Sampling considerations and protocols for assessing groundwater ecosystems

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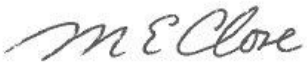

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EXECUTIVE SUMMARY

An Envirolink medium advice grant from the Ministry of Business, Innovation and Employment was sought by Tasman District Council (TDC) and the Institute of Environmental Science and Research Ltd. (ESR), to take the first steps towards standardising a sampling protocol for collection of groundwater ecosystem communities. It is hoped this guidance will be used in the future to continue to assess groundwater ecosystem health. The following manual provides guidance on how to sample the typical biota expected in groundwater monitoring wells and springs including macroinvertebrates (stygofauna or stygobites), meio- and micro-fauna. Guidance is provided for the sampling methodology to adopt as well as the suggested frequency.

ESR are currently involved in several research programmes on groundwater ecosystems and have expanded their knowledge of groundwater ecosystems. This knowledge will be used to determine the varying ecosystem profiles, groundwater ecosystem health and its water resource impacts, including potential risks to public health. This is a new and emerging area of expertise and one which has not yet been transferred to Regional, Unitary Authorities and District Councils for implementation within their routine State of the Environment Monitoring (SEM), analysis and reporting. Knowledge on the ecosystem of groundwater systems can benefit Councils, community and iwi. Understanding how biological diversity varies, aquifer chemistry and recharge can improve overall groundwater management.

The changes in water quality occurring will be seen in changes to biological diversity. Most water in surface water and spring fed system during base-flows are sourced from groundwater discharge. The groundwater ecosystems are the processes that essentially clean up aquifers. However, our understanding of these processes and the organisms involved are limited. The information gained from sampling will improve the science in this area and allow New Zealand to make evidence-informed decisions to enhance and protect our drinking water for future generations. One of the first steps is to transfer knowledge to regional councils on their sampling strategies for assessing the groundwater ecology.

In order to transfer this knowledge from the science, to “real life”, ESR scientists and technicians met with key TDC staff to discuss their SEM strategies for Groundwater Ecosystem Assessment (GEA). ESR offered advice on GEA sampling techniques and protocols for TDC and developed field sampling techniques for the individual catchment setting and selected suitable sites to sample both groundwater and groundwater emergent spring systems. Some aspects may be similar to both, but each system has its own specific setting including geology, hydrogeology and hydrology. Site visits were undertaken to gain an understanding of the context of the catchment situation.

From these meetings and site evaluations, ESR have produced the following suggested sampling manual be considered for future monitoring including coverage to meet TDC’s SEM requirements. ESR also assisted TDC to take samples at suitably identified sites for validation of the sampling plan. Water chemistry, microbiological and macroinvertebrate sampling was undertaken to demonstrate sampling techniques for the different components. The water chemistry samples were analysed externally (Hill Laboratories) and once results have been received these will be combined with ESRs microbiological and macroinvertebrate assessment to provide baseline (pilots scale) data for TDC. As at 30th June 2018 these results were not available and so were not included in this report.

The knowledge gained in this Envirolink will have wide reaching impacts for other regions. Not only will it up-skill regional and district council staff it will also aid in the national discussion required with Government, other RCs, iwi and communities on land-use and its potential impact on groundwater, interrelated surface/spring water quality and potential human health implications. Considerations for assessment of groundwater faunal diversity are made in this report and a suggested sampling manual is included for Tasman District Council as the first step in knowledge transfer.

1. PURPOSE AND SCOPE OF THIS GUIDANCE

This document has been designed for use by Tasman District Council (TDC). The guidance *could* be used by other regional councils but careful consideration needs to be made as to the site specific variables. This guidance is designed to help establish the most appropriate method to sample groundwater systems for biological assessment.

The considerations needed to assess groundwater ecosystems are outlined. In this report we consider predominantly groundwater ecosystems but do include consideration of the wider groundwater dependent ecosystems (i.e. Springs). It is noted that for many regions, groundwater biodiversity is not regularly monitored. The background, recommendations and sampling manual provides guidance and suggested methods to optimise the opportunity to obtain a representative sample across the whole ecosystem. Biological assessment is used to evaluate the condition of other waterbodies (notably rivers, lakes and streams), using surveys and other direct measurements of resident biological organisms (macroinvertebrates, fish, microbiology and plants). Before biological assessments of *groundwater* can be made, baseline surveys and knowledge of key species are required. These baseline studies can then be further developed and combined with long term chemical and biological monitoring surveys to provide a method of assessing groundwater ecosystem health. To demonstrate the suggested sampling protocol, a pilot scale sampling demonstration was undertaken in the Takaka catchment by TDC and ESR. Samples were taken according to the sampling manual at 7 bores and a spring in the catchment. The results of the water chemistry, biological specimens and microbial abundance is underway (results not included in this report).

2. BACKGROUND INFORMATION

The focus on groundwater systems in the past has typically involved problematic organisms, i.e. those that can cause disease. Key processes are known to occur in groundwater that ultimately protect it as a pristine source of drinking and irrigation water (Danielopol and Griebler 2008, Griebler and Avramov 2015). Despite the importance that is placed on groundwater as a source of drinking and irrigation supply, the biota that live within it, are poorly understood and often undervalued. The lack of regular and national data means that any undocumented biodiversity is potentially threatened by anthropogenic changes (e.g. quarrying, mining, extraction, pollution), and long-term hydrological changes that may occur as a result of climate change (Maurice 2009). Without basic evidence that establishes groundwater biodiversity, it is impossible to assess probable impacts (Hancock and Boulton 2009). Land use intensification for industry and agriculture has created greater contaminant loads, which are a threat to groundwater quality. Research has demonstrated that the complex ecosystem present (from microorganisms to macrofauna vis Stygofauna) plays a role in removing contaminants. Each of these fauna have specific function, habitats and interrelationships which are currently poorly understood.

