



Molecular library of groundwater macrofauna

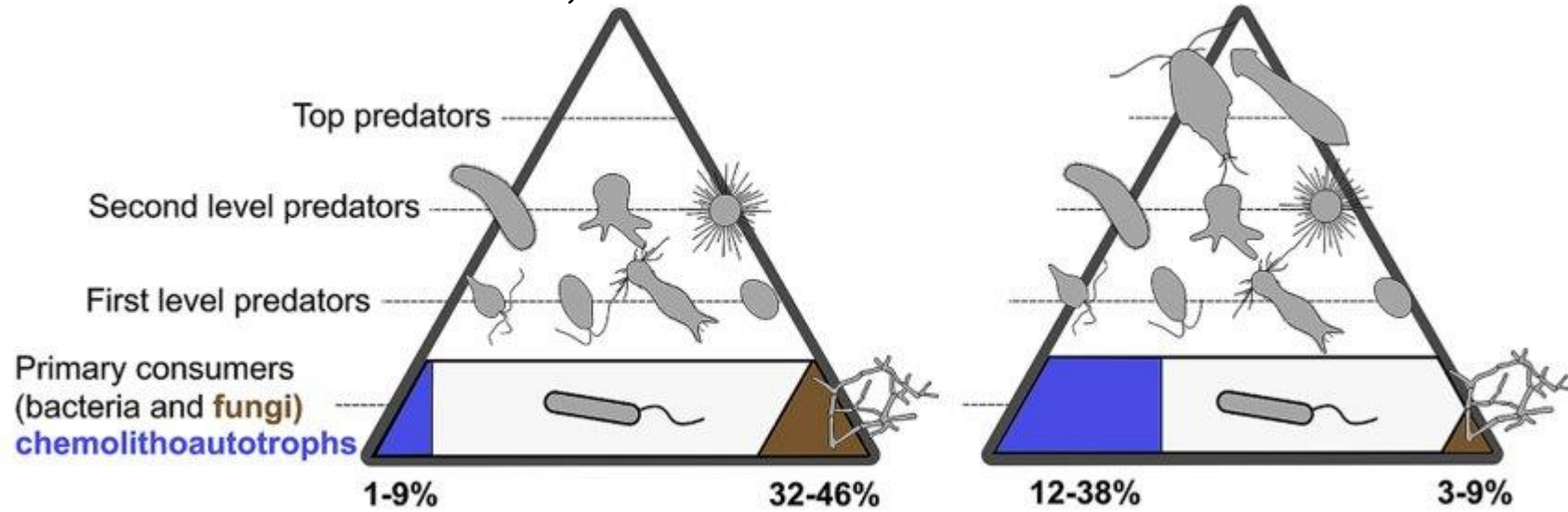
Weaver, L.,¹ Wilkinson, S.,² Bolton, A.,¹ Webber, J.,¹ Smith, B.,³ Gow, P.,⁴ van der Reis, A.,⁵ Hicks, A.,⁶
Handley, K.,⁴ Liggins, L.⁴

¹ ESR, ² Wilderlab, ³ NIWA, ⁴ University of Auckland, ⁵ Massey University, ⁶ Ministry for the Environment

Report prepared for Ministry for the Environment,
Project 25872. June 2024.



