

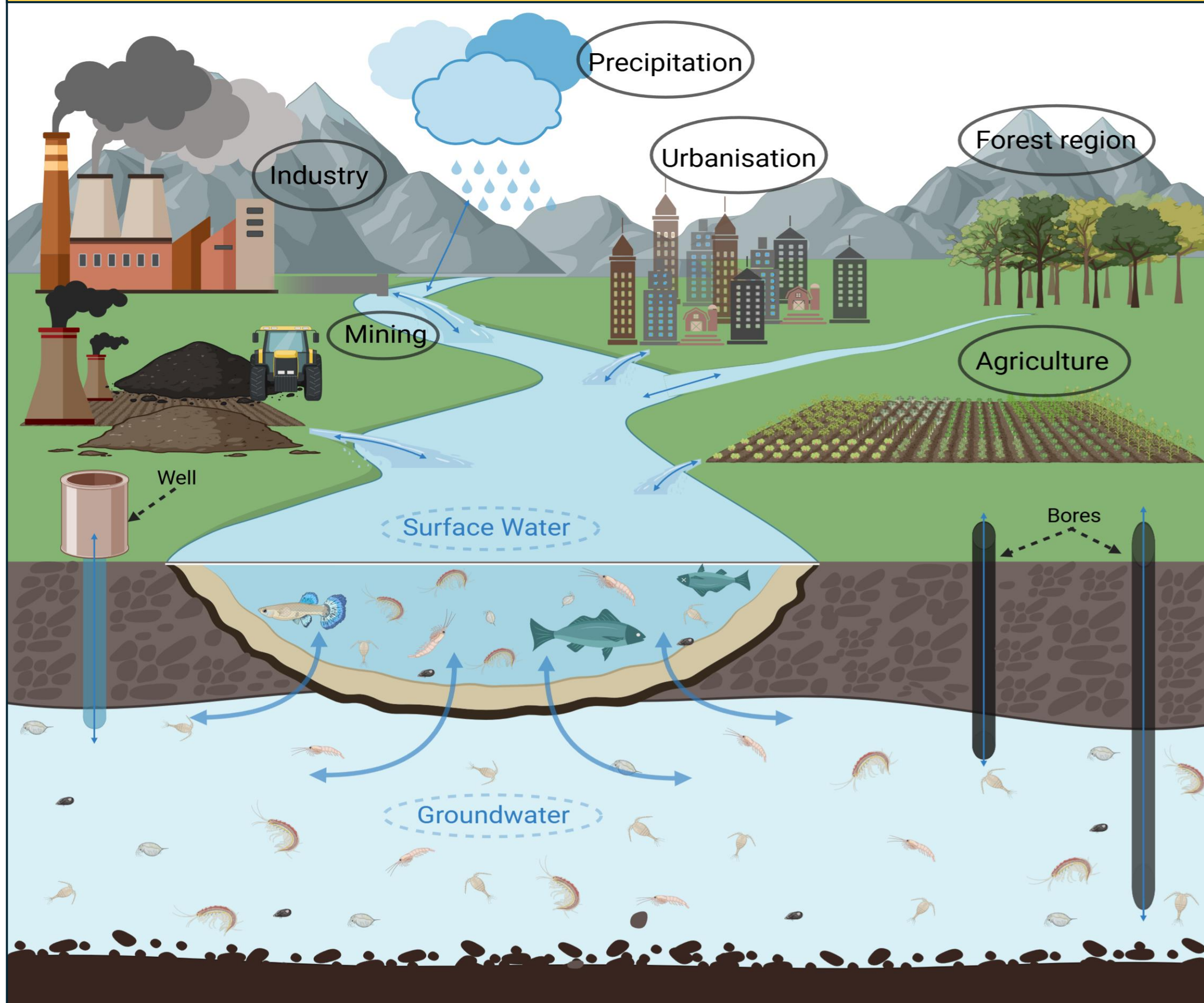
Using eDNA to unveil trophic dynamics of subterranean invertebrates

