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Preliminary Assessment of Groundwater Dependent Ecosystems: Invertebrate Groundwater Fauna, Takaka, Golden Bay, Tasman

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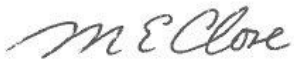		
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DISCLAIMER

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EXECUTIVE SUMMARY

The Tasman District Council is developing a water management plan for the Takaka catchment. The plan includes the karst groundwater system which is linked to the internationally significant Te Waikoropupū Springs, (TWS) (Gall, 2018). At the time of writing this report, a Water Conservation Order (WCO) Environment Court Enquiry was underway. This enquiry was examining the recommendations of the Special Tribunal that heard an earlier application for the WCO in 2018. An application for a WCO of this kind, for a groundwater system, sets a precedent in Aotearoa (New Zealand).

There is a lack of information on the Groundwater Dependent Ecosystems (GDE) that occur in the Arthur Marble Aquifer (AMA) that lies under within the Takaka township and wider Golden Bay. Baseline data on the occurrence, diversity, and significance of GDE in the AMA and the connected water bodies will be useful in assessing any impacts of anthropogenic[1], autogenic[2] or allogenic[3] origin on the Te Waikoropupū Springs.

In this report we provide a preliminary assessment of GDE, specifically stygofauna (both meio-and macro-fauna invertebrates that live within the groundwater system), present in the Arthur Marble Aquifer, using data collected from three sampling rounds over three consecutive years.

One of the first essential components for establishing the presence and diversity of GDE is to develop a robust sampling regime. ESR with TDC, undertook sampling methodological trials in Takaka, Golden Bay, through a previous Envirolink (contract 1861-TSDC143). The sampling methodology has been since updated.

With this methodology, assessment of the GDE present in the Arthur Marble Aquifer can be undertaken systematically. As a first preliminary step, ESR, with assistance from TDC, through ESR SSIF funding have undertaken sampling, over three consecutive years in Takaka, Golden Bay.

No stygofauna were recovered from any wells/bores sampled during the first two sampling rounds (Autumn 2018 and Spring 2019). The recent sampling round (Spring 2020), however, recovered stygofauna, including those which may be endemic to the aquifer. Stygofauna were collected from 7 of 11 samples taken from wells/bores i.e. both from the gravel and marble aquifers. Macroinvertebrates, including stygofauna were also recovered from the springs fed from the karst/gravel aquifer systems. Total abundance and species richness were low, compared to ecosystems examined in other South Island locations e.g., Canterbury, Southland. The last season's results (2020) indicate an endemic ecosystem is present. At present it does not appear to be abundant, but more species could be discovered with more sampling. The karst system in Takaka itself is unique in its evolution and geochemistry and a system that evolves with karst dissolution.

In order to ascertain long term biodiversity in this area, groundwater ecological sampling will need to continue to catalogue the unique groundwater species to this region. With collection of more baseline data, over time, ecological samples can be compared with environmental and chemical data (e.g. water depth, nitrogen-nitrate, conductivity, dissolved reactive phosphorus, dissolved oxygen etc) allowing statistically robust analysis of how natural (allogenic/autogenic) and anthropogenic activity has or may, impact these ecosystems now, and in the future. This information is still lacking across New Zealand at present.

[1] Created by people or caused by human activity

[2] Successional change caused by a change in abiotic environmental conditions i.e., conditions within the karst

[3] Relating to or caused by a change in the environment or an individual organism due to some endogenous factor, i.e., one that comes from within the environment or organism in the environment and *not* human activity

1. Introduction

1.1 BACKGROUND

Tasman District Council (TDC) is developing a water management plan for the Takaka catchment that includes the karst groundwater system. This groundwater system is linked to the nationally recognised Te Waikoropupū Springs (TWS), one of the largest cold-water springs in the Southern Hemisphere. The springs contain some of the clearest water ever measured (Gall, 2018; DOC, 2020). At the time of writing, a Water Conservation Order (WCO) Environment Court Enquiry was underway. The enquiry is examining the recommendations of the Special Tribunal that were heard in an earlier application for the WCO in 2018. An application for a WCO of this kind for a groundwater system would be the first in Aotearoa, New Zealand. There is a lack of information on the Groundwater Dependent Ecosystems (GDE) or subterranean aquatic ecosystems presence, occurrence, abundance in the Arthur Marble Aquifer connected to the TWS. GDE are defined as

> 'ecosystems that need access to groundwater to meet all or some of their requirements to maintain their communities of plants and animals, ecological processes and ecosystem services'.

In this report we refer specifically to microbial[4], meiofaunal[5] and macrofaunal[6] communities that inhabit groundwater aquifers. Current groundwater sampling methodologies include water level/pressure, flow and chemistry parameters but not ecological parameters. The Tasman District council's state of environment regional monitoring strategy for catchments with significant groundwater could in future include GDE in their groundwater assessments. Baseline data would be useful to assess any risk/impacts of allogenic (factors external to the studied sedimentary system), autogenic (factors controlled by processes within the sedimentary system) and anthropogenic (originating from human activity) changes on GDE in the AMA or other catchments where GDE's may occur.

One of the first components for establishing the presence and diversity of GDE is a robust sampling regime. The Institute of Environmental Science (ESR) with TDC, undertook sampling methodological trials in Takaka, Golden Bay. This was supported through a previous Envirolink (contract 1861-TSDC143) and ESR strategic science investment funding (SSIF) in 2018 and 2019. In order to assess GDE, the trial took samples from wells/bores located across the Takaka catchment, focussing on the micro and macro fauna within the screened part of the bore/well bottom and the surrounding aquifer. Since the trial, and publishing of sampling methodology (Weaver et al., 2018), ESR have assessed and simplified the methods for councils to carry this sampling work. The updated methods do not affect the sampling efficacy, both in terms of time and the recovery of groundwater fauna. A further round of sampling in the Takaka region was undertaken with this improved sampling methodology in September 2020.

[4] Organisms microscopic in size i.e., not able to be seen with the naked eye. Normally measured in the range of a millionth of a millimetre (micrometre).
[5] Organisms larger than microbial but smaller than macrofauna. Nominally described as those captured by a 40-micrometre sieve but pass through a 500-micrometre sieve.
[6] Those organisms visible with the naked eye.

This report presents the GDE sampling results found in the Arthur Marble Aquifer from the 2020 season. We note that the previous two rounds did not find any GDE (but did recover a number of surface water invertebrates). There is little information on GDEs in this catchment and the recent work undertaken by Tasman District Council and ESR will enhance the knowledge of GDEs in this aquifer. This collected data can be considered as baseline data and may help to understand groundwater ecosystem and its attributes.

1.2 GROUNDWATER

For the purposes of this report, groundwater dependent ecosystems (GDE) refer to those ecosystems that reside within groundwater (e.g., stygofauna in karst/gravel aquifer systems); and rely wholly or partially on groundwater to maintain an adequate level of ecosystem function as well as maintain community composition over multiple generations (Smith et al., 2006). As there are organisms that are completely dependent on groundwater throughout their entire life cycle, any significant alteration to the groundwater itself could disrupt and potentially irreversibly damage the ecosystem if groundwater was altered beyond its natural variability or range. Examples of groundwater dependent ecosystems include wetlands, vegetation, river base flows, springs and related cave and sub surface aquifer ecosystems.

