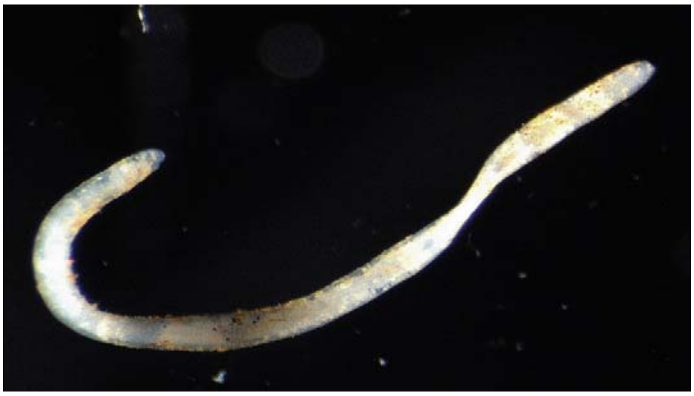
Final Baseline Stygofauna Survey Report for Rockdale

January 2013



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Contents

Executive Summary

This report presents the results of surveys of the groundwater ecosystems of the Pilliga sandstone aquifer for stygofauna and possible biological contamination using rapid assessment techniques. This is the first environmental assessment of the aquatic ecosystems of the Pilliga sandstone aquifer within the Pilliga Forest area. The surveys were conducted beneath the Rockdale pastoral property within the Pilliga forest area approximately 40kms south of Narrabri.

The results reveal the discovery of potentially new species for NSW, belonging to two families of aquatic worms and one aquatic mite family. It is possible that the record of the Enchytraeidae family of aquatic worms may be the first for this family in groundwaters in NSW.

What are stygofauna, why are they important?

Stygofauna are animals that live in underground water. They are generally comprised of crustaceans and other invertebrate groups such as worms, snails, mites and even blind water beetles.

Stygofauna are species that spend their entire lives in groundwater and are generally found nowhere else. As such, these ancient organisms have highly specialised adaptations to survive in the relatively resource-poor aquifers, where there is no light, space is limited, and food is scarce (Humphreys 2008).

Stygofauna are blind, are colourless, have slow metabolism, reduced body size, specialised anatomies and low reproduction rates (Coineau 2000). As there is no photosynthesis below ground, these groundwater environments rely on inputs of organic matter from the surface to provide the basis of the food web on which stygofauna depend (Schneider et al. 2011).

Despite their small size, the cumulative effect of stygofauna activity plays an important part in maintaining groundwater quality. This process is evident in alluvial aquifers where water flowing though sediment particles is cleaned during transit by stygofauna, in much the same way as water moving through slow sand filters or trickle filters in water and sewage treatment (Hancock et al. 2005).

Stygofauna therefore play a functional role in aquifers and are also considered a direct and sensitive indicator of the quality of an underground water source.

Survey results

The surveys recorded three families of stygofauna within the Pilliga Sands aquifer - the first ever known records of stygofauna within this aquifer, which extends from below the Pilliga East forest just west of Narrabri and then west towards Coonamble.

The stygofauna were collected at greater depth than is usual for stygofauna and in a confined aquifer with low dissolved oxygen levels. The stygofauna orders that were recorded during the survey were Aquatic Worms (Oligochaeta) and Water Mites (Acarina). Two families of aquatic worms were recorded - it is possible that the record of the Enchytraeidae family of aquatic worms may be the first record of this family in groundwaters in NSW.

Knowledge of groundwater dependent ecosystems in eastern Australia, including stygofauna, is limited and patchy. The presence of stygofauna in the Pilliga Sands aquifer is considered highly significant because of the conditions in which they were found and the information that this reveals in relation to the quality of the aquifer and its likely connections to surface water.

This bore and the stygofauna within it represent a biodiversity hotspot that indicates moderate to good water quality, connectivity with the adjacent river system and persistent longevity of the community.

Threats to stygofauna

Unlike many surface aquatic species, stygofauna are extremely limited in their ability to disperse and are highly endemic. Aquifers in general are relatively stable compared to surface aquatic environments with little or no daily fluctuations in parameters such as temperature, water level, and electrical conductivity. Therefore, stygofauna are considered very sensitive to any groundwater aquifer changes, including changes in water levels or chemical composition. Most forms of development impacting on groundwater may to lead to their disappearance.

Changes to the quantity or quality of groundwater systems can lead to drastic changes in the diversity of organisms living underground. In particular, such changes can lead to the extinction of stygofauna populations and the penetration of alien species belonging to surface-water communities.

Mining is recognised as a substantial threat to stygofauna communities. Groundwater drawdown and changes to groundwater quality may cause prospective stygofauna habitat to be degraded or lost and cause significant harm to other groundwater ecosystems and communities.

Changes outside the natural range of water quality, water chemistry and levels such as rapid drawdown or changes to water chemistry such as a pollution plumes are likely to have significant impacts on the community composition, biodiversity and overall sustainability of the community.

Groundwater communities also require links to the surface environment to provide organic matter and oxygen. If that linkage is broken or disrupted, the stygofauna community in the area affected could decline over time.

Cumulative effects may result from a number of activities interacting with the environment. In the context of other threats, the proposed CSG activities in a region are growing thus increasing the potential for an impact on the underlying aquifers. The implication of multiple mining actives in one region is that impacts may overlap and result in larger impacts than would be expected for a single mining operation (cumulative effects). Groundwater also supports a large range of other ecosystems including rivers, springs, wetlands and even terrestrial vegetation and marine/estuaries. The cumulative flow on impact to an aquifer will not only impact the water resource for towns and prime agricultural land but the surface environments as well (Serov et al, 2012).

The biological investigation results conclude that the threat posed to stygofauna communities by proposed mining activities within the Pilliga forest area is considered to be high.

Recommendations

Baseline data of groundwater biodiversity and environmental parameters must be collected before mining commences. Aquifers must be monitored for any spatial and temporal changes in parameters of the stygofauna, water quality and water quantity. Monitoring should occur during the construction, operational and post-mining phases of any projects, both within and outside the potential zone of impact from the current and proposed coal seam gas and other mining activities.

The suggested next stage of this project is an examination of water quality and subterranean ecosystem health from as many adjacent bores as possible. This is necessary to provide a benchmarked network of bores for future comparisons.

The sites that have been surveyed and analyzed for water chemistry as part of this project should be regarded as the first benchmarked sites for this aquifer in the area. Benchmarking is necessary and essential in order to characterize the natural distribution and environmental ranges within the aquifer and therefore the requirements of this subterranean ecosystem and the overall health of the aquifer for human consumption.