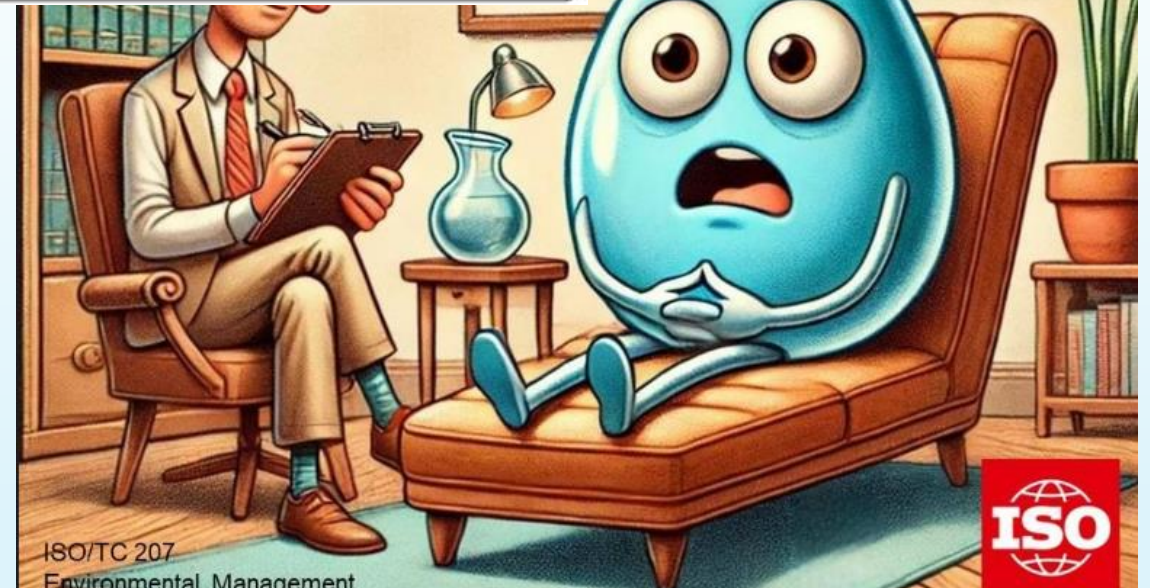
Modified from Hermann et al., 2020



risks and opportunities is
ated factors, including:
use or conflict over safe access
location..." – ISO 14002-2:2023, S.0.2

SO STRESSED
LATELY.

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



Sampling methods

since 1970: first population genetic studies using gel electrophoresis techniques

1974: Williams and Hynes first used the sediment core method

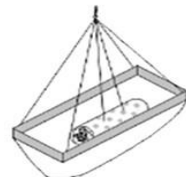


2003 & 2004: Lebert et al. & Barton et al. first applied molecular techniques (DNA sequencing method) and GIS