Over allocation, habitat degradation, land use change and pollution are known to be common anthropogenic issues that can have a substantial impact on the freshwater environment (Woodward et al., 2010). In addition, anthropogenic climate change may exacerbate those stressors further. However, there are few studies that examine the impact of climate change and other anthropogenic impacts e.g. water abstraction on GDE. Current studies show concern for anthropogenic demands and quality requirements for groundwater, with 40% of Aotearoa relying on groundwater as a freshwater resource (StatsNZ, 2017). Emerging literature suggests that GDE may hold more value to the water resource than initially given credit for (Reid and Scarsbrook, 2009; Eamus et al., 2016; Fenwick et al., 2018). Contemporary studies allude that groundwater organisms may provide an array of ecosystem services that contribute to the quality of the water supply, with potential for bioremediation and bio-indication speculated in the academic literature (Malard et al., 1996; Smith et al., 2016; Español et al., 2017; Fenwick, 2018; Fenwick et al., 2018). Yet, many studies currently focus on habitat restoration and the protection of freshwater organisms, and on information gleaned from the surface water environments.

Worldwide, relatively little is known about the characteristics of GDE. Due to the nature of their habitat, their dispersal ability is somewhat limited, and the organisms appear to exhibit high levels of endemism (only existing in one geographic region) within their taxonomic groupings. It is therefore possible to apply general theory, concepts and trends that are consistent worldwide, but site-specific studies should be conducted to properly classify ecosystem potential. In comparison to Australia, North America and Europe, New Zealand's stygofauna has been poorly studied and classified (Scarsbrook et al., 2003). Projected increases of anthropogenic pressures on groundwater ecosystems such as population increase and its impacts, and associated demands on water resources are important reasons to understand and interpret the mechanisms that influence groundwater ecosystems. This includes from both an ecological and biodiversity assessment perspective and using traditional indigenous knowledge where available.

1.3 GROUNDWATER DEPENDENT INVERTEBRATES (MACRO AND MEIO)

The existence of New Zealand's stygofauna was first discovered by Charles Chilton in 1882 (Chilton, 1894) and captured the attention of the international scientific community. Since their discovery, relatively little research has been conducted on GDEs, with New Zealand's description of fauna lacking in comparison to other countries around the world. 'Stygofauna' as a term encompasses a variety of diverse types of organisms that are found in groundwater varying in size. It includes organisms that are obligate (restricted to a particular function or mode of life), groundwater-adapted organisms that complete their entire lifecycle within groundwater aquifers (stygobites), and those that are not specifically groundwater-adapted but are able to survive the harsh conditions in aquifers (stygoxenes and stygophiles), see Figure 1. In addition, stygofauna are part of a much larger interconnected system that includes vast prokaryotic (microbial, archaeal and fungal) communities.

For the purposes of this report, the sizes of stygofauna are determined by the methodology i.e. specific filter or net sizes, reported previously -(Weaver et al 2018). Therefore, micro- represents organisms of (0.22 to 1.2 micrometres or µM[7], meio- is 10 to 65 µM) in size, and macro- are those (over 65 µM). Macrofauna are defined as being seen by the human eye and very well using a light microscope, whereas meio-fauna require more powerful microscopes that can magnify up to 100 times. Microfauna can also be seen with a powerful microscope in the range of 1000 times magnification.

In Aotearoa, stygofauna are dominated by macrofauna such as crustaceans (amphipods, isopods, syncarida, copepods etc) and meiofauna such as acari (mites). Other more familiar invertebrates such as molluscs (snails), coleoptera (beetles), and annelids (segmented worms) also exist in groundwater globally. Within these groups are taxa[8] that are only found in groundwater systems. In aquifers where the sediments provide sufficient pore spaces, micro-fauna such as Turbellaria, Rotifera, Nematoda and Protozoa (Humphreys, 2006) and larger meio-fauna may be present.

Stygobites are typically blind and lack pigment. Several taxa have developed long appendages for navigation without light. They are known to exist in both acidic and alkaline water conditions of either high or low salinity, inside anything from an aquifer to a small fissure or crevice within any rock type. Food sources include allochthonous carbon (carbon from outside the aquatic system), surface water, as well as bacterial biofilms and the predation of other organisms (Fenwick et al., 2018). More than 170 species have been formally identified in Aotearoa, but this is likely to be an underestimate based on global stygofauna biodiversity data.

These organisms are also difficult to collect and identify due to their location (see Section 2.1), unfamiliar taxa, endemism, and lack of taxonomic expertise within Aotearoa. There are now shifts towards using molecular methods to barcode individual samples. However, these approaches lose the detail of organism morphology, function, and ecological role and so a combined approach where possible is desirable.

[7] A micrometre (1 µM) is thousandth of a millimetre, 0.001 mm. For reference, the diameter of a human hair can range from 20 to 200 µM.

[8] A taxonomic group of any rank, such as a species, family, or class.

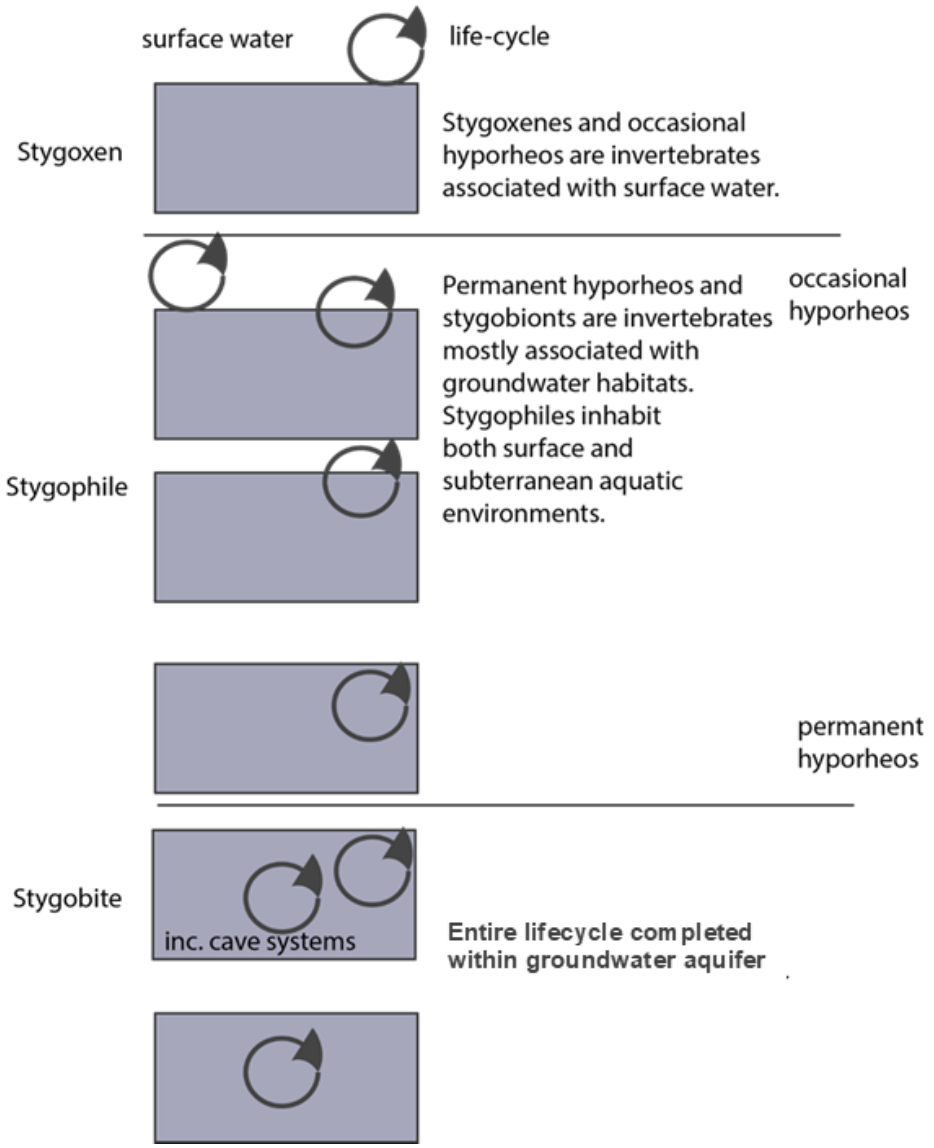

Figure 1: Groundwater habitat where stygofauna life cycle is carried out. Circular arrows refer to organism's life cycle. Groundwater-adapted organisms that complete their entire lifecycle within groundwater aquifers are known as stygobites. Those that are not specifically groundwater-adapted but are able to survive the harsh conditions in aquifers are known as stygoxenes and stygophiles. Hyporheos refers to the connecting space between surface- and groundwater. Its hydrological, chemical, biological and metabolic features are specific of this zone, not belonging truly neither to surface- nor to groundwater (Mugnai et al 2015). Modified from Fig. 1 in Scarsbrook et al., 2003.