Efforts by Local and Regional Councils to improve our understanding of this important resource will help build a better picture of how healthy our groundwater systems are across New Zealand. Groundwater biodiversity and the biological functioning that occurs from the many species living in this environment, play an essential role as providers of ecosystem goods and services. A non-exhaustive list of these services would include:

- nutrient cycling and storage (e.g. carbon, nitrogen, phosphorus)
- organic matter cycling and redistribution
- water treatment (e.g. filtering water to remove toxins) and
- water regulation (e.g. increasing the size of interstitial pore spaces to maintain hydraulic flow pathways and infiltration rates, see Glanville et al. 2016).

Before sampling in any aquifer is undertaken, a desktop review should be completed to ascertain the information available at the geographic setting. Relevant information includes: the likely presence or absence of groundwater fauna (e.g. local geographic setting, hydrology, presence of alluvium and hydrological connectivity, identification of subterranean fauna from previous studies), and assessments of the likely impact on groundwater fauna from direct or indirect occurrences. If there is insufficient information, sampling should be undertaken as a pilot study to better understand the local setting and to determine any groundwater fauna present. There may be occasions when, after the desk top study, the impact on groundwater fauna is deemed unlikely and so emphasis of sampling can be targeted to other more impacted areas.

2.1 PREPARATION FOR SAMPLING

The objective of many studies is to include an assessment of the biodiversity and therefore one aim is to sample as many species as possible[1]. Sampling can be designed to increase the likelihood of capturing the maximum number of organisms using a variety of techniques.

[1] http://nora.nerc.ac.uk/id/eprint/14751/1/OR09061.pdf (Maurice et al 2009)

There is a general consensus that repeated sampling is needed to capture more of the diversity and abundance of groundwater fauna. Different sampling methods (net hauling, pumping, trapping and discrete interval sampling) can affect the results obtained and therefore each individual study should have a unique and carefully thought out sample design that enables the required information to be obtained. Repeated net hauls and pumping appear to be the best methods of capturing the greatest diversity according to the literature on this topic (Hancock and Boulton 2009, Michel et al. 2009). Discrete interval sampling of both chemistry and fauna would be useful in studies aimed at understanding the location of fauna in the aquifer and the relationship between the water chemistry of their habitat.

Groundwater contains many organisms ranging from micro (archaea, bacteria), meio (protozoa, mites etc.) to macro (stygofauna or stygobites). Each of these organisms have an important biological role in keeping groundwater clean and contaminant free. The different organisms that can be found in groundwater are briefly described below. As in other food webs there is a pyramid food web with large numbers of primary producers, i.e. microorganisms to very few top predators, e.g. Stygofauna.

2.2 MICROORGANISMS

Microorganisms present in groundwater ecosystems are the primary producers of the ecological food web. Microorganisms (a.k.a. microbes) in these environments can include bacteria, archaea, and fungi. Bacteria are microscopic single celled organisms that lack a nucleus and have a cell wall. Archaea, also single celled microscopic organisms, were once a branch of bacteria but research has shown them to be a distinct group of organisms. Fungi are larger than bacteria and tend to form long filaments.

Groundwater differs from other aquatic environments in that organic carbon is not replenished by photosynthetic processes but must be supplied from the surface or groundwater environment itself. To have a healthy and fully functioning groundwater ecosystem the balance of these microorganisms is key. The services provided by microorganisms include carbon assimilation, denitrification, sulphate and iron reduction. Microorganisms can influence groundwater quality such as pH, redox status, dissolved oxygen concentration, mineral component composition. Bacteria within the microorganisms' present provide a protection to environmental extremes by growing in a biofilm (Weaver et al. 2015). Organisms within this slime layer (extra polysaccharide substrate, EPS) are protected from extremes such as desiccation and communication (quorum sensing) also occurs within this layer. Higher (larger) organisms graze on microorganisms directly or on the slime layer (EPS) thus preventing overgrowth of the biofilm which could cause clogging of the aquifer. Fungi within the microorganisms' present are more susceptible to environmental conditions, redox status in particular as they require oxygen to function. Bacteria and archaea are less effected by redox status as some can convert to anaerobic respiration and some are obligate anaerobes.

2.3 PROTOZOA

The protozoa are larger than bacteria and possess a nucleus but lack a cell wall. Protozoa are present in both shallow and deep groundwater systems but are dependent on oxygen

and so redox status is important in their survival (Harvey et al. 1995). There is evidence that increases in cyst forming flagellate protozoa occurs down stream of contaminant plumes indicating a protozoan role in contaminant degradation (Zarda et al. 1998, Harvey et al. 2011). Flagellates and amoeba dominate the protozoan communities present in groundwater communities.

Whilst the importance of these organisms is now being recognised internationally there is little research undertaken in New Zealand on groundwater protozoa. In this report and suggested protocol sampling is predominantly designed for microbial and macroinvertebrate taxa. Currently, ESR are investigating whether the suggested protocols offer a suitable strategy for collection and identification of protozoa in groundwater.

2.4 MEIO- AND MACRO FAUNA (STYGOFAUNA/STYGOBITES)

Together the meio- and macro-fauna in New Zealand consist of 8 described taxa (Figure 1), which in terms of abundance, are dominated by acari (mites). These abundances are quite different when compared to studies overseas that show copepoda, acari, amphipoda and isopoda to contain the highest abundance (Scarsbrook et al. 2003). Other species have no doubt been discovered, and few have been described, notably amphipods (Fenwick 2006). It is without a doubt that there are more species awaiting discovery.

