BM Alliance Coal Operations Pty Ltd (BMA)
Blackwater Mine

Stygofauna Pilot Survey

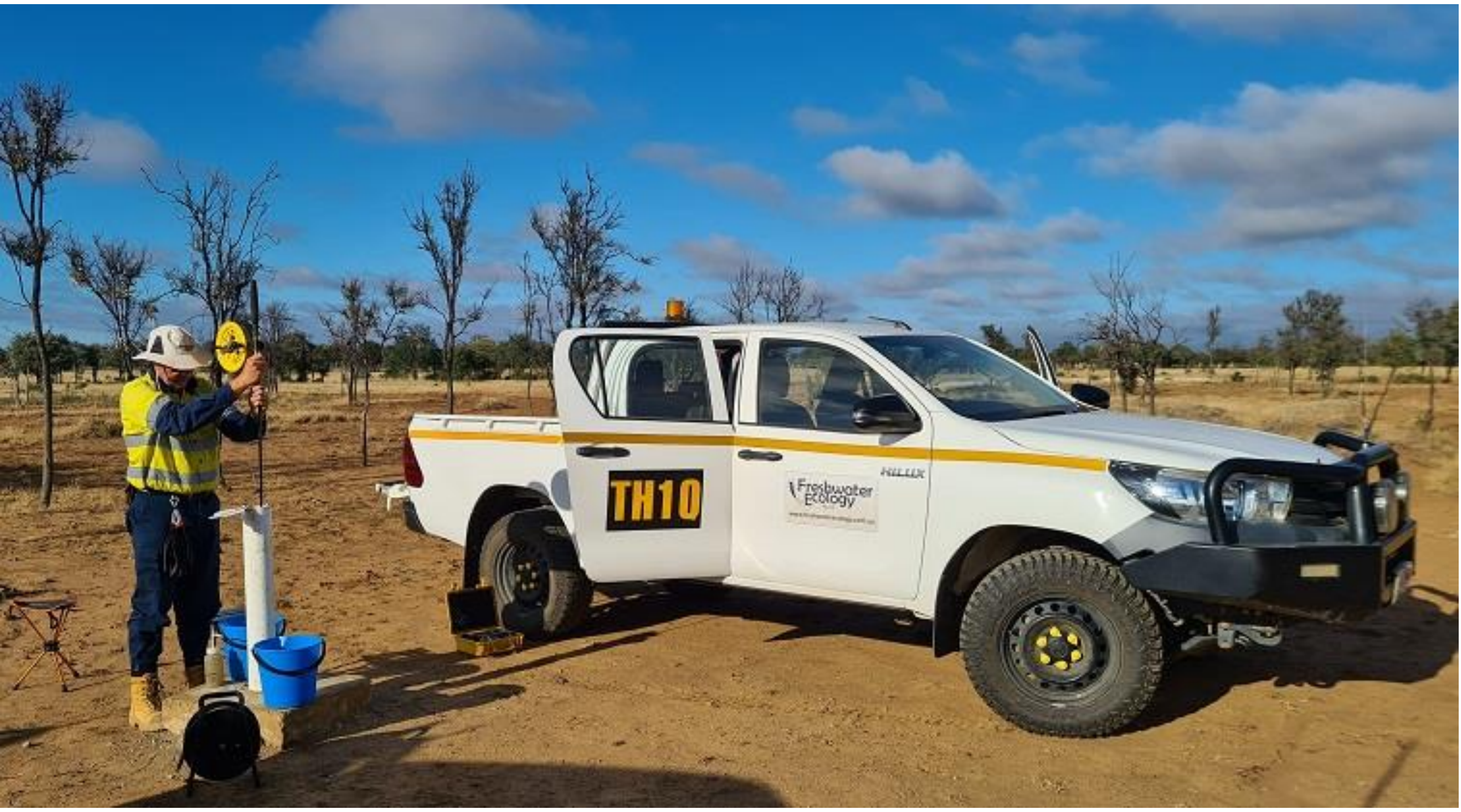

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Table of Contents

Tables

Figures

1. Introduction

The Blackwater Mine is located approximately 25 kilometres (km) south of Blackwater in Central Queensland. Blackwater Mine is owned and operated by BM Alliance Coal Operations Pty Ltd (BMA).

BMA are proposing to extend mining operations on mining lease (ML) 1759 (Surface Area 10) and on ML 1762 (Surface Area 7), located adjacent (to the northeast) to the existing Blackwater Mine.

Freshwater Ecology Pty Ltd (Freshwater Ecology) were engaged to undertake a stygofauna pilot survey for the Blackwater Mine northern extension area 2020. Two field sampling events have been conducted as part of the pilot survey in November 2020 and May 2021.