2. Methods

2.1 COLLECTION OF GROUNDWATER DEPENDENT INVERTEBRATES (MEIO AND MACRO)

Access to bores or wells is the main method to collect biota from groundwater systems. Wells are dug and are typically large in diameter, often consisting of concrete casings and are generally shallower (<8m) than bores. Bores are drilled with a specific diameter and are typically lined with PVC or steel casings[9]. Sections of the borehole can be unlined if the formation is self-supporting, for example in marble or chalk, however, most have screens of varying lengths, defining the flow of water to a specific part of an aquifer. A series of bores/wells were pumped (re: groundwater) and flowing springs were sampled at selected sites (see Table 1 for site details and Figure 2 for location and sampling map) using the protocol described in Weaver et al 2018[10], with modifications described in updated methods.

For bores/wells, 3x (pre-pump) net hauls were collected, followed by 100L of pumped water which was sieved and 3x (post-pump) net hauls following pumping. These samples represent stygofauna from within the bore and the aquifer. In addition to the bores/wells, there were 2 sites where groundwater could be sampled but did not have access into the bore (WWD6013; WWD23720). For those samples the net hauls were omitted. At WWD6013, a modified net was attached to the outlet tap from the artesian well and collected after 48±5 hours. A netted sample was not able to be collected from WWD23720 and only a filtered sample was collected. Two springs (Spittal and Springbrook) were sampled in addition to the wells. For both these sites a modified weighted net was left in each spring beside an upwelling area and collected after 2-4 days respectively.

[9] Modified from https://www.ecpgroup.com/journal2/blog/post?journal_blog_post_id=34

[10] This resource has been updated to reflect new sampling methodology.

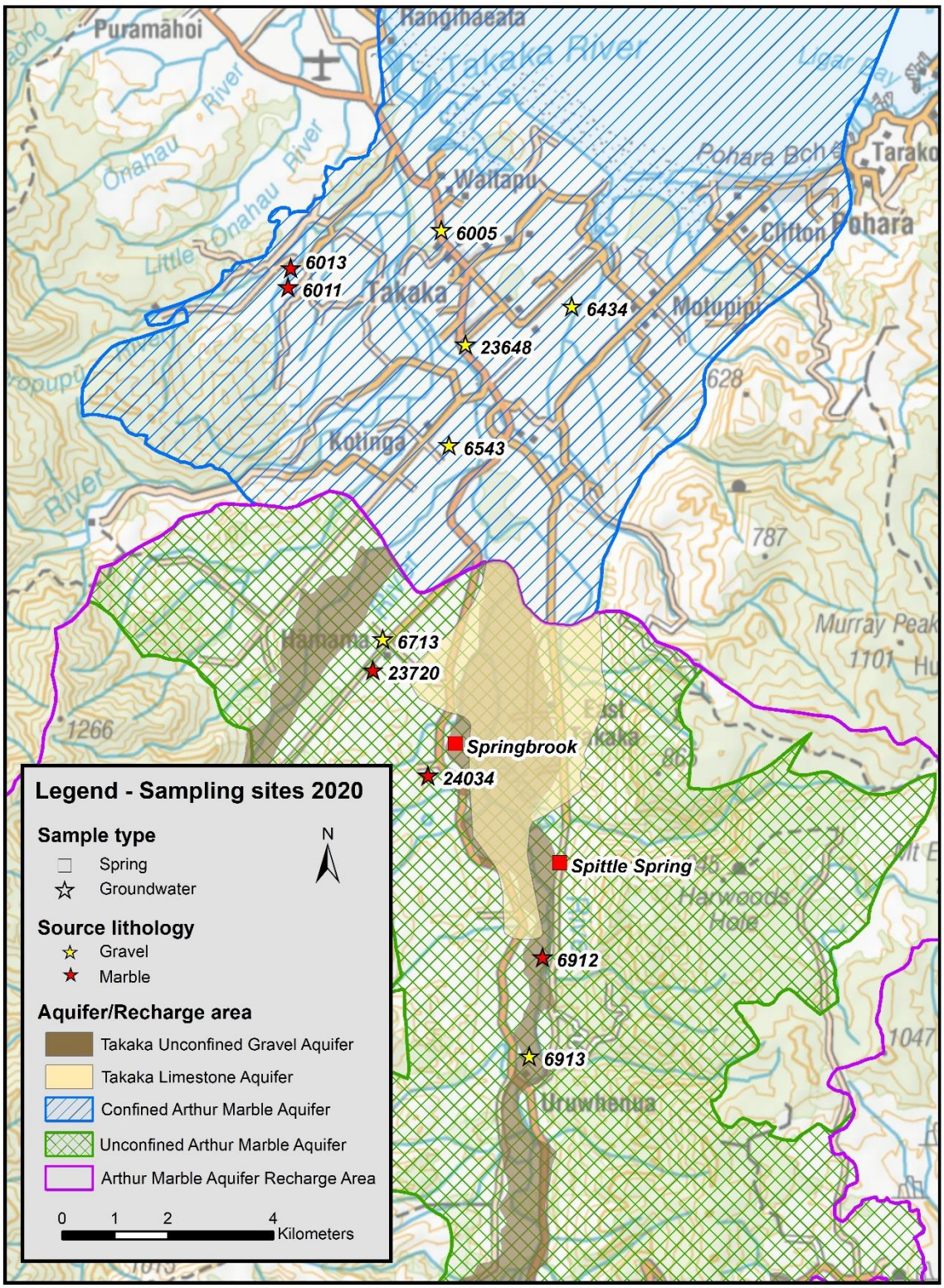

Figure 2: Location of each sampling site. The stars denote groundwater samples collected from wells/bores. The square boxes represent samples collected from Springs. The blue striped area maps the confined Arthur Marble Aquifer, the green crossed area the unconfined Arthur Marble Aquifer. The pink line shows the Arthur Marble Aquifer recharge area. The numbers are the identifiers for each well/bore sampled (note, they lack the precursor WWD to keep text discrete.

There were some notable modifications to the methods which are listed below. Large, modified nets (350, 250, 65 µM) were used to filter the pumped well water in place of sediment sieves (see Figure 3). These nets are inverted at the end of each sampling period and organisms collected, stained with Rose Bengal, and stored in 99% ethanol for identification. Taxa were identified to highest taxonomic level where possible. However, given time and resources were limited this was often at the “Class” or “Order” taxonomic level.