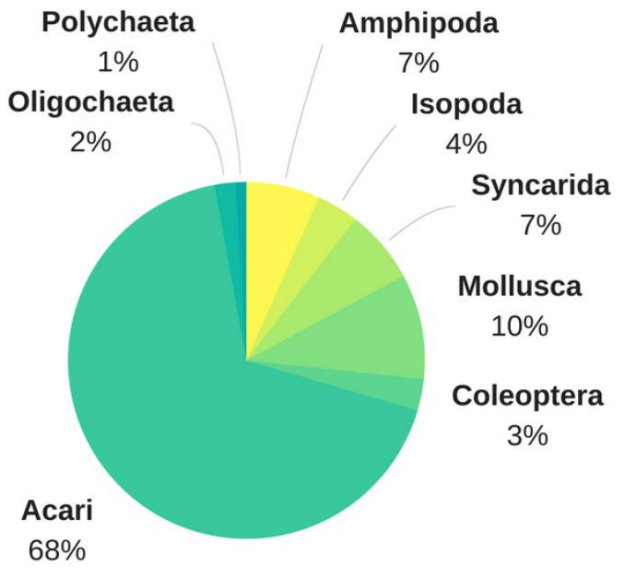

Figure 1: Composition of described groundwater fauna in New Zealand (Scarsbrook et al. 2003)

Meio-fauna are microscopic multicellular motile organisms. Within this grouping of organisms are acari (mites), copepods, oligochaetes and nematodes (worms), tardigrades (water bears) and rotifers. There is a lack of research on these potentially important group of fauna

in groundwater systems. It is known that the abundance of meio-fauna is much greater than macrofauna and that they potentially play an important role in contaminant removal but more research is required in this area to fully understand their role.

Stygobites are subterranean dwelling invertebrates. They include crustaceans such as amphipods, isopod and syncarida, coleopteran (beetles) and molluscs. Their lifecycle occurs completely in groundwater, with no surface stage, and are often differentiated by their lack of eyes and pigmentation. Globally, they are believed to be important indicators of water quality and biodiversity (Korbel, Hancock et al. 2013, Korbel and Hose 2015). It is well known that surface dwelling invertebrates are sensitive to environmental and human change, yet we are still only beginning to understand the importance this relationship when applied to stygofauna.

2.5 SAMPLING STRATEGY

Currently, there is no standard method for sampling groundwater biodiversity in New Zealand. However, numerous studies and ad-hoc reports exist locally and internationally on this subject (Gibert and Culver 2009, Griebler 2009, Gutjahr, Bork et al. 2013). This document has analysed and compiled those, selecting the most commonly used in terms of efficiency and effectiveness, recognising that sampling conditions in New Zealand may be different to other countries. The associated sampling manual suggests an approach to maximise the chance of identifying the biodiversity present across the taxa (microbes to macrofauna). It is likely to be refined as further groundwater ecosystem studies are published, thus improvements or additional samples will improve with time.

In addition, the collection and storage of the information gathered is essential for sharing. In the future we envisage that data collecting from field sampling should be gathered into a central, open source database to allow scientists both in academia, regional councils and others, to analyse the data across several sites in New Zealand. This can be used to examine a number of research questions related to lithology, season, and species richness and also groundwater ecosystem health and ecosystem services across the country. For further reading refer to overseas literature (Danielopol and Griebler 2008, Dole-Olivier, Castellarini et al. 2009, Griebler 2009, Korbel, Hancock et al. 2013, Marmonier, Maazouzi et al. 2013, Griebler and Avramov 2015, Korbel and Hose 2015, Marmonier, Maazouzi et al. 2018).

The sampling protocols in this manual have been developed and modified using existing protocols, in particular from the Department of Environment and Heritage Protection in Queensland, Australia and Dole-Olivier, Castellarini et al. (2009). Each protocol is designed so that it can be separately printed (and preferably laminated) to be taken into the field. It is advised that the user becomes familiar with this sampling manual beforehand. In addition, it is anticipated that in future this manual, or future manuals, will be accompanied with video examples of each sampling technique (on USB) that can be loaded onto a smart phone or tablet, or accessed via YouTube or Vimeo if internet access is available at the field site.

2.6 PILOT STUDY

It is recommended that a smaller pilot scale study is undertaken at the aquifer of interest. This is primarily to address any knowledge or technical issues identified in the desktop

review. The pilot study should also be used to verify the outcomes of the desktop study and be used to further design the routine monitoring sampling strategy. The Australian guidelines (Clifton et al. 2007) suggest that 10 representative bores are studied using the same methodologies as to be used in the routine monitoring. It is suggested in lieu of any alternative that this is the optimal number of bores to be sampled but if not practical, a maximum number of bores are sampled closest to the 10 bores suggested. The same sampling methods should be used in the pilot study and any future routine monitoring. As microbes are ubiquitous in groundwater environments it is suggested that the initial pilot study focusses on the larger macroinvertebrates (Stygofauna) initially. The presence of macroinvertebrates in pilot studies should then initiate a routine monitoring strategy to comprehensively study the diversity present.

