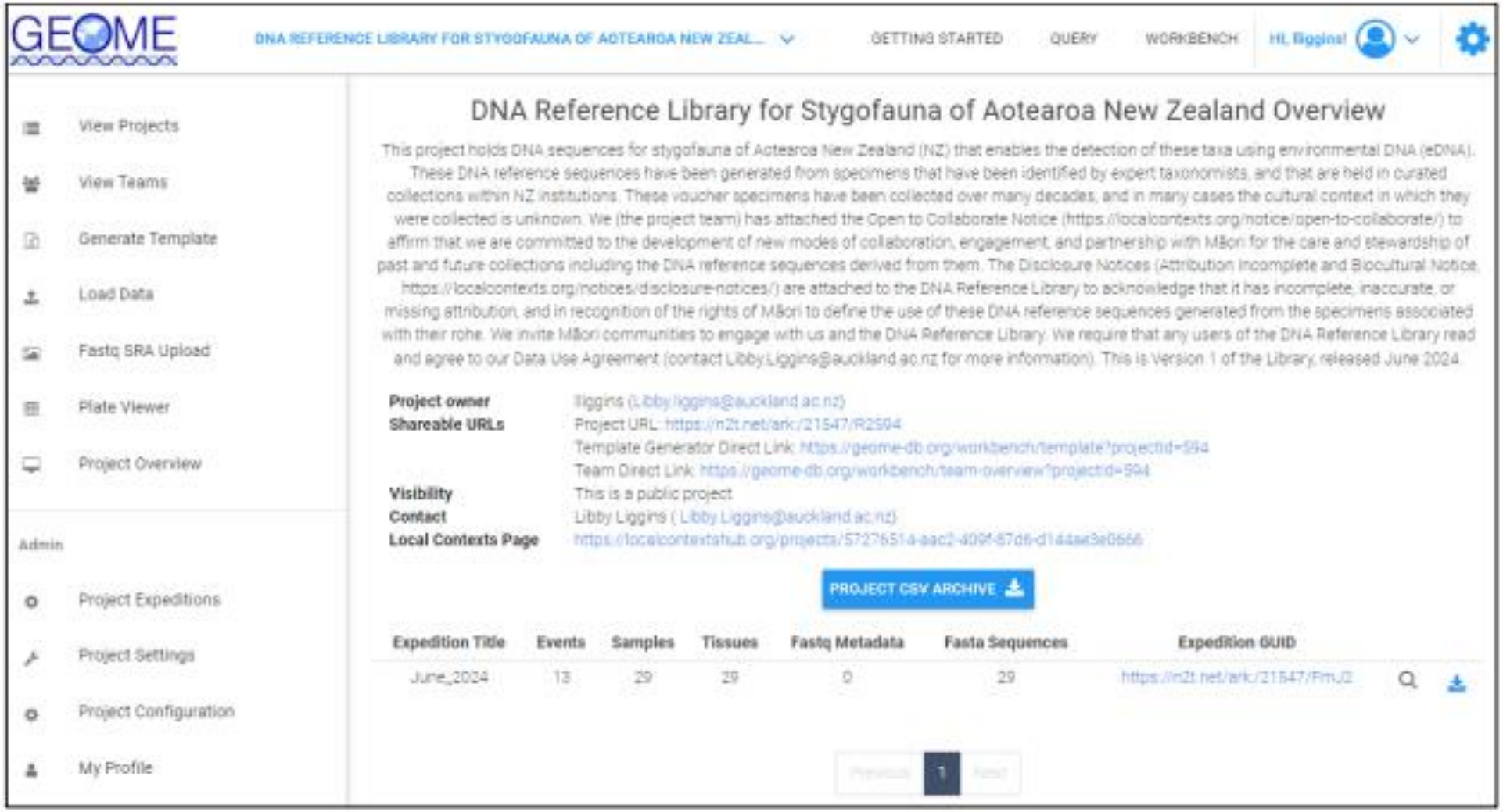
Test of eDNA method on groundwater

- 14 groundwater tested
  - Lots of taxa present! BUT.....
  - Only 1 Stygofauna species identified –Isopod
  - Others potentially Stygofauna – mites, worms

- BUT....
- Lack of taxa in the database NOT failure in the method.

Photograph: Fenwick et al (2007

Results – Database building



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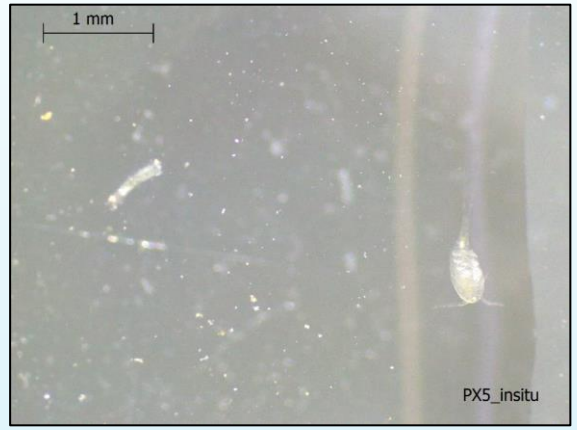
Conclusions

- Specimen curation important:
  - For future DNA extraction
    - Ethanol or RNALater/Lifeguard
  - For future taxonomic ID *and* DNA extraction
    - 70-75% ethanol and propylene glycol or glycerol prior to ID
    - After ID transfer to 100% ethanol or RNALater/Lifeguard
- Commercial eDNA kit not 100% successful at present
- Key outcome is the **lack of a robust Stygofauna database**.....
- Megabarcoding offers promise
  - PhD student is working on this!

Photograph: Fenwick et al (2007)



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Thank you!

References:

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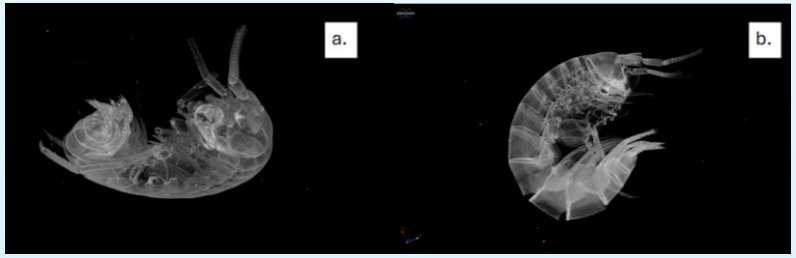
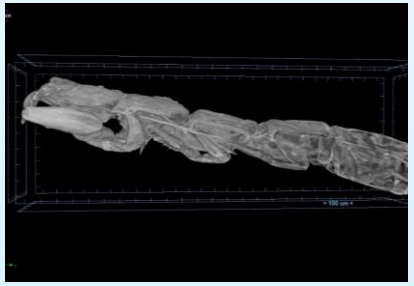
Next Steps

- Refine preservation solutions for Stygofauna
- Optimise long-read PCR assay
- Optimise MinION/GridION sequencing:
  - Long read sequencing across multiple gene regions
- Continue to build molecular database across different aquifer types
- Enhance photographic specimens addition to collections:
  - 3D photogrammetry





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