2005: Hahn presented the baited phreatic trap system



vet
biolo
ven



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

Current field Sampling methods

Netting



Pumping



Biobag



Netting

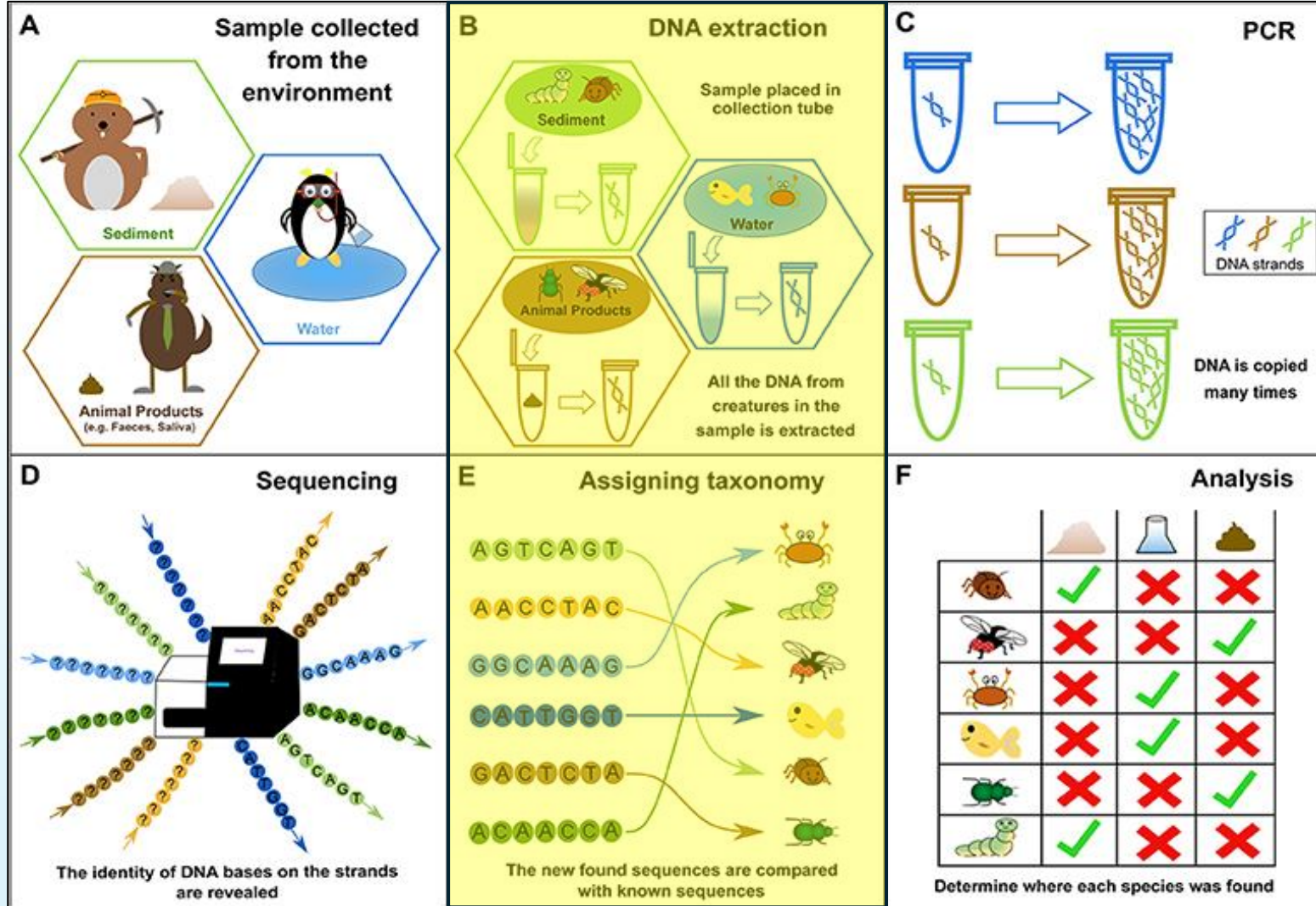


Who are you?

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



Environmental DNA

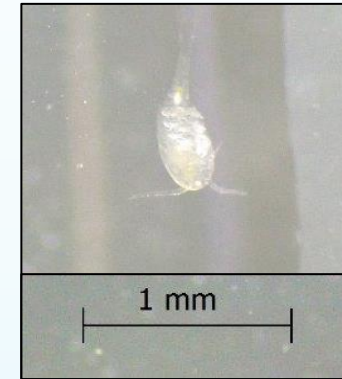


Methods

- 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

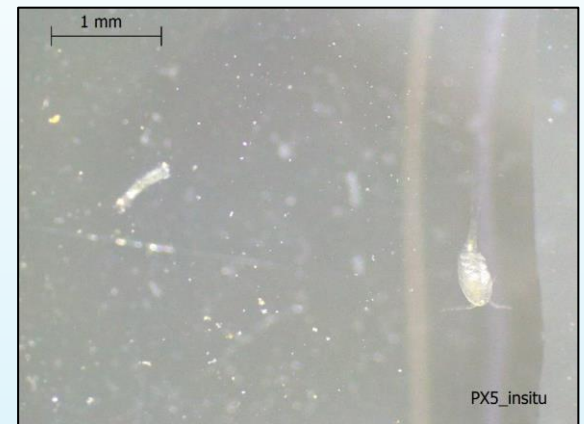


Photograph: Fenwick et al (2007)



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



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

The screenshot shows the GEOME website interface. The top navigation bar includes the GEOME logo, the site title "DNA REFERENCE LIBRARY FOR STYGOFAUNA OF AOTEAROA NEW ZEALAND", and user options for "GETTING STARTED", "QUERY", "WORKBENCH", and a user profile for "HL Liggins".

