Subterranean Fauna Communities that are Groundwater Dependent Ecosystems

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Abstract

Stygofauna are tiny aquatic animals that live in a variety of groundwater systems including limestone, alluvial and fractured rock aquifers from a variety of geological histories and are clearly part of Groundwater Dependant Ecosystems. They are likely to be negatively impacted by activities that reduce groundwater tables. Western Australia (WA) is currently leading the way in Australia, in both surveys and taxonomy and has identified a great diversity of subterranean fauna, with some areas now regarded as Global Hotspots. Although subterranean fauna are routinely assessed in WA as part of the Environmental Impact Assessment process, this is not the case in other states, which often omit stygofauna from groundwater monitoring due to lack of identification information. Currently, Victoria appears to be oblivious to the existence of stygofauna. To overcome this situation, a three staged approach to sampling is outlined, where investigations progressively increase in complexity. The approach includes using low cost sampling of bores and springs to determine stygofauna presence, sorting into major groups and then progression to detailed taxonomic identification. A brief description of sampling methods is provided. The paper also provides information regarding potential subterranean fauna habitat within karst systems.

What are Subterranean Fauna?

A variety of animals have become adapted to living in subterranean habitats and are generally characterised by loss of body pigment and eyes. These include troglofauna which occur in air chambers in underground caves or small voids and stygofauna which are aquatic animals and live in a variety of groundwater systems including limestone, alluvial and fractured rock aquifers from a variety of geological histories. Stygofauna are 100% groundwater dependant (Humphreys 2006a) and as such will be the focus of this paper.

Generally most stygofauna are unpigmented, elongate, small (most less than 0.5mm long) and adapted to living in dark and generally confined spaces. Many have developed elongated feelers. Australian groundwater aquifers support a diverse fauna, largely consisting of crustaceans but it also includes worms, insects, gastropods, mites and fish (Humphreys, 2006a). Photos of some of the species can been seen in the poster (Ingeme, 2009) as part of these proceedings and also on the web sites provided below.

Biodiversity of Australian Stygofauna

Groundwater was once considered to be "biological deserts" but is now proving to be surprisingly rich in species, many of which are new to science (Tomlinson et al., 2007).

Western Australia contains a great diversity of subterranean fauna, with some areas now regarded as Global Hotspots for subterranean biodiversity. It is estimated that the Pilbara contains up to 550 species of stygofauna (DEC, 2008) which far exceeds the 300 stygofauna species recorded from all the cave systems of the United States. The Western Australian Yilgarn and Goldfields region also contain more species of subterranean beetle than anywhere else known in the world (Watts & Humphreys, 2006, in EPA WA, 2008). In some cases the level of biodiversity discovered is a reflection of the level of survey effort.

Humphreys (2006b, pp.2) also states that "while there is an increasing literature on the characteristics, origins and distribution of Australian stygofauna, for most regions of Australia, no data is available. The data show that Australia has a diverse groundwater fauna, but the focus of knowledge is in the rangelands, particularly in Western Australia where subterranean fauna are routinely considered in the environmental review process."

The groups of stygofauna so far known to occur in Australia are shown in Table 1.

Values of Stygofauna

Besides adding greatly to Australia's biodiversity, stygofauna are believed to play an important role in maintaining the quality of groundwater and providing ecosystem services, including keeping pore spaces open to assist movement of groundwater (DEC, 2008). They may prove to be valuable indicator species as measures and early warnings for groundwater health.

Stygofauna are believed to play an important role in maintaining the quality of groundwater as they generally feed on bacteria and biofilm. Bacteria have a recognised role in the bioremediation of some pollutants (Gounot, 1994, in Tomlinson et al., 2007), and therefore the stygofauna grazing on the bacteria and biofilms may stimulate these bioremediation processes (Mattison et al., 2005, in Tomlinson et al., 2007). Stygofauna feeding, movement and excretion are likely to play a role in the transfer of organic matter through aquifers (Hancock et al., 2005, in Tomlinson et al., 2007) and so helping to transform nutrients and maintain water quality (Tomlinson et al., 2007).