Further examinations and benchmarking should result in the aquifer being characterised by:

- The structure/lithology of the aquifer by obtaining bore log/works details of the bores;
- The full water chemistry and water levels of the groundwater (including temperature) over time to establish the natural annual ranges and seasonal fluctuations;
- The aquifer flow paths to determine the connectivity (gaining or loosing) with the associated river above and below the potential area of impact;
- Identify the obligate stygofauna to species (those listed as phreatobites) to determine levels of endemicity of the stygofauna community within the aquifer as this community is the most disturbance sensitive environmental indicators for changes in aquifer conditions;
- Conduct further surveys in other bores and hyporheic zone of the associated river, if available, within this aquifer and adjacent aquifers to determine the range of the species;
- Identify other groundwater dependent ecosystems in the area such as springs and groundwater discharge zones within nearby steams; and
- Conduct water level mapping across the site/aquifer to determine the linkages with the river systems and other users of the groundwater source such as the local community through the need for stock and domestic water and other potential GDE's such as terrestrial vegetation or wetland communities that also rely on consistent water levels and water quality.

Introduction

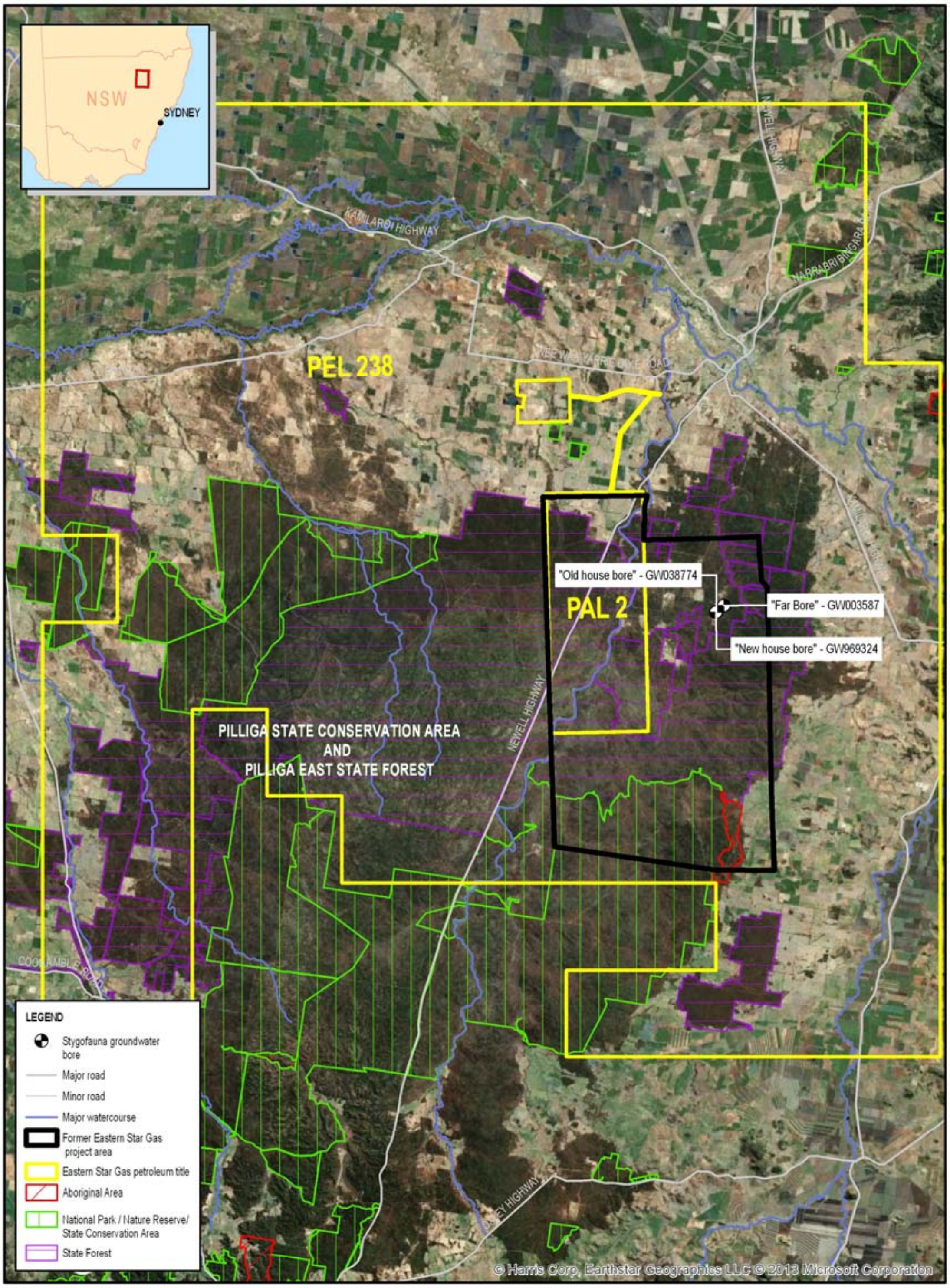
This report presents the results of the biological assessment of the groundwater ecosystems of the Pilliga sandstone aquifer beneath the Rockdale pastoral property within the Pilliga Forest area south of Narrabri. This survey is part of an ongoing investigation being conducted to confirm the presence of stygofauna within confined/semi-confined aquifer within the Pilliga Sandstone formation and continue the examination of the decline in water quality experienced by the property owner. StygoEcologia was commissioned to conduct a baseline biological survey of the bores on the property as an indicator of the groundwater condition to compliment the water chemistry analysis conducted in the same period as well as to provide advice on the possible cause of the water quality change. Three bores were sampled on the 17th May 2012 and then 27th June for stygofauna and possible environmental contamination using rapid assessment techniques in the Pilliga East forest region.

Study Sites

The sites surveyed during the first round of sampling were repeated with the addition of a small seepage located on the northern side of the road leading to the entrance to Rockdale. The seep is positioned on the southern side of a hill and discharges at the surface for approximately 50m downslope. A series of small pools occurs below the discharge point of the seep and these were sampled for aquatic fauna.

Site/Location	**No. species**
"New house bore" - GW969324, Rockdale, pump sample, 17/5/2012	2
"Old house bore" - GW038774, Rockdale, stygofauna, net, 17/5/2012	0
"Far Bore" - GW003587, Rockdale, stygofauna, net, 17/5/2012	3
Roadside seep near entrance to Rockdale	4

Table 1; Locations surveyed on 27th June 2012



Reserves OEH and NSW Dept of Planning. Petroleum titles from NSW Dept of Primary Industries. Watercourses, roads and towns from GEODATA TOPO250K. Groundwater wells from NSW Office of Water. Imagery from Bing.	NORTH 0 km 10 1:600,000 at A4 12/02/2013	Location of the formerly proposed Pilliga Project Gas Field Development Area and stygofauna groundwater survey sites	Figure 1

Map 1, Location sites of the Stygofauna groundwater survey sites in the Pilliga forest.

Method

Each site, except the seep, was sampled using two standardized methods and one nonstandard method.

