CRCCARE
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Final Report

Stygofauna direct toxicity assessment

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Executive summary

BHP Iron Ore leases several sites for mining operations which are potentially impacted by per- and poly-fluoroalkyl substances (PFAS) used in fire training activities. The presence of PFAS is of increasing concern due to the persistence and bioaccumulative nature of the compounds, with ongoing studies for the establishment of threshold levels to human health and the environment. The PFAS detections at BHP sites in Western Australia (WA) are of particular importance to groundwater habitats since this region has an abundance of subterranean fauna, with the Ethel Gorge aquifer stygobiont community representing the primary focus of this study. However, there is currently no directly comparable Commonwealth criteria to assess risks to stygofauna communities and in accordance with the Australian and New Zealand Guidelines for Fresh and Marine Water Quality (ANZG), direct toxicity assessment was required to allow for the derivation of a site-specific guideline/threshold value.

CRC CARE was engaged by BHP Iron Ore to assess the ecotoxicological impacts of PFOS using samples from nominated sites. The research team worked collaboratively with BHP to investigate: (1) contamination levels of PFAS in groundwater samples from nominated sites; (2) status of stygofauna distribution and abundance in groundwater wells; and (3) toxic effects of PFOS on selected stygofauna (*Diacyclops humphreysi*) species. PFOS was the focus of this assessment given the current Commonwealth guidance regarding PFAS toxicity (i.e., PFOS is the primary concern) and it is one of the main compounds found in these BHP Iron Ore sites which are potentially contaminated by PFAS used in fire training activities.

(1) Groundwater analysis:

The groundwater samples were analysed for the occurrence of PFAS and precursors using solid phase extraction and total oxidisable precursor assay [1]. Results indicated trace levels of PFAS in the groundwater samples. All the samples showed concentrations of PFAS below the drinking water guideline values 0.07µg/L (∑PFAS<0.07µg/L) except sample HEC0448 (∑PFAS=0.093µg/L). TOPA analysis showed PFAS precursors in samples W028 and HEC0448.

The groundwater samples had temperatures ranging from 26.2-30.3 °C (mean 28.7 °C), pH ranging from 7.01-8.69 (mean 7.4), EC ranging from 927-6454 µS/cm (mean 2074 µS/cm), and dissolved oxygen ranging from 4.1-51.2 % (mean 29.9 %). Most of the samples contained larger amounts of inorganic carbon than organic carbon. The cations in the groundwater samples indicated Sr and Ba were found in all the samples, and Mn in most samples (Table 7). Sodium was the major cation in all samples.

(2) Stygofauna species

Stygofauna sampling was conducted in March 2021 and a total of 17 groundwater samples were analysed to evaluate the species abundance of stygofauna. Total 252 individual specimen were identified across nine different families. Comparison with previous monitoring results for the same wells indicated variations of stygofauna species, which can be attributed to several potential reasons, including sampling seasons influencing the breeding conditions and life span, temperature/rainfall, groundwater chemistry, groundwater table levels, and the presence of exotic compounds. Long-term monitoring is recommended for the response of the stygofauna community with reference to any changes induced by human activities.

(3) Toxicity study

The live samples of stygofauna (*Diacyclops humphreysi*) were collected for the toxicity study and exposed to different concentrations of PFOS. In total 220 *Diacyclops humphreysi* copepod specimens were used from four groundwater samples, from wells W028, W029, HHS000019M, and T0399. The copepods were acclimatised in filtered groundwater for 48 hours prior to being exposed to different concentrations of PFOS which were prepared using the filtered groundwater as well. The individual specimens were kept in 20 mL solution with lids closed and kept in the dark in a constant temperature room (20ºC). The samples were checked at day 4, 7, 14, 28, 42, 56 for their mobility and mortality, which were further confirmed by investigation under microscope.

The mortality increased with increasing PFOS concentration. The logistic model fitting showed R2=0.95. The LC50 calculated was 238 ± 48.4 µg/L. The values of parameters were used to calculate LC10, which was 139 µg/L. There is a stimulation effect at smaller concentrations (0.1, 1 µg/L), which requires further verification using additional toxicity studies. The results were based on copepods *(Diacyclops humphreysi*) from mixed wells which could also contribute to variations in end point values obtained. The results also indicated increased mortality with time of exposure to PFOS, including in the control sample with no PFOS.

This toxicity study was scored against the Australian and New Zealand Environment and Conservation Council (ANZECC) & Agriculture and Resource Management Council of Australia and New Zealand (ARMCANZ) guidelines which indicated the high quality of this study for the derivation of LC50 values for the groundwater toxicity [2]. Future studies should aim to replicate the results in this study as well as testing the toxicity of PFOS in other stygofauna species and with other PFAS compounds.

In summary, the groundwater wells showed low concentrations of PFAS and below the drinking water guideline values 0.07µg/L (∑PFAS<0.07µg/L) except sample HEC0448 (∑PFAS=0.093µg/L). The low level of precursors was determined in samples W028 and HEC0448. Such results were based on samples collected using bailer along with sampling of stygofauna. Stygofauna identification for the groundwater wells indicated 252 individual specimen belonging to nine families. Several potential reasons may contribute to the variation, including sampling seasons influencing the breeding conditions and life span, temperature/rainfall, groundwater chemistry etc. The PFOS toxicity study performed with the copepod species *Diacyclops humphreysi* indicated this specific species of stygofauna are relevant tolerant to PFOS with the LC levels being around 1000 times higher than the PFAS levels determined. However, this is limited to the current species and groundwater wells obtained in this study. As a large groundwater habitat, further toxicity studies and monitoring programs are recommended to obtain further information on the effect of PFOS to the whole array of stygofauna species present in this habitat. It is recommended that additional information should be gathered on the most abundant, sensitive and representative species on PFOS toxicity to stygofauna. Further studies on screening and toxicity tests that represent the whole array of sensitive species present in this area are needed. It would also be important to investigate the sensitivity of stygofauna species to a greater range of PFAS.

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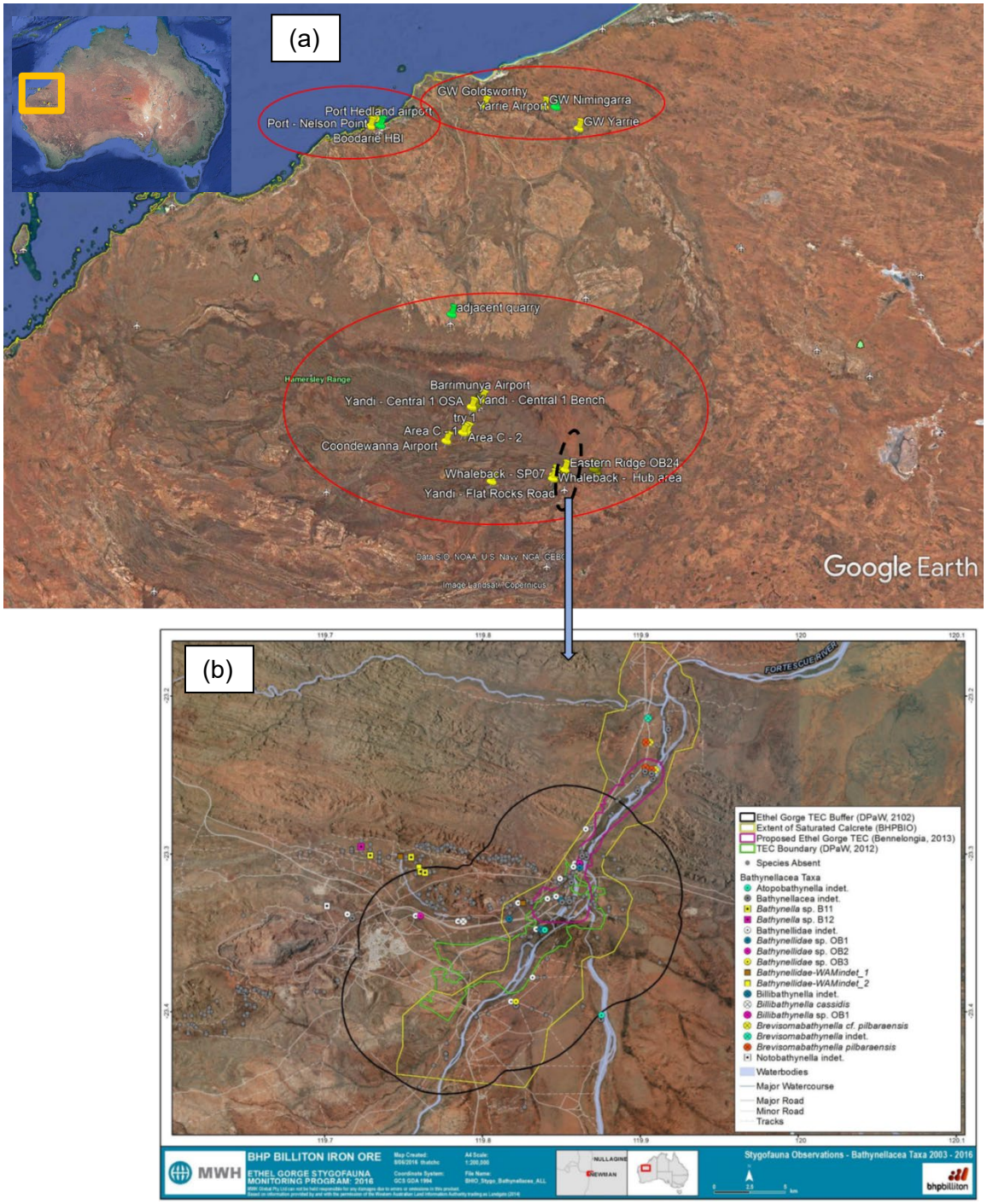
1. Introduction

BHP Iron Ore has several sites which are potentially impacted by per- and poly-fluoroalkyl substances (PFAS). The area of potential impact is illustrated in Figure 1a. These sites have been affected by the historical utilisation of aqueous film forming foam (AFFF) for firefighting activities, e.g. mining sites, airports. These sites have been classified into three categories according to the exposure pathways and potential receptors of the contamination risks according to a previous report [3]: (1) negligible, low or moderate/low potential risk (11 sites); (2) moderate to high risk (7 sites); and (3) moderate to high risk based on limited information (6 sites). However, the risk categories identified in the preliminary site assessment [3] were based on the potential exposure and the probability of completion of an exposure pathway to the receptors and the frequency of exposure. There is a lack of detailed sampling and delineation of PFAS composition and concentration on these sites.

The groundwater resources in the Pilbara are mainly alluvial, sedimentary or fractured rock aquifers [4, 5]. The Pilbara is a large, dry, thinly populated region in the north of Western Australia. It has a population of around 50,000 with a population density of 0.17 persons per square kilometre [5]. It is known for its indigenous people; its ancient landscapes; the red earth; and its vast mineral deposits, in particular iron ore [5]. Ethel Gorge/Ophthalmia Basin has an alluvium calcrete aquifer. Some of the Groundwater Assessment Area lies within the Threatened Ecological Community referred to as the Ethel Gorge Aquifer Stygobiont Community (hereafter Ethel Gorge TEC) (Figure 1). Ethel Gorge TEC resides in the Ethel Gorge (Ophthalmia Basin) alluvium calcrete aquifer on the Fortescue River in the vicinity of the town of Newman. Drawdown within the Groundwater Assessment Area is associated with below water table extensions to the approved mine pits at Orebody 24 and the development of new below water table mine pits at Orebody 25 West.

The contamination of PFAS in soils leads to groundwater contamination as well, which is of great concern to BHP, the wider community, and regulatory agencies. This is important and especially for groundwater ecological systems. There are two types of subterranean fauna, stygofauna and troglofauna, identified in groundwater systems. Troglofauna occur deep underground between the surface soil layers and the water table, while stygofauna live in the groundwater. The Pilbara region in WA is a globally important area for subterranean fauna and has a very rich variety of subterranean species. Some important relictual species and some outstandingly diverse species include those recorded for stygofaunal ostracods and troglofaunal schizomids. A report from Bennelongia Environmental Consultants [6] indicated that the Pilbara supports 500-550 species of stygofauna and more than 650 morphospecies of troglofauna have been collected from the Pilbara to date. The total number of species present has not been estimated but is likely to be much higher. Due to the vulnerability and sensitivity of the species, the presence of potential PFAS contaminants on sites would influence species distribution, types, and density of stygofauna. However, such information is lacking in the current literature and industrial studies.


(a)
GW Goldsworthy
Yarrie Airport
GW Nimingarra
GW Yarrie
Port Hedland airport
Port - Nelson Point
Boodarie HBI
adjacent quarry
Hamersley Range
Barrimunya Airport
Yandi - Central 1 OSA
Yandi - Central 1 Bench
try 1
Area C - 1
Area C - 2
Coondewanna Airport
Eastern Ridge OB24
Whaleback - SP07
Whaleback - Hub area
Yandi - Flat Rocks Road
Google Earth
(b)
FORTESCUE RIVER
Ethel Gorge TEC Buffer (DPaW, 2102)
Extent of Saturated Calcrete (BHPBIO)
Proposed Ethel Gorge TEC (Bennelongia, 2013)
TEC Boundary (DPaW, 2012)
Species Absent
Bathynellacea Taxa
Atopobathynella indet.
Bathynellacea indet.
Bathynella sp. B11
Bathynella sp. B12
Bathynellidae indet.
Bathynellidae sp. OB1
Bathynellidae sp. OB2
Bathynellidae sp. OB3
Bathynellidae-WAMindet_1
Bathynellidae-WAMindet_2
Billibathynella indet.
Billibathynella cassidis
Billibathynella sp. OB1
Brevisomabathynella cf. pilbaraensis
Brevisomabathynella indet.
Brevisomabathynella pilbaraensis
Notobathynella indet.
Waterbodies
Major Watercourse
Major Road
Minor Road
Tracks
MWH
BHP BILLITON IRON ORE
ETHEL GORGE STYGOFAUNA MONITORING PROGRAM: 2016
Stygofauna Observations - Bathynellacea Taxa 2003 - 2016
bhpbilliton

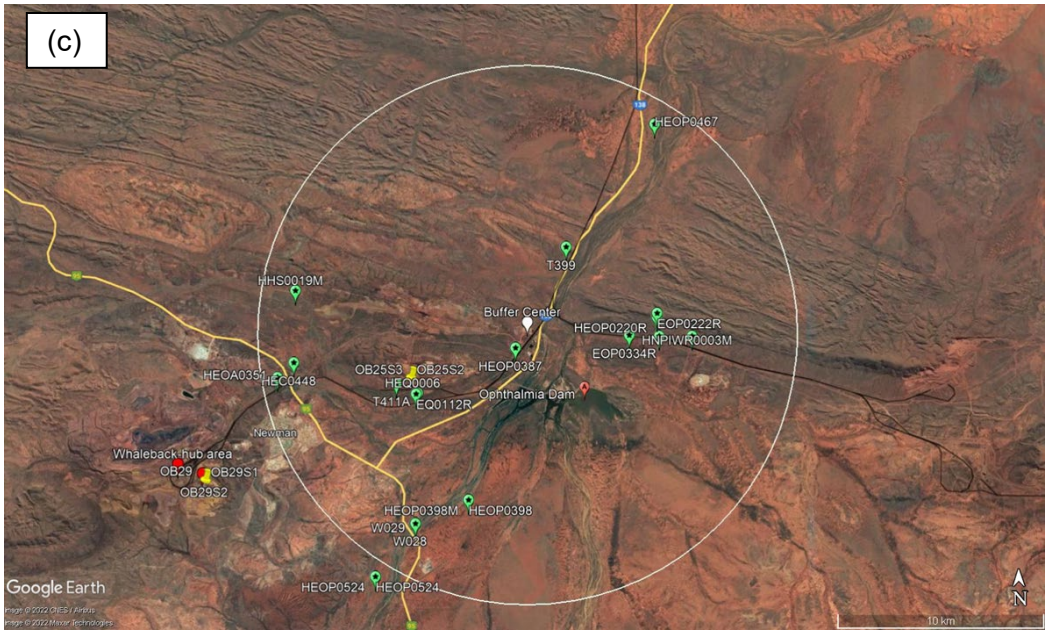


Figure 1 The location of study area. (a) the overall sites that are potentially impacted by PFAS [3] shown by red outlines; (b) the location of the TEC (Threatened Ecological Community) area [7]; (c) the location for sampling area (green – sampling locations in this study; red and yellow - soil samples in other study indicating PFAS occurrence; circle indicating R= 13 km buffer zone)

CRC CARE was engaged by BHP Iron Ore to assess the ecotoxicological status of PFAS on sites, and particularly to conduct ecological studies on the impacts of PFAS on stygofauna. This is a sensitive biological indicator of the groundwater ecosystem's health. Assessment of groundwater ecosystems may not be necessary for routine contaminated site investigations, but nonetheless should be encouraged in situations where groundwater contamination poses a risk to areas of ecological significance and conservation value.

The project aims to determine the characteristics and key factors influencing the toxicity of PFAS in samples originating from BHP contaminated sites. This is achieved through controlled experiments, sampling and analysing of stygofauna from samples in nominated sites. Due to limited sampling events, the report only focuses on the results obtained using the available samples. The main objectives of the project included:

- identifying the concentration of PFAS and precursors in groundwater
- investigating the types, species, and abundance of stygofauna in groundwater samples from Ethel Gorge (Ophthalmia Basin) aquifer in Pilbara region of WA
- toxicity testing using live stygofauna samples obtained.

The results obtained in the project will help BHP develop a strategic approach to managing PFAS at contaminated sites, given the company's need to respond to potential PFAS contamination.

2. Stygofauna distribution in BHP areas

The CRC CARE team was engaged by BHP for the assessment of stygofauna present in PFAS-impacted areas in Western Australia's Pilbara region. The team conducted a desktop review focusing on the two reports provided by BHP (a-b below). Further reports were provided by BHP (c-f) to facilitate the design of the sampling plan. The available literature was also referred to in this section:

a. Ethel Gorge Aquifer Threatened ecological community consolidated taxonomy, by Subterranean Ecology Scientific Environmental Service in 2013

b. Characterisation and mapping of Ethel Gorge Aquifer Stygobiont Threatened Ecological Community, by Bennelongia Environmental consultants in 2013

c. Ethel Gorge Stygofauna Monitoring Program: 2016

d. Ethel Gorge Stygofauna Monitoring Program: 2017

e. Orebody 23/24/25 and Jimblebar Discharge Stygofauna Monitoring 2013 – 2014

f. Technical Review Salinity Tolerance of Ethel Gorge Stygofauna TEC.