This report constitutes the final report for the BMA stygofauna pilot survey and integrates data across both sampling events.

2. General Terminology

In Australia, Groundwater Dependent Ecosystems (GDE's) are defined as 'ecosystems which require access to groundwater on a permanent or intermittent basis to meet all or some of their water requirements so as to maintain their communities of plants and animals, ecological processes and ecosystem services' (Richardson *et al.* 2011). Not all GDE's draw on groundwater directly and not all GDE's are solely reliant on groundwater.

Six types of Groundwater Dependent Ecosystems have been identified in Australia:

- Terrestrial vegetation that relies on the availability of shallow groundwater.
- Wetlands such as paperbark swamp forests and mound springs.
- River baseflow systems where groundwater discharge provides a significant baseflow component to the river.
- Aquifer and cave ecosystems where life exists independent of sunlight (this GDE contains stygofauna and is the focus of the current survey).
- Terrestrial fauna species, both native and introduced, that rely on groundwater as a source of drinking water.
- Estuarine and near-shore marine systems, such as coastal mangroves, salt marshes and sea-grass beds, which rely on the submarine discharge of groundwater.

Until recently, aquifers were considered to be devoid of life, however, recent research in Australia and overseas has highlighted the fact that groundwater systems provide a critical habitat for a diverse range of aquatic fauna called stygofauna (Hose *et al.* 2015; Glanville *et al.* 2016). The term stygofauna encompasses;

- Stygobionts (stygobites) which are defined as being organisms that are obligate groundwater inhabitants for some or all of their life (Sket 2008),
- Stygophiles which are defined as surface-dwelling species that complete some or all of their life cycle in groundwater (Sket 2008), and
- Stygoxenes which are defined as animals found accidentally in groundwater (Sket 2008).

Typically, it is the stygobionts and stygophiles that are referred to collectively as stygofauna (Hose *et al.* 2015) and these definitions will be adopted for this BMA survey.

Section 3 of this report provides a background summary of stygofauna, their ecological requirements, their taxonomic diversity and potential impacts from mining on groundwater ecosystems and stygofaunal communities.

3. What are Stygofauna?

Stygofauna are aquatic subterranean animals that are totally groundwater dependent (stygobites) and found throughout Australian aquifers. Groundwater ecology surveys and studies over the past 20 years in Australia have identified a diverse range of organisms inhabiting groundwater systems, however, whilst the groundwater ecosystem is diverse and unique, this ecosystem is probably the least studied globally. Tomlinson *et al.* (2008) noted that stygofauna are valued as a biodiversity resource, as indicators of groundwater ecosystem health and potential providers of ecosystem services including, nutrient cycling and storage, organic matter cycling and redistribution, water treatment, maintenance of groundwater flow and mineral weathering and formation.

Stygofauna are morphologically and physiologically different from even closely related surface-dwelling species having independently evolved common morphological traits such as lacking eyes, having hardened body parts, lacking body pigments and having worm-like body shapes and enhanced sensory appendages as an adaption to the groundwater environment (Humphreys 2006). Stygofauna in Australia include both microfauna such as Turbellaria, Rotifera, Nematode and Protozoa (Humphreys 2006) as well as larger meiofauna that are generally dominated by crustaceans but may include insects, nematodes, molluscs, oligochaetes and mites. The crustaceans include Copepoda, Syncarida, Amphipoda, Isopoda and Ostracoda (all of which have surface dwelling relatives) as well as groups only found in groundwater such as Remipedia, Thermosbanacea and Speleaogriphacea. Insects are relatively uncommon in groundwater (Humphreys 2006) although diverse coleopteran assemblages have been recorded in some parts of Australia (Watts *et al.* 2007). The diversity of stygofauna in Australia is comparable to that of other regions of the world.

Stygofauna are adapted to groundwater environments and conditions of constant temperature, no sunlight, low nutrients and oxygen content, stable water quality and sediments that provide a limited and narrow pore space (Hose *et al.* 2015). Stygofauna have low metabolic rates and low reproductive rates relative to surface species which enables them to survive in the low energy, low oxygen groundwater environment. Groundwater ecosystems typically have few stygobiont species at any one locality and consequently low diversity. However, the isolation of aquifers and limited dispersal abilities of groundwater organisms has created a fauna dominated by short-range endemic species (Harvey 2002). As stygofauna are adapted to a stable physical and chemical subterranean environment and as species often exhibit narrow geographic ranges, even slight alterations to the groundwater environment (i.e. flow, flux, pressure, level, quality and the transport of nutrients and organic matter) can result in significant changes to the composition and distribution of stygofauna communities and even the potential loss of species. The major pressures on groundwater systems in Queensland, as elsewhere, are from anthropogenic activities (i.e. agriculture, industry and domestic water supply) that modify aspects of the groundwater environment and impact on groundwater quantity and quality. The pressures on groundwater ecosystems are also cumulative (Danielopol *et al.* 2003).