The Phreatic/Hypogean zone

The phreatic zone is the subsurface area within an aquifer where voids in the rock are completely filled with water. This is occupied by phreatobites – i.e. groundwater aquatic invertebrates called 'stygofauna' that are restricted to the deep groundwater substrata of alluvial, fractured rock and karst aquifers (phreatic waters). They have specialized morphology and physiology and occupy a diverse range of niches within the aquifer. These adaptations include the ability to tolerate suboxic conditions (dissolved oxygen concentration (DO) less than 3.0 milligrams per litre) or limited food supply (Malard and Hervant 1999, Hervant and Renault 2002, Datry et al. 2003) and even hypoxia (DO less than 0.01milligrams per litre) (Thomlinson & Boulton, 2008). Dissolved oxygen (DO) concentrations below 1.0 to 0.5 mg/l are the critical threshold for most groundwater fauna (metazoans) (Hahn 2006). The stygofauna community was sampled using two standardised methods.

1. The first technique is the Phreatobiology Net. This is the standard technique that has been used successfully overseas and in Australia (Bou, 1974). The method used conforms to WA guideline [2003 & 2007] requirements. This method involves using a weighted long haul or plankton net with a 150 µm mesh. Sampling consisted of dropping the net down to the bottom of the bore and taking at least three consecutive hauls from the entire water column at each bore. Upon removal from the bore the net is washed of sediment and animals and the contents of the sampling jar (the weighted container at the bottom of the net) are decanted through a 150 µm mesh sieve. The contents of the sieve are then transferred to a labeled sample jar and preserved with 100% ethanol.

2. The second method is the use of a water bailer. A bailer is typically used by hydrogeologists to taken water samples from bores for water quality/water chemistry analysis. The bailer used for this study is 1 meter long by 40mm clear plastic tube with a running ball valve at the bottom. The advantage of using a bailer is twofold. The main reason for using a bailer is that it is able to sample the bottom sediment of a bore that cannot be sampled by a haul net and therefore enables the collection of cryptic invertebrates that do not inhabit the water column or sides of the bore. The second advantage is that in shallow bores down to 5 meters in sediments with low transitivity porosity) a bailer is able to empty the entire contents of a bore and thereby confidently collect all animals within the bore. The contents of the bailer are emptied into a cleaned bucket from which the water is then decanted through a 150 µm mesh sieve. The contents of the sieve are transferred to a labeled sample jar and preserved as above. Following sampling and preservation of the sample and prior to the next sampling all equipment including the bailer, net and sieves must be rinsed clean with clean water via a spray bottle to remove any sediment and animals that may have remained attached to the sampling devices. This is to

reduce the possibility of cross contamination of organisms (stygofauna or bacteria) or pollutants from one aquifer or bore to another.

3. The third (nonstandard) method was used on the "new house bore" only due to access restrictions with the other two methods. This involved pumping water through the house pump to the surface for approximately ten minutes, which removed an estimated two bore volumes. This was drained through a 150µl sieve. The resulting sediment was washed into a container and preserved in 100% ethanol. Three one litre samples were also collected during the collection for water chemistry analysis and processed using the same method. The pump and pipe work was not removed from the bore and therefore the entire water column was not sampled using either the bailer or phreatobiology net as in the other two sites.

4. The seepage pools were sampled using a 250µm gauge sieve. The sieve was passed through the water column and over the bottom substrate. The contents were decanted into a preserving jar, labeled and preserved in 100% ethanol.

Measurement of physico-chemical parameters;

A full water chemistry sampling was conducted prior to the biological sampling with the results pending.

Identification

All samples are preserved in the field with 100% ethanol and returned to the laboratory where each sample was sorted or separated from the collected sediment under a stereomicroscope and stored in 100% alcohol. The preservation of specimens in 100% ethanol enables the specimens to be included in future DNA analysis studies.

Results

Four sites were sampled on the 27th of June 2012 with three registering the presence of fauna. The results are presented in the table below. The old house bore again did not record any insitu fauna, i.e. any fauna that would have been living within the groundwater. The water appeared clean with a water depth comparable to the adjacent new house bore.

The “New House Bore (GW96324) recorded the same two main species recorded in the first survey (May 2012). The species also included a number of fragments of terrestrial fauna such as ants and beetles that have entered the bore and used it as a refuge due to the microclimate contained within bores or accidentally fell in through the small opening in the top of the bore. This result confirms that the two stygofauna, an Oligochaeta (worm) and an Acarina (mite) species, recorded early are active residents within the groundwater at this location. As the specimens of both species were intact and not showing any signs of decomposition they were alive at the time of collection.

The large number of ant remains still present within the “New House Bore” also confirms that they had and may still be occupying the interior of the bore casing although no new, less decomposed bodies were collected.

The “Far Bore (GW003587)” located approximately 900m to the north east of the house also recorded specimens of single species (an Oligochaete) of stygofauna once again confirming the results of the first survey.

The fourth site surveyed the small surface pools created by small roadside groundwater seepage near the entrance to the Rockdale property. The species recorded here belong to the surface aquatic macroinvertebrates. These species are found in a variety of surface water bodies. They included two species of water beetles (Coleoptera) and two species of aquatic flies (Diptera). These groups all have flying adults that are able to easily disperse and colonize new surface aquatic habitats easily. There were no interstitial fauna recorded at this site. All of these species are predators except for the beetle family Hydraenidae, which is an algal grazer. The two fly species occupy the bottom sediments and have moderate tolerance to disturbance although the Tipulidae and the Hydraenidae are generally found in aquatic ecosystems of good water quality (Williams, 1981)

Locality Description	Specimen Condition	Class	Order	Family	Habitat
New house bore" - GW969324	Complete	**Annelida**	**Oligochaeta**	**Enchytraeidae**	***Phreatobite***
New house bore" - GW969324	Complete	**Araneae**	**Prostigmata**	**Halacaridae**	***Phreatobite***
"Far Bore" - GW003587	Complete	**Annelida**	**Oligochaeta**	**Enchytraeidae**	***Phreatobite***
Roadside seep nr Rockdale.	Complete	Insecta	Coleoptera	Dytiscidae	Stygoxene
Roadside seep nr Rockdale.	Complete	Insecta	Coleoptera	Hydraenidae	Stygoxene
Roadside seep nr Rockdale.	Complete	Insecta	Diptera	Ceratopogonidae	Stygoxene
Roadside seep nr Rockdale.	Complete	Insecta	Diptera	Tipulidae	Stygoxene

Table 2; Fauna List of Specimen collected on 27th June 2012
*the bolded rows indicate those species that are regarded as being stygofauna.

Phreatobites

The presence of stygofauna was recorded at two of the bores sampled. This included the "new House Bore" and the "far Bore" again. This is the first known record of stygofauna from the Pilliga Sands Formation and the first known records of stygofauna occurring within an apparently confined aquifer. It is a new and unexpected discovery as the aquifer type from which they were collected from is a confined/ semi confined aquifer and would normally preclude the existence of a subterranean ecosystem due to the low dissolved oxygen levels. They were also collected from a greater depth than is usual for stygofauna.