2.1 The history of BHP stygofauna research

The Ethel Gorge and Newman area (Figure 2) aquifers harbour one of the most stygofauna-rich habitats recorded in Australia as well as internationally. This understanding has been the result of extensive survey efforts conducted over more than 20 years by BHP. Therefore, the taxonomic richness of the Ethel Gorge stygobiont community is probably better characterised compared to any other such diverse subterranean communities in Australia.

The EPA Western Australia (EPA WA)'s conditions for approval to mine below the water table at Orebody 23 (OB23) were granted by the EPA WA in June 1998, subject to a number of conditions relevant to stygofauna (Ministerial statement 478- Attachment B) and includes "Commitment 2" which states:

> "*BHP Iron Ore to continue its support for research into the morphological and molecular variation of stygofauna in the Orebody 23 and expand research into the wider Ophthalmia region*".

This approval to mine below the water table at OB 23 was again granted in January 2006 (Ministerial statement 712).

This condition was supported by BHP as well as many other independent research institutions and researchers, and many papers and reports have been published describing the morphology and molecular genetics of the Ethel Gorge stygofauna since the first collections done by Eberhard and Humphreys [8]. Eberhard and Humphrey's work led to the Threatened Ecological Communities Scientific Committee (TECSC) recommending that the Department of Environment and Conservation (now DPaW) list the Ethel Gorge Aquifer stygobiont community as a Threatened Ecological Community (TEC).

The Ethel Gorge TEC is currently categorised as Endangered. This means in effect an ecological community that has been adequately surveyed and subject to a major contraction in area and/or was originally of limited distribution and is now in danger of significant decline throughout its range or severe compromise or destruction over most of its range in the near future. Of the three criteria for determining endangerment, the Ethel Gorge TEC is listed under B (current distribution is limited) and ii) (there are few occurrences, each of which is small and/isolated and all or most occurrences are very vulnerable to known threatening processes).

As an outcome of reviewing the stygofauna sampling results by Bennelongia Pty Ltd as stated in its 2013 published report, “Characterisation and mapping of Ethel Gorge aquifer stygobiont threatened ecological community”, it is suggested that the stygofauna community in the wider Newman area is richest around OB23 and in the Lower Ophthalmia and Central Ophthalmia areas. Stygofauna sampling results do not indicate the occurrence of a rich community further south where DPaW considers most of the TEC to be.

2.2 Summary of stygofauna distribution

The BHP reports, namely “Characterisation and Mapping of Ethel Gorge Aquifer Stygobiont Threatened Ecological Community” and “Ethel Gorge Aquifer Threatened Ecological Community Consolidated Taxonomy”, were reviewed by the CRC CARE research team to understand the occurrence, abundance and the types of stygofauna present in the Ethel Gorge Aquifer area in the Pilbara region of WA. The Ethel Gorge Aquifer Threatened Ecological Community is illustrated in Figure 1 in the report [9].

Diversity of stygofauna

BHP has undertaken research and monitoring programs on groundwater ecosystems for nearly 23 years. Stygofauna is one of the key ecological indicators that can serve as a legitimate indicator of the health of such ecosystems, since they are very sensitive to changes in their habitat [10, 11]. Surveys and monitoring were carried out to understand and manage the effects of mining in the Ophthalmia Floodplain which hosts the Ethel Gorge Aquifer and its groundwater-dependent stygofauna community. This stygobiont community is listed as a Threatened Ecological Community (Ethel Gorge TEC) [12].

Eighty-two species of stygofauna are said to be listed from the Ethel Gorge Aquifer and/or adjacent local groundwaters in the Newman area. At least 45 of these species are said to be obligate groundwater species (stygobionts). Of the 82 taxa in the consolidated list, 40 species are described in the scientific literature and 42 are undescribed. 95% of taxa from these stygofauna are recognised species or morpho-species/haplotypes. The systematic composition was summarised in the report by Subterranean Ecology and is shown in Table 1 [9].

Table 1 Summary of taxonomic diversity recorded from the Ethel Gorge Aquifer and/or adjacent connected groundwater catchments in the Newman area [9].

Higher classification	Families	Genera	Species
Phylum Platyhelminthes (flatworms)			1
Phylum Aschelminthes			
Class Nematoda (round worms)			1
Class Rotifera (rotifers)			1
Phylum Annelida			
Class Aphanoneura (aphanoneurans)			1
Class Oligochaeta (oligochaetes)	3	5+	16
Phylum Arthropoda			
Class Arachnida			2
Order Acariformes (mites)	2	2	
Subphylum Crustacea			
Class Malacostraca			
Order Amphipoda (amphipods)	1	3+	7
Order Bathynellacea (bathynellaceans)	2	4+	8
Order Isopoda (isopods)	2		2
Class Maxillopoda			20
Subclass Copepoda (copepods)		13	
Class Ostracoda (ostracods)	4	13	23
Total			**82**

Distribution of stygofauna

The report by Subterranean Ecology [9] summarised the distribution of stygofauna in relation to the 20 km buffer zone previously defined by DPaW (Department of Parks and Wildlife) as shown in Table 2 below. Their study area was centred on the Ethel Gorge aquifer in the vicinity of Ethel Gorge / Opthalmia Dam / OB23/OB25 and the surrounding catchment of the Fortescue River and tributaries (Homestead Creek, Warrawand Creek, Shovelanna Creek) within a radius of approximately 20 km [9].

Table 2 Summary of known distribution in relation to the old 20 km TEC buffer zone [9].

Distribution	No. Taxa	As % of total number of taxa	Explanation
Inside 20 km TEC buffer only	39	47.6%	Currently recorded only from inside TEC buffer
Also outside 20 km TEC buffer	40	48.8%	Also recorded outside TEC buffer zone, may be localised SRE or widespread
Unknown	3	3.7%	Not identified to species level so distribution not defined
Total	82	100%	

Mining operations, such as land excavation and dewatering at OB23 and OB25 may be harmful to the Ethel Gorge TEC due to the loss of stygofauna habitat. Furthermore, discharging excess water from the Jimblebar Iron Ore Project and Orebody 29 into the Ophthalmia Dam was identified as a threat that may change the chemistry of groundwater in the Ophthalmia Floodplain aquifer units. As such, a number of conditions relevant to monitoring and management of stygofauna communities potentially impacted by the mining activities were outlined in each Ministerial Statement and Licence to Operate [9].

Mapping species richness

In practice, species-rich bores will typically be identified by the first sample (one sample collects nearly half the abundant and one-quarter of the rare species occurring in the near vicinity of a bore [13, 14]. This is supported by comparing the sample effort and species richness maps, which strongly suggest sample effort is not the key factor driving the observed richness within Ethel Gorge; some well sampled bores have only a few species.

Bennelongia Environmental consultants listed 78 stygofauna species collected in the wider Newman area from 2007-2013 [15]. These 78 species belonged to 11 higher level groups: flatworms, nematodes, rotifers, aphanoneurans, oligochaetes, mites, ostracods, copepods, syncarids, amphipods and isopods (Table 3.1 in their report). Groups that are represented by multiple species include Oligochaeta (16 species), Acariformes (2), Ostracoda (21), Copepoda (19), Amphipoda (8), Syncarida (6) and Isopoda (2). Turbellaria, Nematoda, Rotifera and Aphanoneura were presented as having one species each, but species level identification was not attempted. This is due to the lack of a taxonomic framework for these groups, and it is possible that more species are present.

Stygofauna richness (species per bore) was more prevalent in Ethel Gorge, and upstream to about 1.5 km below the Ophthalmia Dam wall, than in surrounding areas. Some moderately rich bores are located about 4 km downstream of Ethel Gorge.

Limitations to stygofauna mapping: Identifying the existence of a subterranean TEC and mapping its boundary is especially difficult, compared with identifying terrestrial TECs, because subterranean fauna sampling is inefficient, and patterns of species co-occurrence are difficult to determine.

Indicator species for ecological systems of TEC

Ideally, indicator species will occur in sufficiently high abundance across the TEC to be collected in most of the samples gathered within the TEC boundary. No species is likely to meet this criterion well and, in fact, it is doubtful that any species has a range coinciding with the TEC boundary. It might not be possible to find a species which covers a range of TEC, however sentinel or sensitive species can be used for the purpose of an ecological indicator species, and we will be able to protect a whole range of, if not all the stygofauna. The only species with ranges coinciding with the sampling areas making up the Ethel Gorge aquifer stygobiont community (TEC) (Figure 1 b) were the copepods *Nitocrella* OB (B08) and *Fierscyclops* (Pilbaracyclops) *supersensus,* with the latter species occurring in very low numbers according to the report titled, “Ethel Gorge Aquifer Stygobiont Threatened Ecological Community 2013”. Four other species (ostracods *Gomphodella hirsuta*, *Origocandona inanitas*, *Pilbaracandona colonia* and amphipod *Maarrka etheli*) were also collected by Bennelongia Pty Ltd, only across the TEC during wider Newman area sampling in 2013. They are known to have distributions extending beyond the Newman area. Given that four - more widely distributed species - had ranges coinciding with the TEC, it is likely the two copepod species also have wider ranges. The amphipod species *Chydaekata acuminata* might also be viewed as a potential indicator species because it is highly abundant. However, its range extends east of the TEC at OB23.

Identifying the extent of the community through formal multivariate analysis was not possible because of the high proportion of bores that yielded very few species and the very sparse occurrence of nearly all species. Thus, few bores had sufficient species for meaningful calculation of relationships. In such situations most clustering is not biologically meaningful [16]. This problem is characteristic of most stygofauna communities and, despite the large overall sampling effort in the wider Newman area, applies to analyses based around individual samples. The problem is principally the result of the poor method of stygofauna sampling caused by low animal densities [13].

2.3 Summary of PFAS-contaminated sites

PFAS have been manufactured and distributed since the early 1960s [17]. PFAS substances are resistant to biodegradation, atmospheric photooxidation, direct photolysis, and hydrolysis, and are very soluble in water due to their chemical structure [16]. There are thousands of PFAS compounds being detected in contaminated sites resulting from the past use of AFFF formulations, surfactants, solvents, metal plating, aviation and photography. The PFAS compounds include perfluoroalkyl acids (PFAAs) (e.g. perfluoroalkyl carboxylates (PFCAs; $C_nF_{2n+1}COO^-$) and sulfonates (PFSAs; $C_nF_{2n+1}SO_3^-$)), perfluoroalkane sulphonamides (FASAs) (e.g. MeFASAs, EtFASAs), fluorotelomer substances (e.g. n:2 fluorotelomer alcohols (n:2 FTOHs), n:2 fluorotelomer sulfonic acids (n:2 FTSAs), and fluorotelomer carboxylic acids (FTCAs)), and perfluoroalkane sulfonamides (e.g. FASAAs, MeASAAs).

The H atoms on the alkyl chains in PFAS are fully or partially replaced by F atoms. The presence of the strong C-F bond leads to their high chemical and thermal stability, and great oxidative resistance. As such, their persistence and bioaccumulation potential results in their toxicity and concerns over environmental receptors and human health. The typical compounds include perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) which were classified as priority contaminants by the Stockholm Convention in 2001. PFAS behave very differently to other organic contaminants and have generated major concerns for soils and groundwater systems. For example, toxic outcomes for embryo and larval development, reproduction and stress response have been reported in oviparous and viviparous freshwater fish when exposed to PFOS [18-21]. In particular, oxidative stress has been suspected as being one of the chief causes of the observed toxicity in zebrafish embryo development [22].

The current toxicity information is focused on PFOS, PFOA, and to some extent PFHxS in freshwater and marine water organisms. Only limited research has been done on PFAS toxicity to subterranean fauna. Research on the effects of environmental factors modifying the toxicity of PFOS and PFOA is also sparse.

PFAS compounds of current concern in Australia include PFOS (perfluorooctane sulfonate), PFHxS (perfluorohexane sulfonate) and PFOA (perfluorooctanoic acid).

CRC CARE was engaged by BHP to conduct soil sampling and analysis around the Newman area. The results for samples collected near OB25 and OB29 of the BHP Newman area indicated PFAS contamination of soil and groundwater. For example, soils collected from potential source areas near OB25 indicated total PFAS ranging from 5.5 to 13.3 µg/kg, while total PFAS from source areas near OB29 varied from 50.8 to 1630 µg/kg. The two groundwater samples collected from source areas near OB29 have total PFAS as 8.9 and 11.6 µg/L. The sites are closest to the TEC.

Bores located near mining related activities, which may be introducing PFAS contamination in soils and surface water, are shown in Figure 2 and a summary of the presence of stygofauna in these bores is provided in Table 3.

Table 3 Summary of stygofauna occurrence in borewell samples collected in the Pilbara region.

OBJECT ID	Latitude	Longitude	BHPB_DRILL	START DATE	STYGO_ HAUL_ Number	COMMENTS
1	-23.40202	119.84037	EA0285R	20/04/2017	6	Stygofauna Present
2	-23.33632	119.88015	EEX931	19/04/2017	6	Stygofauna Absent
3	-23.318639	119.848889	HEA0121	21/04/2017	6	Stygofauna Present
4	-23.31572	119.85225	HEA0126	21/04/2017	6	Stygofauna Present
5	-23.31688	119.85133	HEA0133	21/04/2017	6	Stygofauna Absent
6	-23.33926	119.76085	HEOP0317M	19/04/2017	6	Stygofauna Absent
7	-23.32787	119.844272	HEOP0387	19/04/2017	6	Stygofauna Present
8	-23.328384	119.844272	HEOP0388	19/04/2017	6	Stygofauna Present
9	-23.39365	119.82127	HEOP0398M	20/04/2017	6	Stygofauna Present
10	-23.32799	119.85805	HEOP0417	19/04/2017	6	Stygofauna Present
11	-23.3258	119.87186	HEOP0425	19/04/2017	6	Stygofauna Present
12	-23.265056	119.886639	HEOP0462M	18/04/2017	6	Stygofauna Present
13	-23.299192	119.865761	HEOP0504	18/04/2017	6	Stygofauna Present
14	-23.42649	119.77696	HEOP0524	20/04/2017	6	Stygofauna Present
15	-23.30616	119.86157	HEOP0574M	18/04/2017	6	Stygofauna Present
16	-23.333925	119.854868	HEOP0798M	19/04/2017	6	Stygofauna Absent
17	-23.32691	119.84976	OB23REG1	19/04/2017	6	Stygofauna Present
18	-23.28426	119.86863	T399	19/04/2017	6	Stygofauna Present
19	-23.34273	119.78778	T411A	20/04/2017	6	Stygofauna Present
20	-23.40333	119.79598	W028	20/04/2017	6	Stygofauna Present
21	-23.24656	119.90713	W116	18/04/2017	6	Stygofauna Present
22	-23.2453	119.90337	W117	18/04/2017	6	Stygofauna Present
23	-23.213889	119.904972	W231	18/04/2017	6	Stygofauna Absent
24	-23.308194	119.86075	WP56	18/04/2017	6	Stygofauna Absent

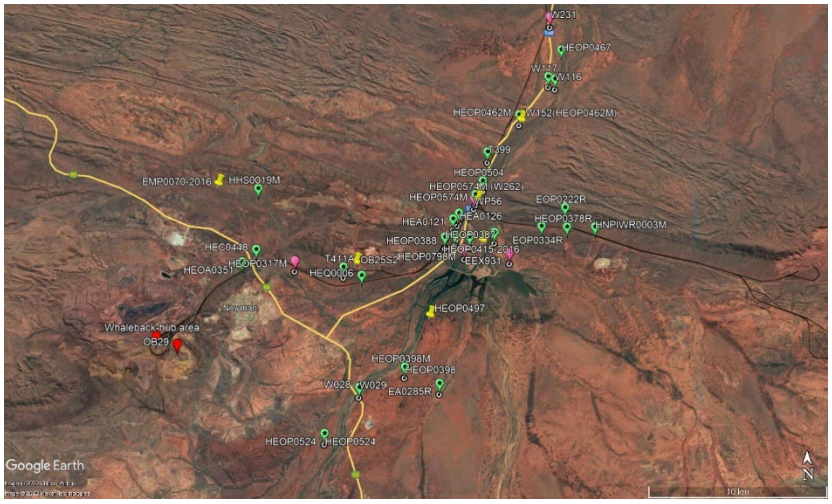


Figure 2. Locations indicating stygofauna sampling and PFAS contamination sampling from previous BHP reports and research studies
(Green – stygofauna presence; pink – stygofauna absence; yellow – soil sampling)

3. Literature review

3.1 Background

Water hidden below the earth's surface accounts for 98% of the planet's freshwater sources [23]. On average groundwater provides one-third of all the freshwater consumed in the world. It is evident from the available literature data that groundwater is being contaminated globally, with many chemicals including pesticides, hydrocarbons and heavy metals both due to anthropogenic as well as geogenic reasons [24-26]. However, current groundwater regulations are not adequate for the protection of human and ecosystem health. The organisms found in groundwater are fauna with primitive or rudiment sensory organisms such as blinded eyes. These organisms are called stygofauna. The term "stygo" originated from the ancient Greek language meaning "boundary river of the underworld", meaning that stygofauna are organisms that live in dark and damp underground environments.

Such groundwater bodies lack light, oxygen and carbon sources [27]. Therefore, the fauna which have lived for a long time in these low energy environments, have many adaptations to suit their habitats. For example these taxa often have lost eyesight or retained primitive eyes, have lost skin pigmentation and rely mainly on sensory tentacles for senses [28]. They also have very low metabolic and reproductive rates which means that culturing them in laboratories is a difficult task. These groundwater-dwelling animals are mainly dominated by crustaceans. These fauna are often highly sensitive to any changes in groundwater quality including contamination by anthropogenic chemicals [29].

Hahn et al. [30] found that changes in groundwater were well captured by monitoring changes in the stygofauna community, including abundance/density and community structure. In fact, hydrogeochemical changes were evident way before other ecological indicators in the surface water system picked up the changes. These results suggested the need for a stygofauna-based groundwater monitoring program. In addition to monitoring hydrogeological parameters, monitoring stygofauna community structure and abundance will provide indications of changes in ground water quality. Biological indicators are better than chemical analyses, as the former can illustrate the effects of bioavailability and toxicity of a chemical, integrating many other factors that influence adverse effects on the ecosystem or human health. These include, for example, pH, antagonistic or synergistic effects of chemicals, environmental conditions, as well as changes in bioavailability of a substance over time [31, 32]. However, currently investigations are lacking on subterranean species worldwide [33, 34].