3.1 Ecological Requirements of Stygofauna

Twenty years ago it was believed that stygofauna only existed within a very narrow physico-chemical parameter range. More recent surveys and studies have shown that this is not the case and that stygofauna may be found across a more diverse physico-chemical range of groundwater systems than was previously commonly assumed. Only recently has the true biological diversity of aquifers begun to emerge, both in Australia and globally.

In 2016, Glanville *et al.*, reviewed a state-wide database which included 755 stygofauna samples from 582 sites in Queensland and the current knowledge on stygofauna biodiversity and biogeography. This study correlated stygofauna discovery against environmental data and reported the following important outcomes:

- Groundwater with a wide range of physico-chemical properties have been recorded as supporting groundwater ecosystems in Queensland.
- Stygofauna have been recorded living in groundwater ranging in depth from 0.1 to 63.2 metres below ground level; electrical conductivity ranging from 11.5 to 54,800 µS/cm; groundwater temperatures ranging from 17.0 to 30.7^{o}C, and groundwater pH ranging from 3.5 to 10.3.
- Stygofauna taxon richness shows a general negative trend with increasing depth to groundwater or electrical conductivity (salinity).
- Taxon richness is highest in neutral to slightly alkaline pH groundwater systems and in water temperatures between 18 and 27^{o}C.
- Taxon richness was shown to decrease sharply with increasing groundwater acidity and alkalinity.

It was acknowledged that the stygofauna preferences identified from the Queensland database may partially reflect the limited sampling effort that has occurred across physico-chemically diverse groundwater systems and that the data was predominantly from sites sampled only once.

Hose *et al.* (2015) also noted a number of key factors determining the presence/absence of stygofauna in aquifers:

- Stygofauna are predominantly found in aquifers with large (mm or greater) pore spaces which are more common in alluvial, karstic and some fractured rock aquifers. The pore spaces within an aquifer matrix are a critical determinant of whether an aquifer can support large-bodied organisms as stygofauna move within an aquifer by either crawling or swimming. The size of the interstitial spaces also influences the hydraulic conductivity and flow of water which ultimately controls the delivery of carbon and oxygen throughout the ecosystem. Hahn & Fuchs (2009) identified that stygofauna were rare or absent in areas with hydraulic conductivity (K_f) less than 10^{-4}cm/s.

- Stygofauna diversity and abundance typically decreases with depth below ground. Stygofauna are rarely found more than 100 m below ground level and are most abundant less than 20 m below ground (Hancock & Boulton 2008).
- Stygofauna are found across a range of water quality conditions (from fresh to saline), but are most common in fresh and brackish water (i.e. where EC is less than 5,000 µS/cm). 4T (2012) in their review of stygofauna data from Australia reported that stygofauna have been found in hypersaline groundwater (86,900 µS/cm), but are most common at salinities less than 10,000 µS/cm.
- Stygofauna are rarely found in hypoxic groundwater where dissolved oxygen concentrations are less than 0.3 mg/L. 4T (2012) reported that stygofauna have been recorded in groundwater with dissolved oxygen concentrations ranging from 0.2 to 15.3 mg/L.
- Stygofauna are more abundant in areas of surface water-groundwater exchange when compared to deeper areas or those further along the groundwater flow path remote from areas of exchange or recharge with poor hydraulic conductivity. Schmidt *et al.* (2007) noted that hydrological exchange between aquifer and surface water can be more important than other hydrogeological conditions in shaping stygofauna assemblages.

Stygofauna were recorded inhabiting a wide range of lithologies, including unconsolidated sedimentary material (e.g. alluvium, sand); consolidated sedimentary rocks (e.g. sandstone) and fractured rocks (e.g. basalt, granite, volcanics). Whilst sampling data are scarce or absent for many lithologies, the results from Glanville *et al.* (2016) suggest that groundwater systems cannot be eliminated as potential habitat for stygofauna based solely on geology or lithology. Stygofauna were also shown to exist across a diverse physico-chemical range of groundwater systems, and as a result, general assumptions of habitat suitability should not be used to guide sampling activities.