In addition to the modified nets, a 10 L sample of the 100 L pumped well water was taken from the first and last bucket of pumped water to collect smaller meiofauna (e.g., ciliates, rotifers etc). Each of these water samples were run through a peristaltic pump (Masterflex I/P Pump, with 9610-73 tubing, Masterflex) and filtered through a track etched polycarbonate 10 µM pore size membrane (47 mm) at the minimum flow rate (set to number 1, approx. 1 LPM (Litres Per Minute)). The filter was placed before the pump head which in previous laboratory experiments were found to minimise the loss of live microorganisms. The filtered membranes were transferred into falcon tubes containing a 50:50 mixture of groundwater and a traditional hay and milk broth to maintain meiofauna prior to identification.

Figure 3: Modified nets (350, 250, 65 µM) used for collection of stygofauna. Nets are stacked and used to filter pumped well water.

Samples were refrigerated at 4°C until they could be examined in the laboratory. The samples were sieved and sorted by size (250, 100, 65 µM) and examined under a light microscope. Specimens were removed from the main sample and identified to highest taxonomic level where possible. For the live meiofauna, after 22-25 days of incubation in the dark at 12(±2) °C, the samples were examined under a light microscope (x400 -1000 magnification) for presence or absence of live organisms. Additional broth was added to the samples every week and viewed under the microscope following a further 6-9 days. The 2020 protocol reflects the modifications described in this section.

Table 1: Site location and well details. Aquifer material is designated depending on where sample was collected from.

Site Code/Tasman District Council Well Number	Source	Well/bore depth (m)	Well/bore diameter (mm)	Screen depth (m)	Screen slot width (mm)	Recharge/ Non-recharge area	Aquifer material
WWD23648	Bore	36.5	150	31-35.5	2.5	N	Gravel
WWD23720	Bore	117	300	76-117		Y	marble
WWD24034	Bore	36	125		8	Y	marble
WWD6005	Well	5.34	1050	No screen, concrete well	No screen	N	Gravel
WWD6011	Bore	114	125	No screen, cased to 12.1m (into marble)	No screen	N confined AMA	marble open hole
WWD6013	Bore	20	125	No screen, cased to 35 m	No screen	N confined AMA	marble open hole
WWD6434	Well	6.12	1050	No screen, concrete well	No screen	N	Gravel
WWD6543	Bore	9	250	5-7	4	N	Gravel
WWD6713	Bore	50	150	24.5-49.5	3	Y	Gravel (top of marble)
WWD6912	Bore	64	125	No screen, casing down to marble	No screen	Y	marble
WWD6913	Bore	48	150			Y	Gravel
Spittle Spring	Spring	0	-		N/A	Y	marble
Springbrook	Spring	0	-		N/A	Y	Gravel

3. Results and Discussion

3.1 TOTAL ABUNDANCE OF SAMPLES BY LITHOLOGY

Previous sampling rounds have shown nominal recovery of organisms (Table 2). However, the retrieval of nauplii, free swimming forms of crustacean larvae, at a marble aquifer location does indicate the presence of crustaceans. Interestingly, there were no significant organisms recovered in the gravel sites in 2018 and 2019.

The following section describes the taxa recovered from each of the sites in 2020 by lithology.

3.1.1 Gravel

A total of 32 groundwater invertebrates were recovered from five of the six gravel aquifer sites (Figures 4 to 6). The sites with the most abundance in order of the highest first were: WWD6434 (n=18), WWD6543 (n=3), WWD6005 (n=8), WWD6713 (n=2), WWD6913 (n=1). These counts do not include samples that were indeterminate. Many of the invertebrates recovered were smaller meiofauna (<50 µM) and included copepods and copepodites (most abundant, 15/43), acarina (n=10/43), and gastropods (n=1/43).

The largest sized invertebrates were worms and amphipods, but these individuals were not abundant. Of notable value was the recovery of two amphipod specimens from site WWD6713. These individuals were identified to be of the genus Paracrangonyx. On closer inspection these crustaceans were validated to be a yet, undescribed species (personal communication, Graham Fenwick). This genus is strongly laterally compressed and presumably feeds on underground microbial films. Larger protists (e.g., ~30 µM) were also present in two of the gravel samples (WWD6005 and WWD23648). In terms of macroinvertebrates recovered historically in this study region, amphipods have been collected that include several from the Paraleptamphopus genus. Previous reports have suspected additional species and genera in this group (Watson, 1972; Chapman and Lewis, 1976; Bousfield, 1983).

The genus Paraleptamphopus is one of the most widespread and common of the freshwater amphipods. Paraleptamphopus are abundant in many slow-flowing, soft bottom streams around New Zealand (Manaaki Whenua, 2021). Studies indicate they are collector-gatherers, feeding on deposited organic matter and biofilms growing on submerged surfaces. There are two described species within this genus: *P. caeruleus* which is an epigean (surface) species found in the central and southern portion of the South Island (Hurley 1975) and *Paraleptamphopus subterraneus* a blind, subterranean species that was first described in 1882 from Canterbury aquifers (Chilton, 1894). The historic samples collected include the stygobite *Paraleptamphopus subterraneus* (Pupu springs) (Fenwick 2016). Exact locations of these sample collection are unclear in the reference. The source of the water collected could be local shallow water or groundwater or input from nearby Fish Creek. From the reference it is difficult to ascertain the actual location the specimen was collected from i.e. from the Arthurs Marble aquifer, overlaying gravel aquifer or surface water.

3.1.2 Marble

A total of 41 groundwater invertebrates were recovered from two of the five marble aquifer sites (Figures 2 and 5). These were: WWD6013 (n=24) and WWD6011 (n=17). Note that WWD6013 is an artesian well and therefore the sampling method was altered to allow specimen collection. Briefly, samples were taken through a valve and filtered into 63 µM mesh over a 36-hour period. Most invertebrates recovered from WWD6013 were meio-fauna (<50 µM) and included acarina (most abundant, n=15/24). Site WWD6013 also recovered a number of arachnids but these were thought to be contaminated from the surrounding environment. The presence of copepodites in the same sample (WWD6013) does, however, support the presence of organisms from within the aquifer. Their exact origin cannot, however, be identified at present. At WWD6013 samples collected from the bore could have originated from the Marble or could be from the overlaying gravel aquifer. It is noted that no groundwater invertebrates were found in marble bores WWD24034 andWWD23720 (in the AMA recharge area).

3.1.3 Natural Springs

Spring Brook

Amongst several freshwater surface invertebrates[11] (see footnotes), acarina (mites) were recovered. Further identification is required to determine whether these organisms are phreatic (strictly subterranean), freshwater or soil mites.

Spittal Spring

Similar to Spring Brook, an ostracod was recovered from the spring. Further identification is required to determine whether that organism is strictly subterranean.

In addition, to invertebrates found in the spring (Figure 6) live meiofauna were recovered from several sites and are included within Tables 3 and 4 and displayed in Figure 7.

[11] **Other invertebrates recovered included Mayflies (Ephemeroptera): 2 x *Deleatidium* spp., 1 x *Zephlebia dentata;* Caddisflies (Trichoptera): 2 x *Hydrobiosella* sp.; Flies (Diptera): 1 x Orthocladiinae; Mites (Acarina): 1 x Oribatidae; 1 x indeterminate mite**

Table 2: Taxon abundance per site, sampling method and lithology for sampling years 2018 and 2019.