At present, most government authorities such as WaterNSW are concerned about the quality of surface and groundwater in terms of their physicochemical parameters, such as pH, dissolved organic carbon, electrical conductivity, among several others. However, it is important to note that there exists a unique subterranean microfauna just as important as the above ground ecosystems that can be affected by the impacts of contaminants and other environmental perturbation. Due to the lack of knowledge concerning the effects of environmental changes on the stygofauna community, and the lack of conservation efforts thereof, the risks of these stygofauna becoming extinct is great [8, 35]. Western Australia's government has recognised the need for ecological testing and risk assessment of groundwater systems and has made extensive changes to its policies and groundwater

monitoring programs to include stygofauna monitoring [36].

The research team conducted a literature review on stygofauna and toxicity studies to summarise the factors influencing the viability of stygofauna, adverse effects of hydrogeological parameters and groundwater chemistry to stygofauna, and implications for the further groundwater risk assessment. The information provided here will help all stakeholders understand two aspects: first, exposure levels of stygofauna to contaminants; and second, risk implications for groundwater ecosystems.

3.2 Factors affecting the viability of stygofauna

Stygofauna are important [37] to biodiversity and for an ecosystem balance locally and globally, because they:

- Comprise an inconspicuous but important component of biodiversity
- Contribute ecosystem services via nutrient cycling and as indicators of groundwater health
- Represent examples of adaptation and ongoing evolutionary processes
- Contain many ancient lineages of high scientific value and conservation significance
- Have many species with small distribution ranges, i.e. Short Range Endemics (SREs)
- Are vulnerable to extinction from environmental changes and human activities
- Include species and communities protected under State and Commonwealth environmental legislation
- Need to be considered as a factor in environmental assessment and approval for economic development projects in most Australian states and territories.

In the last two decades, BHP has become involved in many scientific research and biological surveys, which are often associated with environmental impact and risk assessment from mining-related activities and groundwater management practices. These activities have collectively led to a better scientific understanding of Western Australian stygofauna [8, 11].

Most stygofauna are invertebrates, predominantly crustaceans, but also include worms, snails, water mites, and diving beetles, while others are vertebrates (fish) [11]. There are many factors affecting the viability of a stygofauna species, given that they are sensitive to any changes in the quality or quantity of groundwater they inhabit, for instances changes in the water–surface barrier, and any alterations to their habitat [38, 39]. These factors include:

- Urban water supply development (groundwater pumping, surface water collection)
- Agricultural works (irrigation, fertilisation)
- Below water table drilling/mining (dewatering)
- Tailings production and storage
- Excavation works including mining
- Dust suppression
- Seepage
- Waste rock storage
- Backfilling and site rehabilitation work
- Water diversions and surface sealing
- Water storage
- Hazardous goods storage
- Salinity changes
- Level of the water table
- The direction of flow of the aquifer
- Water table level fluctuation frequency and timings
- Spring water pressure
- Physico-chemical properties of the groundwater
- Introduction of contaminants into the groundwater
- Groundwater interactions between subsurface systems and between groundwater and surface systems.

Aquifer depressurisation from works which intersect aquifers as well as a drop in water levels may limit access to groundwater, changes in surface water flow, or reduction in groundwater quality [39]. Declining water tables can lead to the substrate becoming unsaturated, which will further reduce where the stygofauna can live and breed [40].

Changes to groundwater quality can result from alterations in the groundwater table which lead to the influx of surface water contaminated with metals and organic contaminants (e.g. PFAS). Such contaminated plumes can change the direction of flow depending on many factors including hydrogeology of the aquifer and the level of the water table.

Climate change is an additional factor that can affect the viability of the groundwater-dependent ecosystems through alterations in the water table. Furthermore, anthropogenic activities such as mining can have an influence on groundwater quality. Therefore understanding the effects of mining activities or other industrial developments should not be considered in isolation [11].

Since the groundwater habitat can be perturbed by various factors, appropriate sampling is required to minimise any influences on the ecosystem and to obtain representative samples. Several important criteria have been described for a successful selection of groundwater wells for stygofauna sampling [41], as shown below:

- The well having an aperture of ≥50 mm diameter
- The well must intersect with the water table
- If lined – it should be slotted through the water column
- The wells should be vertical with a drilled depth of < 200 m
- There should be reference bores in the vicinity
- Coverage of all hydrogeological units present
- The wells can be of varying age in excess of six months and preferably undisturbed as it can affect Stygofauna viability, i.e. it should not be regularly pumped or purged
- Bores are supposed to have a level of salinity less than 5000 µS/cm EC (preferably less than 1500 µS/cm), a dissolved oxygen (DO) concentration >1 mg/L and pH range 6.5 to 7.5.

The Pilbara region is characterised by very hot summers, mild winters and low and variable rainfall. It is classified as hot desert in northern and inland areas and hot grasslands in the north-west [42]. During summer and early autumn (December to March), average daily temperatures regularly exceed 30°C across the region, with average daily maxima exceeding 35°C from October to March. In northern inland areas, such as Marble Bar, average maxima exceed 40°C during summer and temperatures higher than 45°C are common [42]. During the winter months (June to August), average temperatures are around 20°C throughout the region [42]. It is well documented that temperatures play a major role in stygofauna viability and some stygofauna can tolerate temperature changes to some extent [43]. Castaño-Sánchez et al. [44] observed 50% of groundwater crustacean population died at 6.9 °C above the ambient aquifer temperature for copepods, and more than 10°C for syncarids in Australia.

Rainfall is spatially and temporally variable in the Pilbara region, Western Australia, Australia [42]:

- Annual rainfall declines from 300–350 millimetres (mm) in the north-east to less than 250 mm in the south and west.
- Elevated areas in the Hamersley Range average more than 500 mm.
- Rainfall is greatest during summer and autumn and least during winter and spring.
- Rainfall in the eastern Pilbara is most influenced by tropical and monsoonal drivers, which are predominantly active in summer and autumn.
- Rainfall in the western Pilbara is also influenced by southern mid-latitude drivers, such as frontal systems, during autumn and winter.

Over the last 40–60 years, average annual temperatures have risen across most of Western Australia [42]. For Australia's coastal waters, between 10.5°S and 29.5°S, this

warming has already resulted in southward shifts of climate zones by >200km along the eastern coast, and by ~100 km along the western coast [45]. There is huge evidence for ocean warming both at the surface and through the water column, which is supported by global compilations [46-49]. Climate projections show very high confidence [42] for substantial temperature increases to continue in the Pilbara, with the north-west of WA warming more than elsewhere in Australia. Annual average temperature is projected to increase by 0.6–1.5°C by 2030 for all emission scenarios [42].

The groundwater resources in the Pilbara are mainly alluvial, sedimentary or fractured rock aquifers [4, 5]. Stygofauna have adapted to survive the restricted conditions of aquifers; in Australia stygofauna exist within alluvial, karstic, calcrete and certain fractured rock aquifers.

Stygofaunal activity such as burrowing and feeding assist in maintaining aquifer flow paths; therefore, an increase in microbial activities may help to maintain water quality [50]. Greater knowledge of stygofauna and their ecosystems will continue to improve our understanding of the steady aridification of Australia [51]. It is thus critical to protect the stygofauna system and groundwater ecosystem given their hydrogeological importance.

3.3 Ecotoxicity studies with stygofauna

Using stygofauna for ecotoxicity testing is a relatively “young” scientific topic and there exists challenges in using stygofauna for such testing. Given the great sensitivity of stygofauna to environmental contaminants it is imperative to develop ecotoxicity thresholds based on the studies conducted using that fauna. A few studies have investigated the ecotoxicity of stygofauna exposed to pollutants such as trace elements [52, 53]. Nevertheless, challenges exist in maintaining and culturing stygofauna under laboratory conditions. Some of these challenges include diversity in the species of stygofauna found in different geologies and the lack of knowledge on the life span/biology of each stygofauna species, and the lack of information on their susceptibility and tolerance to toxic chemicals. For example, amphipods and syncarids found in Western Australia and NSW have a shorter life span ranging from a few days to a few weeks under laboratory conditions, whereas copepods could live for months [54, 55].

Due to these challenges many toxicological studies for groundwater fauna were carried out using data obtained from surrogate freshwater fauna, such as Daphnia for which there is adequate data on their life span, culture conditions and sensitivity to environmental factors. This has aroused some concerns among the scientists who claim that such extrapolations would be erroneous as these fauna have different life styles and physiological characteristics and sensitivity compared to those in surface water habitats [52]. With their longer life spans, along with available experimental data, copepods currently remain the most suitable fauna which can be used for testing the toxicity of chemicals in groundwater [53, 56].

Copepods are reported to be a useful taxon for ecotoxicological studies as they are found in aquifers of different geologies around the globe. The study conducted by Hose et al. [53] tested the sensitivity of obligate groundwater copepods to metal contaminants including arsenic (III) (As), chromium (VI) (Cr) and zinc (Zn). They found that copepods are sensitive to As, Cr and Zn to varying degrees while they were most sensitive to Cr across all taxa. Avramov et al. [57] tested the toluene toxicity in groundwater using the amphipod *Niphargus inopinatus*. Due to the natural scarcity of these test organisms, only

a small number of animals were used per each assay at a time (which resulted in n<10 for each toxicant level in the test). The LC_{50} value where the test organisms showed 50% mortality was at 23.3 mgL^{-1} Toluene. Sofia et al. [58] investigated acute toxicity of copper sulfate and potassium dichromate on stygobiont *Proasellu* spp. and the freshwater standard species *Daphnia magna*. *Proasellus* spp. were remarkably more tolerant to chronic (long-term) exposure to dichromate than *D. magna*. However, the less groundwater adapted species *Daphnia magna* were revealed to be more tolerant to acute toxicity of both the compounds above.

Hose et al. [59] investigated the toxicity of As, Cr and Zn in the stygobitic syncarid. They found As was the most toxic to the syncarid with a 14 day LC_{50} of 0.25 mg As/L. The test organisms were collected from a fractured sandstone aquifer at Somersby, NSW, Australia. Lorenzo et al. [52] conducted an ecotoxicity study of the β-blocker propranolol using the copepod *Diacyclops belgicus*. Their results showed that propranolol did not pose a risk to groundwater bodies in Europe at the concentrations investigated in their study. Burton et al. [60] and Di Marzio et al. [56] noted the sensitivity of copepods, such as *Bryocamptus zschokkei* and *B. praegeri* as potential bio-indicators for metal pollution. They concluded these potential test organisms would be more suitable to protect meiofaunal communities which are small invertebrates that live in both marine and fresh water environments [56].

The available ecotoxicity studies that used stygofauna as the test species are summarised in Table 4. Currently no ecotoxicological studies used stygofauna in Australia as the test species in assessing the toxicity of PFAS, which is particularly relevant to the sites identified in WA and other sites of concern with abundance of stygofauna. The bioavailability / bioaccumulation and ecotoxicological studies for PFOS/PFOA to surface and marine biota from other parts of the world are summarised in Table 5.

One ecotoxicity study from Canada using stygofauna [61] was performed to test toxicity to perfluorinated acids (PFAs) degradation products using Amphipod, *Hyalella Azteca.* The perfluorinated acids (PFAs) counterparts/degradation products used include 6:2, 8:2, and 10:2 saturated (FTsCA) and unsaturated (FTuCA) fluorotelomer carboxylic acids. They found that the *H. azteca* was most sensitive to the 8:2 FTsCA and 10:2 FTuCA, with LC_{50}s of 5.1 and 3.7 mg/L [61]. Another study with PFAS compounds using stygofauna was performed using the amphipod, *Hyalella Azteca* conducted in Ontario, Canada [62]. They noticed the amphipod survival was significantly reduced at 97 mg/L (42-d LC_{50} = 51 mg/L PFOA), but also found growth and reproduction to be more sensitive endpoints (42-d EC_{50} for both endpoints = 2.3 mg/L PFOA) [62].

Another study [63] conducted in Australia investigated the toxicity of PFOS and PFOA to Water flea (Daphnia carinata). The results indicated PFOS exhibited higher toxicity than PFOA. The 48 h LC_{50} values (confidence interval) based on acute toxicity for PFOA and PFOS were 78.2 (54.9–105) mg/Land 8.8 (6.4–11.6) mg/L, respectively [63].

BHP sites in the TEC area are of great ecological value due to the presence of stygofauna and are also affected with PFAS contamination from the decades-long use of AFFF in fire training activities. Thus, investigations on the presence and ecotoxicity of PFAS to stygofauna in identified bores are of great interest to the research community, regulatory bodies and BHP. This project will provide useful data on the occurrence of PFAS and stygofauna in BHP sites and the potential for PFAS effects on stygofauna species.

Table 4 Ecotoxicity studies using stygofauna

Stygofauna species	Stygofauna taxon	Chemical/physical parameter tested	Toxicity endpoints observed	Location studied	Reference
Diacyclops belgicus	Copepoda	Propranolol	LC50 (CI 95%) - 4.99 mg propranolol/L and LC10 – 2.00 mg propranolol/L	Medio Valdarno, Tuscany, Italy	[52]
Two Cyclopoid species One harpacticoid species	Copepoda	As (III), Cr (VI), zinc	28 days EC_{50} Budderoo cyclopoid 3.63 mg As/L, 0.27 mg Cr/L, 0.77 mg Zn/L. 28 days EC_{50} Somersby cyclopoid 0.25 mg As/L, 0.22 mg Cr/L, 0.50 mg Zn/L. 28 days EC_{50} Somersby harpacticoid – As –N/A mg/L, 0.02 mg Cr/L, Zn – N/A.	Budderoo, NSW Somersby, NSW	[53]
Two stygobiont species *Proasellus lusitanicus* and *Proasellus assaforensis*	Isopods	Potassium dichromate ($K_2Cr_2O_7$) and copper sulfate ($CuSO_4$)	48 hours EC_{50} *Proasellus lusitanicus* 1.12 mg $K_2Cr_2O_7$/L 48 hours EC_{50} *Proasellus assaforensis* 17.99 mg $K_2Cr_2O_7$/L 48 hours EC_{50} *Proasellus lusitanicus* 6.21 mg $CuSO_4.5H_2O$/L For *Proasellus assaforensis* only 20% mortality was recorded for the maximum concentration of $CuSO_4$ tested (52 mg/L)	*P. lusitanicus* was from Aliviela cave, Central Portugal *P. assaforensis* from Assafora cave, Central Portugal	[58]
Niphargus inopinatus	Amphipod	Toluene	Test 1 21-34 day LC_{50} 32.1 mg toluene /L Test 2 21-23 day LC_{50} 37.8 mg toluene/L	Germany	[57]
Stygobiotic Syncarid (Syncarida: Bathynellidae)	Syncarid	As, Cr and Zn	14 days LC_{50} to the syncarid, 0.25 mg As/L, 0.51 mg Cr/L, 1.77 mg Zn /L.	Somersby, NSW	[59]

Stygofauna species	Stygofauna taxon	Chemical/physical parameter tested	Toxicity endpoints observed	Location studied	Reference
Diacyclops belgicus	Copepod	Temperature	The D. belgicus did not show significant variations in the oxygen consumptions under a temperature change of 3°C	A phreatic well in Tuscany, Italy	[64]
Bryocamptus zschokkei	Copepod	Chromium Cr^{6+} Aldicarb (Pesticide) α-Endosulfan (Pesticide) Ammonia	96-h lethal concentrations 1.85 mg Cr^{6+}/L, 2.47 mg Aldicarb/L, 0.07 mg α-Endosulfan/L, 18.63 mg ammonia/L	Presciano spring system, L'Aquila, Italy	[56]
Bryocamptus minutus	Copepod	Chromium Cr^{6+} Aldicarb (Pesticide) α-Endosulfan (Pesticide) Ammonia	96-h lethal concentrations 3.56 mg Cr^{6+}/L, 2.5 mg Aldicarb/L, 0.2 mg α-Endosulfan/L, 18.22 mg ammonia/L	Presciano spring system, L'Aquila, Italy	[56]
Bryocamptus pygmaeus	Copepod	Chromium Cr^{6+} Aldicarb (Pesticide) α-Endosulfan (Pesticide) Ammonia	96-h lethal concentrations 3.48 mg Cr^{6+}/L, 2.42 mg Aldicarb/L, 0.2 mg α-Endosulfan/L, 18.22 mg ammonia/L	Presciano spring system, L'Aquila, Italy	[56]
Attheyella crassa	Copepod	Chromium Cr^{6+} Aldicarb (Pesticide) α-Endosulfan (Pesticide) Ammonia	96-h lethal concentrations 3.82 mg Cr^{6+}/L, 3.17 mg Aldicarb/L, 0.247 mg α-Endosulfan/L, 17.8 mg ammonia/L	Presciano spring system, L'Aquila, Italy	[56]
Bryocamptus echinatus	Copepod	Chromium Cr^{6+}	96-h lethal concentrations 1.26 mg Cr^{6+}/L, 2.71 mg Aldicarb/L, 0.095 mg α-Endosulfan/L, 14.61 mg	Presciano spring system,	[56]

Stygofauna species	Stygofauna taxon	Chemical/physical parameter tested	Toxicity endpoints observed	Location studied	Reference
		Aldicarb (Pesticide) α-Endosulfan (Pesticide) Ammonia	ammonia/L	L'Aquila, Italy	

Table 5 Bioavailability/bioaccumulation and ecotoxicological studies for PFOS/PFOA to surface and marine biota

Biota	Biota species/ taxon	Chemical/physical parameter tested	Toxicity endpoints observed	Location studied	Reference
Zebrafish embryos	Family: Cyprinidae	LC_{50} and EC_{50} values following the exposure to PFOS.	The LC_{50} at 120 hpf (hours post-fertilization) was 2.20 mg/L and the EC_{50} at 120 hpf was 1.12 mg/L	Oregon, USA	[65]
Marine mussel	*Perna viridis*	PFOS single and PFOS-NP (Nanoplastics) co-exposure	More structural damage to the gills and gonads were observed after PFOS-NP (Nanoplastics) co-exposure at 1000 µg/L than single PFOS/NPs exposures.	Guangdong Province, China	[66]
Stygofauna	*Hyalella azteca* (amphipod)	Perfluorooctanoic acid (PFOA)	42-d LC_{50} = 51 mg/L PFOA 42-d EC_{50} for both growth and reproduction = 2.3 mg/L PFOA	Burlington, ON, Canada	[62]