Stygofauna are adapted to a low nutrient (particularly carbon) and oxygen environment. For aquifers to sustain stygofauna there must be a continuous vertical flow of dissolved organic carbon (DOC) from the surface to the aquifer. It is this carbon plus dissolved nutrients that are the basis of the simple food web that sustains bacteria and fungi which stygofauna can feed on (Humphreys 2006). It is largely for this reason that stygofauna diversity and abundance decreases with depth and distance along groundwater flow paths as nutrient supplies decline.

Stygofauna are rarely found more than 100 m below ground level, nor where dissolved oxygen concentrations in the groundwater are less than 0.3 mg O_2/L (Hose *et al.* 2015).

When groundwater in an aquifer that sustains stygofauna is drawndown, the stygofauna become stranded within the pore spaces. Generally, stygofauna can survive in unsaturated sediments for periods of around 48 hours, and survival decreases with decreasing sediment saturation. We do know from limited studies (Tomlinson 2008; Stump & Hose 2013) that some stygofauna can move vertically within the pore spaces and follow the decline in groundwater levels, however, this is only possible if drawdown is slow (perhaps <1.0 m/day), allowing time for the stygofauna to migrate. Rapid drawdown is particularly detrimental for stygofauna and does not allow time for vertical movement to keep pace with the loss of groundwater. Stumpp & Hose (2013) also demonstrated that stygofauna with legs that were able to crawl (e.g. amphipods) were more successful at moving within pore spaces and following the declining groundwater level than some microcrustacea (e.g. copepods) which move within aquifers by swimming.

3.2 Stygofauna Diversity

Hose *et al.* (2015) reports that in 2000 there were over 7,800 known stygofaunal species globally, however, large research efforts in Australia and Europe have shown that this number is an underestimation. Guzik *et al.* (2010) reported some 770 stygofauna taxa were known from Western Australia alone, however, this value was estimated to be only 20% of the true number of stygobiont taxa. True richness for the region may be in excess of 4,000 stygobitic species. Based on these values, and that the diversity of stygofauna in the eastern states is largely unexplored, it is likely Australia is globally significant in terms of stygofauna diversity (Hose *et al.* 2015).

Many of Queensland’s stygofauna communities are unstudied or understudied, hampering both global and local comparisons. Queensland is known to host at least 24 described families and 23 described genera of stygofauna across 9 of the 17 major stygofaunal taxonomic groups. Undescribed families have also been recorded across a further three major stygofauna taxonomic groups (Glanville *et al.* 2016). The composition of stygofauna in Queensland is broadly consistent with the world average with the notable exception of high richness of oligochaetes and syncarids and low numbers of molluscs. Despite indications that a significant diversity of stygofauna is likely to exist across Queensland groundwater systems, stygofauna biodiversity largely remains undocumented due to limited sampling effort, limited taxonomic resolution and the tendency for stygofauna to exhibit morphological similarities (Glanville *et al.* 2016).

3.3 Knowledge Gaps Regarding Stygofauna

In 2015, Hose *et al.* published a report commissioned by ACARP entitled “Stygofauna in Australian Groundwater Systems: Extent of Knowledge”. This report identified a number of emerging issues where knowledge is lacking with regards to risks to aquifer ecosystems from extractive industry operations such as coal and CSG mining. In particular, Hose *et al.*

(2015) identified a very limited ability to understand and subsequently predict impacts of dewatering/depressurisation of aquifers on stygofauna communities. Additional knowledge-deficient areas were identified as:

- The role of coal seams as stygofauna habitat;
- Water quality tolerance of stygofauna – toxicants and physico-chemical stressors;
- Groundwater foodwebs as a pathway to impact stygofauna;
- Taxonomy and distribution of stygofauna species, and
- Links between hydrological modelling and impacts on stygofauna

4. BMA Sampling Program for Stygofauna

A total of 10 groundwater bores were selected by BMA for stygofauna sampling. All bores developed for the BMA northern extension area were sampled (BMA *pers com*). The location and characteristics of each bore and hydrostratigraphy are presented in Tables 1 and 2 below. Sampling was conducted for this project from 7th to 10th December 2020 and from 10th to 12th May 2021. *Freshwater Ecology* conducted the field assessment with field support provided by ALS.

Table 1: Location of Groundwater Bores Selected for Stygofauna Sampling.