2.7 ROUTINE MONITORING STRATEGY

First, based on the desktop assessment, a site specific strategy should be devised depending on the question asked. For a general diversity assessment efforts need to be made to cover all the geological formations present but concentrating on areas where full diversity is likely (or has been demonstrated) to be present. This will optimise efforts to identify all species present and give a good picture of the overall diversity. Sampling should be undertaken initially for at least two seasons with sampling occurring at least three months apart to minimise previous sampling affecting results of subsequent sampling. With the aim of sampling representative bores the following needs to be considered:

- Groundwater macrofauna would have access to the borehole/well if the screen has slots >2 mm.
- The bore is at least six months' old
- The bore has groundwater present

When sampling for complete diversity assessment i.e. microbes to macroinvertebrates considerations need to be made with multiple sampling strategies employed e.g. net hauls, pumping.

In addition, for sampling of macroinvertebrates the bore should **not** be purged prior to sampling to maximise the chance of collection of specimens. However, if the bore has not been purged or sampled in the past year, or longer, it should be purged 6 – 8 weeks prior to sampling.

2.8 SKILLS, TRAINING AND EXPERIENCE

Skills, training and/or experience required to understand and/or undertake this method include training and experience in groundwater sampling. Other training or experience that may be useful includes collection and identification of river and stream freshwater invertebrates.

2.9 PERMITS AND APPROVALS

Permits, permissions to access private and Māori land, and consultation with local iwi should be granted prior to field sampling. Summaries of the key findings should be given to the relevant stakeholders.

2.10 HEALTH AND SAFETY

Before following the methods outlined in this document, a detailed risk management process (identification, assessment, control and review of the hazards and risks) must be undertaken. All work carried out must comply with the District or Regional Council's Work Health and Safety legislative obligations.

The equipment required will depend on the type of sampling. At a minimum, for health and safety reasons, the following equipment should be taken:

- First aid kit (preferably one team member will be basic first aid trained and/or field first aid trained)
- A fieldwork buddy (fieldwork alone is not advised)
- Notification of planned field trip, times, location, contact number and planned returned time. Field workers should confirm their return with nominated buddy who is not working in the field (i.e. in the office, located close by).
- Communication device that will work in the field
- Water and food
- Sun protection (if sunny)
- Study shoes or steel toe cap boots (depending on whether heavy machinery is required)
- Suitable clothing, including gum boots in case of inclement weather
- Hi-vis vest or coat
- Vehicle suitable for the terrain
- Any personal medicines/medical devices (e.g. insulin, inhaler, epinephrine)
- Map
- Head torch
- Emergency contact details/location of closest medical centre
- Fire extinguisher

2.11 DATA MANAGEMENT

Any data collected during groundwater diversity assessment should be provided and stored in a suitable format to enable uploading into a wider database in the future. At a minimum the date sampled, site location (including coordinates), sampling method(s) used, geological formation and lithology sampled, water quality measurements, field measurements (temperature, pH, salinity, total borehole depth and depth to water table), taxa identified and abundance of each taxon.

2.12 EQUIPMENT

Appendix 1 provides a suggested equipment checklist which can be amended as appropriate for the specific sampling plan.

3. PROCEDURE

3.1 PREPARATION FOR SAMPLING

Ensure all equipment is functioning and not faulty, all nets are complete (i.e. hole free) and microbiological equipment is sterilised.

3.2 SITE DETAILS

Well/bore selection is an important part of establishing a reliable GW ecosystem monitoring site. Construction details of the well/bore should be known in advance. A site card should be taken in the field recording:

Well/bore number	Casing material	RL (GL and MP)
Depth	Screen position	MP above GL
Diameter	GPS location co-ordinates	Owner contact details
Photo	Location map	Historical water level/field meter info

If no previous records have been taken collect details to complete site card for future reference. Record, WL (metres below ground level, mbgl), well surrounds, land use, weather, photograph site record and any other factors that may influence the sample.

IMPORTANCE OF ASEPTIC SAMPLING

Aseptic sampling is a technique that ensures both the sampler and the sampling equipment do not contaminate or cross contaminate the sample. Decontamination procedures should be followed to minimise risk of cross-contamination between boreholes and sites. At a minimum 70% ethanol should be used to clean equipment. Optimally equipment should be soaked in Decon90 solution (according to manufacturer's instructions) and rinsed with 70% ethanol. In the field in between boreholes and sites decontamination of equipment should take place with 70% ethanol. Nitrile or latex gloves should be worn when undertaking microbiological sampling.

FIELD MEASUREMENTS AND WATER CHEMISTRY SAMPLING

Record field parameters (temp, DO, ORP, specific conductivity, etc. by placing the hose into a weir system to reduce re-aeration of the sample and sample with a field multiprobe.

Water chemistry samples (e.g. Nutrients, Dissolved Organic Carbon, metals etc.) should be collected straight from the hose into the sampling containers once stygofauna sampling has finished (i.e. after 100 L has been collected in sample buckets).

3.3 NET HAUL METHOD

The net procedure aims to sample benthic (within the sediment) and pelagic (within water column) meio and macrofauna from the groundwater ecosystem. The net design comes from (Clifton, Cossens et al. 2007). It consists of a weighted glass McCartney vial with the bottom removed and attached to a 63 µM (or smaller) pore sieve mesh. The cap has a hole punched, also covered with a 63 µM mesh that is secured when the cap is screwed to the bottle (Figure 1a).

[OPTIONAL FOR LIVE SAMPLES] – For immediate field observation live samples can be collected. This requires some pumped water from the well. Empty some well water into a bucket (approximately 200 mL), then open the contents of the bottom net and allow contents to empty (Figure 1c). Submerse the rest of the net into the bucket so that all the sides drain into the bucket. Pour the bucket contents into a suitable container, label as live samples (refer to processing sheet for live samples). Live samples should be transferred to a cool box in the dark until they can be processed in the lab.

Ensure that the net and bottom mesh of the McCartney vial is clean and in place. Connect the top of the net to the line and reel.