The species composition of the site would indicate (and confirm) the existence of an unconsolidated aquifer (which is probably a palaeochannel of an ancient river bed) consisting of inter-bedded medium to course grained sands and gravels. The finding also indicates that the aquifer is only semi-confined with a connection to surface water ways or the upper unconfined aquifers. The aquifer will have moderate to high transmissivity and connectivity throughout the system and is likely to be connected with the associated river system in some locations. It will also support baseflow within local streams including the hyporheic zone, terrestrial vegetation through the upper porous (sandy) soils and shallow perched aquifers.

The species also indicate moderate water quality. The presence of only Oligochaeta (worms) and Acarina (mites) and the absence of the normally dominant groups such as the crustaceans and molluscs, may be an indicator of either naturally moderate to high acidic groundwater conditions or a rapid change to these conditions that has eradicated the other more sensitive groups . These two groups have previously been found to be the only fauna within mild (ph 5-6) to highly acidic (ph 4-2) groundwater environments (see discussion).

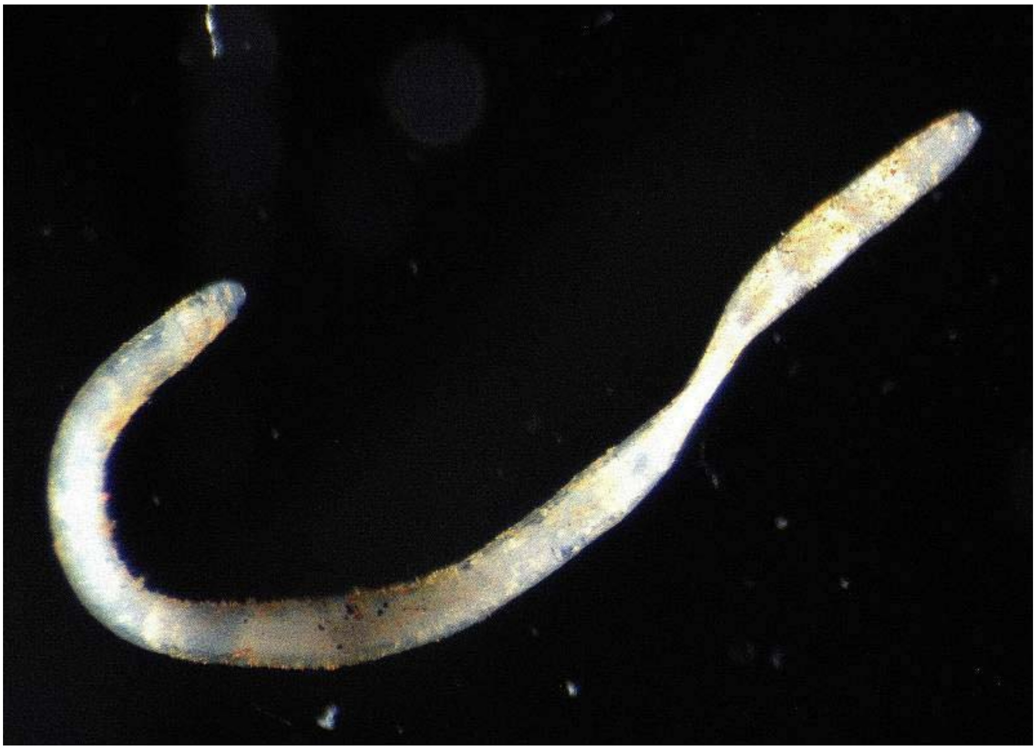


Photo 1; the worm family ©Stygoecologia2012

Apart from the significance of the new find, it demonstrates a direct connectivity within the aquifer between the two bores i.e. the new bore and far bore as well as a strong connection between the aquifer and the surface environments. That is, both bores appear to be connected to the same water source and the water source appears to be connected to the either or both the overlying shallow unconfined aquifers or the surface water bodies. The implication of this is that if the aquifer had been impacted by a general contamination it would be detected in both bores unless it is a localized, point source impact/ contamination or if the flow path precludes one or other bore. As the same fauna was detected in the two bores it is expected that any contaminants will also be detected in all bores.

The obligate groundwater fauna is characterised by the two Oligochaete Families, the Enchytraeidae and Naididae (from the previous survey) and the Astigmata water Mites. The Enchytraeidae is a small family of aquatic worms that are poorly known although they have been found in freshwater environments in Victoria, NSW and recently in groundwaters in Queensland. They are a poorly known group that requires further taxonomic work (Pinder & Brinkhurst, 1994). This is possibly the first record of this family in groundwaters in NSW.

The other species of stygofauna collected belongs to the Acarina or water mites. There is at least one species of water mite present belonging to the Family Halacaridae. Although subterranean water mites are classed as stygobites they have their highest biodiversity within the riverine, hyporheic zones and are classed as members of the “permanent hyporheos or the community that occurs within the deep sand and gravel beds associated with areas of groundwater discharge (Gilbert, 1994). They have however, been frequently found in unconsolidated aquifers coastal sandbed aquifers as well (Serov, unpublished data).

Photo 2; Aquatic Water Mite collected at New Bore ©Stygoecologia 2012

Water mites typically characterize the transition zone between the temporary or shallow hyporheic ecotone and the groundwater hypogean environment. *(*Boulton & Hancock, 2006, Gilbert, 1994, Humphreys. 2006, Serov, *et al*, 2011.). It is therefore unusual to find this group within the deep phreatic zone (deep groundwater). It is another indication that this aquifer is or has been connected to surface water sources as a discharge source where the discharge can be either point source springs or diffuse discharge through a moderate to course grained substrate such as sand or gravels (Gilbert, 1994). The presence of both of these species/groups within the phreatic or deep groundwater zone is therefore a direct indicator of groundwater connectivity not only between the "New House Bore" (GW969324) and the "Far Bore" (GW003587) but also between the local rivers systems and shallow unconfined aquifers.

Discussion

Knowledge of groundwater dependent ecosystems in eastern Australia is limited and patchy (e.g. Eberhard and Spate 1995, Thurgate et al 2001, Hancock 2002, 2004, Hancock & Boulton 2008, Hose et al 2005, SMEC 2006, Watts et al 2007). With the exception of a small number of studies in the sandstone environments of the Blue Mountains and the Upper Nepean areas (Hose 2008, SMEC, 2006) and the coastal sands of the mid North coast (Serov Unpublished data) and recent work in the Maules Creek and Namoi catchments (Serov et al, 2009, Thomlinson and Boulton, 2008) we are unaware of other studies of groundwater dependent ecosystems in porous sandstone aquifers in eastern Australia. Even internationally, studies of groundwater ecosystems in porous rock are scarce.

The striking features of the fauna collected in this series of surveys are:

1. The presence of stygofauna at all;
2. The very low diversity of the stygofauna;
3. The fauna composition consisting of disturbance tolerant groups that have been found in other studies to be able to tolerate and preferential occupy habitats that have moderate to very high acidic conditions (3-5 ph units), very low dissolved oxygen, as well as being able to tolerate high salt loads.

This discussion will present a brief review of studies that have been conducted in NSW and general discussion of the use of stygofauna as indicators of environmental condition and change.