Bore Code	Easting (GDA94:Z55)	Northing (GDA94:Z55)	Formation	Date Drilled	Dates Sampled
MB19BWM02A	690127	7390182	Siltstone (Weathered Rewan)	15/11/19	08/12/20 11/05/21
MB19BWM01P	690037	7390281	Aries Coal Seam	12/11/19	08/12/20 10/05/21
MB19BWM07A	689279	7376877	Alluvium	29/11/19	08/12/20 11/05/21
MB19BWM25P	689259	7376879	Sandstone (Weathered Rewan)	29/11/19	08/12/20 11/05/21
MB19BWM27P	688958	7376559	Aries Coal Seam	18/12/19	08/12/20 11/05/21
MB19BWM03P	688454	7383473	Aries Coal Seam	7/11/19	08/12/20 11/05/21
MB19BWM04R	688315	7383604	Sandstone (Rewan)	11/11/19	08/12/20 11/05/21
MB19BWM05A	688501	7383611	Claystone (Weathered Rewan)	7/11/19	08/12/20 11/05/21
MB19BWM06P	697680	7379450	Aries Coal Seam	5/11/19	08/12/20 11/05/21
MB19BWM08P	691542	7370739	Aries Coal Seam	16/10/19	09/12/20 11/05/21

Table 2: Bore Hole Characteristics (mBGL - metres below ground level; mBTOC - metres below top of casing; EoH – end of hole; SWL – standing water level).

Bore Code	**Depth to EoH (mBGL)**	**SWL (mBTOC) *Dec. 2020***	**SWL (mBTOC) *May 2021***	**Bore Diameter (mm)**	**Slotted Depth (m)**
MB19BWM02A	17	7.87	7.89	50	12-15
MB19BWM01P	192	12.98	13.34	50	168-171
MB19BWM07A	7	Bore Dry	Bore Dry	50	4-7
MB19BWM25P	20	11.52	11.79	50	17-20
MB19BWM27P	198	4.18	4.44	50	180-189
MB19BWM03P	234	31.37	28.71	50	222-232
MB19BWM04R	80	36.02	35.87	50	71-80
MB19BWM05A	15	Bore Dry	Bore Dry	50	9-15
MB19BWM06P	192	11.57	11.57	50	180-186
MB19BWM08P	198	13.21	13.37	50	184-190

5. Project Methodology

5.1 Sampling Team

Field sampling at BMA Blackwater was conducted by Mr Garry Bennison and Dr Tim Howell from *Freshwater Ecology*. Both staff are professional aquatic ecologists and experienced in stygofauna sample collection and analysis. Garry Bennison has in excess of 40 years' experience as an aquatic ecologist and Tim Howell has in excess of 20 years' experience as an aquatic ecologist. Garry has over 15 years' specific experience working on groundwater ecology projects throughout Australia. *Freshwater Ecology* was supported in the field by Denver O'Grady from ALS Rockhampton.

5.2 Stygofauna Sampling

Sampling was conducted by *Freshwater Ecology* during the pre-wet season in December 2020 and the post-wet season in May 2021. A total of 10 groundwater bores were sampled for stygofauna in accordance with the methods defined in Queensland Environment Protection (Water) Policy 2009 – Monitoring and Sampling Manual: 'Sampling Bores for Stygofauna' (QEPA 2018) and 'Background information on Sampling Bores for Stygofauna' (QEPA 2018) and following established sampling techniques used elsewhere in Australia and overseas (Hancock & Boulton 2008, Dumas & Fontanini 2001, WA EPA Guidance Statements 54 and 54a 2003 & 2007).

A 40mm diameter phreatobiological net was used for stygofauna sampling in all groundwater bores that were 50mm in diameter (net design and construction conformed with WA EPA Guideline [2003 & 2007] specifications). Nets were made of 50 µm nybolt mesh material and weighted at the bottom with a brass fixture and an attached plastic collecting jar. The net was lowered to the bottom of the bore, bounced three to five times to dislodge any resting animals, and slowly retrieved. At the top of each haul, the collecting jar was rinsed into a 50 µm mesh brass sieve and the net lowered again.

Once six hauls were completed (the aim was always to collect between 3 and 6 hauls with all hauls reaching the bottom of the bore), the entire sieve contents were transferred to a labelled sample jar and preserved in methylated spirits as DNA testing of aquatic specimens was not required. A small amount of Rose Bengal, which stains animal tissue pink, was added to each sample to aid in sample processing.

All field equipment was of high quality and fit for purpose, well maintained and operated in accordance with the manufacturer's specifications. It is noteworthy that stygofauna sampling was conducted three weeks following pumping of the groundwater bores by ALS for routine monthly water quality monitoring for both the December 2020 and May 2021 sampling events.

All field data was recorded on-site using specialised field sampling sheets and photos were taken of each bore sampled, including surrounding land use.