Biota	Biota species/ taxon	Chemical/physical parameter tested	Toxicity endpoints observed	Location studied	Reference
Freshwater fish	*Pimephales promelas* (fathead minnow)	Perfluorooctanoic acid (PFOA)	Fathead minnows exhibited no significant effects in all endpoints with the exception of uninflated swim bladder, which was significantly higher at 76 mg/L PFOA (15%) than controls (0%).	Burlington, ON, Canada	[62]
Water flea (Daphnia)	*Daphnia carinata*	The toxicity of two major PFAS, namely perfluorooctanoic acid (PFOA) and perfluorooctanesulfonate (PFOS)	PFOS exhibited higher toxicity than PFOA. The 48 h LC_{50} values (confidence interval) based on acute toxicity for PFOA and PFOS were 78.2 (54.9–105) mg L^{-1} and 8.8 (6.4–11.6) mg L^{-1}, respectively.	NSW, Australia	[63]
Freshwater algae Amphipod	*Chlorella vulgaris* and *Pseudokirchneriella subcapitata* *Hyalella azteca*	Saturated (FTsCA) and unsaturated (FTuCA) fluorotelomer carboxylic acids	*C. vulgaris* was generally the most sensitive species, with EC_{50}s of 26.2, 31.8, 11.1, and 4.2 mg/L for the 6:2 FTsCA, 6:2 FTuCA, 8:2 FTuCA, and 10:2 FTsCA, respectively. *H. azteca* was most sensitive to the 8:2 FTsCA and 10:2 FTuCA, with LC_{50}s of 5.1 and 3.7 mg/L.	Canada	[61]

Biota	Biota species/ taxon	Chemical/physical parameter tested	Toxicity endpoints observed	Location studied	Reference
Ice amphipod, polar cod, black guillemot and glaucous gull	Ice amphipod (*Gammarus wilkitzkii*), polar cod (*Boreogadus saida*), black guillemot (*Cepphus grylle*) and glaucous gull (*Larus hyperboreus*)	PFOS	Increase in PFOS concentration was observed from amphipods and fish to black guillemot and glaucous gull (Tukey's HSD, $p < 0.05$ for all but one pairwise comparison). A non-linear relationship was established when the entire food web was analyzed (ANOVA on GAM; df = 1.89, Chi square = 18.7, $p < 0.001$). When excluding the ice amphipod samples from the model, the relationship between PFOS and trophic level was significantly linear ($\beta_1 = 2.68$, adjusted $R^2 = 0.48$, $F_{1,36} = 35.0$, $p < 0.001$).	Barents Sea east of Svalbard, an archipelago in the Arctic Ocean.	[67]
Majority were fish samples	The majority of specimens were fish from the Centrarchidae family (bass and sunfishes), accounting for 77% of whole fish samples. Other species collected in the study included representatives from Poeciliidae (gambusia and mollies, 8%), Cambaridae (crayfish, 5%), Cyprinidae (carps and minnows, 5%), and Ictaluridae (catfish,	Six PFAS were monitored	The highest concentration of PFOS in whole fish was 9349 ng/g dry weight, with 15% of samples exceeding what is believed to be the maximum whole fish concentration reported to date of 1500 ng/g wet weight.	Bossier City, Louisiana (USA)	[68]

Biota	Biota species/ taxon	Chemical/physical parameter tested	Toxicity endpoints observed	Location studied	Reference
	5%).				
25 fish samples (9 different species)	The most abundant fish was the Iberian Gudeon (*Gobio lonzanoi*) present in all points. The Black Bass (*Mycroptero salmoides*) and the Pumpkinseed Sunfish (*Lepomis gibbosus*) are invasive species, and Eel (*Anguila anguila*) is an endangered species	21 perfluoroalkyl substances (PFASs: C4–C14, C16, C18 carboxylate, C4, C6–C10 sulfonates and C8 sulfonamide) were assessed in water, sediment, and biota	Mean PFAS concentrations detected in sediments (0.22–11.5 ng g^{-1}) and biota (0.63–274 µg kg^{-1}) samples were higher than those measured in water (0.04–83.1 ng L^{-1}), which might suggest (bio) accumulation.	Jucar River (E Spain)	[69]
Fish	Main species captured were *Barbus graellsii* and *Cyprinus carpio*. One sample of *Micropterus salmoides* was also captured	The occurrence and sources of 21 perfluoroalkyl substances (PFASs: C4–C14, C16, C18 carboxylate, C4, C6–C8 and C10 sulfonates and C8 sulfonamide) were determined in water, sediment, and biota	In general, mean PFAS concentrations measured in sediments (0.01–3.67 ng g^{-1}) and biota (0.79–431 µg kg^{-1}) samples were higher than those found in water (0.01–233 ng L^{-1}), which might suggest (bio) accumulation.	Llobregat River ecosystem, Mediterranean area, NE Spain	[70]
Archived polar bear (*Ursus maritimus*) liver tissue samples.	*Ursus maritimus*	Perfluorocarboxylic acids (PFCAs) from carbon chain length C8 to C15, perfluorohexane sulfonate, PFOS, the neutral precursor perfluorooctane sulfonamide (PFOSA), as well as 8:2 and 10:2	Concentrations of PFOS and PFCAs with carbon chain lengths from C9 to C11 showed an exponential increase between 1972 and 2002 at both locations. Doubling times ranged from 3.6	Two geographic locations in the North American Arctic	[71]

Biota	Biota species/ taxon	Chemical/physical parameter tested	Toxicity endpoints observed	Location studied	Reference
		fluorotelomer acids and their α,β unsaturated acid counterparts.	± 0.9 years for perfluorononanoic acid in the eastern group to 13.1± 4.0 years for PFOS in the western group		
Marine biota	Purple sea urchin (*Strongylocentrotus purpuratus*) Mediterranean mussel (*Mytilus galloprovincialis*) opossum shrimp (*Americamysis bahia*) bioluminescent dinoflagellate (*Pyrocystis lunula*)	Perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA)	For PFOS and PFOA, the order of species sensitivity, starting with the most sensitive, was *M. galloprovincialis*, *S. purpuratus*, *P. lunula*, and *A. bahia*. The range of median lethal or median effect concentrations for PFOS (1.1–5.1 mg L^{-1}) and PFOA (10–24 mg L^{-1}).	USA	[72]

3.4 Summary

Stygofauna are important subterranean organisms that are critical for healthy groundwater ecosystems. The stygofauna are sensitive to environmental changes including, groundwater table, aquifer quality, and temperature. The protection of stygofauna from environmental changes relies heavily on regular and enforced monitoring of groundwater quality and the stygofauna community. There is currently a lack of groundwater ecotoxicity threshold values based on stygofauna. The current use of extrapolation of toxicity data of surface water crustaceans and invertebrates to groundwater stygofauna, is not a suitable strategy. Furthermore, even interpolation of toxicity data among different species of stygofauna can be erroneous.

Available toxicity studies suggest that we have only limited knowledge on the toxic effects of various contaminants to stygofauna, and there is no study on toxic response of stygofauna following exposure to PFAS. Due to the rising concerns about PFAS contamination in groundwater ecosystems in WA, it is necessary to investigate the potential toxic effects of PFAS to sensitive stygofauna in these systems.

4. Sampling and analysis plan

The project aimed to determine the characteristics and key factors influencing the toxicity of PFAS in ground water samples from BHP sites, which was achieved through a series of controlled experiments, sampling, and analysis of stygofauna. The sampling events were facilitated by BHP. However, the COVID-19 pandemic and subsequent limited access to the sites and travel restrictions, delayed sampling for 6 months. The CRC CARE research team prepared the sampling plan in discussion with BHP and Stantec to obtain samples from sites selected by BHP. The following sample analysis and toxicity testing were based on samples received from BHP.

4.1 Sampling methods

The sampling of stygofauna was conducted following the approach described in the technical report by the Environmental Protection Authority in 2016 and the CRC CARE technical report No. 21 [73, 74] with support from Stantec Australia Pty Ltd, a global design and delivery firm (see Appendix A). One-time sampling was conducted instead of two different seasons per year (due to covid related issues), which was conducted at the end of March 2021. A licence to collect fauna for scientific purposes was required under Regulation 17 of the Wildlife Conservation Act 1950, which was sought by Stantec. Regulation 17 licences were sourced from the Department of Environment and Conservation. The bore sampling was based on the assumption that the species in bores are representative of all those in the surrounding aquifer. The bores were selected and identified by BHP, where PFAS contamination and stygofauna presence are of interest. The sampling locations are shown in Figure 3 with detailed sampling locations shown in Appendix B.

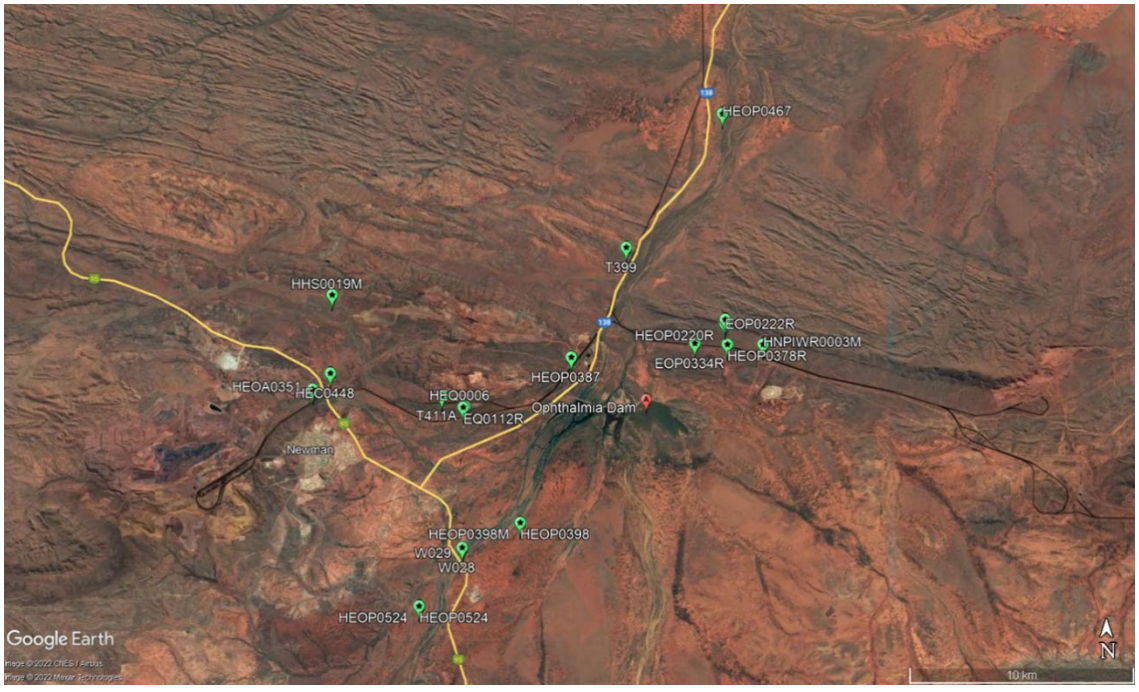


Figure 3 Sampling locations

PFAS-free materials were used at all stages of the sampling processes and specifically when performing the methods used to collect water for water quality purposes.

The sampling events include:

(1) Analysis of groundwater physicochemical parameters:

Prior to fauna sampling, groundwater physicochemical parameters such as electrical conductivity (EC), pH, reduction-oxidation potential (redox) and dissolved oxygen (DO) were measured in water from each well. The data were shared by Stantec to the CRC CARE team which enabled the conduct of further research activities.

(2) Groundwater sampling using a bailer:

The groundwater samples were collected using a bailer to obtain 2-4 L of water, which were added into 2L polypropylene (PP) containers. These samples were taken from the same wells used for stygofauna sampling. The water was used to acclimatise stygofauna after filtering. The water was also used for any chemical analysis including TOC, cations, PFAS after preparation. The samples were collected prior to stygofauna analysis.

(3) Stygofauna sampling using haul net method:

A haul net method was used to conduct the stygofauna sampling, as shown in Figure 4 [73], made from either 50 or 150 µm mesh, with a glass collecting vial at the base within a brass weight [75]. The base of the glass vial was removed and replaced with 50 µm mesh to improve water flow through the net. At each well, a stygofauna sample was collected by lowering the net to the end of the well, bouncing the net several times to stir the sediment and slowly retrieving it.

The net hauling method requires relatively little equipment and can be performed quickly and works equally well for all depths of bore. However, it can only be used in vertical bores and is a relatively inefficient method of sampling: several hauls must be made to obtain a sample of the stygofauna present at the time of sampling. The net is lowered and retrieved six times as recommended [76], with the operator being aware that, in most cases, the majority of animals will be near, or in, the sediments at the base of the bore. As a consequence, the stygofauna yield will increase if the sediments are vigorously agitated.

A large mesh size of 150 µm net was used three times to collect larger size organisms, while a small mesh size (about 50 µm) net can be used three times for reliable collection of the smaller species of stygofauna, since many stygofauna are <0.5 mm in length and elongated in body form. The contents of the net were emptied after each haul because any organisms present are likely to escape as the net is dropped back down the bore.

The contents of the vial were then transferred into filtered bore water or 100% ethanol depending on the need for the live or preserved collection for stygofauna, respectively. Three hauls with the 50 µm mesh net and three hauls with the 150 µm mesh net were made for each sample. After each sampling, nets were washed in ethanol, and rinsed using bore water to prevent the transfer of stygofauna between bores during the survey.

The groundwater well was not purged in order to sample groundwater water, and this was to avoid disturbance for the groundwater biota ecosystem.

Figure 4 Stygofauna nets of different diameters showing the machined brass weight fitted to the bottom of the net and McCartney vial, with mesh base, that fits into the brass weight [58] (photo provided by Stantec)

4.2 Sample preservation

Samples were preserved in the field and returned to the laboratory for sorting under a dissecting microscope. The samples were preserved using two methods based on the purpose of use, including: (1) preservation in 100% analytical grade ethanol for speciation; and (2) preservation in cool conditions in an insulated container for live samples collected for toxicity testing. The detailed preservation methods are described below.

- *Live samples*

  In the field, the stygofauna and water samples from the bore were placed in a polypropylene plastic bottle, with lid tightened and placed in a zip lock bag. The containers were wrapped in bubble wrap and placed on a layer of freezer blocks which had a layer of bubble wrap on top of the freezer blocks, to avoid excessive temperature change.

- *Preserved samples*

  The samples on the haul net were rinsed with 100% ethanol and rinsed into a container containing 100% ethanol. The well closed containers were placed into individual plastic bags, and then this whole thing was wrapped with bubble wraps to make sure the containers are not damaged, and samples are not leaked out. Samples were clearly labelled, and a small note was placed into each sample's bag.

4.3 Analysis

4.3.1 Stygofauna identification

The preserved samples were inspected under an Olympus SZ61 stereomicroscope and compound microscopes. All stygofauna taxa collected were identified to the lowest taxonomic rank possible using published and informal keys, the aim being to achieve species or morpho-species identification. Where necessary, animals were dissected and examined under a compound microscope to achieve identification. The numbers of individuals of each taxon present were recorded.

Several samples were treated for SEM analysis using methods modified from Felgenhauer [77] and Huys & Boxshall [78]. Briefly, the copepods were fixed in 3% glutaraldehyde at room temperature for 3 hours in 0.1 M phosphate buffer at pH of 7.0. These specimens were rinsed with 0.1 M phosphate buffer at pH of 7.0, three times for 5 minutes each with gentle agitation to remove excess fixative. Then the samples were dehydrated two times at each concentration of ethanol (50%, 75%, 95% and 100 %) for 10 minutes with agitation. Chemical drying was performed by keeping the samples in mixtures of EtOH (ethanol) and HMDS (Hexamethyldisilazane) for 15 minutes each consecutively at the EtOH:HMDS combinations of 2:1, 1:1, 1:2 and then 15 minutes each in HMDS alone for 3 times. The last round of samples in HMDS was allowed to evaporate slowly in near closed condition. The dried samples were mounted on SEM specimen stages and the SEM imaging was performed using Zeiss Sigma VP.

Photos were taken from an Olympus SZ61 stereomicroscope and SEM for representative stygofauna samples. Figure 9 exhibits some of the photos of the stygofauna collected from the wells in WA.

4.3.2 Groundwater chemistry

The groundwater samples were analysed for the presence of PFAS, including PFSA, PFCA and their precursors. A total oxidable precursor assay (TOPA) was conducted to identify any unidentified PFAS compounds [1]. The presence of cations in the groundwater samples was also analysed to facilitate discussion on the toxicity study. Cations (calcium (Ca), potassium (K), sodium (Na), magnesium (Mg), etc.), dissolved organic carbon, PFAS and precursors were examined using the ICP-MS/OES, IC, TOC analyser, and LC-MS-MS techniques.

Sample pre-treatment and analytical methods are included below.

(1) PFAS analysis

PFAS concentrations in groundwater samples were analysed using LC-MS-MS after being concentrated and cleaned through solid phase extraction (SPE). The detailed procedure for SPE is provided in Appendix C. The concentrated samples were then analysed using LC-MS-MS, with detailed information provided in Appendix C.

(2) TOPA analysis

TOPA aims to detect PFAS precursor compounds by oxidising samples to determine whether or not they develop into regulated (and easier to detect) PFAS compounds, which would be concern to human health and the ecological system. Detailed information on TOPA analysis is provided in Appendix C. In brief, the water samples were mixed with potassium persulfate and sodium hydroxide (NaOH) prior to being heated for reaction. The solution was treated through SPE prior to being analysed by LC-MS-MS. The analysed sample was concentrated 100 times compared to the original water sample.

(3) Metal analysis

Metal(loid) concentrations in groundwater samples were analysed using the Agilent 7500c (Agilent Technologies, Tokyo, Japan) inductively coupled plasma mass spectrometer (ICP-MS) coupled with an auto-sampler (ASX-520, CETAC Technologies) after filtering through 0.45 µm cellulose acetate filters. LOR: 0.25 mg/L (ICP-OES), 0.5 µg/L (ICP-MS), CCV 101.7%±3.4%.