Drivers of groundwater ecology

(Extract from Tomlinson, M., Boulton, A. 2008)

"Dissolved oxygen is a key environmental parameter in interstitial environments (Danielopol et al. 1994, Ward et al. 1998), although Malard and Hervant (1999) state that dissolved oxygen is not a limiting resource for all animals in groundwater, as faunal distribution in many studies does not match oxygen gradients, and further, not all groundwater habitats have low dissolved oxygen. However, Hahn (2006) found oxygen concentrations of one milligram per litre to be a critical limit for subsurface fauna.

Although a correlation between an easily-measured variable, such as dissolved oxygen, and a measure of community condition, such as species richness, would be ideal for the purpose of management, such a relationship is seldom apparent or consistent. Some studies show weak correlations between individual water quality variables and stygofaunal community composition or species distribution (Dumas et al. 2001, Hahn 2006, Castellarini et al. 2007b). Others studies illustrate contrasting results. For example, Mauclaire et al. (2000), studied a glacio-fluvial aquifer some 20 kilometres east of Lyon, France, found that, while bacterial activity and abundance were correlated with dissolved organic carbon (DOC) concentrations, faunal abundance was relatively homogeneous and only weakly correlated with DOC.

However, in the same aquifer, but at sites closer to the Rhône River, Datry et al. (2005) reported that groundwater invertebrate assemblages were more abundant and diverse in sites artificially recharged with storm water compared with reference sites recharged by rainfall infiltration. Concentrations of dissolved organic carbon (DOC) were significantly higher in the recharge sites than in reference sites, where the thickness of the vadose zone was less than 10 meters in all sites, although mean concentrations of DOC were no greater than one milligram per litre at any site. In contrast, Masciopinto et al. (2006) reported that, in wells affected by artificial recharge of waste water in southern Italy, increased DOC at similar concentrations to the Datry study was associated with a decline in faunal biodiversity. DOC concentration in bedrock zone groundwater is typically quite low; Wetzel (2001) cites a median DOC content of groundwater as 0.65 milligrams per litre. This is comparable to a median value of 0.7 milligrams per litre recorded in a survey of 100 bores and springs in 27 states of the US (Kaplan and Newbold 2000).

These results might indicate differences in the quality rather than quantity of DOC (Sobczak and Findlay 2002). The bioavailability of DOC varies, and depends on its source and chemical composition. DOC consists of an extremely complex mix of organic compounds of varying structure and molecular weight. The more labile, simpler compounds are metabolized more rapidly by bacteria, although there is some evidence that more complex compounds support higher bacterial numbers over longer time periods (McDonald et al. 2007). Although organic matter supply may be necessary to sustain life, species richness, faunal community composition and spatial patterning are likely to be determined by multiple interacting factors: transmissivity, oxygen, dissolved organic carbon, redox and pH accounted for 52 per cent of the variability in faunal abundance in two French alluvial riverbank aquifers (Mauclaire and Gibert 2001). The conflicting results could also be due to the limitations of measuring water quality variables from pumped groundwater in which mixing effects mask small-scale heterogeneity in aquifer conditions (Strayer 1994).

Physico-chemical variables are also unlikely to be the sole determinants of species distributions and community assemblages. Dispersal constraints (Belyea and Lancaster 1999), such as hydrological disconnection (Sheldon and Thoms 2006), could isolate parent populations from which populations observed at any particular sampling time are derived. Lag effects are likely, so that the species presence and abundance data collected at any sampling time could result from previous rather than current physico chemical conditions. There might also be multiple points of population or community stability due to varying influences of different combinations of driving variables as environmental conditions change.

Chemical elements cycle through organisms and their abiotic environment in a series of reactions termed biogeochemical cycles (Clapham 1973, Brewer 1988) of which the carbon, nitrogen and phosphorus cycles are most pertinent from the perspective of this review. As most subterranean food webs are heterotrophic, transfer of carbon from particulate and dissolved organic matter to invertebrates is mediated by biofilms coating sediment particles and rock surfaces (Bärlocher and Murdoch 1989, Chafiq and Gibert 1996, Claret et al. 1998, Findlay and Sinsabaugh 1999). Biofilms transduce nutrients and energy (Battin et al. 2003) through processes including abiotic adsorption of molecules to the biofilm matrix and biological uptake by enzymatic hydrolysis. The bacterial uptake and repackaging of carbon and nutrients constitutes a microbial loop (Sherr and Sherr 1988) through which dissolved and particulate organic matter is made available to grazing protozoans and invertebrates. Carbon and nitrogen cycles are linked because most nitrogen in aquatic systems is bound in organic matter and is unavailable until it is mineralised to ammonium ($NH4+$) by the breakdown of organic matter (Duff and Triska 2000).

Microbially-mediated geochemical cycles involve the transfer of electrons between compounds. The rate and direction of geochemical cycling depends on the availability of electron donors and acceptors. Under aerobic conditions, oxygen acts as an electron acceptor, but under anaerobic conditions other compounds are used as donors in a reduction sequence of nitrate, manganese, iron, sulphate and carbon dioxide (Wetzel 2001). Different reactions occur in oxic and anoxic conditions, and the co-occurrence over small spatial scales of coupled processes contributes to the characteristic patchiness of SGDEs.

Microbially-mediated nitrogen cycling can occur as coupled nitrification-denitrification reactions along gradients of oxygenation (Baldwin and Mitchell 2000). Phosphorus dynamics are closely related to the cycling of iron, and therefore require anaerobiosis (Baldwin and Mitchell 2000). Rates of biogeochemical transformations are affected by factors such as temperature, pH or the presence of heavy metals. Nitrification is the bacterial oxidation of ammonium ($NH4$ +). Ammonium is produced by excretion or the decomposition of organic matter. Denitrification is the bacterial reduction of nitrites and nitrates (NOx) either back to ammonium, or to nitrogen gas, which is then lost from the system. (Wetzel 2001)

The spatial availability of electron donors is determined by patterns of water flow, which in turn, are driven by hydrologic connectivity and hydraulic conductivity (Baker et al. 2000a), key factors in our proposed typology. Water is a transport agent (Bakalowicz 1994) that percolates through the vadose zone, or pulses through the hyporheic zone, to deliver dissolved and particulate organic matter and dissolved oxygen to biofilms. Microbial activity is typically highest near the source of recharge and declines along a gradient with distance from it (Kaplan and Newbold 2000). In aquifers connected to surface waters, the hyporheic zone is a crucial interface for fluxes of nutrients (Boulton et al. 1998, Dahm et al. 1998, Fischer et al. 2005).

Flood pulse inundation in a semiarid catchment in New Mexico altered rates of nutrient retention and organic matter processing in floodplain groundwater (Baker et al. 2000b). Local lateral exchange processes such as cycles of bank discharge and recharge can also play an important role in the timing and direction of nutrient processing in floodplains (Lamontagne et al. 2005b). In unconfined alluvial aquifers with fluctuating watertables, a significant portion of organic carbon metabolism can occur in oxic–anoxic cycles in the zone of intermittent saturation (Vinson et al. 2007).