5.3 Laboratory Processing of Field Samples

Field samples were logged into a Laboratory Information Management System to record and track sample processing details. Stygofauna sample containers were drained of methylated spirits and stain and washed gently into channelled Sedgwick-Rafter counting trays to create a thin layer of sediment spread across the bottom of the tray. Samples were then sorted under a stereomicroscope with 10x objective lenses and a zoom capability of between 6.3x and 60x. All aquatic animals present were removed (stygofauna and non-stygofauna) and identified to Order/Family level (or lower taxonomic rank if visually possible) in accordance with standard DES ToR and placed in labelled, polyethylene containers filled with 100% Analytical Reagent Grade ethanol for long-term storage.

5.4 Groundwater Quality Sampling

Water samples were collected from each bore using a bailer lowered by hand to approximately 3 m below the water surface (SWL) prior to stygofauna sampling. Water was measured for temperature (°C), pH (units), electrical conductivity (µS/cm), dissolved oxygen (mg/L) and turbidity (NTU) using a multi-parameter water quality meter to provide a general estimate of standing groundwater quality. The field meter was calibrated in the laboratory prior to its use in the field, with calibrations regularly cross-checked in the field.

Depth to groundwater (SWL) was measured from the top of each bore casing using an electronic dip probe provided by ALS.

Groundwater sampling preceded biological sampling to ensure the groundwater contained within the bore was undisturbed.

6. Results

In-situ groundwater quality monitoring results are presented in Table 3. The groundwater bores ranged in depth from 7 m to 234 m bgl and included a range of standard water quality profiles, although all bores recorded quite high salinities with the exception of MB19BWM25P and MB19BWM08P. High pH values were recorded at bore MB19BWM01P (Dec'20 and May'21) and an unusually high turbidity value was recorded for bore MB19BWM04R in May 2021 when compared to the same bore in December 2020.

Table 3: Groundwater Quality

Bore Code	Temperature (°C)		Dissolved Oxygen (% satn)		Turbidity (NTU)		pH (units)		EC (µS/cm)		Volume Sampled (L)
	Dec'20	May'21	Dec'20	May'21	Dec'20	May'21	Dec'20	May'21	Dec'20	May'21	
MB19BWM02A	26.6	27.98	12.1	22.0	22.0	7.24	6.33	6.29	37,251	36,962	2
MB19BWM01P	26.6	26.17	24.1	52.0	20.4	18.1	11.52	11.70	21,201	20,870	2
MB19BWM07A	-	-	-	-	-	-	-	-	-	-	Bore Dry
MB19BWM25P	27.4	28.7	18.6	22.4	3.1	8.01	7.38	7.17	4,360	5,015	2
MB19BWM27P	27.3	28.27	26.6	21.8	12.0	15.63	7.53	7.22	13,060	13,994	2
MB19BWM03P	26.9	24.96	29.6	24.9	49.4	43.4	8.23	8.05	12,260	13,177	2
MB19BWM04R	27.2	23.31	26.0	22.3	10.6	58.6	7.28	6.96	33,890	34,092	2
MB19BWM05A	-	-	-	-	-	-	-	-	-	-	Bore Dry
MB19BWM06P	-	-	-	-	-	-	-	-	-	-	No Sample*
MB19BWM08P	24.7	28.31	25.4	26.5	14.9	9.93	7.88	7.53	5,790	5,420	2

{No Sample* - Bore MB19BWM06P was damaged and the hand bailer could not reach the groundwater (SWL 11.57m) to collect a water sample. The dip probe and stygofauna net were both able to pass through the constriction.}

The quality of stygofauna samples collected across 10 groundwater bores in December 2020 and May 2021 is summarised in Table 4. The sampling method aimed to collect between four and six replicate hauls off the bottom of each bore in order to be classified as a good sample. Overall, high quality stygofauna samples were collected from 7 of 8 bores that contained water (88%) which indicates both a significant and successful sampling effort.

Table 4: Summary of Stygofauna Sampling Effort and Sample Quality.

Bore Code	No. Replicate Samples		Sample Quality
	Dec 2020	May 2021	
MB19BWM02A	6	5	Good samples with all hauls off the bottom of the bore.
MB19BWM01P	4	3	Generally good samples with all hauls off the bottom of the bore.
MB19BWM07A	-	-	Bore Dry. No sample
MB19BWM25P	6	5	Good samples with all hauls off the bottom of the bore.
MB19BWM27P	3	3	Average samples with stygofauna net clogging with colloidal clays. Not all samples off the bottom of the bore.
MB19BWM03P	4	3	Generally good samples with all hauls off the bottom of the bore.
MB19BWM04R	6	4	Good samples with all hauls off the bottom of the bore.
MB19BWM05A	-	-	Bore Dry. No sample
MB19BWM06P	4	4	Good samples with all hauls off the bottom of the bore.
MB19BWM08P	4	4	Good samples with all hauls off the bottom of the bore.

