This project involved a broad spatial scale pilot survey of groundwater bores in the Beetaloo Sub-basin and Roper River region in the Northern Territory to characterise stygofauna and microbial communities within subterranean groundwater-dependent ecosystems.

**KEY FINDINGS**

- Northern Territory aquifers support diverse stygofauna, including new genera and species.
- All Beetaloo stygofaunal communities sampled were dominated by crustaceans.
- All atyid specimens (shrimps) comprise a single species, *Parisia unguis*.
- This species was found across 260 kilometres indicating significant groundwater connectivity.
- Potential spill risks need to be adequately mitigated to prevent contaminants from entering this connected aquifer.
- Diverse microbial communities were obtained from bore samples, dominated by aerobic heterotrophic bacteria.
- Denitrifying bacteria and sulfate-reducing bacterial populations were present in many wells.
- These microorganisms are likely to be colonising well bore casings as part of complex biofilm communities.

**Community concerns**

The recent Scientific Inquiry into Hydraulic Fracturing in the Northern Territory highlighted community concerns about the potential of the onshore gas industry to affect the quality and quantity of groundwater.

The inquiry identified the need for a regional assessment of groundwater-dependent ecosystems within the Beetaloo Sub-basin and the Roper River region.

This study provides the first description of stygofauna in an otherwise little-studied region of Australia and includes discovery of new genera and species.

This was a collaborative project with CSIRO scientists working with researchers from Charles Darwin University (CDU) on all aspects of the project, including fieldwork, data analyses and interpretation.

This baseline data is essential for biodiversity conservation and the maintenance of the ecological integrity of high value groundwater-dependent ecosystems in the region.

The Beetaloo Sub-basin, which underlies the Cambrian Limestone Aquifer (CLA), is regarded as one of the most prospective areas for shale gas in Australia. The CLA provides baseflow to the Roper River and is an important domestic and pastoral water resource.
Subterranean fauna collected from NT aquifers

D-F: Amphipoda: Melitidae
G: Syncarida: Bathynellaceae: Brevisomabathynella sp.
H-J: Ostracoda: Podocopida: Candonidae
K: Harpacticoida: Ameiridae: Nitokra lacustris
L: Gastropoda: Caenogastropoda
M-N: Cyclopoida: Cyclopidae
O: Annelida: Aeolosomatidae: Aeolosoma sp.
Groundwater-dependent ecosystems

Groundwater and groundwater-dependent ecosystems such as aquifers and surface springs have significant ecological and cultural value and are important for agriculture, tourism and community water supply.

Groundwater biota, in addition to their biodiversity values, provide an indication of aquatic health of aquifers and are integral to the ecosystem services (the benefits to humans) provided by these systems.

As a consequence, protection of subterranean groundwater-dependent ecosystems have been recognised at the federal level for over 20 years.

Collecting and analysing samples

CSIRO and CDU scientists sampled 26 groundwater wells (bores) and two springs in August and October 2019, across a distance of approximately 500 kilometres, from the sub-tropical Mataranka region in the north to the semi-arid Barkly Tablelands (Barkly Stock Route) in the south.

The depth to the water table, electrical conductivity (EC), pH and water temperature (°C) were measured at every site.

The scientists used a range of sampling devices, including plankton nets and motorised pumps, depending on the type and size of bore hole. All live stygofaunal samples were filtered through a 50 μm mesh-sized net, stored in 70 per cent ethanol and subsequently analysed by microscopy in the lab.

For DNA analysis, scientists collected and preserved 300ml of water for subsequent filtering and DNA extraction in the lab. The cytochrome oxidase I gene (COI) was used to determine the presence of stygofauna and the 16s ribosomal gene (16sRNA) to gain a fingerprint of all bacteria present. Genetic material from shrimp tissues was used for COI barcoding.

Results

All Beetaloo stygofaunal communities sampled were dominated by crustaceans: shrimps; amphipods; ostracods; copepods; and syncarids.

This fauna showed little affinity with the stygofauna recorded from the more extensively studied Pilbara and Yilgarn aquifers in Western Australia, indicating that new genera and species are present in the Beetaloo.

The atyid shrimp, *Parisia unguis*, is large relative to all other taxa collected and its predatory behaviour places it at the top of the Beetaloo subterranean aquatic food web.

This shrimp has previously been recorded from the Cutta Cutta caves near Katherine, NT.

Microbial communities

Diverse microbial communities were obtained from bore samples which were dominated by aerobic heterotrophic bacteria.

Denitrifying bacteria and sulfate-reducing bacterial populations were present in many wells. These microorganisms are likely to be colonising well bore casings and other bore infrastructure as part of complex biofilm communities growing on hard surfaces.

Aquifer connectivity

DNA analysis indicates that all atyid specimens collected comprise a single species, ranging across a geographic distance of ~260 km. Low genetic divergence among specimens - less than 2.2 per cent - suggests groundwater connectivity in recent times.

The relatively wide distribution of the shrimp, *Parisia unguis*, supports recent CSIRO groundwater tracer studies that found that the Cambrian Limestone Aquifer is highly connected (i.e. is conducive to the movement of water, matter and organisms between different parts of the aquifer).

Risk of contamination by pollutants associated with shale gas and other industries in the region must be mitigated sufficiently to prevent contaminants from entering this subterranean aquifer of high connectivity.

The magnitude of this risk is dependent on a range of factors including:

- probability of a spill occurring
- containment, removal and rehabilitation at the spill site
- degradation/adsorption of residual chemicals in the soil by microflora
- dilution of residual chemicals by large flow volumes during the monsoon, and
- further degradation of chemicals in the aquifer itself.
Next steps

The final report for this study suggests the following additional steps:

- Undertake further sampling, particularly the northern region between Larrimah and Katherine where the water table is close to the surface.
- Develop standardised sampling methods.
- Formally describe new species.
- Establish a comprehensive DNA sequence library to enable future monitoring programs to use eDNA metabarcoding.
- Develop assessment and monitoring protocols in areas where onshore gas extraction may affect groundwater quality and quantity in the Beetaloo Sub-basin.

This was a collaborative project with CSIRO scientists working with researchers from the Research Institute for Environment and Livelihoods, Charles Darwin University. The project was co-funded by the Australian Government and the Northern Territory Government (38%); CSIRO (20%); and by Origin Energy, Santos and Pangaea (25.8%). An in-kind contribution was provided by Charles Darwin University.