Hydraulic conductivity also determines the availability of electron donors for biogeochemical processes. Interstitial storage of dissolved organic matter and the availability of dissolved oxygen are influenced by particle size and pore size (Maridet et al. 1996). Larger particle size and high porosity allow higher flows and higher availability of oxygen but reduce entrapment and retention of nutrients.

In fractured rock and karstic aquifers, uneven porosity due to the distribution of fissures, fractures and solutional conduits creates preferential flow paths, which create spatial heterogeneity in biogeochemical cycling (Ayraud et al. 2006). Spatial and temporal variability in groundwater flow paths is also influenced by surface microtopography (Pfeiffer et al. 2006) and by stream channel morphology (Dahm et al. 1998). The functional diversity of subsurface ecological processes is thus determined by shifting gradients in oxygen, nutrients and physico-chemical conditions, which create pockets of oxia and anoxia, nitrification and denitrification.

As in other ecosystems, heterogeneity in subsurface environments is a critical determinant of ecosystem function (McCarty et al. 2007). Disturbance to the groundwater regime, including disruption of patterns of hydrological connectivity (Baker et al. 2000b) and sediment wetting/drying cycles (Baldwin and Mitchell 2000), might potentially alter spatial and temporal patterns of groundwater flow, flux and quality, with implications for rates of organic matter mineralisation and nutrient cycling. Pumping from a fractured rock aquifer in north-west France caused physical disturbance to water flux in the aquifer, reduced groundwater residence time and subsequent drastic modification to the water chemistry resulting in less active biogeochemical processes (Ayraud et al. 2006).

Prolonged desiccation of sediments caused by watertable drawdown is likely to alter the balance between aerobic and anaerobic processes and change the composition of microbial populations, reducing the incidence or rate of anaerobic metabolism. Fischer et al. (2005) concluded that carbon and nitrogen cycling in hyporheic sediments were central to the metabolism of a large lowland river in Germany, and designated the hyporheic zone as the 'river's liver'. Disturbance to the groundwater regime can alter the rate and nature of subsurface ecological processes, resulting in reduced availability of carbon, nitrogen and phosphorus, with flow-on effects for biodiversity and ecosystem services, not only within the aquifer, but also in connected ecosystems including rivers, riparian zones and estuaries."

Changes in biological diversity

The quantity and quality of the various kinds of pressures on GW systems are able to induce drastic changes in the diversity of organisms living underground. Two types of such changes can impact an aquifers water quality parameters and its associated ecosystem, namely (1) decline in GW-dwelling organism populations leading to species extinctions and (2) penetration of alien species belonging to surface-water communities. Both processes determine changes in the functioning of GW systems, generally reducing the efficiency of some ecosystem processes.

Microbes are highly abundant in subsurface waters. Although little is yet known about microbial diversity, contamination in almost every case causes a shift in the composition of the microbial community (Bekins *et al.* 1999; Ludvigsen *et al.* 1999; Rooney-Varga *et al.* 1999). Loading of the aquifer by hazardous chemicals also leads to a decrease or increase in abundance (Haack & Bekins 2000). Changes in abundance and diversity of GW organisms are also suggested in the case of overpumping, which also induces structural changes in the water-saturated habitat.

A reduction in the self-purification potential and therefore water quality was observed within the bank filtration area along the Danube at Vienna after the major part of the sediment-associated microbial biomass was removed together with the habitat, namely the fine sediment fraction, by overpumping of the water (Frischhertz 1979). Intensive withdrawal of GW for irrigation purposes leads to local decline in animal communities as in coastal aquifers of Greece or alluvial aquifers in southern France (Danielopol 1981; Dumas 2002). The overexploitation of the GW of the Balcones Fault Zone in the Edwards artesian aquifer (Texas) endangers one of the world's most species-rich subterranean assemblages, including many endemic stygobitic crustacean, fish and amphibian species (Longley 1992). Plans for dewatering of local confined aquifers for ore exploitation in Western Australia could lead to the extinction of a unique subterranean crustacean fauna of great scientific value (Humphreys 1999). Organic loading of the subterranean environment may lead, to extinctions of stygobitic animals (Elliott 2000).

Apparently, subterranean animals are sensitive to toxic chemicals like pesticides (chlorophenols), various salts (potassium chloride, potassium nitrate) and heavy metals (Notenboom *et al*. 1992; Mösslacher 2000). In organically polluted habitats located close to surface water there is also potential invasion of cosmopolitan surface dwelling species, which outcompete or temporarily replace the stygobites. Such is the case with a karstic stygobitic fauna in southern France near Montpellier, where the arrival of organic liquid waste caused the colonization of the subsurface system by ubiquitous surface-dwelling species like the tubificid worm *Tubifex tubifex* or the crustacean copepod *Acanthocyclops robustus* (Malard 2001). At the unimpacted sites, many stygobitic species continued to exist.

Groundwater habitats along large rivers, like the Rhône or the Danube, which are polluted not only by organic matter but also by toxic heavy metals, display low biological diversity and are represented mainly by surface-dwelling taxa. The interstitial fauna of the riverbanks in the city of Lyon represent such a case (Gibert *et al.* 1995). River regulation combined with the negative effect of organic pollution alter GW habitats; for example, through stronger siltation and oxygen depletion of the interstitial voids, the free- moving crustaceans (such as stygobitic copepods and isopods) are replaced by assemblages dominated by epigean animals such as nematodes and oligochaetes (Danielopol 1976, 1983). Arid climates like those prevailing in Northern Africa or in Arizona (USA), determine the drying of streams and the interruption of water infiltration into adjacent-shallow subsurface areas. The fauna of hyporheic habitats in such cases is represented by a few epigean species that can survive the dry period until the next rewetting (Boulton & Stanley 1995).

Survey data from studies from within eastern Australia provide useful insight into the characteristics of Australian and more, specifically, eastern Australian groundwater ecosystems. The first study I will highlight was conducted by Jiwan & Serov (2002) on the coastal sands aquifers of the Lime Burners creek area north of Port Macquarie. This is a fine grained, unconfined aquifer that has direct links with Maria River estuary. This study revealed a stygofauna community dominated by Oligochaetes. The water chemistry is characterised by low to very low pH ranging from 6.39 down to 3.01 with most values averaging between 3-4 ph units. Salinity levels were generally very high ranging from 43958 µS to as low as 101 µS and averaging between 5000-14000 µS. The high values were associated with bores in close proximity to the estuary. Dissolve oxygen in the groundwater associated oligochaete and mites with ranged from 3.42 to 0.34 mg/l with an average range from 1.0-2.0 mg/l. Serov et al, 2009 highlighted the use of stygofauna community composition as an indicator of water quality/water chemistry change within the Maules Creek Catchment within the Naomi Valley.