1. Lower the net to the bottom of the bore (Figure 1b) using a line and reel.
2. Once the net has reached the bottom of the bore, raise the net up and down to dislodge any fauna attached to the bottom of the bore. The net should be drawn up and down a distance of approximately 0.5 m and a total of four times.
3. Reel the net up in a smooth and steady motion (~0.1-0.2 m/sec) to avoid a bow wave and losing any fauna captured.
4. Place the bucket sieve into a dark coloured plastic bucket. Once the net is clear of the bore, remove the collecting vial and pour the contents into the 50 µM mesh sieve in the bucket. Ensure the net does not touch the ground.
5. Hold the net over the sieve and wash using water from a wash bottle.
6. Repeat steps 1 to 6 above four times in total, once before pumping and three more times after pumping has been completed.
7. Collect all washings into one pre labelled sample bottle (Figure 1d). The bottle label should record the bore number, collection date, sample number and sample type (net or pump) and the sampler's initials and surname.
8. Tilt the sieve and wash the contents of the sieve into a sample jar (Figure 1d). Preserve the sample with 90-100% ethanol.

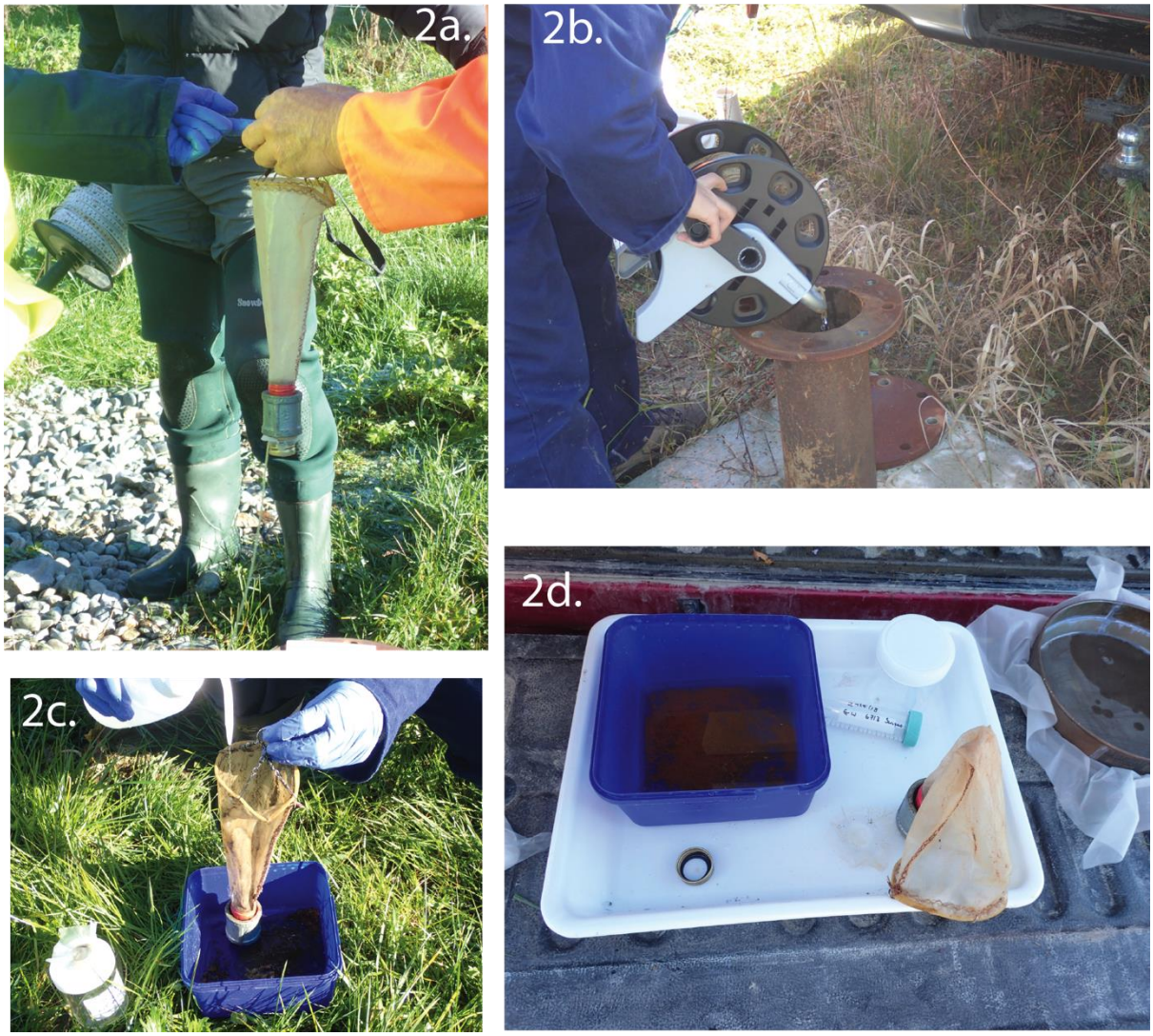

Figure 2: Net sampling images.

Figure 2a shows the net with the end cap sampling container; figure 2b demonstrates a reel set up for lowing and raising the net; Figure 2c shows washing of the net; Figure 2d shows the sample washed from the net in the blue container prior to pouring into wide necked sample container (white lid).

3.4 PUMPING THROUGH A SIEVE

Pumping aims to sample meio and macrofauna from the screen and aquifer sediments immediately outside the well. It is preferable to use a mechanical piston type pump as impeller driven pumps are more likely to damage fauna during collection. In the literature (Hancock, Eberhard et al. 2007) the Bennett Pump (a reciprocating piston pump) has been shown to be an effective pump for GW meio and macrofauna sampling at a range of depths, although the inlet screen must be removed before use (Figure 2a). Hand inertia pumps can also be used at shallow depths. All pumps should be used as per manufacturer's instructions.