Stygofauna Habitat

Characteristics that make good stygofauna habitat include pore spaces in the rock and disolved oxygen in the water ($0.5-1mg/L^{-1}$) (Hahn, 2006). Ground water salinity needs to be less than 60,000mg/L⁻¹ (note that freshwater lying above a hypersaline lens may support stygofauna) (EPA WA, 2008). They may occur in very deep ground water. Metazoan communities, comprising obligate subterranean animals, are known to occur in aquifers to a depth of at least one kilometre (Humphreys, 2002)

Stygofauna are highly likely to occur in limestone aquifers, but are also known to occur in alluvial and fracture rock aquifers from a variety of geologies. But as

Table 1	Known	Australian	stygofauna	taxa
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Taxon	No. of Families	Further info. on Families or species	Description
Platyhelminthes: Turbellaria	1	or species	
Annelida: Polychaeta	1	Phyllodocida (Nereididae)	worms
Annelida: Oligochaeta	2	Tubificida	small aquatic earthworms snails
Mollusca: Gastropoda	1	Neotaeniglossa (Hydrobiidae), Basommatophora (Planorbidae)	snails
Arachnida: Acarina	2	Momonidae,Mideopsidae	mites
Insecta: Coleoptera	1	Dytiscidae	diving beetles
Vertebrata: Pisces	2	Blind cave eel (Ophisternon candidum), Blind Gudeon	eels
		(Milyeringa veritas)	fish
Crustaceans			
Syncarida	4	Parabathynellidae, Bathynellidae, Anaspidacea	Tubular animals with unformed legs
Remipedia: Nectiopoda	1	Speleonectidae	
Copepoda: Calanoida	3		Tiny
Copepoda: Cyclopoida	1		prawn-like crustaceans
Copepoda: Harpacticoida	7		
Copepoda: Misophrioida	1		
Ostracoda: Myodocopida	1		Small seed- like animals
Ostracoda: Podocopida	5		with calcified shells
Peracarida: Spelaeogriphacea	1	Spelaeogriphidae	
Peracarida: Thermosbaenacea	1	Halosbaenidae	
Amphiopoda	9	Perthiidae, Hadziidae Neoniphargidae, Hyalidae, Paramelitidae, Eusiridae, Paracalliopiidae, Melitidae, Bogidiellidae	Shrimp like
Isopoda	8	Oniscidea, Tainisopidea, Amphisopididae,Asellota, Phreotoicidea, Cirolanidae, Tainisopidea	Slater like
Decapoda	3	Atylidae	



a starting point for potential habitat a map, (Fig. 1) indicates the known karst areas of Australia and the global biodiversity "hot spots" for stygofauna.

Threats

Stygofauna are clearly part of Groundwater Dependent Ecosystems, and are likely to be negatively impacted by activities that reduce groundwater tables such as extraction and harvesting or dewatering for mining along with outright habitat removal through rock extraction.

Threats to Stygofauna therefore include water abstraction, artificial filling and contamination of aquifers (including the clogging of pore spaces by mobilisation of fine sediments (Hancock, *et al.* 2005, in Humphreys, 2006b).

Depending on the location of the aquifer containing stygofauna, additional threats may also include sewage or effluent, pesticides, fertilizer, hazardous materials via accidental spills or deliberate dumping (Lewis, 2002).

Perhaps the most significant threat currently operating is the complete ignorance of the stygofauna's existence by land managers and regulatory agencies causing them to be ignored in assessment processes. This stems from the fact that very few areas have documented the presence of stygofauna (Humphreys, 2006b).

Case in Hand – An Application For a Groundwater Bulk Entitlement

In 2008, a Victorian water authority applied to the Water Minister for a bulk entitlement to annually extract an *average* of 7000 million litres of groundwater from a borefield. This is a large volume of water, enough for 35,000 homes a year. To date the environmental assessments have only considered the "terrestrial" Groundwater Dependant Ecosystems (GDEs) (D.S.E., 2008) The ecosystems that are 100% dependant on groundwater, the stygofauna, were not included in the assessment process.