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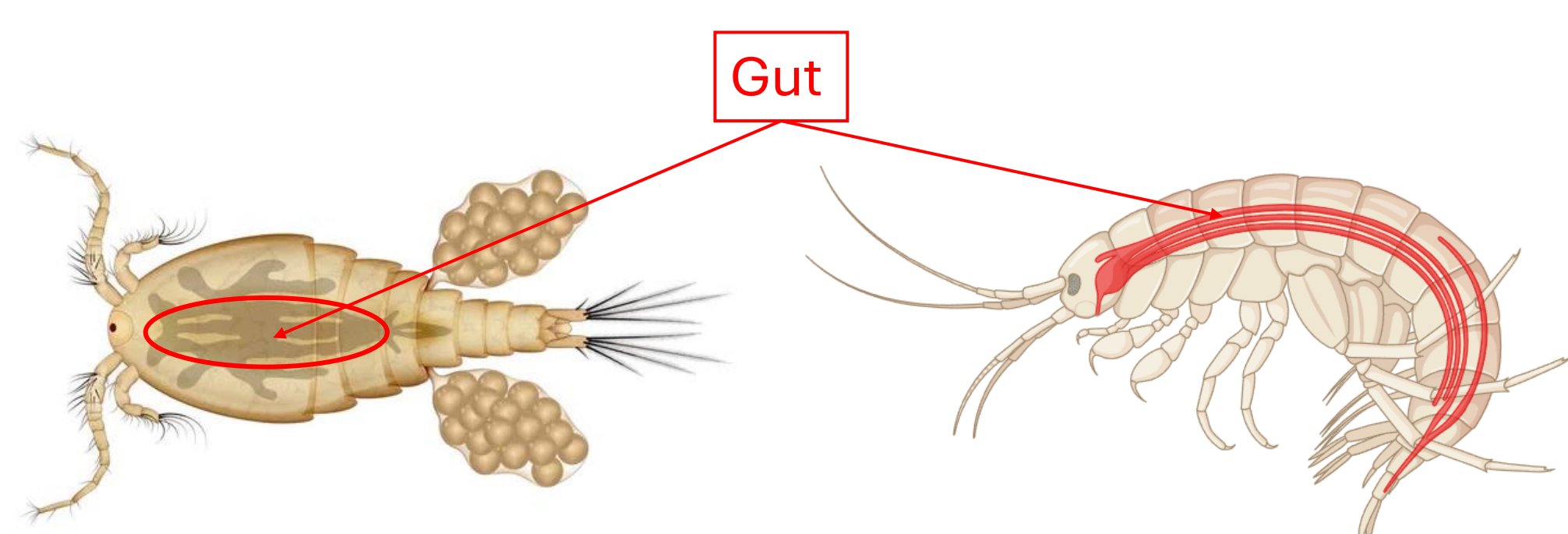
Introduction



- Subterranean ecosystems are unique environments that host diverse and cryptic species, such as stygofauna, which play key roles in ecosystem functions (Humphreys, 2009).
- Little is known about their diets and trophic interactions due to the challenges of studying these habitats (Culver & Pipan, 2019).
- Environmental DNA (eDNA) methods provide a powerful, non-invasive approach to studying trophic interactions by identifying dietary components from genetic material extracted from gut contents and the surrounding environment (DiBattista et al., 2023).
- Potential dietary sources for stygofauna include plant material, bacteria, fungi, other stygofauna, troglifauna (organisms falling into or transferred to water), and guano inputs (Venarsky et al., 2023).
- Loss of individual components, caused by anthropogenic activities and climate change can have a considerable negative impact (Saccò et al., 2020).

Objectives

- By exploring spatial and temporal patterns in stygofaunal diets this research aims to provide new insights into the trophic links of subterranean ecosystems, contributing to their conservation and management.
- **Primary objectives:**
 - To identify the trophic links of stygofauna in subterranean ecosystems by analysing gut contents using eDNA.
- **Specific Objectives:**
 - To compare the diets of stygofauna species that are common across all three sites (Barrow Island, Robe River Valley, and Ethel Gorge).
 - To investigate dietary differences between unique species found in specific locations.
 - To identify and characterise the trophic levels of various stygofauna.
 - To assess temporal variations in dietary patterns across 2-3 years of data collection.
 - To evaluate the potential influence of environmental factors on trophic interactions within subterranean ecosystems.