1. Prior to lowering the pump run blank down the well to check for obstructions.
2. Feed the sampling hose into the bore until it reaches the bottom of the bore casing. Then lift hose so that it sits either midway in the screened section or approximately 0.5 m above the bottom of the bore.
3. Set out a row of ten x 10L buckets, equivalent to a total of a 100 L sample (Figure 2b). Sit the buckets onto tarpaulin as a clean site for collection of samples. If the area surrounding the bore is heavily vegetated, clear the vegetation to provide flat surface. A weed trimmer/brush cutter maybe required for thick vegetation.
4. On the top of the first bucket place the ring and collar, ensuring it is snuggly fitted. Place the sieve (65 µM) on top of the collar and then the top collar on (Figure 2c).
5. Start the pump and hold the end of the hose close to the sieve. Once the bucket is full move the collar and sieve apparatus to the next bucket and continue to sieve (Figure 2d).
6. Fill buckets sequentially and try to minimise splashing and overflows.
7. Once 100L has been collected, remove the sieve and wash the sieve contents into a pre labelled jar.
8. Record bore number, water level depth, collection date, sample number and sample type (i.e. net or pump) on a label and add label to jar.
9. Water chemistry samples (e.g. Nutrients, Dissolved Organic Carbon, metals etc.) should be collected straight from the hose into the sampling containers once stygofauna sampling has finished (i.e. after 100 L has been collected in sample buckets).
10. Sample bottles must be labelled and the sample name, site, data and time and sampler name must be recorded in a notebook or equivalent.
11. Record field parameters (temp, DO, ORP, specific conductivity, etc. by placing the hose into a weir system to reduce re-aeration of the sample and sample with a field multiprobe.

Figure 3: Sieve sampling images.

Figure 3a shows the Bennett pump; figure 3b indicates a suggested 100 L sampling layout (note 20 L buckets were used in this case); figure 3c shows the sieve set up with high collar over the sieve to prevent sample overflow; figure 3d demonstrates moving of sieve apparatus onto the next bucket.

3.5 PRESSURISED FIELD FILTERING METHOD

Pressurised field filtering aims to collect a representative groundwater sample of the microbial diversity. In addition, ESR are currently investigating the potential for field filtration for protozoa assessment. Pressure filtration involves using compressed air to force large volumes (>20 L) of water from a pressure pot through a filter housing sequentially containing a 1.2 µM and 0.22 µM filter papers. The residue on the filter paper is what is submitted for microbial diversity analysis.

This method uses high pressure air (~35 psi) safety glasses and PPE must be worn. Compressor should be used as per manufacturer's instructions.

1. Collect 20 L pumped water into a clean sterile bucket.
2. Aseptically pour this 20 L into the sterile pressure pot (capacity of this device) (Figure 3a)
3. Aseptically place 0.22µM and 1.2 µM pore size filters with the 0.22 filter on the bottom of the filter housing and the 1.2 µM filter on the top. (Figure 3b and 3c)
4. Fit the top of the filter housing tightly and connect the hose from the pressure pot to the filter housing.
5. Start the compressor and allow 20L groundwater to pass through the filters by recording the volume collected after filtration i.e. the filtrate.
    a. **Note**: if more than 20 L is required repeat steps 1 to 5 above.
6. Once 20 L has passed through the filters aseptically place filters into a sterile pre labelled container and cover with preservative (LifeGuard™ or RNALater™[2]) (Figure 3d to 3f).
7. Label container with sample name, site, date and time and sampler name.
8. Store immediately in dark at <10deg.

[2] Lifeguard™ Soil Preservation Solution, Qiagen, Melbourne, Australia; RNALater™ Solution, Thermo Fisher Scientific, Baltics, UAB.