Site Taxa/Method	1 Pre-pump net haul	2 Pumped (sieved) sample	3 Post-pump net haul	4 Initial pumped sample	5 Final pumped sample	6 Other method	Total	Gravel	Marble	Spring/ Cave
	Bore bottom	Aquifer	Bore bottom	Aquifer	Aquifer	Aquifer				
YEAR = 2018										
WWD24034 nauplii (crustacean larvae)		1							●	
Subtotal		**1**					**1**			
WWD6011 collembola		1							●	
Subtotal		**1**					**1**			
Spittal Spring chironomidae indeterminate						1 1				●
Subtotal						**2**	**2**			
YEAR = 2019										
GW6011 Indeterminate	1								●	
Subtotal	**1**						**1**			

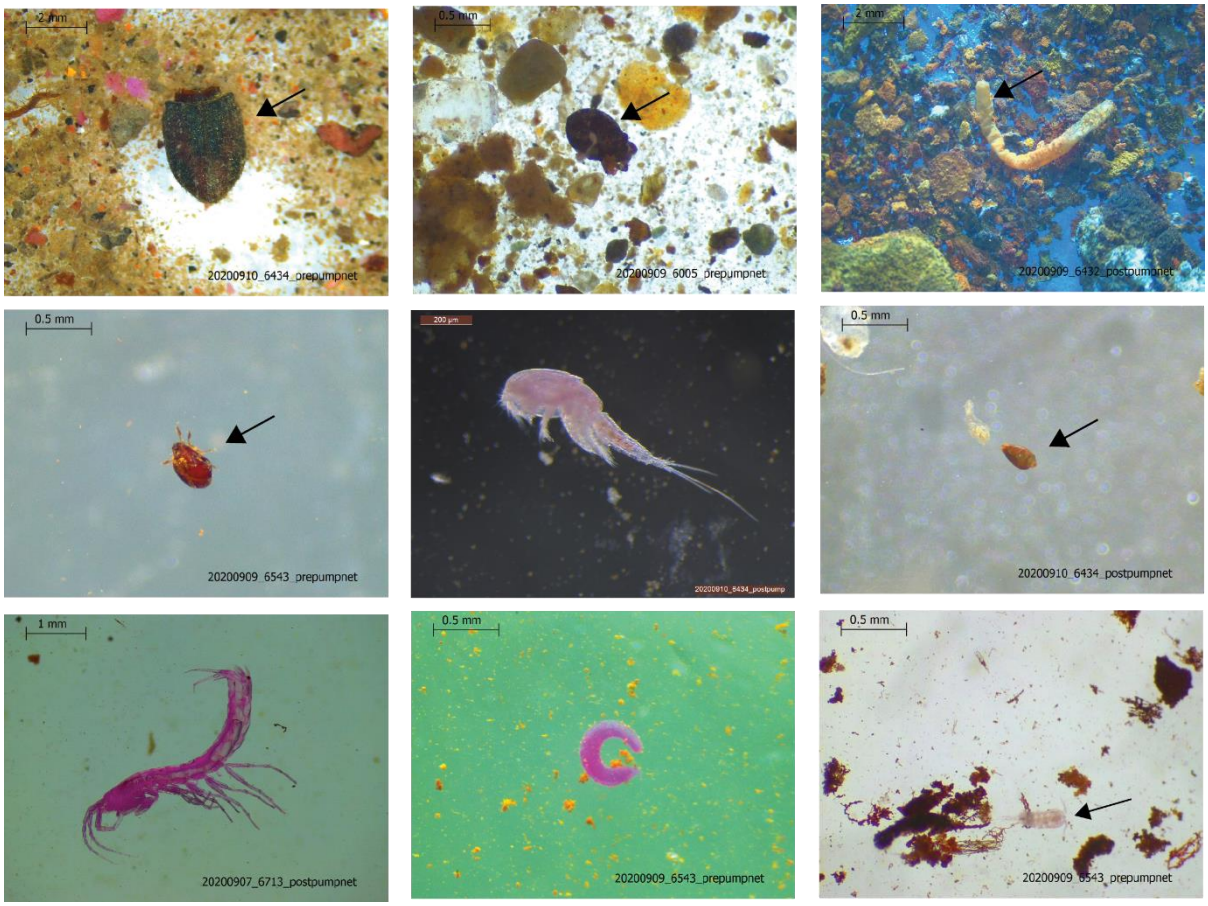

Figure 4: Selected groundwater invertebrates (macro and meio) collected from the gravel aquifer sites, 2020. Note that (some) organisms have been stained pink to ease identification. Images show from 1st row left to right: coleoptera (beetle) casing, acarina (mite), worm; 2nd row from the top, left to right: acarina (mite), copepod, gastropod (snail), 3rd row from the top, left to right: amphipod, worm, copepod.

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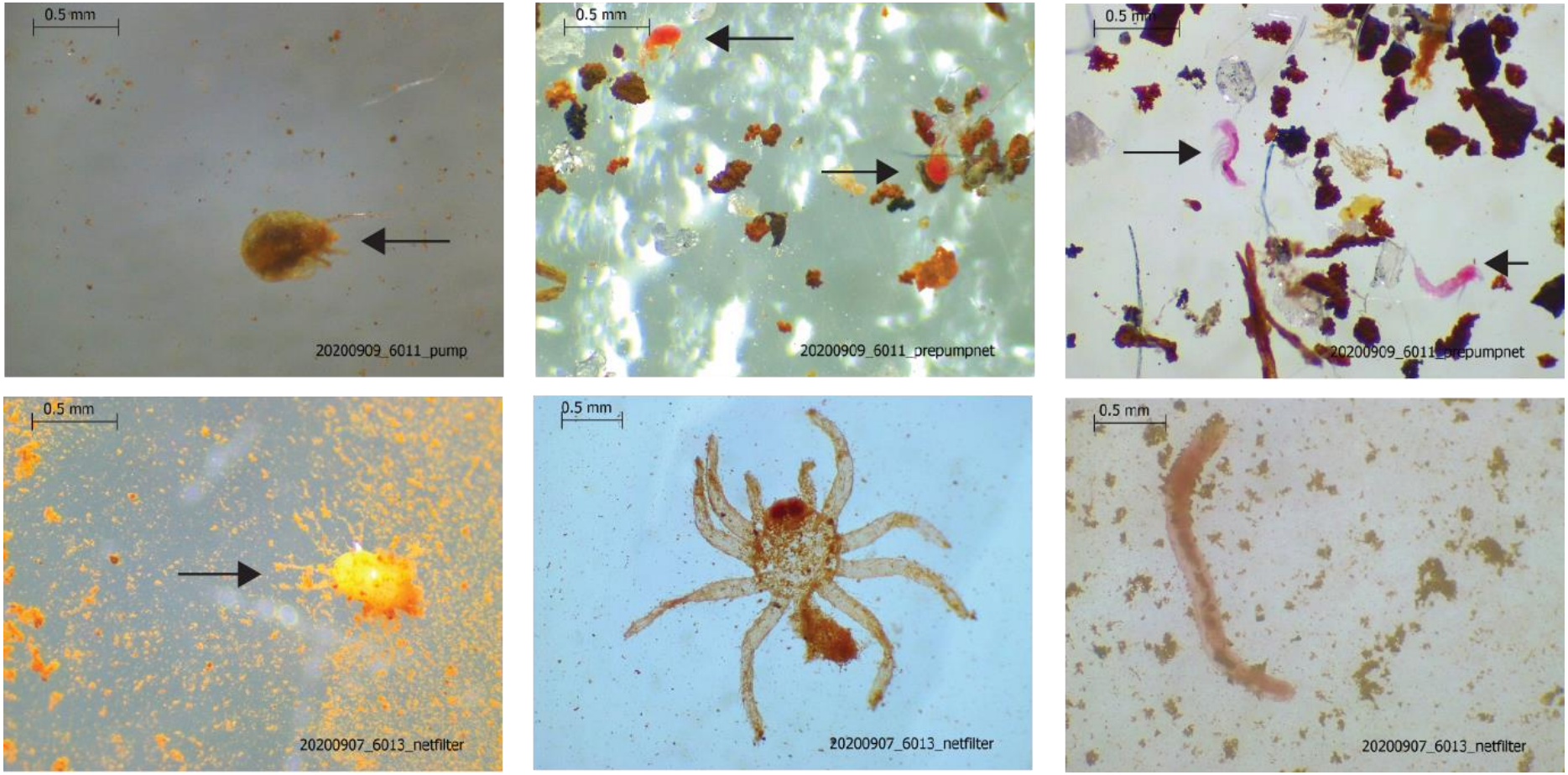

Figure 5: Selected groundwater invertebrates (macro and meio) collected from the marble aquifer sites, 2020. Images show from top left to right: acarina (mite), acarina (mite), nauplii (crustacean larvae); 2nd row from the top, left to right: acarina (mite), arachnid (spider), worm.