DNA Reference Library for Stygofauna of Aotearoa New Zealand Overview

This project holds DNA sequences for stygofauna of Aotearoa New Zealand (NZ) that enables the detection of these taxa using environmental DNA (eDNA). These DNA reference sequences have been generated from specimens that have been identified by expert taxonomists, and that are held in curated collections within NZ institutions. These voucher specimens have been collected over many decades, and in many cases the cultural context in which they were collected is unknown. We (the project team) has attached the Open to Collaborate Notice (<https://localcontexts.org/notice/open-to-collaborate/>) to affirm that we are committed to the development of new modes of collaboration, engagement, and partnership with Māori for the care and stewardship of past and future collections including the DNA reference sequences derived from them. The Disclosure Notices (Attribution Incomplete and Biocultural Notice <https://localcontexts.org/notices/disclosure-notices/>) are attached to the DNA Reference Library to acknowledge that it has incomplete, inaccurate, or missing attribution, and in recognition of the rights of Māori to define the use of these DNA reference sequences generated from the specimens associated with their rohe. We invite Māori communities to engage with us and the DNA Reference Library. We require that any users of the DNA Reference Library read and agree to our Data Use Agreement (contact Libby.Liggins@auckland.ac.nz for more information). This is Version 1 of the Library, released June 2024.

Project owner | liggins (Libby.liggins@auckland.ac.nz)
Shareable URLs | Project URL: <https://n2t.net/ark:/21547/R2594>
Template Generator Direct Link: <https://geome-db.org/workbench/template?projectId=594>
Team Direct Link: <https://geome-db.org/workbench/team-overview?projectId=594>

Visibility | This is a public project
Contact | Libby Liggins (Libby.Liggins@auckland.ac.nz)
Local Contexts Page | <https://localcontextshub.org/projects/57276514-aac2-409f-87d6-d144ae3e8666>

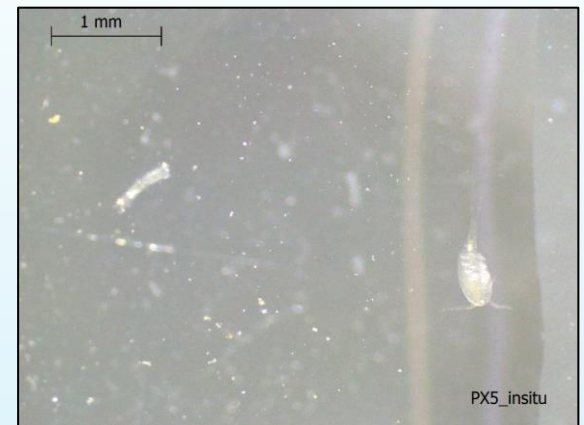
PROJECT CSV ARCHIVE [Download icon]

Expedition Title	Events	Samples	Tissues	Fastq Metadata	Fasta Sequences	Expedition GUID
June_2024	13	29	29	0	29	https://n2t.net/ark:/21547/RmJ2 [Search icon] [Download icon]

Navigation: [Previous] 1 [Next]

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!



A large, dynamic splash of clear blue water against a white background, creating a sense of movement and freshness.

Thank you!

References:

FENWICK, G. D., GREENWOOD, M. J., HOGG, I. D. & MEYER, S. J. 2021. High diversity and local endemism in Aotearoa New Zealand's groundwater crustacean fauna. *Ecology and Evolution*, 11, 15664-15682.

GELLER, J., MEYER, C., PARKER, M. & HAWK, H. 2013. Redesign of PCR primers for mitochondrial cytochrome c oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. *Molecular ecology resources*, 13, 851-861.

SCHALLENBERG, L., WOOD, S. A., POCHON, X. & PEARMAN, J. K. 2020. What Can DNA in the Environment Tell Us About an Ecosystem? *Frontiers for Young Minds*.

Louise.Weaver@esr.cri.nz

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