(4) DOC analysis

In the groundwater samples, the dissolved organic carbon (DOC) was estimated by a TOC analyzer (Shimadzu: TOC-L CSH, Kyoto, Japan). These values for the groundwater samples are included in Appendix E. LOR: 1 mg/L, CCV 99.3%±5.0%.

During analysis, the USEPA QA/QC protocols were followed. In summary, the following has been included in the analysis:

a) Calibration of the instrument using calibration standards ensuring strongly significant relationship ($R^2 > 0.99$) between dose and instrument response.
b) Every batch of samples included initial blank runs.
c) Following every 10-samples run, there was a blank run and calibration verification standards (CCV).

4.3.3 Ecotoxicity test

For the toxicity study, we sampled 17 groundwater wells with the help of scientists from Stantec Australia Pty Ltd to investigate the effects of PFOS on stygofauna. *Diacyclops humphreysi* was the most abundant copepod found in the groundwater samples, which provided sufficient numbers for the ecotoxicity test. Those sorted from groundwater wells W028, W029, HHS0019M, and T0399 were used for the toxicity test, using a modification of the method employed by Di Lorenzo et al. [52]. The detailed procedure for toxicity testing is given below.

The live animals were sorted under an Olympus SZ61 stereomicroscope, with copepods placed into separate containers for each well for acclimatisation prior to toxicity testing. The bore water was filtered through a 0.7 μm glass fibre filter prior to being used for toxicity testing to ensure the removal of other animals and potential malicious organisms such as fungi. No food was provided to the copepods during acclimatisation (48 h), followed by the test. At the end of acclimatisation, only actively swimming individuals in the filtered groundwater were used for the final test. A stock solution of PFOS was prepared using Milli-Q water and corresponding filtered bore water. The PFOS stock solution was prepared by dissolving PFOS potassium salt (98%, sigma) in methanol. Different concentrations of PFOS solutions were prepared by dilution of stock solution employing filtered bore water. Eleven nominal concentrations of PFOS in a geometric series with a factor not exceeding 2.2 were prepared, including 0, 0.05, 0.1, 1, 5, 25, 50, 100, 200, 500, 1000 μg/L.

The individual copepod specimens were used for toxicity testing. Individual specimens were placed into 20 mL of PFOS solution in a PP container. There were 20 replicates for each concentration. The copepods were retained in the PFOS-spiked solutions prepared from the corresponding bores. In total 220 specimens were used for ecotoxicity testing from the four wells. The copepods samples were not disturbed during the experiments, no food and nutrients were supplied, and the solutions were static.

The mortality was the endpoint to determine LC10, and LC50 (lethal concentration that killed 50% of test animals). The experiment lasted for 56 days when the control samples showed 50% mortality. Counting and observation of mortality were performed on Days 4, 7, 14, 21, 28, 42 and 56. The record for the mortality counting is shown in Appendix H. Each PP container was closed with a screw cap immediately after specimen loading. The samples were kept in dark conditions at 20±0.2°C without disturbance. Each container was observed under a stereomicroscope for the presence of dead animals (specimens showing no movement after gentle shaking of the container for 15s, and no movement under microscope) on the above-mentioned observation and counting days. The number of dead animals were recorded and used to calculate LC values. The mortality endpoints were calculated with the toxicity data, where they are deemed valid according to OECD acute toxicity guidelines [79]. A condition of validity of this acute toxicity test included that the mortality in the controls should not exceed 10% at the completion of the test [79].

5. Analysis of groundwater samples

5.1 Groundwater samples and chemical analysis

A total of 17 groundwater samples were sampled at the end of March 2021, including 13 wells that were sampled for live stygofauna samples and 4 wells that were sampled for preserved stygofauna samples. The groundwater samples were collected prior to sampling of stygofauna using a bailer. Precautions were taken to eliminate any cross-contamination. The water samples were collected without purging. The groundwater samples were analysed as received. A summary of samples analysed is shown in Table 6. The geochemical properties of groundwater from the sampling areas are indicated in Appendix D.

Table 6. Summary of groundwater samples and stygofauna samples

GW No.	Sample ID	latitude	longitude	Standing Water Level (meters)	Water samples	Stygofauna samples
1	W028	-23.403285	119.795948	4.91	2 × 2L	Live
2	W029	-23.40367	119.796144	4.84	2 × 2L	Live
3	HHS0019M	-23.302086	119.740546	35.63	1× 2L	Live
4	HEC0448	-23.333318	119.739392	19.62	2 × 2L	Live
5	HEA0351	-23.339639	119.731578	18.66	2 × 2L	Live
6	T0399	-23.284239	119.868514	5.65	2 × 2L	Live
7	HEQ0006	-23.347071	119.797855	16.55	2 × 2L	Live
8	HNPIWR0003M	-23.3234	119.9278	30.3	2 × 2L	Live
9	EOP0378R	-23.3233	119.9122	12	2 × 2L	Live
11	EOP0334R	-23.3228	119.8981	14	2 × 2L	Live
12	EOP0222R	-23.3134	119.9113	24.92	2 × 2L	Live
13	HEOP0398	-23.3936	119.8214	5.85	2 × 2L	Live
14	HEOP0524	-23.4265	119.777	6.25	1× 1L	Preserved
15	EOP0220R	-23.3152	119.9113	20.5	1× 1L	Preserved
16	T0411A	-23.3423	119.7879	21.61	1 × 1L	Preserved
17	EQ0112R	-23.3474	119.797	31	1 × 1L	Preserved
18	HEOP0467M	-23.2312	119.911	5.3	2 × 2L	Live

The groundwater samples had temperatures ranging from 26.2-30.3°C (mean 28.7 °C), pH ranging from 7.01-8.69 (mean 7.4), EC ranging from 927-6454 µS/cm (mean 2074 µS/cm), and dissolved oxygen ranging from 4.1-51.2 % (mean 29.9 %). The detailed information is shown in Appendix D. The total organic carbon (TOC), total carbon (TC), and inorganic carbon (IC) content of the groundwater samples are shown in Figure 6. Most of the samples contained larger amounts of inorganic carbon than organic carbon. The cations in the groundwater samples were also determined, as summarised in Table 7 and Figure 7. The analysis of cations indicates that strontium (Sr) and barium (Ba) were found in all the samples, and Mn in most samples (Table 7). Sodium was the major cation in all samples.

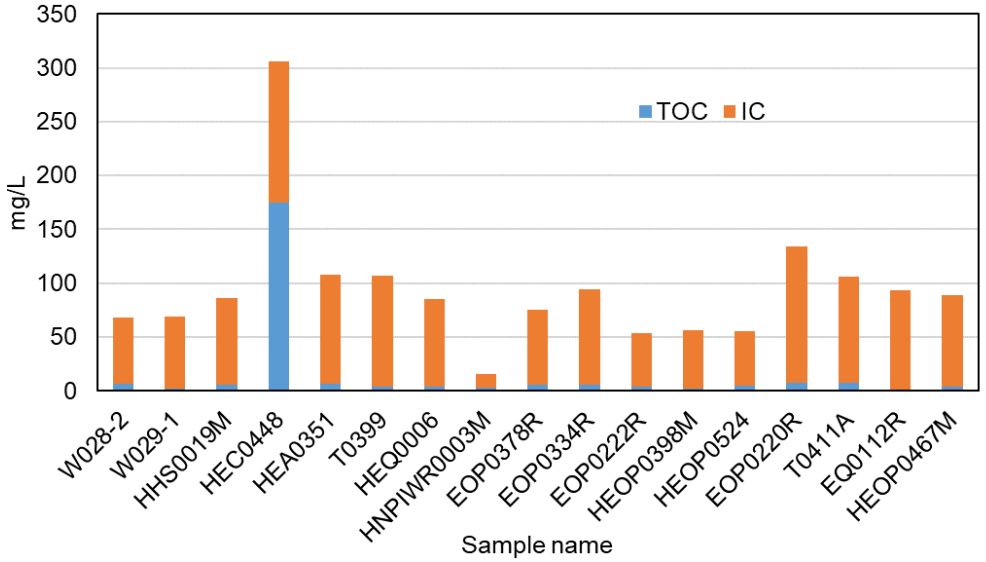


Figure 5. TOC (total organic carbon) and IC (inorganic carbon) content of groundwater samples collected from Newman sites in Western Australia

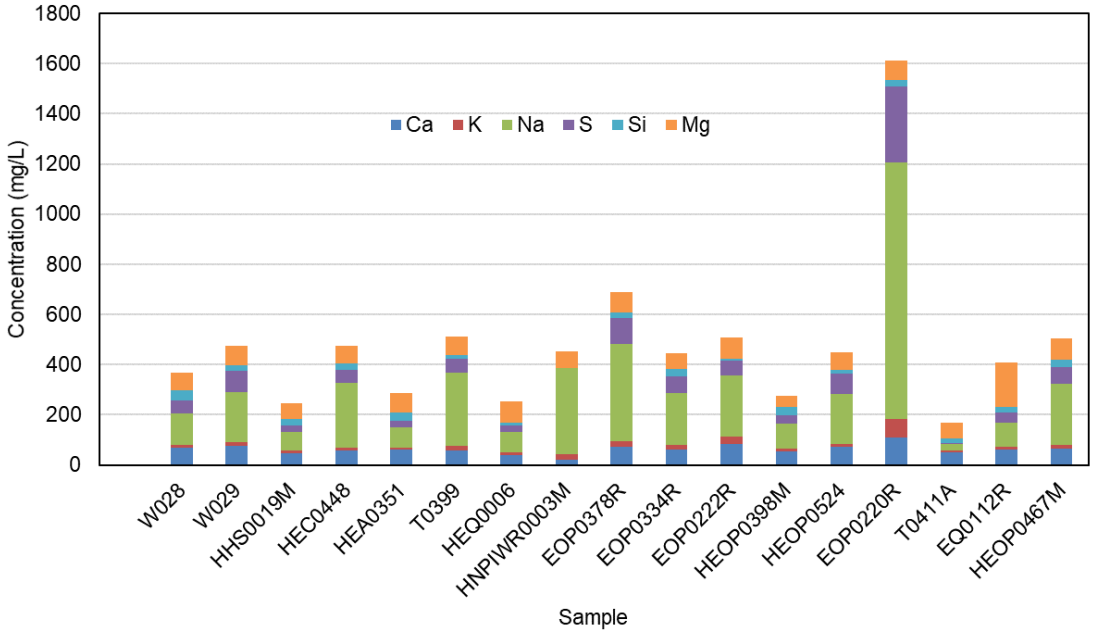


Figure 6. Concentration of inorganic elements (cations) measured in groundwater samples from Newman sites in Western Australia.

Table 7. Concentration (µg/L) of heavy metals in groundwater samples collected from Newman sites in Western Australia

	Sample/site ID	V	Cr	Mn	Fe	Co	Zn	As	Se	Sr	Mo	Ag	Cd	Sb	Ba	Pb
LOR	**µg/L**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**
GIL	**µg/L**	**ID**	**1***	**1900**	**ID**	**ID**	**8**	**13****	**11**	**ID**	**ID**	**0.05**	**0.2**	**ID**	**ID**	**3.4**
1	W028-2	20.40	ND	ND	ND	<LOR	ND	2.15	5.02	540	3.54	<LOR	<LOR	<LOR	46.40	<LOR
2	W029-1	7.74	ND	4.22	3.55	<LOR	ND	ND	2.24	591	4.57	ND	<LOR	ND	38.47	<LOR
3	HHS0019M	1.43	ND	ND	ND	<LOR	ND	<LOR	ND	164	ND	<LOR	ND	ND	8.22	<LOR
4	HEC0448-1	0.94	ND	157.92	4.60	<LOR	ND	<LOR	ND	345	ND	<LOR	<LOR	<LOR	27.39	ND
5	HEA0351-2	5.25	ND	1.15	ND	ND	ND	<LOR	3.21	244	<LOR	ND	ND	ND	11.17	<LOR
6	T0399-2	1.00	ND	17.84	14.29	<LOR	ND	ND	1.63	502	ND	ND	<LOR	ND	27.95	<LOR
7	HEQ0006-1	<LOR	ND	109.29	1.40	<LOR	ND	<LOR	2.49	116	ND	<LOR	<LOR	<LOR	10.37	<LOR
8	HNPIWR0003M-1	<LOR	ND	46.44	<LOR	ND	ND	ND	ND	155	ND	ND	<LOR	ND	6.33	<LOR
9	EOP0378R-2	2.21	ND	2.00	ND	ND	ND	ND	1.83	730	3.26	ND	<LOR	ND	36.55	<LOR
11	EOP0334R-2	0.71	ND	ND	ND	ND	ND	ND	3.85	263	ND	ND	<LOR	ND	24.41	<LOR
12	EOP0222R-1	0.84	4.72	ND	<LOR	<LOR	ND	ND	ND	745	ND	<LOR	<LOR	ND	55.80	<LOR
13	HEOP0398M-2	16.21	0.09	3.40	ND	<LOR	105.83	<LOR	ND	350	2.02	ND	<LOR	ND	47.26	<LOR
14	HEOP0524-1	1.89	ND	103.76	0.67	ND	ND	ND	<LOR	420	1.87	ND	<LOR	ND	24.50	<LOR
15	EOP0220R	1.61	ND	238.69	0.67	ND	ND	0.72	1.78	1226	<LOR	ND	<LOR	ND	41.22	<LOR
16	T0411A	0.87	ND	28.84	ND	ND	ND	ND	ND	218	ND	ND	ND	ND	16.73	<LOR
17	EQ0112R	2.35	ND	0.76	ND	ND	ND	ND	<LOR	248	ND	ND	ND	ND	24.15	0.51
18	HEOP0467M-2	4.47	ND	<LOR	ND	ND	ND	<LOR	0.80	374	1.20	ND	ND	ND	26.11	<LOR

Note: LOR: limit of reporting; ND: not detected

GIL: Groundwater Investigation Levels (GILs) for fresh water. These investigation levels are taken from the 95% species protection values of the Australian and New Zealand Guidelines for Fresh and Marine Water Quality (ANZECC & ARMCANZ 2000)[80, 81].
ID: Insufficient data to derive an investigation level.

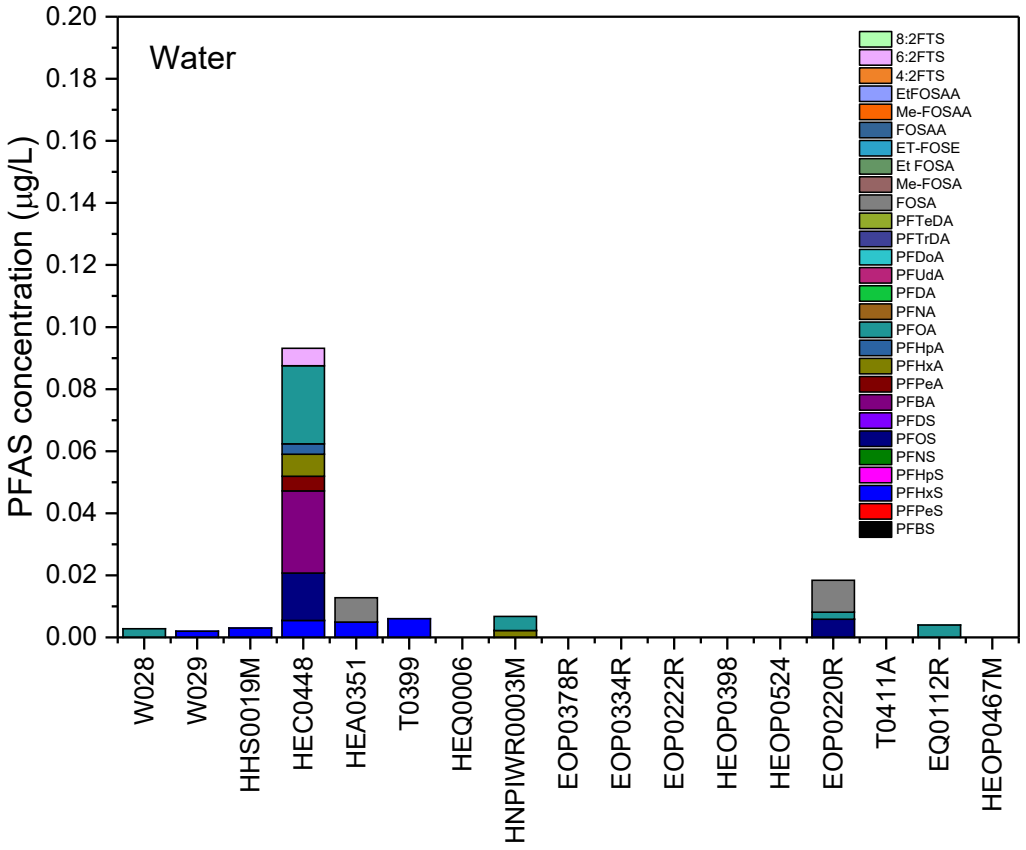
* as Cr (VI)

** as As (V)

5.2 Distribution of PFAS and stygofauna in groundwater samples

5.2.1 PFAS and precursors in groundwater

The groundwater samples were analysed for the occurrence of PFAS and precursors using solid phase extraction and total oxidisable precursor assay [1]. The results indicated low levels of PFAS in the groundwater samples. All the samples showed concentrations of PFAS below 0.07µg/L (∑PFAS<0.07µg/L) except sample HEC0448 (∑PFAS=0.093µg/L). TOPA analysis showed PFAS precursors in samples W028 and HEC0448 (Figure 7). Since the water samples were collected concurrently with the stygofauna samples, this may introduce cross-contamination for PFAS analysis. It is worth noting that groundwater sampled here may not represent the typical collection of groundwater samples on-site, as our samples contained some sediment particles. The water samples were from the top surface of the groundwater well without purging for groundwater sampling. Any contamination from the surface may occur. Further investigations of a PFAS sampling program are recommended following proper sampling methods for PFAS compounds. This was not performed in this study due to limited sampling time arrangement. There is a lack of hydrogeology for the site, further discussion on PFAS analysis in relation to potential contaminated sites near the Newman area is required, to establish a PFAS and stygofauna monitoring program.