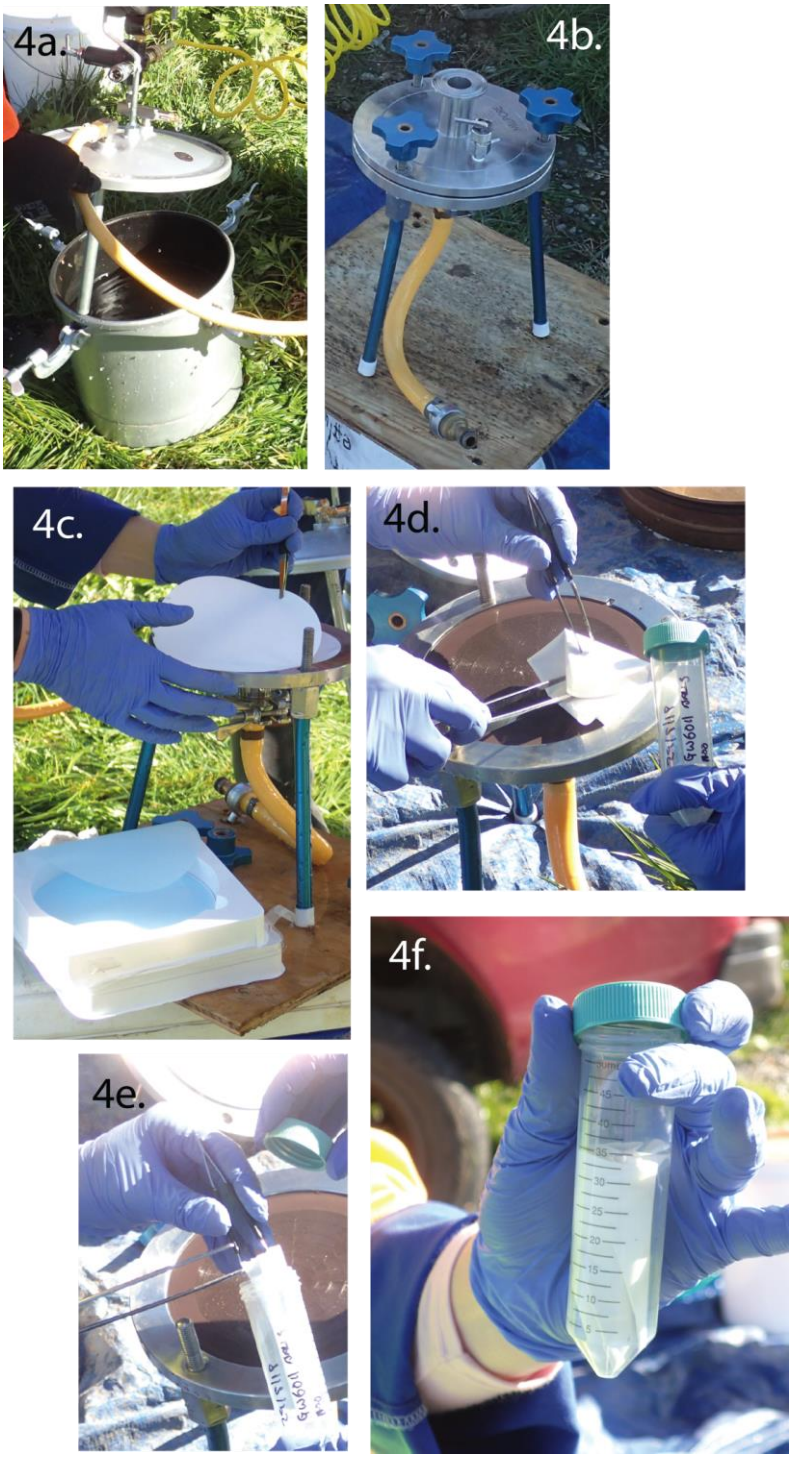

Figure 4: Apparatus and procedure for microbial sampling with in-field pressure filtration for collection of large volumes.

Figure 4a shows the pressure pot with sample before being closed; figure 4b shows the filtration housing; figure 4c shows placement of the filters onto the open filter housing; figure 4d demonstrates folding of the filter before placement into the collection tube; figure 4e shows placement into the collection tube; and figure 4f shows the filter in the tube with preservation fluid added.

3.6 COMPLETION OF SAMPLING

After sampling is completed, remove hose from the bore and pump and empty it of water. Pump a solution of 70% ethanol or Decon90 through the hose to decontaminate it, then pump thoroughly with tap water.

Wash the outside of the hose and wipe dry with a towel to prevent grass and dirt sticking to the hose and contaminating the next bore.

3.7 *IN SITU* BIOFILM SAMPLERS

The *in situ* biofilm sampler aims to collect a representative GW ecosystem community sample that has established over time. The microbial biofilms will grow on the gravel or other aquifer substrate and larger organisms are also captured around the bags and in the water contained in the sampler. It involves placing 63 µM mesh fabric bags containing sterile fine gravel or aquifer substrate in a PVC cartridge that is deployed down the well for a period of about 6 months (Williamson, Close et al. 2012, Weaver, Webber et al. 2015). The bags are later harvested and sent for microbial diversity analysis.

3.7.1 DEPLOYMENT

1. Take the *in situ* sampler and place biofilm bags into the sampler (Figure 4a to 4b).
2. Attach two different coloured cords with the same length as the total depth of the well. Note and label on the cords which cord is attached to the inner sleeve and which to the outer sleeve.
3. Holding both sets of cord separately slowly lower the sampler in to the well.
4. Once at the water table move the sampler up and down to allow it to fill with water and then continue to drop the sampler into the well.
5. Once at the bottom of the well (non-screened well) or midway in the screened section raise the cord attached to the inner sleeve of the sampler so the screened section is above the outer sleeve (Figure 4c).
6. Secure both cords at the top of the well.

If the well is pumped or sampled between deployment and retrieval it is important to remove the in situ sampler, store in a column of well water while the sampling or pumping is taking place and then replace the in situ sampler back down the well.

3.7.2 RETRIEVAL

1. Label sterile 250 mL wide necked pot with date, time, site/location, well/spring, sampler.
2. Open lid of pot and place lid so inside is facing up i.e. not touching the ground next to pot.
3. Put on latex (or equivalent) gloves.

4. Reel up biofilm bags taking care not to touch the bag.
5. Place the bag into pot and cut the string/fishing line off bag.
6. Put lid back on pot – do not touch the inside of the pot/lid or touch the bag.
7. Place pot into chilly bin with ice packs immediately and keep in dark.
8. Once sampling has been completed add label to outside of chilly bin and send to:

FAO: Judith Webber/Louise Weaver
ESR Ltd.
Christchurch Science Centre
27 Creyke Road
Ilam
ChC
8045