Methodology

Study Area:

Barrow Island:

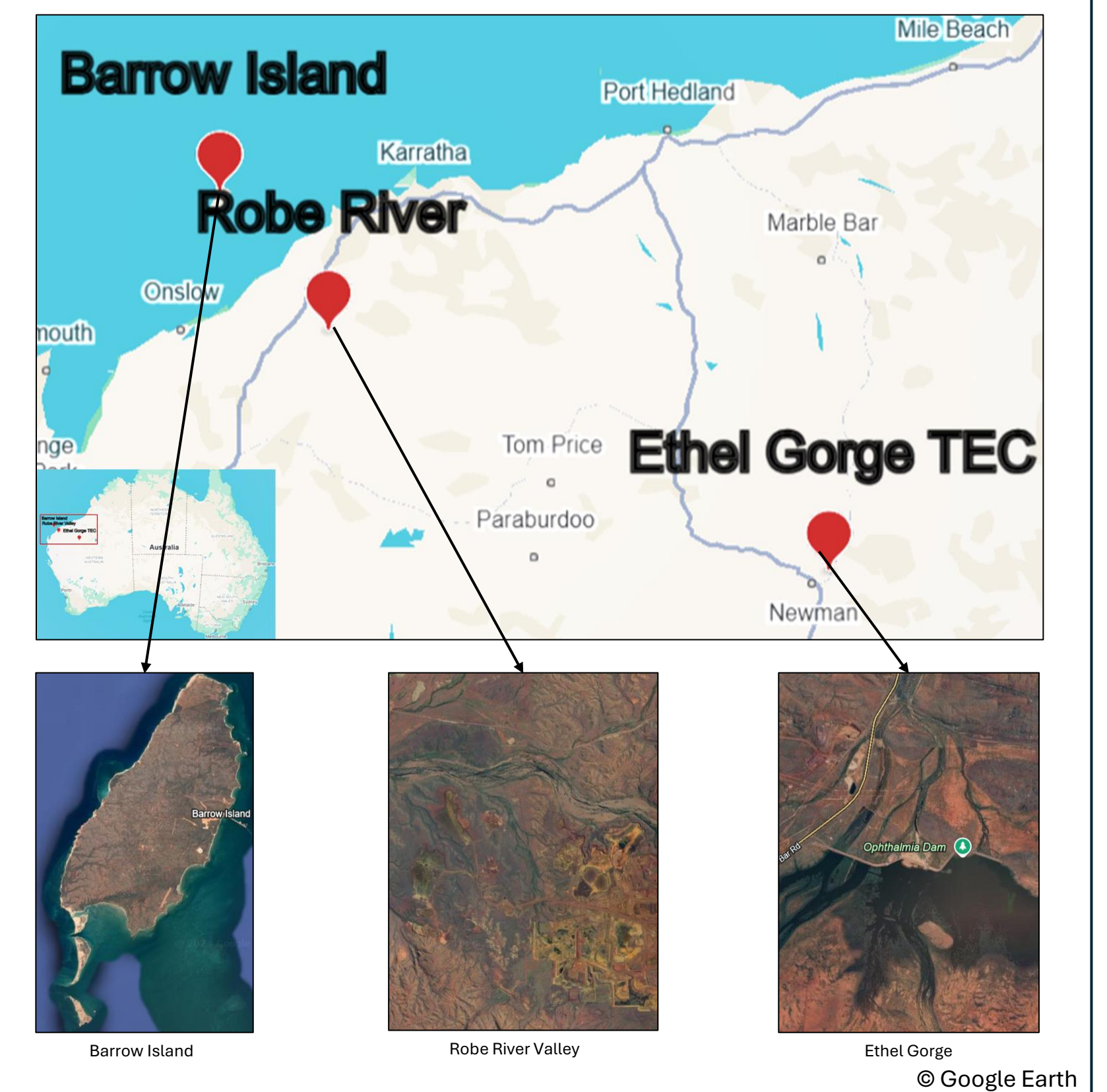
- Island anchialine aquifer on a Class A Natural Reserve.
- Samples provided by Chevron
- Collection dates: Nov 2020, Oct 2021, Jun 2022

Robe River Valley:

- Inland alluvial aquifer within a mining region.
- Samples provided by Rio Tinto and Biologic
- Collection dates: April-May and Sept 2021, Mar and Aug 2022