Results from the analysis of the groundwater samples for the presence of stygofauna are presented in Table 5 below. No stygofauna (stygobites or stygophiles) were recorded from any of the 10 groundwater bores sampled in December 2020 and May 2021. A total of 12 non-stygofauna taxa (stygoxenes) were recovered from four groundwater bores in December 2020 including 11 Isoptera (termites) and one Oribatida (soil mite). Sampling in May 2021 recovered five non-stygofauna taxa (stygoxenes) from three groundwater bores including three Thysanoptera (thrips) and two Collembola (springtails).

Figure 1 shows the presence of Isoptera from groundwater bore MB19BWM03P in December 2020 and Figure 2 shows the presence of Thysanoptera and Collembola from bores MB19BWM25P and MB19BWM04R in May 2021.

Table 5: Analysis of Groundwater Samples for the Presence of Stygofauna. Sampling was conducted in December 2020 and May 2021.

Bore Code	**Dates Sampled**	**Stygofauna Taxa** (Dec'20/May'21)	**Non-Stygofauna Taxa** (Dec 2020)	**Non-Stygofauna Taxa** (May 2021)
MB19BWM02A	08/12/20 11/05/21	0	4 Isoptera	1 Thysanoptera
MB19BWM01P	08/12/20 10/05/21	0	3 Isoptera 1 Oribatida	0
MB19BWM07A	08/12/20 11/05/21	Bore Dry	Bore Dry	Bore Dry
MB19BWM25P	08/12/20 11/05/21	0	0	2 Thysanoptera
MB19BWM27P	08/12/20 11/05/21	0	0	0
MB19BWM03P	08/12/20 11/05/21	0	3 Isoptera	0
MB19BWM04R	08/12/20 11/05/21	0	0	2 Collembola
MB19BWM05A	08/12/20 11/05/21	Bore Dry	Bore Dry	Bore Dry
MB19BWM06P	08/12/20 11/05/21	0	0	0
MB19BWM08P	09/12/20 11/05/21	0	1 Isoptera	0

Figure 1: Isoptera (termites) recorded from Bore MB19BWM03P in December 2020 (Photo: Chris Pietsch)**.**