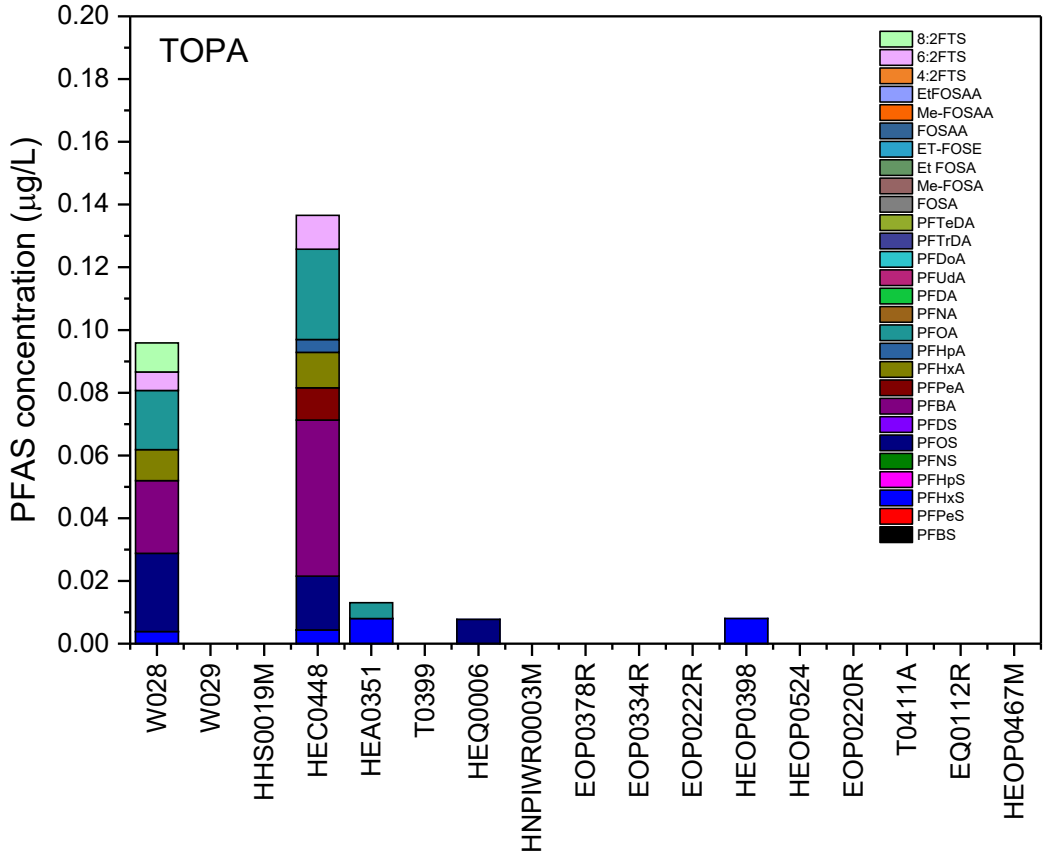


Figure 7. Concentration of PFAS for groundwater samples before and after TOPA analysis

5.2.2 Stygofauna species

During the sampling of 17 groundwater wells, 10 species belonging to 9 higher level groups were identified: amphipods, isopods, myodocopids, acariformes, trichoptera, cyclopoids, harpacticoids, thysanopteras and oligochaetes. Amphipods represented multiple species including *Neoniphargus* sp, *Hypogastrura* sp and *Wesiphargus nichollsi*. Oligochaetes, Trichoptera, Cyclopoida, Haplotaxida, Isopoda, Myodocopida, Harpacticoida and Thysanoptera were presented as having one species each. The detailed information is presented in Appendix G. The stygofauna samples used for toxicity testing were identified, as documented in Appendix G. The morphology of some samples is shown in Figure 9. The numbers of stygofauna present in the wells are summarised and compared with previously available reports (Table 8).

Table 8. Presence of stygofauna in groundwaters from Newman sites in Western Australia at different years.

GW No.	Site ID	Latitude	Longitude	Stygofauna abundance in each monitoring round (number of individual specimen)		
				2016	2017	2021
				Ethel Gorge stygofauna monitoring program: 2016	Ethel Gorge stygofauna monitoring program: 2017	Ecological assessment of PFAS in groundwater at contaminated sites in Western Australia (current report)
1	W028	-23.403285	119.795948	59	20	140
2	W029	-23.40367	119.796144	NM	NM	65
3	HHS0019M	-23.302086	119.740546	NM	NM	4
4	HEC0448	-23.333318	119.739392	NM	NM	0
5	HEA0351	-23.339639	119.731578	NM	NM	0
6	T0399	-23.284239	119.868514	102	69	6
7	HEQ006	-23.347071	119.797855	NM	NM	1
8	HNPIWR0003M	-23.3234	119.9278	NM	NM	0
9	EOP0378R	-23.3233	119.9122	NM	NM	0
11	EOP0334R	-23.3228	119.8981	NM	NM	3
12	EOP0222R	-23.3134	119.9113	NM	NM	0
13	HEOP0398	-23.3936	119.8214	1	2	1
14	HEOP0524	-23.4265	119.777	10	2	5
15	EOP0220R	-23.3152	119.9113	NM	NM	3
16	T0411A	-23.3423	119.7879	0	3	1
17	EQ0112R	-23.3474	119.797	NM	NM	3
18	HEOP0467M	-23.2312	119.911	NM	NM	3

*NM – Bores not monitored

There are changes in the abundance of total stygofauna numbers in each monitoring round, in some cases a slight change and in other instances drastic changes (Table 8). For example, the stygofauna abundance in well W028 in 2021 was higher than that of 59 in 2016 and 20 in 2017. However, the total abundance of 6 was far less in well T0399 during the 2021 monitoring round compared to 102 for the 2016 monitoring round and 69 in the 2017 monitoring round. The total number of stygofauna remained more or less the same during all three rounds in well HEOPO398, whereas in well HEOPO524, the total abundance dropped from 10 to 2 from 2016 to 2017, and in the 2021 round it rose to 5. In well T0411A, the total abundance of 0 in 2016 increased to 3 in 2017, but again decreased to 1 in the 2021 round. Some other wells which were not monitored in the 2016 and 2017 rounds were monitored in the 2021 round. They exhibited stygofauna abundance varying from 0-65. These wells and their abundance (within brackets) are W029 (65), HHS0019M (4), HEQ006 (1), HNPIWR0003 (0), EOP0378R (0), EOP0334R (3), EOP0222R (0), EOP0220R (3), EQ0112R (3) and HEOPO467M (3). These changes might be due to the level of sampling effort or the different approaches by different research groups who conducted these surveys each year. In addition, the different

sampling times for each year might have resulted in different abundance due to the life cycle of stygofauna. The onsite activities might also have had an impact on the water levels in the wells. Regular monitoring is recommended for the wells to demonstrate the seasonal changes, and variation due to other hydrogeological and chemical concerns. The regular monitoring results are suggested to be interpreted along with the regular PFAS monitoring in order to derive any effect from potential PFAS stress.

Copepods used for the toxicity testing from bores W028 and W029 belonged to the Cyclopoid copepod, *Diacyclops humphreysi.* Their abundance in the 2021 monitoring amounted to 136 in bore W028, and 63 in W029, respectively. When observing the abundance of each stygofauna species from Ethel Gorge Aquifer, it was noted that some taxons such as copepods have increased in number during the 2021 monitoring round, compared to that of the rounds conducted in 2016 and 2017, respectively (Table 2 in Appendix A). The abundance of copepod, *Diacyclops humphreysi,* in bore W028 in 2016 and 2017 was 45 and 8, respectively. However, it should be noted that the 2016 and 2021 rounds showed higher abundance of 45 and 63, and were performed in the month of March. Conversely, the 2017 monitoring round was done in November which is the last month of spring. The results may be influenced by sampling methods among other factors as discussed above and therefore interpretation should be exercised with caution.

Other factors including pH, electrical conductivity (EC), dissolved oxygen (DO), and redox potential (Redox) that could affect stygofauna abundance were examined. It was noted that in 2016 the groundwater parameters in the bore W028 were EC of 2028 µS/cm, pH 7.24, DO of 1.01 mg/L, and redox of 320.7 mV, whereas in 2017 they were 1955 µS/cm, 7.45, 10.1 mg/L and 143.5 mV, respectively. These results may suggest that high oxygen amounts in 2017 in bore W028 hindered the abundance of these copepods, although all other parameters apart from redox potential were in virtually the same range. It is widely accepted that the most widespread physiological adaptation of the copepods is their ability to adopt to seasonally unfavourable conditions. They do this by changing their metabolic rates and entering diapause in either the egg or late copepodid stages. Diapause is a period of suspended development in an insect, other invertebrate, or mammal embryo, especially during unfavourable environmental conditions [43]. Diapause may be induced by changes in oxygen concentration [82] or environmental temperature [83]. These resting stages exhibit high tolerance to extreme temperatures and desiccation [43].

Female cyclopoid copepods are more tolerant to anoxia or the absence of oxygen compared to males, while smaller species resist anoxia better than larger species [43]. This tolerance to anoxia might explain high numbers observed in the 2016 round despite lower DO levels.

Samples used for toxicological testing were also analysed morphologically, Figure 9 illustrates pictures of organisms sampled from W028, W029. The imaging from the Olympus SZ61 stereomicroscope (Figure 8) highlights the absence of eyes and pale translucent bodies of the stygofauna, while SEM images (Figure 10) were taken after treating stygofauna with chemicals in several steps including fixing and dehydration (section 4.3.1). These SEM images confirmed the presence of appendages and structures in detail. Mixed male and female specimens of *Diacyclops humphreysi* were used in this study as there were limited sample numbers. Adult copepod was used for the toxicity study while no adult life stage was differentiated.

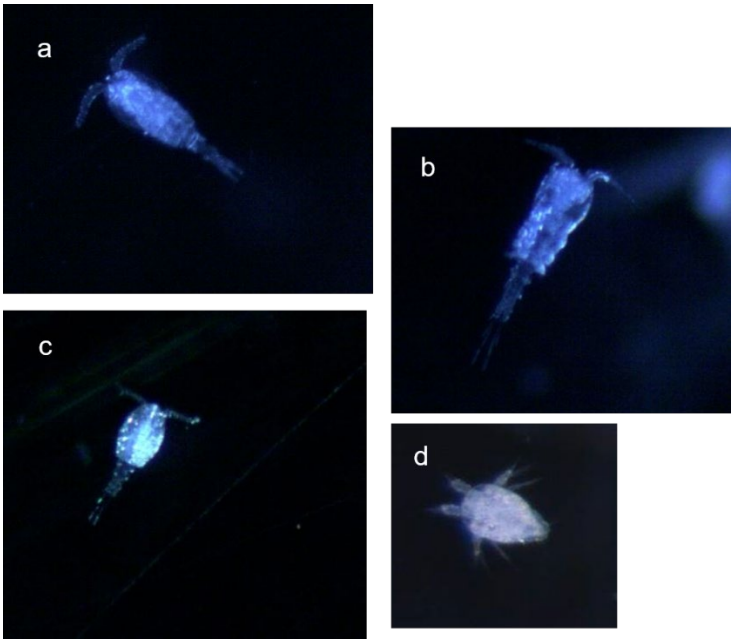


Figure 8 Stereomicroscope images of stygofauna copepod specimens.
Specimens collected from wells W028 (a and b) and W029 (c), and the copepod nauplii stage obtained from T0399 (d) during March 2021 in groundwater from Western Australian wells

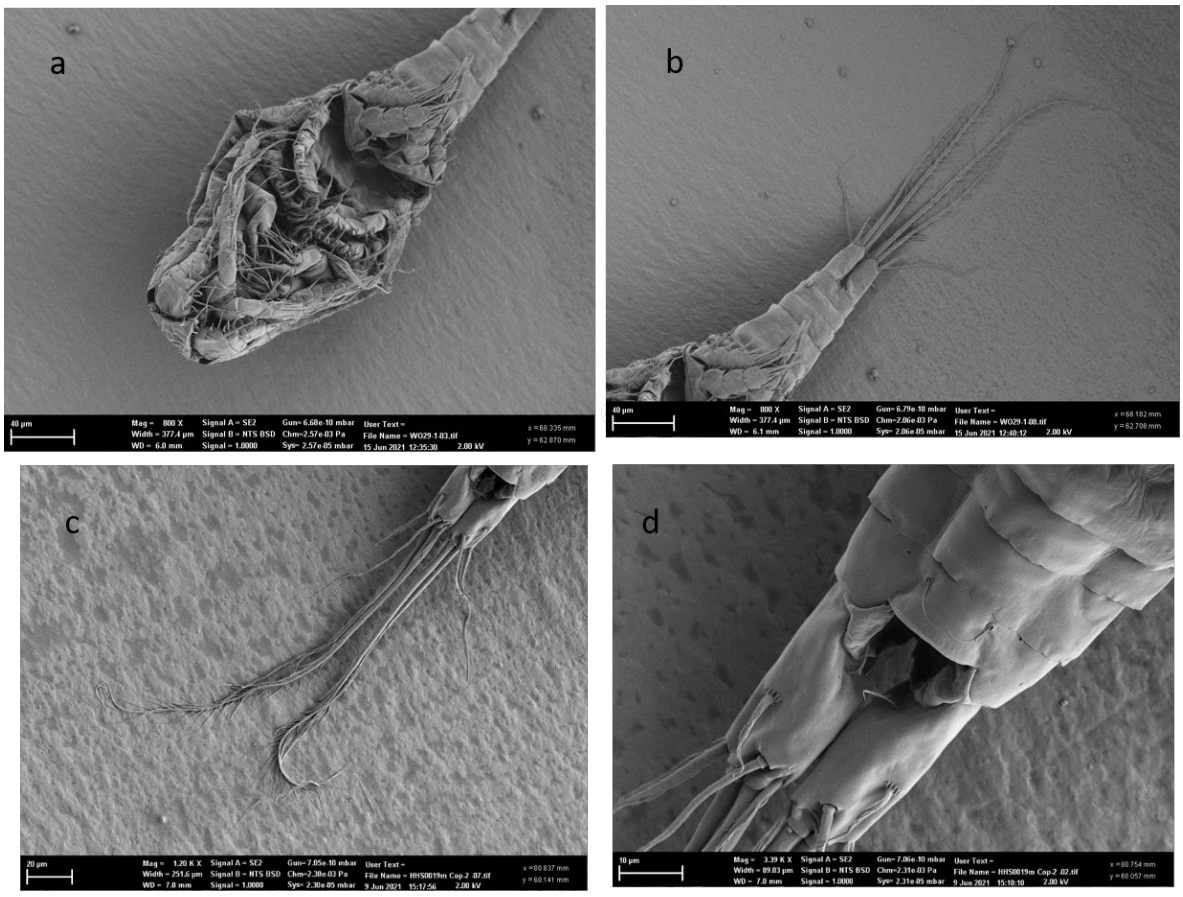


Figure 9 SEM (scanning electron microscope) images of copepod specimens.
Ventral view of the copepod (a) antennas, legs and genital segment and (b) part of the legs, urosomal segments, caudal ramus, lateral caudal seta and terminal caudal setae of a copepod from well W029, and dorsal view of the copepod (c) terminal caudal setae and (d) part of urosomal segment and caudal ramus of the copepods from well HHS0019m.

The term Copepoda comes from the Greek words “kope” for “oar” and “podos” for “foot”, denoting their swimming legs [43]. Copepod reproduction is sexual. It reaches its full development through 12 stages of life. This involves the first six stages which are termed “nauplii”, while the next five stages are referred to as “copepodids” [43]. Generally, the copepod body is an elongated, segmented body with an exoskeleton as shown in Figure 12. The first antennae or antennule, serve many functions related to feeding, locomotion and reproduction [43]. They have both chemoreceptors and mechanoreceptors on them which aid in identifying prey, mates, predators and harmful substances like pollutants. The first antennae in male copepods are geniculae which helps in mating. Their abdomen terminates into two caudal rami that are variously armed with setae and spines. The anus is located at the posterior end of the urosome just above the caudal ramus. Copepod females are generally larger compared to males [43].

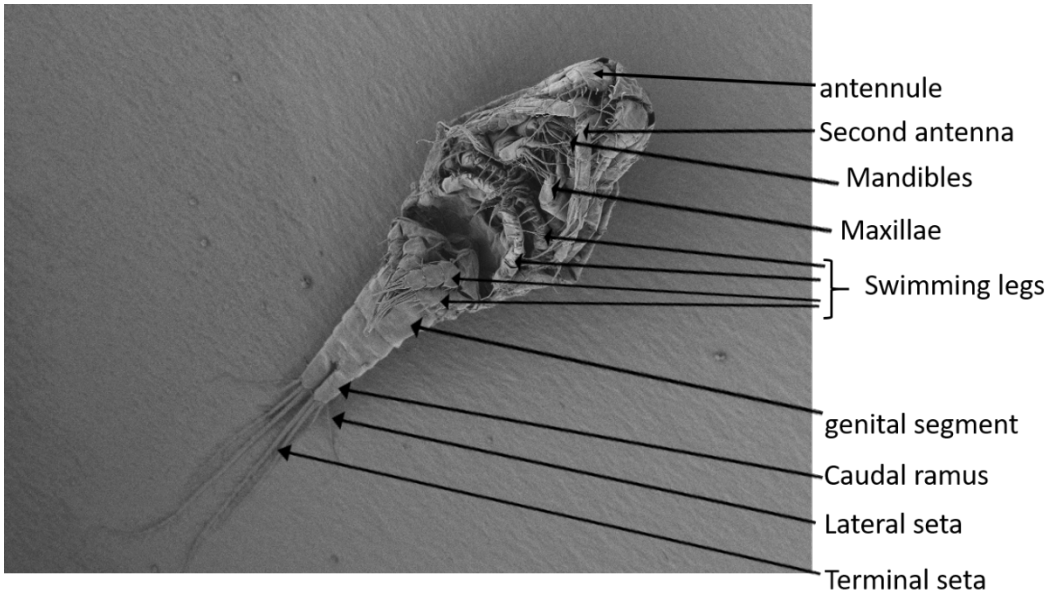


Figure 10 Ventral view of a copepod from the well W029 using SEM (scanning electron miscroscope)

The presence of copepods and other species of stygofauna can vary according to several factors. An increase in the abundance of cyclopoid copepod *Diacyclops humphreysi* was evident in March 2021, compared to that of monitoring rounds 2016 and 2017, for groundwater well W028. This might suggest successful groundwater management practices implemented in mitigating impacts from the potential decline of groundwater levels as a result of mine dewatering of OB23 and OB25. On the other hand, reasons for this increase in copepod numbers might have been due to the level of sampling effort or approaches by different research groups who conducted these studies each year. Also, the seasonality of the sampling times might have had an impact on the water levels in the wells.