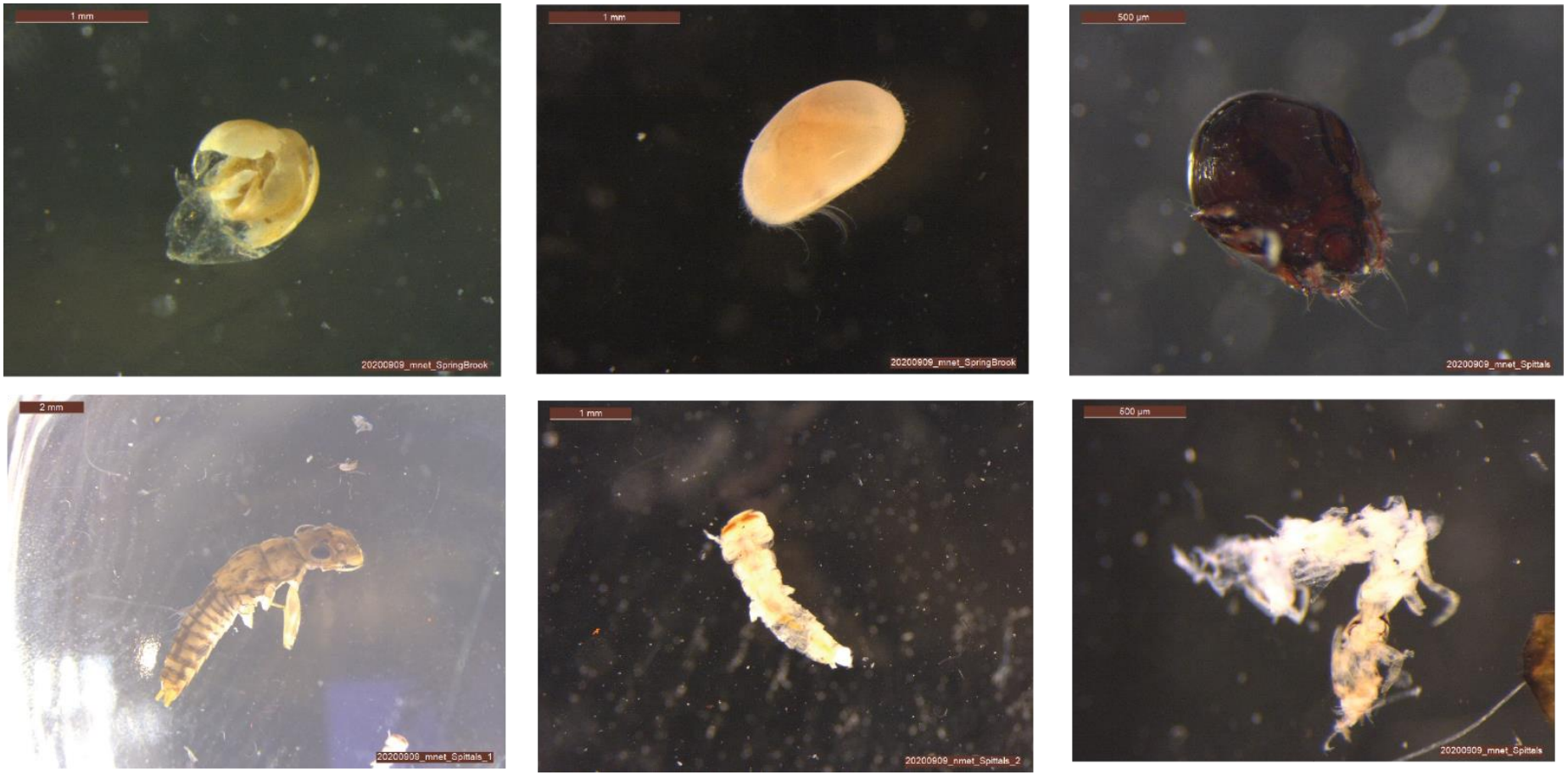

Figure 6: Selected groundwater microfauna collected from selected springs, 2020. Images show from top left to right: Indeterminate organism, ostracod, acarina (mite); 2nd row from the top, left to right: Mayfly larvae, caddisfly larvae, indeterminate organism.

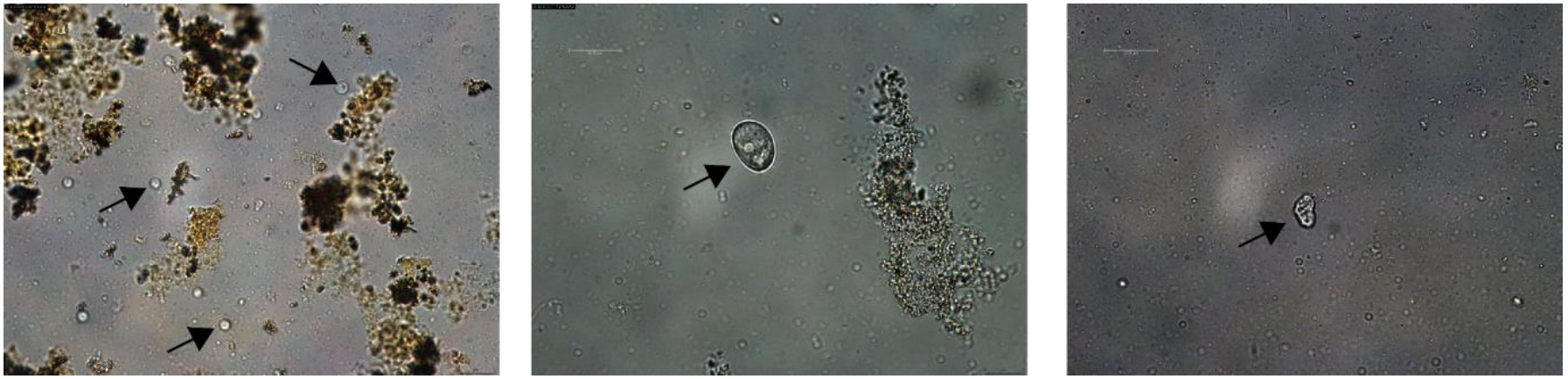

Figure 7: Selected groundwater micro-fauna collected from all sites in 2020. Images show from top left to right: WWD6005 small protist, WWD23648 unidentified ciliate, WWD6005 unidentified ciliate.

Table 3: Taxon abundance per site, sampling method and lithology for sampling year 2020.