Extensive groundwater abstractions have led to decreasing groundwater levels in many aquifers around the world, particularly in semi-arid catchments. This abstraction can have severe impacts on flow in streams that are hydraulically connected to the aquifers being pumped. These impacts range from a reduction in base flow to a change from a gaining to a loosing stream or to a complete cessation of flow. The consequences in terms of stream flow are obvious, particular in regards to the ecology of the instream surface water ecosystems. In recent years it has been realised that these changes may also cause changes in groundwater chemistry. E.g. dissolved and particulate organic matter in the stream water may percolate into the streambed and the aquifer and may lead to a consumption of oxygen and reducing conditions. However, the consequences on the groundwater ecosystem in the vicinity of the stream are largely unknown.

All aerobic organisms require a specific range of conditions in order to survive and function including a physical living space, an energy source or food, and oxygen. If these specific parameters for life are changed then a change to the community structure of an ecosystem is to be anticipated. Surface aquatic invertebrate communities for example have long been recognised as being ideally suited for the assessment of environmental health and condition in riverine ecosystems as they are 1) diverse, 2) occupy every available niche within a water body, 3) are one of the major contributors to the processing of energy through an ecosystem and 4) responds directly to physico-chemical changes within the aquatic environment. 5) The composition of these communities reliably reflects both natural and threatening processes operating within a catchment. 6) The specific range of habitat requirements of each species dictate the distribution of each component of both the species and community levels, which 7) enables their diversity to be used as an indicator of a water body's connectivity and condition within a catchment.

The subsurface stygofauna communities possess all of the above features and more. It has long been acknowledged that they are intrinsically adapted to their specialised environment both in terms of their specialised morphology, physiologies, habitat requirements and long life cycles. Therefore the link between flow conditions, geochemical conditions and the abundance, diversity and composition of the stygofauna community should be anticipated and utilised. There have, however, been few studies that have tried to determine the environmental requirements of these communities and fewer studies that have used them as indicators of hydrological groundwater-surface water connectivity or their responses to environmental change. Riverine aquifers have been referred to as Macro Ecosystems due to the aquatic linkages within between the phreatic, hyporheic and epigean environments.

These linkages were investigated along a 1 km reach of Maules Creek, in semi-arid western New South Wales, Australia. Maules Creek is a small, essentially, ephemeral, tributary catchment of the Namoi River. The investigation area includes two tributaries, Maules Creek and Horse Arm Creek that join upstream of Elfin Crossing (a road crossing) and extend below the crossing. This reach was chosen because of the presence of a small number of pools that were reported permanent (P. Laird, pers comm.), in an otherwise dry creek system. A preliminary physicochemical investigation suggested the pools were the result of upwelling groundwater in the up-stream section. The groundwater connection was also strongly indicated by the presence of obligate groundwater species being found in a preliminary survey of the shallow hyporheic zone.

Vertical streambed profiles of hydrochemistry and stygofauna were collected at five different locations along the creek. Hydrochemistry were sampled from the streambed and down to 1.7 m at 10 to 30 cm intervals using a 10 mm diameter steel probe with a 50 mm perforated screen at the end. Bore water samples were pumped to the surface using a 60 ml syringe and O_2, pH, EC and Eh were measured in an inline flow cell. Fe^{2+} and alkalinity was analysed in the field, whereas samples for major and minor cations, anions and DOC were preserved and analysed at a later date. Streambed (hyporheic) fauna was sampled next to the chemical profiles at 20 cm intervals using a 20 mm diameter open ended steel pipe with perforations in the lower 10 cm. 30 L of pore water, fine sediments and organisms were pumped to the surface at each interval using a hand bilge pump and sieved through a 150 µm sieve. The retained fraction was washed poured into sample vials and preserved with 100% ethanol for later species identification.

The results of the stygofauna sampling demonstrated a heterogenous and complex ecosystem with relatively consistent downstream gradients, in terms of overall numbers of animals, number of species, composition of the community and the size of the individual specimens from the upper sites to the lower sites. The total number of specimens and number of species and size of specimens were high at the three upstream sites whereas specimen numbers and species diversity and size decreased along the river towards the lower sites. The upstream sites were almost exclusively dominated by a rich, abundant, endemic stygofaunal or obligate groundwater faunal community throughout the substrate sampling column. This suggests that this is a very stable, long term environment, both hydrologically and chemically, with an active groundwater discharge.

The stygofauna community consisted predominantly of crustaceans and a small number of other groups including Oligochaetes and Flat worms. Within the crustaceans, the dominant taxa consisted of stygobitic Copepoda, Syncarida and Amphipoda and Ostracoda. All obligate stygofauna are blind and colourless. The middle sites in both streams recorded a mixture of the above stygofauna with a number of surface aquatic macroinvertebrates indicating a mixing of groundwater/surface waters to depths of at least 100cm although most species were confined to the upper 40cm. The lower site below Elfin crossing were essentially devoid of life to the depth of the sampling except for an occasional terrestrial soil invertebrate or oligochaete suggesting that this is an unstable, highly fluctuating environment.

These changes are directly reflected in the changes in water chemistry results. The major ion distributions showed constant levels from the stream surface water and down into the streambed at all five sites revealing a hydraulic contact between the stream and the streambed sediments. Dissolved redox sensitive chemical species (O_2, NO_3^-, Fe^{2+} and Mn^{2+}) revealed more complex patterns. Generally the streambed pore waters became more reduced in a downstream direction. The three upstream sites were generally oxic to slightly anoxic containing either O_2 or NO_3^- throughout the profiles. The two downstream sites showed a more pronounced redox sequence over depth with O_2 in the upper 20-40 cm replaced by NO_3^- and finally by Mn^{2+} and Fe^{2+} deeper in the profile. The DOC was surprisingly uniform between all five sites and over depth (~ 1.0 mg C/L) and DOC is clearly not in itself a good indicator of stygofauna abundance or diversity.

Surprisingly, there was a weak inverse correlation between specimen numbers (or species diversity) and dissolved O_2 concentrations. Frequently high specimen numbers (and species diversity) were found at sites with low dissolved O_2 (< 0.14 mg/L), indicating that a number of these stygofauna species can thrive or function at low dissolved O_2 levels or even at sub-oxic conditions. One of the specialised physiological features of stygofauna, particularly the crustaceans, is an adaptation to living in low dissolved O_2 environments that would normally preclude surface water invertebrates. Consequently dissolved O_2 seems to be a poor quality indicator for stygofauna abundance and biodiversity.

A much clearer inverse correlation was found between specimen numbers and diversity and pore water concentrations of Fe^{2+} and Mn^{2+}. Specimens were rarely found when Fe^{2+} and Mn^{2+} concentrations surpassed 0.1 and 0.5 mg/L, respectively. This suggests that higher invertebrate ecosystem functioning seems to break down only when the system becomes truly anoxic i.e. when iron- and manganese-oxides are being reduced. In turn the distribution of Fe^{2+} and Mn^{2+} seems to correlate to the overall flow patterns of the system with upwelling of relative oxic groundwater in the upper reaches with relatively fast residence times in the streambed sediments preventing the development of reducing conditions ie. a consistent groundwater flow through a porous medium – groundwater discharge zone.