Figure 11 shows the relationship between pH, EC and total PFAS levels with the abundance of stygofauna in the wells sampled in March 2021. It is difficult to predict any trends for the abundance of stygofauna with pH, EC or PFAS levels, given the very limited data available and uncertainties associated with PFAS analysis. Long-term monitoring for water quality and stygofauna abundance would help document definite trends in changes of environmental settings and the stygofauna ecosystem.

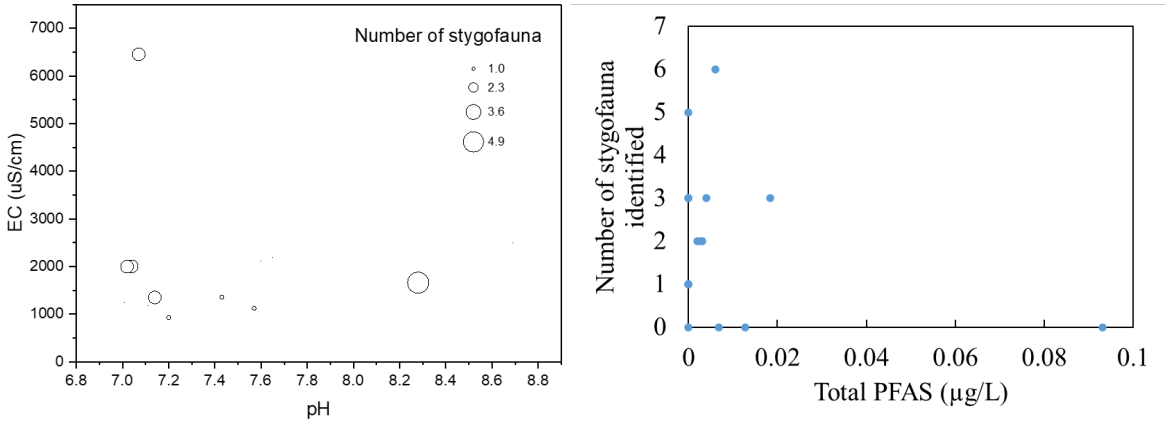


Figure 11 The relationship between abundance of stygofauna with the EC (µS/cm), pH and total PFAS levels of groundwater from Western Australia.

5.3 Toxicity of PFOS on mortality of stygofauna

Standard solutions of PFOS were prepared and spiked (0, 0.05, 0.1, 1, 5, 10, 50, 100, 200, 500, 1000 µg/L) in the groundwater samples from W028, W029, HHS0019M and T0399. Concentrations were measured after spiking before exposure to copepods. The recovery of PFOS in spiked samples was around 87.7±22.4%. The mortality of copepods after exposure to PFOS solutions was recorded and is presented in Figures 12-14.

The survival rate varied for copepods at different concentrations and days of exposure (see Figure 12). Generally, the survival rate declined with increasing exposure time from 0 to 56 days, including the control sample with no PFOS. The survival rate in the control sample significantly decreased at Days 42 and 56 in comparison to Day 28, which was constant till Day 28. The control maintained less than 10% mortality till Day 28, which is the threshold to validate toxicity testing. No significant differences in survival rate for concentrations up to 200 µg/L at two weeks' exposure were found. However, the survival rate diminished after two weeks.

At Day 28 of exposure to PFOS, 10%, 15% and 20% mortality rates were observed in the 0.05 µg/L, 0.1 µg/L and 1 µg/L PFOS concentrations, respectively, whereas in both the 5 µg/L and 25 µg/L PFOS concentrations the mortality rate was 10% (Figure 12). In the 500 µg/L PFOS concentration, the mortality rate was nearly 60% and this reflected the highly toxic effects on the copepods. The increasing mortality rates from 0.05 µg/L to 1 µg/L PFOS concentrations may be due to the uptake of more PFOS contaminated water by copepods.

The mortality at Day 28 was used for fitting, using the logistic model (Figure 15), as 95% of the control is alive while the toxic effects from other concentrations are obvious. The mortality increased with rising concentrations from 0.05 to 1 µg/L while it fell at 5 µg/L prior to increasing again at 25 µg/L. The fluctuation in mortality ended at around 100 µg/L and then increased up till 1000 µg/L. This fluctuation could be induced from the stimulation effects at a smaller concentration. However, future replication experiments are required to confirm the results reported here. The logistic model simulates the dose-response relationship between mortality and PFOS concentrations (nominal) and can be used for estimating LC values.

$$y = a + \frac{b - a}{1 + (\frac{x}{x_0})^p}$$

x = the independent variable, the concentration of PFOS; and **y** = the dependent variable, the mortality of copepods. The four parameters estimated are as below:

b = the minimum value that can be obtained (i.e. what happens at 0 dose), calculated as 12.37 ± 2.03%

a = the maximum value that can be obtained (i.e. what happens at infinite dose), calculated as 63.81 ± 5.67%

x_0 = the point of inflection (i.e. the point on the S-shaped curve halfway between a and b), 237.67 ± 48.38 µg/L

p = Hill's slope of the curve (i.e. this is related to the steepness of the curve at point x_0), calculated as 4.12 ± 3.44.

The model fitting showed R^2=0.95. The LC50 calculated was 237.67 ± 48.38 µg/L. The values of parameters were used to calculate LC10, which was 139.43 µg/L. However, further investigations on the toxic effects with different groups of stygofauna are required, as are replication experiments to determine the threshold values. The NOEC (no observed effect concentration) value is determined as 100 µg/L for 28 days exposure.

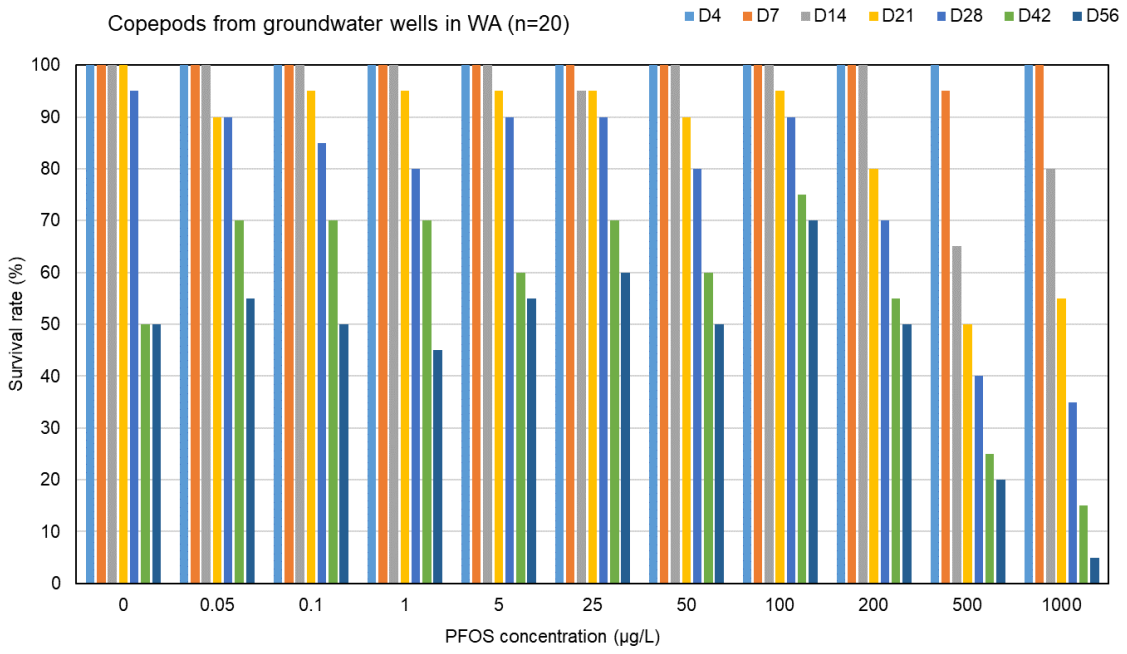


Figure 12 Survival rate of copepod after exposure to PFOS for different periods of time. D1 – D56 indicating the days for mortality.

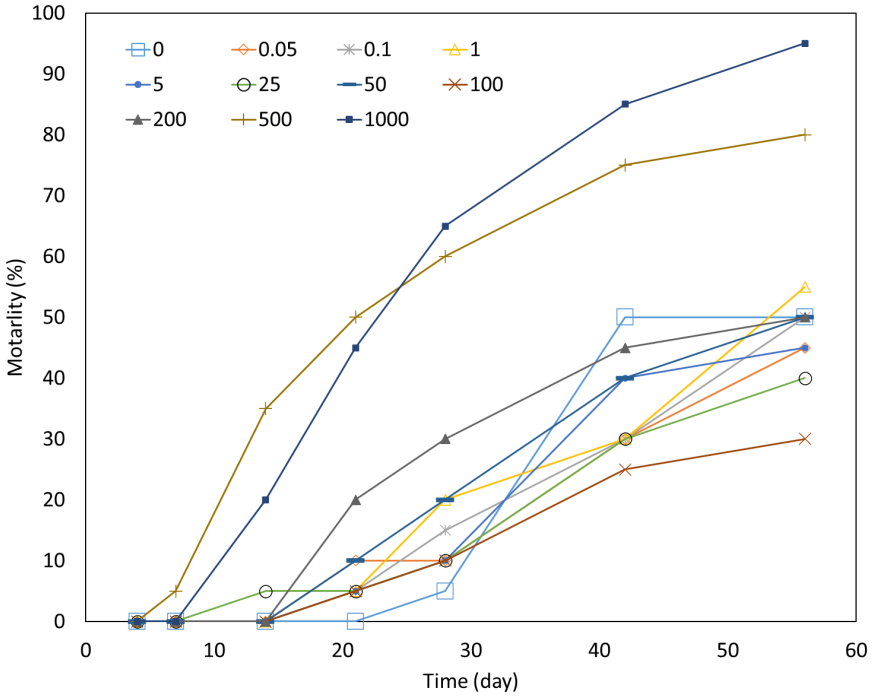


Figure 13 Mortality of copepods after exposure to PFOS at different concentrations for different periods of time. Different lines represent different dose of PFOS.

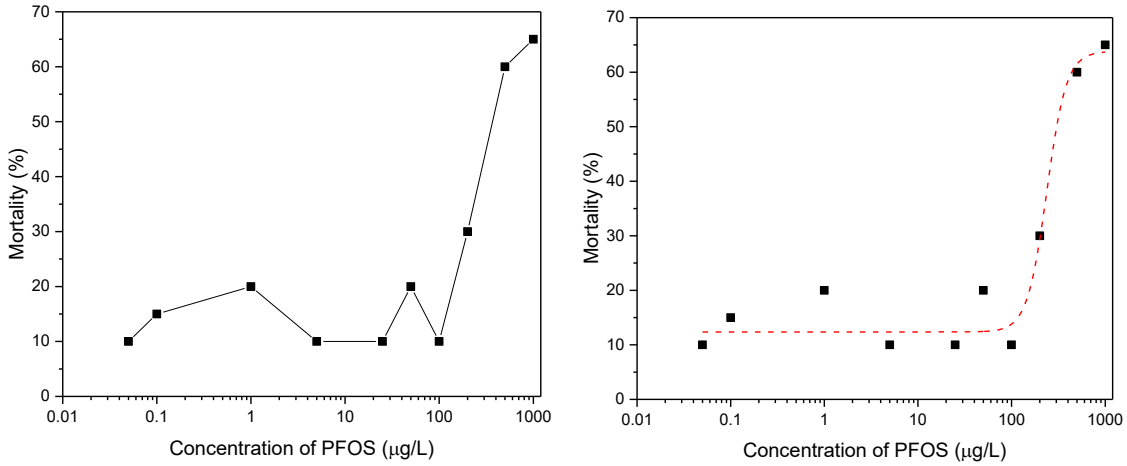


Figure 14 Mortality of copepods after exposure to PFOS for 28 days

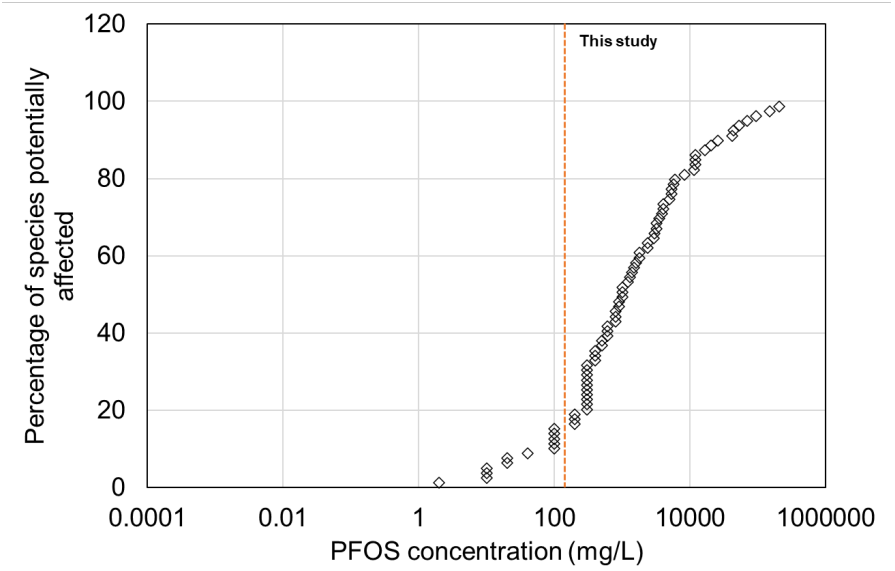


Figure 15 Compilation of NOEC/LC/EC10 values with aquatic and marine species [84]

The toxicity testing was scored against the ANZECC&ARMCAN guidelines [85], which is shown in Appendix I. The total score was 81.9% indicating high quality of data from this study. The NOEC and LC10 values for *Diacyclops humphreysi* exposed to PFOS are determined as 100 µg/L and 139.43 µg/L respectively, which is in the range of various guideline values for drinking water, reaction water, surface water and wastewater levels in Australia and other countries (0.13 – 700 µg/L) (Appendix J).

There have been no records on LC_{50} values for PFOS in benthic invertebrates in Australia. The only related information at present is an ecotoxicity study from Canada [61] using Amphipod, *Hyalella Azteca* to study ecotoxicity from PFAs (perflorinated acids) degradation products . The PFAs (perflorinated acids) degradation products used include 6:2, 8:2, and 10:2 saturated (FTsCA) and unsaturated (FTuCA) fluorotelomer carboxylic acids. They found that the *H. azteca* was most sensitive to the 8:2 FTsCA and 10:2 FTuCA, with LC_{50}s of 5.1 and 3.7 mg/L [61]. Another study with PFAS compounds using stygofauna was performed using the amphipod, *Hyalella Azteca* conducted in Ontario, Canada [62]. They noticed the amphipod survival was significantly reduced at 97 mg/L (42-d LC_{50} = 51 mg/L PFOA), but also found growth and reproduction to be more sensitive endpoints (42-d EC_{50} for both endpoints = 2.3 mg/L PFOA) [62]. Compared to this study our study found that the 28-d LC_{50} value to be 0.24 ± 0.04 mg/L PFOS. Since PFOS are generally more toxic to organisms than PFOA, these LC_{50} values, although from two different taxa of stygofauna, are relatively comparable as both of them are groundwater dwelling organisms.

Another study [63] conducted in Australia investigated the toxicity of PFOS and PFOA to water flea (*Daphnia carinata*). The results indicated PFOS exhibited higher toxicity than PFOA. The 48 h LC50 values (confidence interval) based on acute toxicity for PFOA and PFOS were 78.2 (54.9–105) mg/L and 8.8 (6.4–11.6) mg/L, respectively [63]. A compilation of aquatic and marine toxicity values (NOEC/EC/LC10) for PFOS is plotted in Figure 17 using data from [84]. The current study for PFOS toxicity end points is at the medium-high sensitivity level for above ground freshwater and marine biota (Figure 17). However, further information for toxicity studies using benthic biota would be more relevant as they are from similar habitats.

5.4 Summary

The groundwater and stygofauna abundance and distribution were analysed in BHP sites. The groundwater samples were analysed for the presence of cations, TOC/IC, and PFAS. The samples showed a low level of PFAS with PFOS < 0.07 µg/L. The groundwater samples were filtered prior to being used for acclimatisation and toxicity testing and minimal background PFAS levels were present compared to the spiked values. The species analysis of stygofauna confirmed that changes occurred in the number of stygofauna present in the groundwater wells compared with previous monitoring years. Various factors could have contributed to the variation in abundance and distribution, e.g., sampling seasons that guide breeding conditions and life span, temperature/rainfall, groundwater chemistry, groundwater table levels, and the presence of exotic compounds. Long-term monitoring is recommended for the response of the stygofauna community with reference to any changes induced by human activities.

Based on the PFOS toxicity studies performed, the LC50 value was found to be 238 ± 48.38 µg/L whereas the LC10 value was 139 µg/L. Our results suggest that Cyclopid copepod *Diacyclops humphreysi* can tolerate PFOS contaminations to a certain extent. However, further experiments are needed with more replicates to confirm these findings. Furthermore, the sensitivity of other types of stygofauna presents in these bores for PFOS and other PFAS toxicity should be tested.

6. Conclusions

Stygofauna are recognised as sensitive biological indicators of the groundwater ecosystem's health. Studies describing the effects of various contaminants on the mortality of stygofauna are limited. CRC CARE was requested by BHP Iron Ore to conduct ecotoxicological assessment of PFAS using stygofauna samples collected from nominated BHP mining sites. This project was aimed at understanding: (1) PFAS levels in groundwater samples from nominated BHP mining sites; (2) status of stygofauna in groundwater wells; and (3) the toxic effects of PFOS on a major stygofauna species (copepod). The investigations were based on samples obtained with support from BHP and staff from Stantec.