Site Taxa/Method	1 Pre-pump net haul	2 Pumped (sieved) sample	3 Post-pump net haul	4 Initial pumped sample	5 Final pumped sample	6 Other method	Total	Gravel	Marble	Spring/ Cave
Sampling position (within bore/well or in aquifer)	**Bottom**	**Aquifer**	**Bottom**	**Aquifer**	**Aquifer**	**Aquifer**				
WWD6005										
acarina	2		4				6			
ciliate					P		P			
Amoeba					P		P	●		
cysts				P			P			
Unidentified	1						1			
worm	1						1			
Subtotal	**4**		**4**	**P**	**P**		**8**			
WWD6011										
acarina	13	1					14			
Copepodites	2						2		●	
indeterminate	1						1			
Subtotal	**16**	**1**					**17**			
WWD6013#										
Acarina						15	15			
Arachnid						6	6			
Crustacean egg						1	1		●	
Indeterminate meiofauna						1	1			
worm						1	1			
Subtotal						**24**	**24**			
WWD6434										
Coleoptera	1						1			
Copepoda			14				14			
Gastropoda			1				1	●		
indeterminate	1						1			
Poss crustacean		1					1			
worm	1		1				2			
Subtotal	**3**	**1**	**16**				**20**			
WWD6543										
Acarina	4						4			
Copepoda	1						1	●		
indeterminate	7	1					8			
Subtotal	**12**	**1**					**13**			
WWD6713										
Amphipoda (Paracrangonyx spp.)			2				2	●		
Subtotal			**2**				**2**			
WWD6913										
indeterminate	1						1	●		
Subtotal	**1**						**1**			
WWD23648										
ciliate				P			P	●		
Subtotal				**P**			**P**			
Spittal Spring$										●
Acarina (1 x Oribatidae; 1 x indeterminate mite)						2	2			
Subtotal						**2**	**2**			
Spring Brook*										●
ostracoda						1	1			
Subtotal						**1**	**1**			

P = presence; A = absence and refers to water samples that were filtered in the field at 10 µm and less, cultured in the laboratory and observed after 22-25 days; # refers to a sample that was filtered with a 63 µm mesh sock into a bucket and left for 24 hours; $ refers to a sample from a spring, situated next to a marble cave; *=refers to a natural spring located in a cordoned off small, ponded area next to a dairy farm. Note, no stygofauna were collected from WWD23720, WWD6912 or WWD23034 and so are not in the above table.

Table 4: Taxon abundance per site by sampling method and lithology, normalised to 100 litre water volume[12], sampling year 2020.

Site Taxa/Method	1 Pre-pump net haul	2 Pumped (sieved) sample	3 Post-pump net haul	4 Initial pumped sample	5 Final pumped sample	6 Other method	Total	Gravel	Marble	Spring/ Cave
Sampling position	**Bottom**	**Aquifer**	**Bottom**	**Aquifer**	**Aquifer**	**Aquifer**				
WWD6005										
acarina	4		14				18			
ciliate					P		P			
ameoba					P		P	●		
Cysts				P			P			
Unidentified	4						4			
worm	4						4			
Subtotal	**4**		**4**	**P**	**P**		**26**			
WWD6011										
acarina	1	1					2			
Copepodites	-						-		●	
indeterminate	-						-			
Subtotal	**1**	**-**					**2**			
WWD6013#										
Acarina						-	-			
Arachnid						-	-			
Crustacean egg						-	-		●	
Indeterminate meiofauna						-	-			
worm						-	-			
Subtotal						**-**	**-**			
WWD6434										
Coleoptera	3						3			
Copepoda			39				39			
Gastropoda			3				3	●		
indeterminate	3						3			
Poss crustacean		3					3			
worm	3		3				6			
Subtotal	**9**	**3**	**45**				**57**			
WWD6543										
Acarina	4						4			
Copepoda	1						1	●		
indeterminate	7	1					8			
Subtotal	**12**	**1**					**13**			
WWD6713										
Amphipoda (Paracrangonyx spp.)			-			-	-	●		
Subtotal			**-**				**-**			
WWD6913										
indeterminate	-						0	●		
Subtotal	**-**						**-**			
WWD23648										
ciliate				P			P	●		
Subtotal				**P**			**P**			
Spittal Spring[$]										●
Acarina (1 x Oribatidae; 1 x indeterminate mite)						-	-			
Subtotal						**-**	**-**			
Spring Brook*										●
ostracoda						-	-			
Subtotal						**-**	**-**			

[12] To normalise, the volume of groundwater in the wells/bores per litre was calculated and multiplied up to 100L to match the volume collected by pumping. This multiplication factor was used to re-calculate the abundance of fauna in the bores/wells per 100L. Where '-' appears, it reflects where there was less than one sample in the well/bore when multiplied.

P = presence; A = absence and refers to water samples that were filtered in the field at 10 µm and less, cultured in the laboratory and observed after 22-25 days; # refers to a sample that was filtered with a 63 µm mesh sock into a bucket and left for 24 hours; $ refers to a sample from a spring, situated next to a marble cave; *=refers to a natural spring located in a cordoned off small ponded area next to a dairy farm. Note, no stygofauna were collected from WWD23720, WWD6912 or WWD23034 and so are not in the table.

3.2 COMPARISON OF DIFFERENT METHODS

At all the sites, more individuals were recovered from inside the bores/wells than the surrounding aquifer even after sites were compared on a per litre basis[13]. There were not enough data to determine whether the first or last net haul recovered more or less individuals or more diverse taxa, given that recovery rates were low. The observation of higher abundance from net hauls is consistent in some literature (Hahn and Matzke, 2005; Bork et al., 2008) but not others (Hancock and Boulton, 2009). In their groundwater samples, Hakenkamp and Palmer, (1992) and Danielopol, (1999) found higher densities per litre in the first sample volume and inside the bores, respectively. Both studies suggested that bores are preferentially colonized. An increased abundance from the net hauls (inside and the bottom of wells/bores) is possible because sediment is also collected in the nets, increasing the probability of collecting invertebrates that may be living in both the bottom substratum and those that are swimming in the water column. Pumped samples also assume that invertebrates cannot swim away and/or are small enough to pass through the screen sizes of some wells/bores, which in this sampling round, ranged between 2.5 to 8 mm wide. Pumping wells/bores also assumes that the velocities generated are sufficient to entrain the invertebrates in both gravel and marble aquifers.

On a per site basis, higher abundances were collected from both spring samples and site WWD6013. When adjusted on a per litre basis, and compared to all samples retrieved, the spring samples recovered were negligible. There are two likely reasons for this initial higher abundance. First, these samples were left for 43 hours, such that the volume of water extracted from this sample far exceeded comparable volumes from other sites sampled. Second, there was room for contamination, as demonstrated by the presence of more likely terrestrial and/or surface water specimens.

Although our experimental methods have been modified from previous years (e.g. the addition of v-shaped nets for filtering) we do not believe they are the reason for the increased recovery of stygofauna in this sampling round (year 2020). Most of the samples collected were through net hauls, the type and size of which remain the same.

3.3 FINAL DISCUSSION

The results presented in this report show that there are stygofaunal species present in both the gravel aquifer (both overlying and not overlying the Arthur Marble Aquifer e.g. in the lower Takaka valley and around Takaka township) and the Arthur Marble aquifer. There are major caveats that need to be considered in interpreting this data. First, noticeable numbers of stygofauna were collected from few wells/bores on only one sampling occasion. As such there is significant uncertainty regarding the extent of stygofaunal diversity and abundance in either of the aquifer systems. Second, although there is a low presence of stygofauna (including meiofauna) in both aquifers, there is limited temporal data to ascertain any trends in diversity or abundance present, nor any influence of land use on the diversity or abundance present. Instead, the data presented in this report should be considered as a baseline. More comprehensive studies would need to be conducted to ascertain abundance, occurrence and temporal variability or trends. It is noted that two bores in the upper valley recharge area of the AMA and within the marble did not recover any stygofauna.