An adequate environmental assessment process is critical to make informed and appropriate management

decisions. A sound understanding of the possible species and communities present within and reliant on the aquifer is necessary to determine possible threats posed by actions such as extraction. This information is required to develop mitigation measures to prevent or minimize the threats.

Current knowledge and understanding of stygofauna

Humphreys (2006b) provides a number of references recognising the diversity of stygofauna on the western plateau of Australia with Thurgate *et al.* (2001a, 2001b, in Humphreys, 2006a) collating data from south-east Australia.

While the knowledge and understanding of Australian stygofauna is largely coming from the great diversity of species of the Pilbara and Yilgarn regions, limited research elsewhere in Australia indicates that significant stygofauna occur in other parts of WA and in NT, SA, TAS, QLD, NSW and Christmas Island (Humphreys, 2006b). Victoria is notable in its absence from this list. It is very likely that it is the lack of survey effort that is resulting in this lack of records. Seek and you shall find!

In a report by Humphreys (2006b) to the Australian State of Environment Committee, he notes that the focus of stygofauna research and hyporheic systems over the years have been respectively the Western Australian Museum and University of New England, with it broadening in recent years to include SA Museum, WA CALM, Queensland Natural Resources & Mines, Universities (Western Australia, Adelaide, Flinders) and consultancy bases. The focus of groundwater fauna research is present or developing in NSW, Queensland and SA. Victoria is again a notable absence from these research lists

A current study being undertaken by Flinders University SA, has found a wealth of stygofauna including new species belonging to Amphipoda (Neoniphargidae Bogidiellida, Chiltonidae, Melitidae), Isopoda (Heterias), Syncarida (Bathynellacae, Anaspidacae), Gastropoda (Hydrobiidae), Copepoda (Cylopoidea, Harpacticoidea),



Figure 2. Groundwater Stygofauna sampling sites in South Australia showing where stygofauna have so far been found. **Legend** Yellow Stygofauna present Green No stygofauna found

(Map provided by Remko Leijs – Flinders University SA, January 2009)

Ostracoda and Oliochaeta (Roudnew and Leijs, 2008). Many of the survey sites are close to the Victorian border (Roudnew et al., 2008) (Fig. 2). A similar diversity may therefore be expected to occur in Victoria in the same or similar aquifers.

Legislation

WA is currently leading the way in Australia, in both surveys and taxonomic identifications, but also from a legislative point of view where subterranean fauna assessments and surveys are part of the Environmental Impact Assessment process with the WA EPA developing Guidance Statements. The EPA WA (2003, pp 5) Guidance Statement No. 54 states that "The EPA will require proponents to undertake a survey for stygofauna when a project may potentially have a significant impact on groundwater levels, groundwater quality, or subterranean cave and void systems."

Water resource policy in Australia is co-ordinated through the Council of Australian Governments (COAG), which agreed that water must be allocated for environmental use in 1994 (ARMCANZ, 1996, in Tomlinson *et al.*, 2007). There is national agreement within Australia to manage groundwater better (COAG Task Force on Water Reform, 1996, in Humphreys, 2006a) with one of the key principles being that natural ecological processes and biodiversity are sustained and that water uses are managed in way that recognises ecological values (ARMCANZ, 1996, in Humphreys, 2006a). Obligate subterranean aquatic species, the stygobites, are clearly

part of the most dependant groundwater ecosystem (Hatton and Evans, 1998, in Humphreys, 2006a) and need to be considered.

It is now becoming urgent to ensure that these Groundwater Dependant Ecosystems are fully assessed before they are threatened with extinction. Climate change and the current reduced rainfall and associated reduced groundwater recharge and increasing demand and extraction of groundwater are potential threats to these ecosystems. Groundwater allocations must be carefully assessed and regulated to maintain ecological sustainability.

