# Surveying stygofauna and groundwater microbes video - Transcript

**Voice over:** Groundwater supports unique and often diverse communities of microbes, fungi and groundwater invertebrates, also known as stygofauna.

These communities contribute to crucial ecosystem processes such as, purifying water through biogeochemical cycling, maintaining flows in aquifers and supporting the health of connected surface waters such as wetlands, rivers and streams.

Biomonitoring of groundwater communities is becoming increasingly common to assess how human activities might cause environmental harm. By monitoring changes in community structure over time, we may detect early warning signs of human impacts.

**Professor Grant Hose:** Groundwater ecosystems can be monitored in a variety of ways. The traditional approaches, using nets and bailers can be combined with newer environmental DNA techniques to detect the presence of stygofauna. Environmental DNA (also known as eDNA) methods are designed to collect short fragments of DNA which are shed by an organism in the environment. This DNA material can be extracted from the air, soil or water, and is used for identifying different organisms in the environment. In groundwater, one DNA sample can be used to detect both stygofauna and microbes.

**Voice over:** This video, presented by staff at Macquarie University, demonstrates practical ways to survey stygofauna and groundwater microbes for environmental assessment and routine biomonitoring. It is part of a broader study commissioned by the Independent Expert Scientific Committee on Unconventional Gas Development and Large Coal Mining Development (IESC).

**Professor Grant Hose:** This study focused on using nets and high flow pumps to sample the groundwater. Low flow pumps could be used for this purpose but more research is needed to test the outcome for using this approach.

The first step to any sampling strategy is bore selection. This is essential to ensure representative sampling of groundwater.

Features that you are looking for in bore section include: spatial coverage, accessibility, the aquifer type, the bore construction, the bore depth and age, and also the water level.

**Tess Nelson:** The first thing we do when we get to a bore is to measure the standing water level, and we do this because the biota can vary with depths.

**Voice over:** Depending on your studies objectives, stygofauna samples can be directly collected from the bore using a net before the bore is purged. If you are looking simply for presence of stygofauna in a pilot study, this may be the only step required.

However, for more detailed assessments, this method alone may not be sufficient. Our studies showed that netting alone, can fail to record common groundwater taxa and can overestimate total abundances.

**Dr Kathryn Korbel:** So the net lowered into the bore slowly until it reaches the bottom. When it’s at the bottom we agitate the sediment a few times and then slowly wind it upto the top and collect the sample.

The contents are emptied into a container, with ethanol as the preservative. The process is repeated 5 times with a fine 50 or 63-micron mesh net, and then with a coarse 150 micron mesh net.

Net sampling can be a much slower process than sampling the well with a pump. However, results for pre-purged samples do not show any differences between net and pump sampling in this step.

**Voice over:** When taking water samples, eDNA and detailed community analysis, setup generally involves placing a pump on the well and then inserting the pump tubing into the well.

As we are collecting DNA samples, it is essential that the outside of the tubing is wiped with an ethanol or bleach wipe as it is being inserted to prevent contamination from other DNA not in the Groundwater.

It is also essential that the tubes have been rinsed with bleach between sites to remove all contaminants. This is usually done at the end of the sampling to ensure a clean tube is ready for the next site.

Here you can see the pump attached to the bore, water meters ready and several buckets ready to collect water samples.

Purging is recommended to estimate total richness and to detect specific taxa. Purging is also required before eDNA and water chemistry samples are taken. If unpurged water is sampled it will not represent the wider aquifer.

**Loren Pollitt:** To purge the bore, a high-flow pump is attached. Then 3 times the volume of water is removed from the bore. For example, this shallow bore is 50 mm in diameter and has 5 m of standing water which means it has a bore volume of around 10 litres, so to purge the bore fully,around 30 litres is being pumped. Also note the buckets are given a quick rinse using a small amount of bore water**.** BUT DO NOT THROW OUT THE PURGE WATER!

The bore water collected during this process is likely to contain a high abundance of stygofauna.

As seen here, the water is filtered for stygofauna through a 63 micron sieve, and then preserved in ethanol. This sample will give an indication of whether stygofauna exist within groundwater, and will also give a rough indication of stygofauna richness, that is, the number of species present.

However, more sampling effort is required to complete a full assessment of stygofauna community structure, including richness and relative abundance.

**Tess Nelson:** and once the bore is purged, you can now collect your environmental DNA and water quality samples. We can collect these sampled in 2 ways, but both require the use of sterile equipment and gloves to ensure DNA from the external sources does not enter the sample.

The first method uses a pump which is fast and involves pumping water directly into a container for eDNA analysis and collecting additional water for chemical analysis and measurement of physico-chemistry (such as electrical conductivity, temperature and pH). When using this method, the tubing must be bleached, and sterile bottles used. eDNA bottles should be sterilised, but also triple rinsed with groundwater before the final sample is collected.

The second method, that can be used if you don’t have a pump, is using a sterile bailer. The bailer is lowered into the bore and the contents emptied into a sterile, triple rinsed, 4-5L container for eDNA. Then physico-chemical properties of the water are measured such as pH and further samples can be collected for analyses in the lab if required.

Regardless of the method, all samples for eDNA analysis must be placed in the dark on ice or in a portable fridge- with water samples being treated depending on what is being measured.

**Voice Over:** Additional sampling by pumping a further 90L after purging is required to get a more accurate indication of stygofauna richness and relative abundance. This gives us information on stygofauna community structure.

**Dr Kathryn Korbel:** This is a typical set up, with 10-L buckets arranged for sampling. Water is pumped into buckets and then passed through a sieve. This is completed for 9 buckets or 90L, after purging has occurred.

The sieve contents are then collected into one container and preserved in ethanol to represent post-purge stygofauna samples.

**Voice Over:** Samples are then taken back to a lab or accommodation, where the groundwater is filtered onto membranes, the membranes frozen and then transported to other deep freezers to await further processing. This should be done as soon as possible.

**Loren Pollitt:** So, we’re currently undertaking the sterilisation process, in which the equipment and bench tops are bleached to remove any traces of DNA, other equipment (such as forceps) are also sprayed with ethanol and flamed for sterilisation.

This process ensures no microbes or DNA remain on the equipment.

It is important that sterility is maintained for any surface that comes into contact with the sample - this includes the inside the filtration equipment, membranes, forceps and petri dishes.

We are filtering our sample, we also sample a field blank, which is DNA-free water for the control, but the process is the same for all samples.

The equipment is first thoroughly rinsed with sample to ensure no bleach residue remains. A sterile membrane is added and the sample poured into the funnel.

Once the sample has been vacuum-pumped through the membrane, it is removed, placed into a sterile petri dish secured with parafilm and frozen.

**Professor Grant Hose:** From this point, DNA must be extracted from the samples and sequenced for either stygofauna, microbes or both. There are a growing number of research and commercial laboratories that can provide these services.

**Voice over:** This is a brief outline of the sampling protocols for groundwater biota. For full details of the recommended practices for a particular sampling technique please refer to the full report which can be found at iesc.gov.au. There is also a summary in the factsheet on the same website.