There is a lack of detailed information even in global literature on stygofaunal diversity. Most studies conducted to date have used different sampling methods, making global

comparisons difficult. In some studies, spot locations have been studied, with limited temporal data. In terms of the sensitivity of these fauna to land use changes, the international literature (1977 to 2018) shows 18 studies have been undertaken investigating the toxicological effects on stygofaunal species (Di Lorenzo et al 2019). Within those studies, the authors highlight limitations including studying only adult life-stages and predominantly being limited to amphipoda, isopoda and copepoda (only one study reported on oligochaeta and one decapoda). Table 5 lists the species studied and the chemical elements or compounds on which those species were tested against (from Di Lorenzo et al 2019). Examining Table 5, very few of those studies were conducted on both a broad range of species, life stages or chemicals. Further studies are needed for specific chemicals, of relevance to Aotearoa, such as nitrogen and phosphorus species. The few studies that have examined the effect of nitrogen compounds on stygofauna do not provide, at present, conclusive evidence. For example, the literature provides a varied response to nitrogen compound presence, some showing a detrimental effect (e.g. Menciá et al 2014, Marmonier et al 2018, Di Lorenzo et al 2014) but others no effect (e.g Di Lorenzo and Galassi 2013, Boutin et al 1995, Korbel et al 2013). It is also important to consider multiplicative or additive effects on organisms of interest, if likely.

Stygobites are deemed to be extreme energy savers, with slow metabolisms due to the environment they reside and the restrictive nature of nutrient availability. Di Lorenzo et al (2015) showed that subterranean copepods used 7-fold less oxygen compared with surface copepods. Other studies have shown similar findings in other stygofauna, isopods and amphipods consuming half the oxygen as their surface relatives (Issartel et al 2005, Simičič et al 2005, Wilhelm et al 2006). In addition, Hervant et al (1996) suggest a reduced enzymatic activity (metabolism) compared with surface water relatives.

In consideration of these points, it is currently difficult to make conclusions regarding the impacts of contaminants on groundwater ecosystem biota in Aotearoa. Numerous publications do suggest that using a *surface* water index for assessing groundwater ecosystem health is inappropriate (Griebler and Avramov 2015, Di Lorenzo et al 2014, Di Lorenzo et al 2019). Therefore, there is a need for a national and global based assessment of any likely impact of land use (anthropogenic) on groundwater ecosystems, particularly given the significant knowledge gaps that currently exist. In a complex karst system such as Takaka, natural variability and allogenic and autogenic process will also need to be considered. For this to be achieved, long term studies are required in the field coupled with laboratory assessments of toxicological effects on endemic and key species, both for short- and long-term impacts.

Table 5: Stygobiotic species (total 22) tested in 18 studies identified from 1977-2018, along with life stage (A: adults; C: copepodites NR: not reported) and the chemicals tested per species. MLSS: maturated lead secondary smelting slags. (Di Lorenzo et al. ,2019).

Species	Taxon	Life stage	Chemicals
Metacrangonyx spinicaudatus	Amphipoda	A	NH_4^+, Cd^{2+}, Cu^{2+}, Pb^{2+}, Zn^{2+}
Niphargus aquilex	Amphipoda	A	Cd^{2+}, Zn^{2+}
Niphargus rhenorodanensis	Amphipoda	A	NaAsO2, Cr^{6+}, $C_{15}H_{22}ClNO_2$ (S-metholachlor), $C_6H_{10}ClN_5$ (desethylatrazine),
Niphargus rhenorodanensis	Amphipoda	A	S-metholachlor + desethylatrazine, MLSSS
Niphargus inopinatus	Amphipoda	A	C_7H_8
Caecidotea bicrenata	Isopoda	NR	NaClO, $CdCl_2$, $ZnSO_4 \cdot 7H_2O$
Caecidotea stygia	Isopoda	A	Cd^{2+}, Cu^{2+}, Cr^{6+}
Proasellus cavaticus	Isopoda	A	Cd^{2+}, Zn^{2+}
Proasellus strouhali strouhali	Isopoda	NR	$C_{21}H_20Cl_2O_3$ (permethrin), NaClO
Proasellus lusitanicus	Isopoda	A	$K_2Cr_2O_7$; $CuSO_4 \cdot 5H_2O$
Proasellus assaforensis	Isopoda	A	$K_2Cr_2O_7$; $CuSO_4 \cdot 5H_2O$
Proasellus slavus vindobonensis	Isopoda	A	KCl, KNO_3
Typhlocirolana haouzensis	Isopoda	A	Cd^{2+}, Cu^{2+}, Pb^{2+}, Zn^{2+}
Orconectes australis australis	Decapoda	A	NaClO (free chlorine, total residual chlorine)
Parastenocaris germanica	Copepoda	A and C	$CdCl_2 \cdot 2H_2O$, $ZnSO_4 \cdot 7H_2O$, $C_6H_4Cl_2O$ (3,4-dichlorophenol)
Parastenocaris germanica	Copepoda	A	$C_7H_{14}N_2O_2S$ (Aldicarb), C_6HCl_5O (pentachlorophenol), $C_6H_{12}N_2S_4$ (Thiram)
Budderoo cyclopoid	Copepoda	NR	$NaAsO_2$, $K_2Cr_2O_7$, $ZnSO_4 \cdot 7H_2O$
Somersby cyclopoid	Copepoda	NR	$NaAsO_2$, $K_2Cr_2O_7$, $ZnSO_4 \cdot 7H_2O$
Somersby harpacticoid	Copepoda	NR	$NaAsO_2$, $K_2Cr_2O_7$, $ZnSO_4 \cdot 7H_2O$
Diacyclops belgicus	Copepoda	A	NO_3NH_4, CH_4N_2O, $C_{15}H_{19}N_3O_4$ (Imazamox), Ariane II[a], Imazamox + NO_3NH_4
Diacyclops n. sp	Copepoda	A	KCl
Diacyclops sp. aff. disjunctus	Copepoda	A	KCl
Fabaeformiscandona wegelini	Ostracoda	A	KCl
Trichodrilus tenuis	Oligochaeta	A	Cd^{2+}, Zn^{2+}

[a] The herbicide Ariane II is a mixture of Fluroxypyr, Chlorpyrlid and MCPA.

3.4 RECOMMENDATIONS

Although most of these sites have been sampled more than once, it was only during the third sampling round that a noticeable number of taxa were recovered from some bores/wells. With more baseline data, over time, ecological samples can be compared with environmental data (e.g. water depth, nitrogen-nitrate, conductivity etc) allowing statistically robust analysis of this groundwater ecosystem. In conjunction with microbiological data, we could develop a better understanding of the ecosystem health of this area by combining data across the food web/ecosystem to include both microbiological and meiofauna data e.g. bacteria and protists.

We recommend that to improve knowledge on GDE's:

- Groundwater ecological sampling continues in order to catalogue the unique groundwater species in this region;
- Inclusion of meiofauna sampling continues to provide a more complete picture of groundwater ecosystem present;
- Stygofauna samples collected are taxonomically identified to the lowest level possible (bearing in mind that there may be new species present);
- DNA barcodes are generated from taxonomically identified stygofauna in order to develop a database for inclusion in eDNA identification in the future;
- Microbiological data is linked with the stygofauna data to determine any potential relationships/ecological significance;
- Experimentation for optimal microscopy identification - A number of individuals recovered had stained well with Rose Bengal. However, it did not stain all organisms identified. A combined series of staining solutions to increase observations of organisms under the microscope could be beneficial.

A national programme on GDE's encompassing the above recommendations for the nations aquifers will also aid in the understanding of the significance of GDE's and their abundance, occurrence and diversity including temporal variability both locally and regionally.

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