In contrast, the lower reaches experiences infiltration of surface water into the streambed presumably containing a higher load of reactive organic matter and fine sediments that clogs the pore spaces of the sediments thus slowing the flow rate that in turn, triggers a sequence of redox reactions. Thus the data tends to suggest that changes in catchment water management such as reducing baseflow through abstraction in a hydraulically connected system can hugely impact streambed and aquifer ecosystems by inducing changes in water chemistry.

This study highlights the direct correlation between water management, water chemistry and ecosystem function and highlights some of the relationships between groundwater and surface water systems in hydraulically connected environments. The study also indicates that stygofauna can be used as biological tracers of groundwater discharges. The results of this study have major implications for the management of both surface ecosystems and groundwater ecosystems.

Management

The surveys identified the presence of a significant subterranean fauna within this aquifer, notably within Bores GW969324 and GW003587. These bores and the fauna within it represent a biodiversity hotspot and indicates moderate to good water quality, connectivity with the adjacent river system and persistent longevity of the community (Danielopol, *et al*, 2003, Serov, *et al*, 2011.). The presence of these species also strongly suggests there may have been a water chemistry change that could have adversely impacted the stygofauna community significantly reducing by the eradication of the normally dominant crustacean groups. The community within this aquifer may therefore have had or has in other parts of the aquifer a higher biodiversity covering a larger area within suitable habitats than have not as yet been impacted.

Key points from the Biological Investigation so far:

All three bores appear to be accessing the same water source/aquifer based on the presence of the same stygofauna species within the “New House Bore” and “Far Bore”. It is not known why at this point stygofauna were not collected within the “old Bore”, except that it is likely to be a connectivity issue in terms of substrate porosity. As the sampling has been completed twice and there is still no fauna collected it is reasonable to conclude that this section of the aquifer has an impervious boundary that precluded the movement of stygofauna. It is also quite a common occurrence for very close bores that appear to be drawing from the same aquifer to have completely different survey results due to a number of causes such as the complexity of the subterranean environments, including slightly different water chemistry or a lack of appropriate pore space (fine sediment lenses) to allow the invertebrate to pass through the matrix.

- Stygofauna are present within the water bearing zone. Stygofauna indicate moderate to high water quality across the aquifer i.e. overall good aquifer health.
- The large accumulation of ants and smaller numbers of frog bones are all well decomposed, which could have occurred at the same time but is not possible to determine. However, given the fact that no new, (non-decomposed) ant bodies were collected in the second survey it can be suggested that the large number of ants within the new bore is the result of an asphyxia or fumigation event.
- As there was no active monitoring of water levels or water chemistry occurring at the time of either of the episodes of water quality decline any evidence collected may only be circumstantial. It is therefore advised that an ongoing monitoring program be established using insitu water level, water quality and air quality (in bore) probes be installed.

Therefore the threat posed to stygofauna communities by proposed mining activities within the Pilliga forest area is considered to be high.

Mining developments, in which stygofauna are considered to be a relevant environmental factor, need to be closely assessed with respect to the extent of the proposed groundwater drawdown zone and the likely impacts on groundwater quality. Both of these activities, over time, may cause prospective stygofauna habitat to be degraded or lost with the potential for significant impact on groundwater communities. Stygofauna are able to tolerate natural fluctuations in water parameters such as water level, electrical conductivity, and temperature, and this has been demonstrated experimentally (Tomlinson *unpublished*) for stygofaunal amphipods, copepods, and syncarids. However, changes outside the natural range of water quality, water chemistry and levels such as rapid drawdown or changes to water chemistry such as a pollution plumes are likely to have significant impacts on the community composition, biodiversity and overall sustainability of the community.

Groundwater communities also require links to the surface environment to provide organic matter and oxygen. If that linkage is broken or disrupted, the stygofauna community in the area affected could decline over time. A high degree of endemism can occur in aquifers, even within the same system or between adjacent systems (Hancock and Boulton 2008). However, providing there is sufficient hydrological connectivity within and along the flow path of the aquifer, and the physico-chemical conditions are suitable and remain stable, the distribution of species will not be restricted to small parts of an aquifer.

Cumulative Impacts

Cumulative effects may result from a number of activities interacting with the environment. The nature and scale of these effects can vary significantly, depending on factors such as the type of activity performed, the proximity of activities to each other and the characteristics of the surrounding natural, social and economic environments (Brereton and Moran, 2008). They may also be caused by the synergistic and antagonistic effects of different individual activities, as well as the temporal or spatial characteristics of the activities. Importantly, cumulative effects are not necessarily just additive (SKM, 2010). The proposed CSG activities in a region are growing thus increasing the potential for an impact on the underlying aquifers. The implication of multiple mining actives in one region is that impacts may overlap and result in larger impacts than would be expected for a single mining operation (cumulative effects).

Suggested Actions

The recommendation of this report is therefore to monitor the aquifers for the spatial and temporal changes in parameters of the stygofauna, water quality and water quantity during the construction, operational and post-mining phases of both projects, both within and outside the potential zone of impact from the current and proposed CSG and other mining activities.

The suggested next stage is:

1. An examination of water quality and subterranean ecosystem health from as many adjacent bores as possible. Even if no contamination is found it will provide a benchmarked network of bores for future comparisons.
2. The establishment of a monitoring program on both the Rockdale property and surrounding properties in order to ensure appropriate measurements are recorded if this event occurs again. It is much easier to determine cause and effect if there is reliable time series data before and after an impact.

The sites that have been surveyed and analyzed for water chemistry should be regarded as the first benchmarked sites for this aquifer in the area. Benchmarking is necessary and essential in order to characterize the natural distribution and environmental ranges within the aquifer and therefore the requirements of this subterranean ecosystem and the overall health of the aquifer for human consumption.

The aquifer should be characterised by:

- The structure/lithology of the aquifer by obtaining bore log/works details of the bores;
- The full water chemistry and water levels of the groundwater (including temperature) over time to establish the natural annual ranges and seasonal fluctuations;
- The aquifer flow paths to determine the connectivity (gaining or loosing) with the associated river above and below the potential area of impact.
- Identify the obligate stygofauna to species (those listed as phreatobites) to determine levels of endemicity of the stygofauna community within the aquifer as this community is the most disturbance sensitive environmental indicators for changes in aquifer conditions;
- Conduct further surveys in other bores and hyporheic zone of the associated river, if available, within this aquifer and adjacent aquifers to determine the range of the species.
- Identify other groundwater dependent ecosystems in the area such as springs and groundwater discharge zones within nearby steams.

Conduct water level mapping across the site/aquifer to determine the linkages with the river systems and other users of the groundwater source such as the local community through the need for stock and domestic water and other potential GDE's such as terrestrial vegetation or wetland communities that also rely on consistent water levels and water quality.

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