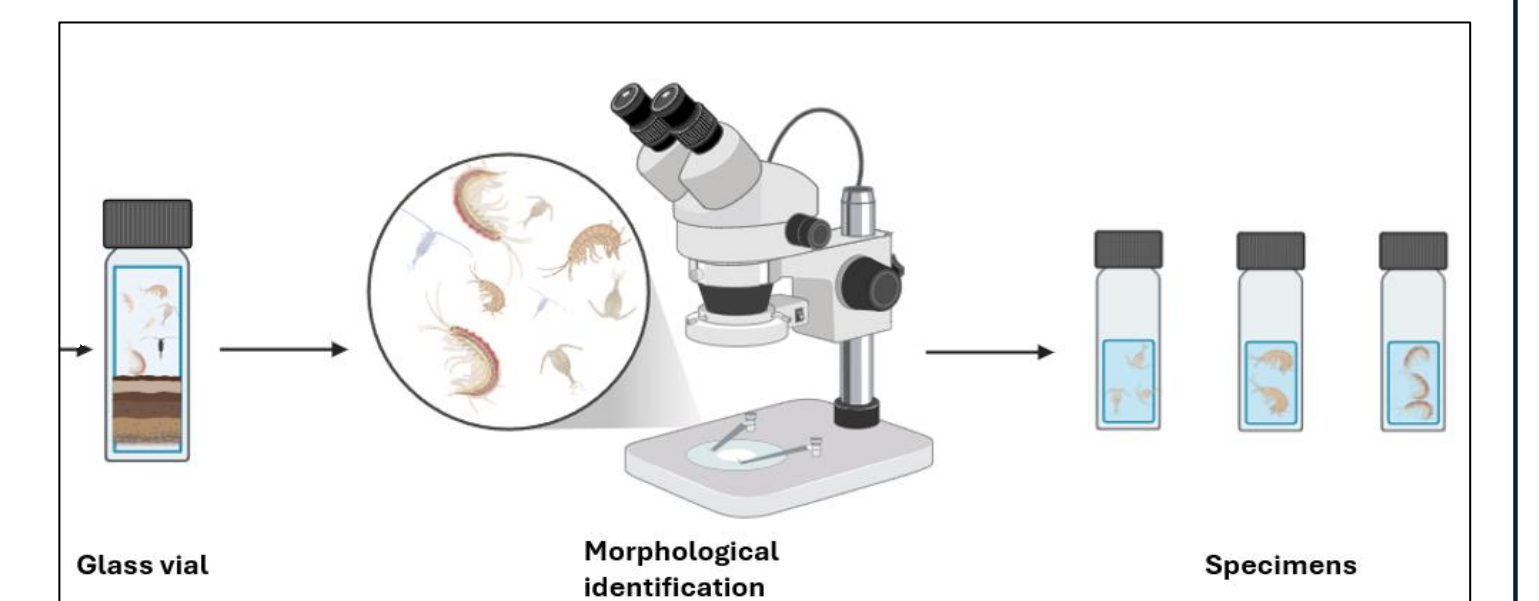
Ethel Gorge:

- Inland calcrete aquifer classified as a Threatened Ecological Community (TEC).
- Samples provided by BHP.