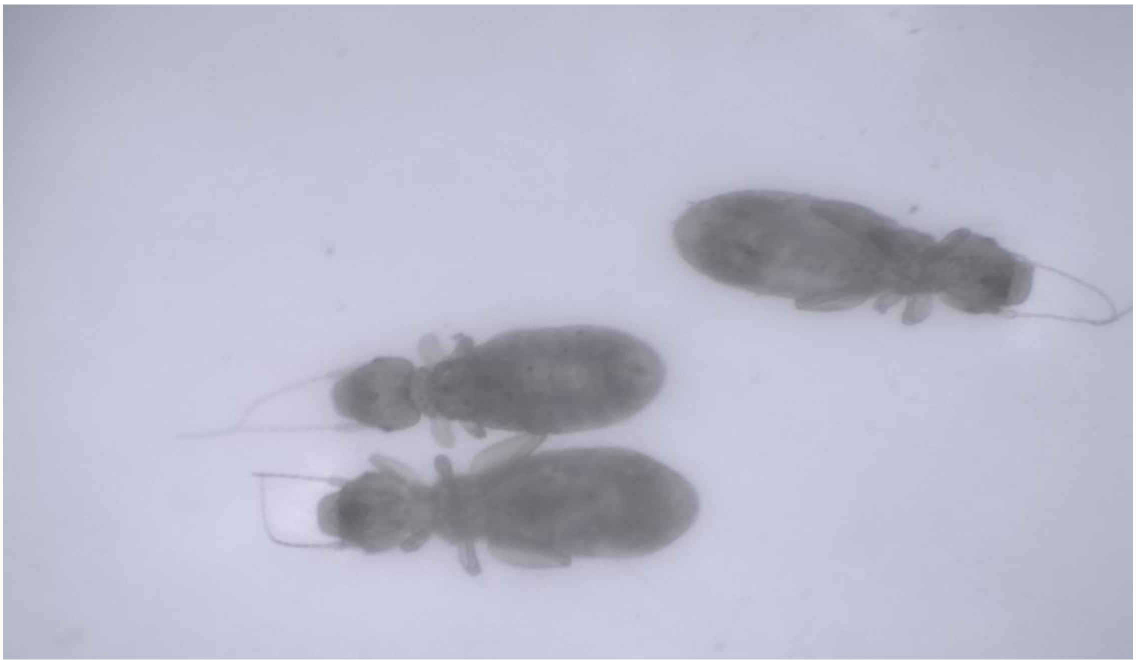
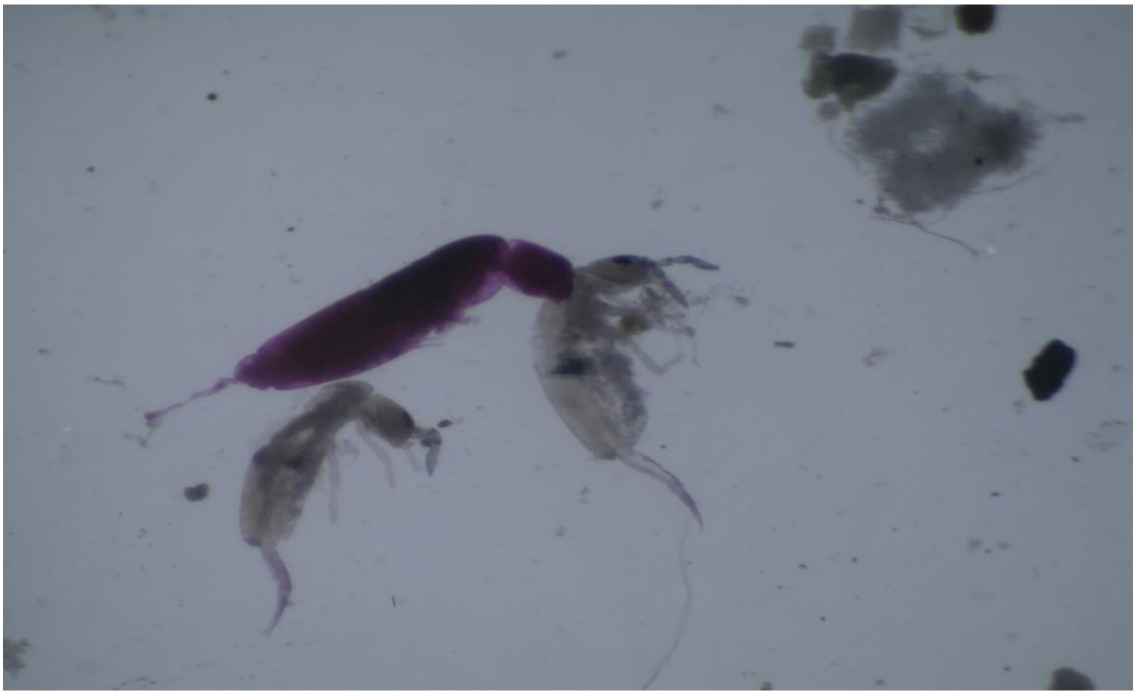


Figure 2 2: Thysanoptera (thrips) and Collembola (springtails) recorded from Bores MB19BWM04R and MB19BWM25P in May 2021 (Photo: Chris Pietsch)**.**



7. Conclusion

Two stygofauna sampling events were conducted by Freshwater Ecology at the BMA Blackwater Mine in December 2020 (pre-wet) and May 2021 (post-wet). Ten groundwater bores, selected by BMA, were sampled on each occasion in accordance with the methods defined in Queensland Environment Protection (Water) Policy 2009 – Monitoring and Sampling Manual: 'Sampling Bores for Stygofauna' (QEPA 2018) and 'Background information on Sampling Bores for Stygofauna' (QEPA 2018) and following established (standard) sampling techniques used elsewhere in Australia and overseas (Hancock & Boulton 2008, Dumas & Fontanini 2001, WA EPA Guidance Statements 54 and 54a 2003 & 2007). A significant sampling effort produced a total of 68 high quality samples across both sampling events.

No stygofauna (stygobites or stygophiles) were recovered from any of the 20 samples collected across two sampling events covering pre-wet and post-wet seasons. Two of the 10 bores that were sampled exhibited *in-situ* water quality conducive to the presence of stygofauna, in particular, relatively low salinity (<5,500 μS/cm), pH between 7 and 8 units, low turbidity (<10 NTU) and a dissolved oxygen concentration between 19% and 27% saturation. Six of the 10 sampling sites recorded the presence of non-stygofauna taxa (stygoxenes). The presence of the stygoxene taxa do not add any significant information to this Pilot Survey.

It is important to note that the lack of stygofauna recovered from these two sampling events does not necessarily mean stygofauna do not exist in aquifers associated with the BMA Blackwater Coal Mine. Sampling intensity across different seasons and across a range of aquifers present, with an emphasis on alluvial aquifers, is important.

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