"Escalating groundwater exploitation is outstripping social and scientific understanding of the sustainability of this resource" (Tomlinson et al., 2007, p 1317). A three staged approach for sampling has been put forward as a means of progressing our knowledge and understanding of the biodiversity of these ecosystems.

A Three Staged Approach To Sampling Stygofauna

Tomlinson et al. (2007) advocate a three staged approach to sampling, described below, where investigations progressively increase in complexity. It is seen as a means of overcoming the current situation of omitting stygofauna from groundwater assessment and monitoring because there is insufficient information for the interpretation of survey results.

1. This involves firstly establishing the presence of stygofauna using simple low cost sampling methods, monitoring bores, wells and springs. This should be the minimum compulsory assessment. Assessment of bore water is viewed as providing a representative sample of the associated groundwater aquifer. Hahn and Matzke (2005) undertook tests comparing the first water pumped from within the bore cavity and that sucked in from the aquifer as pumping continued. The results strongly suggested that bores contain all species found in the aquifer, albeit in different proportions.

2. The second stage is to identify the main types/ groups of invertebrates present and the diversity of species present, even if it is just grouping the specimens and identifying them as species 1, 2, 3, etc. This is very useful, as early indications are that these groups differ in their tolerance to water-table fluctuations.

3. The final stage involves more detailed quantitative sampling and using taxonomic specialists to identify the specimens collected.

1. Establishing the presence of stygofauna

Using simple low cost sampling methods to monitor bores, wells and springs. This can be achieved using weighted plankton nets lowered down into a well, water pumped through a fine mesh, or simple "stygofauna traps" being left for several days. More information regarding sampling methods is available from the EPA WA web site, or the web page for the PASCALIS project, viewed 2009.

Weighted plankton/haul nets The net is lowered to the bottom of the bore, agitated vigorously to stir up the bottom sediments and benthic dwelling animals and then slowly retrieved. At least six hauls are required, and to increase the chances of capturing the size range of fauna (most between 0.3mm to 10mm (DEC, 2008)), half the hauls should be undertaken with $50\mu m$ net and the other half with $150\mu m$ net. The contents of each net haul should be emptied to prevent loss of specimens. Washing down the walls of the net ensures all specimens are collected. Net diameters should be two thirds the diameter of the bore to allow upward movement of fauna when lowering the net.

All samples collected should immediately be preserved in at least 70% ethanol (100% analytical grade ethanol if undertaking DNA analysis) (EPA WA, 2008).

Pumping and filtering This method may recover more animals but it is more time consuming, requires expensive equipment, is not suitable for very deep bores and may damage the animals collected. Different pumps cause different levels of damage to specimens. EPA WA (2008) provides more detailed information.

It is recommended that the volume of water pumped and filtered is either 300 L or three times the bore volume (eg. the purging water), whichever is greater (EPA WA 2008) Hancock and Boulton (2007, in EPA WA, 2008) have shown through sampling in eastern Australia that 300 L yields at least equivalent numbers of stygofauna to net-hauling.

Stygofauna traps are rarely used because they are more time consuming. They need to be set and left several days before collection, and so require more trips to be made. They generally consist of a suitably weighted and baited container being lowered into the bore and left for a few days. There is a risk of taxonomic bias as they appear to preferentially capture larger animals such as isopods and amphipods and generally miss the animals in the sediments (Hancock, 2007, in EPA WA, 2008).

In all cases, all samples collected should immediately be preserved in the field in at least 70% ethanol (100% analytical grade ethanol if undertaking DNA analysis) in fully labelled containers and taken back to the laboratory for detailed and careful sorting under dissecting microscope to ensure that nothing is missed (EPA WA, 2008). The EPA WA (2008) web page contains useful information on how to make sorting a bit easier.

The collected fauna can often be seen with the naked eye, so their existence, and that of GDEs can be determined in the field. This small effort would help alert the public to the presence of life in aquifers, confirm GDEs, and stimulate research and management attention towards groundwater ecology.