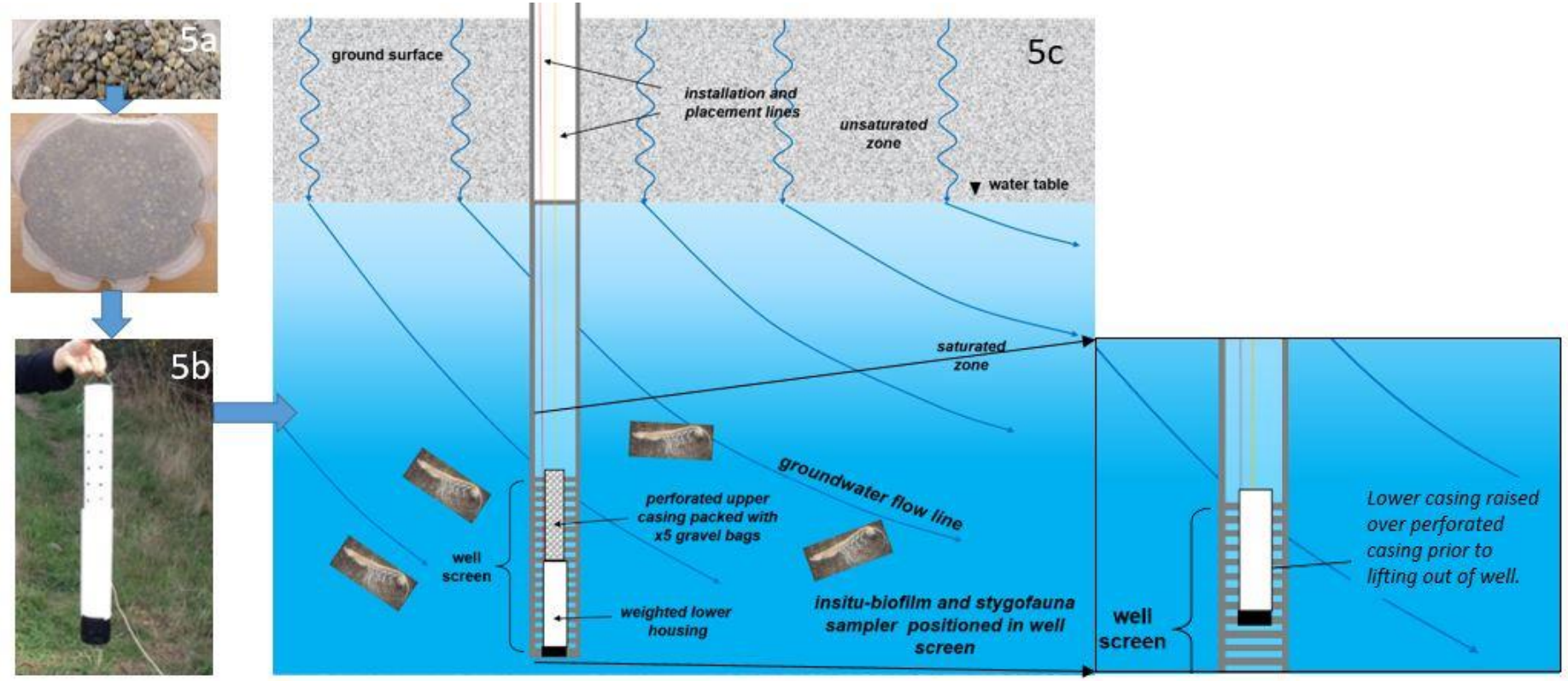

Figure 5: Schematic of the biofilm bag preparation and placement in the well.

Figure 5a shows typical material from the bore to be sampled and placement in the biofilm bag; figure 5b shows the in situ sampler containing biofilm bags before deployment; figure 5c shows placement of the in situ sampler within the screened section of the well with the outer casing lower than the perforated casing while biofilm is establishing. Prior to sampling the outer casing is raised over the perforated casing BEFORE the in-situ sampler is raised out of the well. Note: Stygofauna are **not** to scale.

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APPENDIX A: SAMPLING EQUIPMENT

Equipment	✓
Weighted phreatobiological nets (63-*μ*m mesh) (See Figure 1a). Check for holes[3].	
Waterproof markers, pencils etc	
Sample containers: wide necked large (>250 mL) pots, sterile Falcon tubes (or similar), bottles for water chemistry	
Single use pipettes	
Video equipment or camera (ipad is OK)	
70% ethanol	
Nitrile or latex gloves	
Rinse bottle filled with 70% ethanol	
Rinse bottle filled with demineralised water	
Chilly bins with ice packs	
Tissues and wipes	
Pumping equipment	
Power supply	
Compressor	
Hoses and reel	
Buckets (at least 10 L) to give total volume collection of 100 L	
Field probes	
Sieves	
Tweezers	
Dark open containers for net and sieve samples	

[3] http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2427.2007.01878.x/full

APPENDIX B: FIELD SAMPLING NOTES

Site ID: _____ Analyst:_____

Date:_____

Location Name:_____

Location and sampling notes (include weather conditions):

Well depth (m):_____Height of collar:_____

Does casing extend entire length of bore?:_____

Is the bore screened?:_____

Time started sampling:_____Time ended sampling:_____

Water depth (mbgl):_____

Field parameters read: Time:_____

GW temperature (deg C):_____

GW specific conductivity (µS/cm):_____

GW dissolved oxygen (DO%):_____

GW DO (mg/l):_____

GW pH:_____

GW ORP (mV):_____

Water chemistry taken? Y?N, if Y sent to (address & quote number):_____

_____

Photographs Taken: YES ☐ NO ☐

Photographs notes/ID:

Sample ID	Purged (y/n)	Netting (y/n)	Pumping (y/n)	Type of pumping	Volume pumped for sieving (L)	Volume pressure filtered (L)	Number of filters used	2nd netting (y/n)	Biofilm bags deployed/ collected? (y/n)	Comments
Date										
Time started										
Time ended										

Extra notes:

Faunal Sample:

Other data:

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