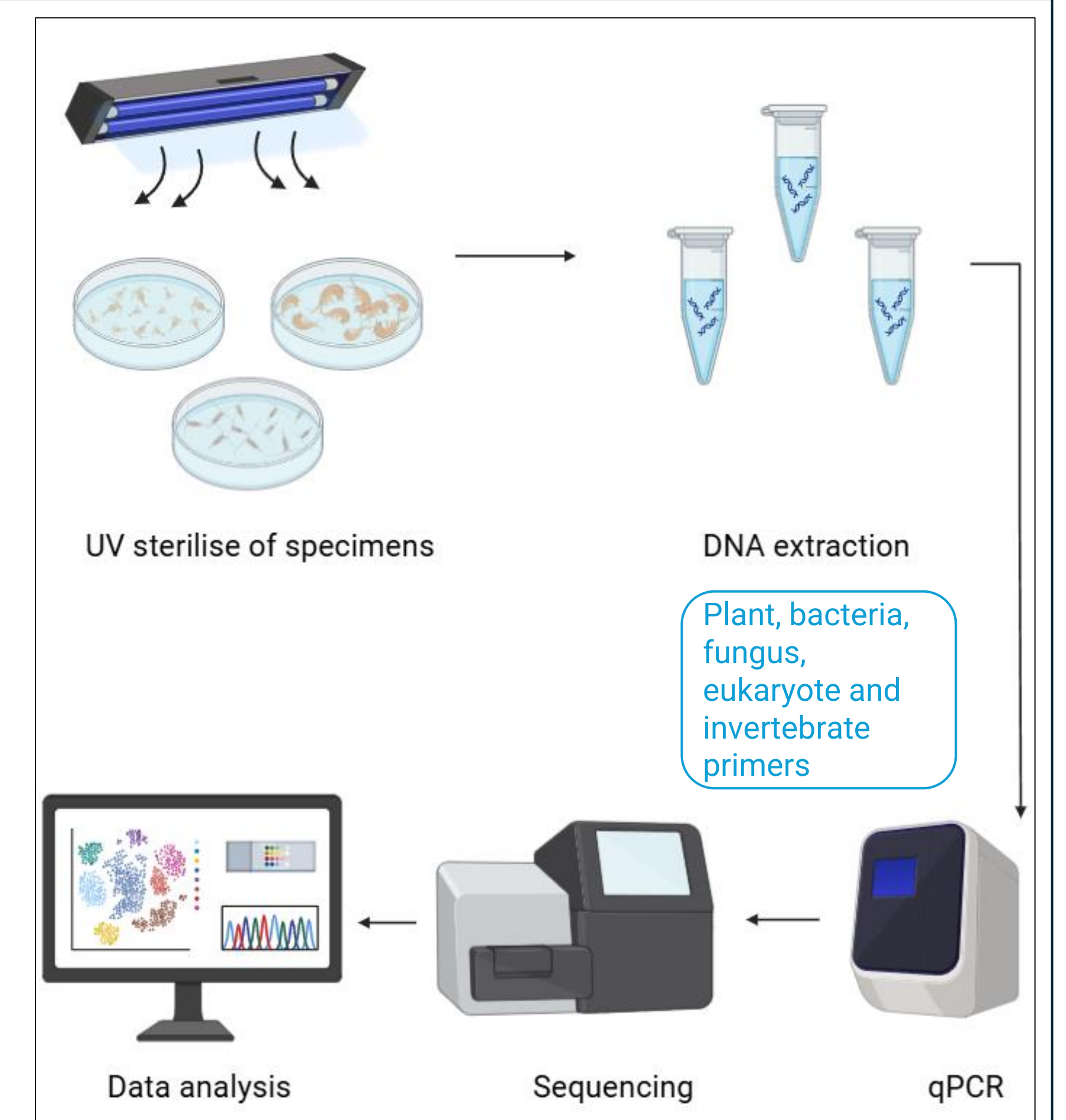
Sample collection:

- Stygofauna were collected during routine monitoring of the sites over a 2–3-year period.
- Includes common species across all locations and unique to specific sites.



DNA Extraction and Sequencing:

- Whole stygofaunal specimen will be used for eDNA extraction.
- Extraction will be performed using DNeasy Blood and Tissue Kit (Qiagen).
- Primers that will be used aim to capture a broad range of dietary sources: bacteria, algae, fungi, other stygofauna, and plant.
- Sequencing will be performed on Illumina MiSeq.
- Raw reads will be processed using an in-house bioinformatics pipeline.
- Dietary composition will be reconstructed by matching sequences to reference databases.



Planned Statistical Analysis:

- Comparative analysis of gut and water eDNA:
 - Taxa detected in stygofauna gut contents will be compared with those identified in water samples collected during the same time.
- Dietary composition analysis:
 - Diversity matrices will be calculated to assess dietary richness and evenness across sites and species.
 - Taxonomic profiles will be analyzed to identify dominant prey groups and their relative abundance in the diet.
- Temporal and spatial analysis:
 - Variability in diet across years and differences between sites will be measured.
- Multivariate analysis such as NMDS, and PCA will be used to visualize dietary patterns.
- Trophic interactions will be visualised as networks.

Significance

- Essential baseline knowledge of the diet of different stygofaunal species.
- Provides a detailed trophic map of the subterranean ecosystem using eDNA.
- Advancement of knowledge of the subterranean stygofaunal food web structures.
- Help understand the ecological dynamics critical for protecting these fragile ecosystems in areas impacted by activities such as mining.
- Demonstrate the use of eDNA for dietary analysis for studying the cryptic macroinvertebrate species.

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Images created in <https://BioRender.com>

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