2. Identify the main invertebrate types/groups

Under a microscope, the preserved animals can be sorted into similar looking types or "morphotypes". The number of morphotypes provides some indication of the diversity and also allows broad comparisons to be made over time in an area. With some training, it is possible to distinguish the major groups of invertebrates. Some web sites and references are provided at the end of the paper to assist with identification and grouping.

3. Undertake more detailed studies using specialist taxonomists and quantitative sampling.

These more detailed studies are required to determine conservation issues and truly assess the biodiversity. This data is required to address issues such as: spatial and temporal variations of species abundance and diversity; correlations of community composition with physical and chemical variables; and application of the data to determine environmental water requirements.

Sampling for stygofauna in caves

Before any collection of fauna occurs, you need to ensure that all necessary fauna collection permits have been obtained from the relevant authorities, such as Department of Sustainability and Environment in Victoria and possibly Parks Victoria depending on land status. Populations of invertebrates in caves are often very small so collection may have a big impact. Initially photos may be the lowest impact method for the initial stages of recording what fauna occurs within caves.

Cave systems may support both stygofauna and trolgofauna that may then form part of groundwater dependant ecosystems. Caves may provide a variety of habitats for these species. Using the Victorian cave in far south west Victoria, Jones Ridge Cave DD4, as an example, the types of habitats that it provides include a flowing permanent stream, a waterfall that helps aerate the water, several pools that provide additional refuge habitat during low flow and particularly during drought, moist mud substrate the provides both food and shelter, and a sump that again provides refuge habitat during low flow and drought, but also plays a significant role influencing the climate (air flow and humidity) within the cave. Other caves may also contain root mats that provide both food and habitat within the cave, bringing additional nutrients into the cave.

Troglofauna and stygofauna in caves can be found with careful searching of the habitats mentioned above. Sampling might also involve the use of troglofauna traps, baiting, or using nets etc in streams or pools. Stygobiont animals that find refuge in the benthic layer may be captured by disturbing the sediment and filtering the water immediately down stream of the disturbed area. This can be achieved using a Surber sampler, a Hess sampler, or even a pond net (Fig. 3).

Caves and Habitat Protection

As cavers and speleologists, we need to be aware what else may be living in the cave and how our actions may adversely impact on those species. This may include stirring up mud in the stream, increasing the turbidity, the settling sediments may then smother niche habitats such as under and between rocks and may also impact the overall health of species. Artificially removing or damaging significant features within the cave, such as sumps, may have very adverse impacts by altering the hydrology and climate within the cave.

Detailed assessment including biological assessments should be undertaken and evaluated before rash ad hoc damage and destruction to caves, such as removal of sumps, or digs, is planned and undertaken.

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Surber sampler

Figure 3. Aquatic invertebrate sampling equipment. (Source: http://groundwater-ecology.univ-lyon1.fr/ nouveau/methodes-souterraines.htm)

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Useful Web Sites and References Survey and Monitoring Methods

PASCALIS Protocols for the Assessment and Conservation of Aquatic Life In the Subsurface (ie monitoring protocols) <u>http://www.pascalis-project.com/.</u>

http://groundwater-ecology.univ-lyon1.fr/nouveau/ methodes-souterraines.htm.

WA EPA Environmental Impact Assessment Guidance Statements <u>http://www.epa.wa.gov.au/docs/1720_GS54.pdf.</u>

Identification of Invertebrates, Subterranean Fauna

International Symposium of Subterranean Biology (held in Fremantle WA 2008) <u>http://www.issb2008 org.</u> au/gallery/ISSB/Stygofauna.html.

www.mdfrc.org.au/bugguide/resources/macroinverteb ratetutorial.htm

http://www.mdfrc.org.au/bugguide/display.asp?type= 1&class=19

Amphipods

http://www.mdfrc.org.au/bugguide/display.asp?type= 3&class=18&subclass=33&Order=34&couplet=0.

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Pilbara Stygofauna Image Sheet

http://www.dec.wa.gov.au/images/stories/nature/ science/pilbara/stygofauna/pilbara%20stygofauna%20s heet.pdf.

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