An initial literature review was conducted to understand BHP sites and the abundance of stygofauna on the sites in Western Australia. The review enabled an understanding of the historical monitoring of stygofauna and supported the preparation of a sampling plan. The Pilbara region and the TEC area are of great environmental importance in terms of biodiversity and ecology due to the richness of stygofauna present. The long-standing mining activities by BHP may affect the diversity and habitat of stygofauna in the region. Continuous monitoring programs and research activities are needed to investigate the influences of, firstly, mining activities and secondly, associated changes in environmental conditions and water quality on the presence, distribution and abundance of stygofauna.

A literature review on stygofauna toxicity testing was performed to understand the existing information on the sensitivity of stygofauna and groundwater risk assessment. The review showed that there exist limited published studies on the toxic effects of groundwater contaminants on the mortality of stygofauna. The most commonly used stygofauna group comprises copepods which are relatively abundant worldwide and can endure environmental changes. Available toxicity studies focused on the effects of selected heavy metals on copepods, but no investigation has been done on PFAS compounds.

A total of 17 groundwater wells were sampled for live and preserved stygofauna samples. The groundwater samples were collected along with the stygofauna sampling and TOC, IC, cations/trace elements, and PFAS levels were measured. The stygofauna samples were identified under microscopes. The variation of stygofauna abundance and species in sampling wells among the different sampling rounds from previous studies were found, which can be attributed to several potential reasons. For example, sampling seasons that guide breeding conditions and life span, temperature/rainfall, groundwater chemistry, groundwater table levels, and the presence of exotic compounds. Long-term monitoring is recommended for the response of the stygofauna community with reference to any changes induced by human activities.

The live copepod *Diacyclops humphreysi* samples were used for a toxicity study after acclimatisation and exposure to different concentrations of PFOS. In total, 220 copepod specimens *Diacyclops humphreysi* were used from four groundwater samples, from wells W028, W029, HHS000019M, and T0399. The results indicated increased mortality of the copepod, *Diacyclops humphreysi,* with time of exposure to PFOS, including the control sample with no spiked PFOS. The mortality rose with an increase in the PFOS concentration. The LC50 value (concentration at which 50% of organisms died) was found to be 238 ± 48.4 µg/L whereas LC_{10} was 139 µg/L. These values are comparable to or

higher than those reported LC_{50} values for surface water benthic organisms as there is limited stygofauna toxicity studies for PFAS. The results indicated copepod *Diacyclops humphreysi* might represent medium to highly sensitive groundwater species comparing the results of this study with toxicity data from fresh water and marine water biota PFOS studies. However, such a comparison is not based on biota from similar habitats. Further studies are required to reveal the toxic effects to the groundwater ecosystem. The toxicity values are within the range of PFOS screening levels determined for drinking water, reaction water, surface water and wastewater levels in Australia and other countries (0.13 – 700 µg/L). There is a lack of PFAS toxicity studies on groundwater biota, which requires further research efforts.

The results show that Cyclopoid copepods *Diacyclops humphreysi* can tolerate PFOS contaminations to some extent. There is a stimulatory effect at smaller concentrations (0.1, 1 µg/L), and this demands further verification using more toxicity testing. The toxicity study was evaluated against the ANZECC & ARMCANZ guidelines which indicated the high quality of this study and derived LC50 values for the groundwater toxicity.

The groundwater analysis for the samples collected indicated a much lower PFAS concentration, 1000 times lower, compared with the NOEC, LC_{10}, and LC_{50} levels. This large discrepancy indicates a PFOS impact on Ethel Gorge stygofauna is not expected unless environmental conditions change. The current stygofauna census in this study and showed a large variance from a previous report, it does not appear that this is due to the presence of PFAS. However, there is a lack of regular monitoring of both PFAS and stygofauna in this region as well as a lack of sufficient toxicity data for other species in groundwater. Further studies are required to confirm the toxic effects.

Our study results were based on copepods from mixed wells (four wells), which could also contribute to variations in LC_{50} values in the data obtained. This is the first time a PFOS toxicity study was conducted with stygofauna sampled from a monitoring program that began in 2009. Although one species of stygofauna, namely Cyclopoid copepod *Diacyclops humphreysi* showed relative tolerance to PFOS, it should be noted that there could be other stygofauna which could be more sensitive to PFAS compounds. Furthermore, the site had several other PFAS compounds and toxicity assessment should be performed for a mixture of PFAS present at the site. Therefore, toxicological experiments should be continued to confirm the data obtained and investigate PFAS toxicities in more detail using other stygofauna species and mixtures.

The information provided in this project is useful for the scientific community to understand the toxic effects of PFOS in subterranean ecosystems. Such information is needed for the risk assessment and development of remediation strategies for groundwater. The major PFAS of current concern in Australia, namely PFOS (perfluorooctane sulfonate) was investigated for toxicity in an invertebrate model. Other PFAS such as PFHxS (perfluorohexane sulfonate) and PFOA (perfluorooctanoic acid) need to be tested for their toxicity in the future.

Overall, the groundwater wells showed concentrations of PFAS below the drinking water guideline values 0.07µg/L (∑PFAS<0.07µg/L) except sample HEC0448 (∑PFAS=0.093µg/L). The identification of stygofauna species showed variance comparing with previous monitoring results, which can be attributed to several potential reasons, including sampling seasons influencing the breeding conditions and life span, temperature/rainfall, groundwater chemistry etc. PFOS toxicity study performed with the

copepod species *Diacyclops Humphreys* indicated this specific species of stygofauna are relevant tolerant to PFOS, with the LC levels being 1000 times higher than the PFAS detected in groundwater samples in this study. However, this is limited to the current species and groundwater wells obtained in this study. As a large groundwater habitat, further toxicity studies and monitoring programs are recommended to obtain further information on the effect of PFOS to the whole array of stygofauna species present in this habitat. It is recommended more information should be gathered on the most abundant, sensitive and representative species on PFOS toxicity to stygofauna. Further studies on screening and toxicity tests that represent the whole array of sensitive species present in this area are needed. It would also be important to investigate the sensitivity of stygofauna species to a greater range of PFAS.

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APPENDIX A. Sampling plan

Document A-1 Scope of work (prepared by CRC CARE)

Scope of Work

Sampling of Stygofauna in Newman area (TEC) – BHP

Background and objectives:

This scope is to provide requirements for sampling of groundwater samples and stygofauna, in sites in Western Australia with the support of Stantec. The major objectives for this SoW include:

1. Sampling stygofauna for species identification (preserved)
2. Sampling stygofauna for ecotoxicity study (alive samples)
3. Sampling groundwater samples for PFAS analysis

The SoW was prepared in consultation with BHP representatives and reviewing of previous site investigation reports as shown in Table 1.

Table 1 Reports on TEC area

Year	Name of the report	Prepared by
December 2013	Ethel Gorge Aquifer Threatened Ecological Community Consolidated Taxonomy	Subterranean Ecology
December 2013	Characterisation and Mapping of Ethel Gorge Aquifer Stygobiont Threatened Ecological Community	Bennelongia Pty Ltd
June 2014	Orebody 23/24/25 and Jimblebar Discharge Stygofauna Monitoring 2013 - 2014	Subterranean Ecology
June 2016	Ethel Gorge Stygofauna Monitoring Program: 2016	MWH
September 2016	TECHNICAL REVIEW Salinity Tolerance of Ethel Gorge Stygofauna TEC	MWH
November, 2017	Ethel Gorge Stygofauna Monitoring Program: 2017	MWH (now part of Stantec)

Sampling procedures:

In conjunction with the SoW the basic information of sampling sites is to be provided by staff on-site as a record. Included are the following:

- Bore ID and description while sampling
- Bore locations and sampling date

- Bore surface water level, groundwater depth, bore construction (either 50 mm plastic tubes, or 10-20 cm big steel tubes, or hole in the ground with caps)
- Site analysis of groundwater geochemical properties using multimeter, including pH, EC, DO, redox potential, temperature, etc.

1) Groundwater quality parameters assessment

The standing water level (SWL) and depth of the groundwater well will be measured using a Solinst 101 water level meter.

Basic groundwater physicochemical data (pH, water temperature, dissolved oxygen (DO), electrical conductivity (EC), total dissolved solids (TDS) and reduction-oxidation potential (Redox)) will be recorded in the field from a water sample. It will be collected by a bailer from the upper surface of the bore column using a YSI water quality meter. The equipment will be calibrated in the laboratory prior to the field trip, according to the manufacturer's instructions. These field parameters will be recorded on field data sheets. General observations of the water quality will also be documented including colour, turbidity, and odours.

These procedures can be modified according to staff on-site.

2) Sampling stygofauna for ecotoxicity study
Haul net method – For live samples used for toxicity studies

Haul net method has been widely used in monitoring programs in TEC area which was found to be the most efficient retrieval method (Allford et al. 2008). Sampling was consistent with the procedures outlined in the Guidance Statement No. 54a (EPA 2007). The sampling method was referred to Report <Ethel Gorge Stygofauna Monitoring Program: 2016>:

1. Samples will be collected using two weighted haul nets with mesh sizes of 150 µm and 50 µm. Each net will be fitted with a collection vial.
2. The 150 µm net will be lowered first, near the bottom of the hole.
3. Once at the bottom, the net will be gently lifted up and down to agitate the sediments.
4. The net will then be raised slowly to minimise the ‘bow wave’ effect that may result in the loss of specimens, filtering the stygofauna from the water column on retrieval.
5. This process will be repeated three times with the 150 µm net and three times using the 50 µm net.
6. To keep stygofauna alive to observe under the microscope, the stygofauna will be transferred to the filtered bore water (150 µm); use only the water pressure from the splash bottle for this transfer, do not use fingers or put pressure on the samples as this may damage the live animals.
7. In the field, place these Stygofauna + water from the bore in a plastic bottle, tighten the lid then place it in a zip lock bag and place it in the cool box/Esky with ice.
8. To prevent cross-contamination, all sampling equipment was washed thoroughly with bore water from sites or stilled water, Decon 90 or tap water is not to be used as it will damage the stygofauna;

9. For live samples to be packed the following procedure will be employed:
    - Plastic containers to be used due to their light weight and low risk of breakage, e.g. 2 L plastic container;
    - To avoid significant temperature changes for the live samples, the stygofauna will be kept alive at a constant temperature that is similar to the bore water temperature. Exposure to heat in summer can be fatal for stygofauna;
    - The samples are to be collected in plastic containers (e.g., 1 L or 2 L) as in steps 5-6; tape around the mouth of the container well; Wrap the containers in bubble wraps.
    - Prepare eskies: place the freezer blocks at the bottom of eskies; put one layer of insulation (bubble wrap/papers) on top of freezer blocks to avoid samples suddenly having excessive temperature changes.
    - Keep the samples on the insulation layer, then close the lid of the eskies.
    - These live samples will be transferred to a refrigerated environment on-site at the end of each survey day; and shipped immediately on the same day or the day after. It is essential to book a courier/flight earlier to facilitate the shipment. Delay in shipping increases the likelihood of stygofauna mortality.
10. Samples will be couriered back to the GCER laboratory, NSW, maintaining the cool chain, where they will be refrigerated at approximately 2-8°C prior to conducting the toxicity study.

3) Sampling stygofauna for species identification
Haul net method – for preserved samples set aside for species identification

Stygofauna will be sampled using haul nets, which are the most efficient retrieval method according to Allford et al. (2008). Sampling was consistent with the procedures outlined in the Guidance Statement No. 54a (EPA 2007). The sampling method is as follows:

1. Samples will be collected using two weighted haul nets with mesh sizes of 150 µm and 50 µm. Each net will be fitted with a collection vial.
2. The 150 µm net will be lowered first, near the bottom of the hole.
3. Once at the bottom, the net will be gently lifted up and down to agitate the sediments.
4. The net will be then raised slowly to minimise the ‘bow wave’ effect that may result in the loss of specimens, filtering the stygofauna from the water column on retrieval.
5. Once retrieved, the collection vial will be removed, the contents emptied into a 250 ml polycarbonate vial, and preserved with 100 % undenatured ethanol.
6. This process will be repeated three times with the 150 µm net and three times using the 50 µm net.
7. To prevent cross-contamination, all sampling equipment was washed thoroughly with potable water after each site; —as the use of detergent is to be avoided during PFAS sampling, it is suggested to not use detergent during sampling events.

8. In the field, tightly closed samples will be wrapped in bubble wraps and placed into eskies with ice bricks prior to being transferred into a refrigerated environment on-site at the end of each survey day; and
9. Samples will be couriered back to the GCER laboratory in NSW, where they will be stored in 100% ethanol for speciation.

4) Collecting groundwater using bailer for rinsing stygofauna and toxicity study

The groundwater samples can be collected on site in conjunction with the stygofauna sampling using a bailer.

1. Drop the bailer into the groundwater well and collect the water sample.
2. Repeat this several times to collect enough water.
3. Then sieve it through a 150 µm sieve into another container.
4. Aqueous samples are collected in 2L polypropylene containers and stored at 4°C (in eskies filled with ice).
5. This water will be used to acclimatise stygofauna.

5) Collecting groundwater samples for PFAS analysis

The groundwater sample will be taken from the well using bailer and placed in 5 L polypropylene containers. The equipment that will be used for this collection will be free from PFAS. The measurement of the parameters will be recorded after the readings stabilised. This will provide a cross-check and ensure representative inflow of the water that is collected.

The sample containers will be filled and capped immediately. Following sampling, the sample containers will be placed in a chilled cooler box to be transported to the laboratory for analysis.

To summarise:

- Before sampling, water quality parameters and characteristics of the aquifer will be collected for groundwater modelling
- Water samples will be collected after aquifer parameters are stable
- Low-yield well groundwater levels will be allowed to recover before sampling
- Low flow methodology will be used for groundwater sampling
- Collected groundwater samples will be chilled for storage and transportation

For PFAS sampling, the following precautions should be made.

When handling samples, no Teflon-coated materials or aluminium foil was used. All re-usable sampling equipment was made from high-density polyethylene (HDPE) or stainless steel and decontaminated prior to use. The sampling equipment will be rinsed with deionised water and allowed to air dry. No detergents will be utilised unless the detergent is confirmed as PFAS-free. All equipment will be washed again after each sampling day. During field sampling of PFAS, the sampling personnel adhered to the sampling recommendations ((DER, 2017), followed by (HEPA, 2020)), which included the following:

- No brand-new field clothing was worn.
- No waterproof clothing (e.g. GoreTex, Teflon or Tyvek clothing).
- No fast-food wrappers/containers or pre-wrapped foods or snacks.
- No use of self-sticking notes or similar office products.
- No reusable chemical or gel packs were used to cool samples. Instead food-grade ice contained in polyethylene bags was utilised to cool the samples.
- Natural sunscreens and insect repellents were utilised.

The detailed sampling requirements are referred to section 18.5 in HEPA (2020), which may vary by laboratory and sampling staff according to availability at the sites. The following are included:

- Use polypropylene or HDPE sample containers. Glass containers with lined lids are not suitable for PFAS analysis.
- Decontamination of drilling equipment must avoid the use of detergents unless they have been confirmed to be PFAS-free. Use tap water (tested to ensure it is PFAS-free) or deionised water instead.
- Sampling must include submission of representative sample(s) of water used for drilling/ decontamination purposes.
- Avoid using equipment (such as pumping equipment, water meters, etc.) containing PTFE unless it has been confirmed not to impact water quality.
- Use class 18 u-PVC casing with a lower section of slotted screen (also minimum Class 18 u-PVC). PVC casing should not be reused.
- Prior to well development, any personnel handling decontaminated well development equipment that directly contacts bore water must wash their hands with plain soap and rinse thoroughly in tap water before donning a clean, new pair of disposable nitrile gloves. A new pair of nitrile gloves must be worn for each well developed. Decontamination soaps must not be used unless confirmed to be free of fluoro-surfactants.
- Following the completion of well development, purged groundwater must be treated as PFAS-contaminated waste (i.e. assumed to be contaminated until verified, and then managed accordingly).

- Equipment recommended for obtaining groundwater samples includes low-flow peristaltic pumps using silicone or HDPE tubing or polypropylene HydraSleeves (or similar products). Consumable sampling equipment must not be reused.
- Rinsate samples should be collected if there is any doubt about whether or not materials or personnel are PFAS-free, including when detergents are being used and secondary containers.
- Larger sample volumes may be necessary if the required LOR are ultra-trace and/or a TOP Assay or TOF Assay analysis is to be performed on the same sample. We would require 5 L samples.

Sampling locations

The TEC area bore wells are monitored and sampled in 2013-2014, 2016, and 2017. A list of recent sampling (2016, 2017) with bore information and total number of stygofauna identified are shown in Table 2. Potential wells for sampling live stygofauna samples are highlighted as suggestions. In particular, *Diacyclops humphreysi* were found at bore code HEOP0415 (WP 105 or W 105) (found 200 specimens), bore code W116 (found 300 specimens), and bore code W152 (found 100 specimens) in 2016. However, this is not guaranteed as shown in the 2017 sampling events.

Table 2 Total stygofauna specimen identified in 2016 and 2017 monitoring program

			2016	2017
Bore Code (Previous code/s)	**Latitude (DMS)**	**Longitude (DMS)**	**sum**	**sum**
EA0285R (W196)	23°24'07"S	119°50'25"E	1	
EEX931	23°20'11"S	119°52'49"E	0	
EMP0070	23°17'48"S	119°43'02"E	200	
EQ0125R	23°20'42"S	119°48'42"E	4	
EQ0212DM4	23°20'42"S	119°48'42"E	15	
HEA0121 (WP23-12i)	23°19'07"S	119°50'56"E	30	21
HEA0126 (WP14S)	23°18'57"S	119°51'08"E	81	16
HEA0133 (P20S)	23°19'01"S	119°51'05"E	7	0
HEC0339	23°20'19"S	119°49'01"E	60	
HEOP0317M (W013)	23°20'21"S	119°45'39"E	0	0
HEOP0387 (W078)	23°19'40"S	119°51'18"E	85	11
HEOP0388 (W79D)	23°17'47"S	119°51'44"E	18	46
HEOP0398M (W088)	23°23'37"S	119°49'17"E	1	2
HEOP0415 (WP105 or W105)	23°19'37"S	119°51'51"E	251	
HEOP0417 (W107)	23°19'41"S	119°51'29"E	26	15
HEOP0425 (W115 or WP115)	23°19'33"S	119°52'19"E	20	65
HEOP0497	23°21'54"S	119°50'04"E	0	
HEOP0504 (W193D)	23°17'57"S	119°51'57"E	0	58
HEOP0524 (UNKNOWN3)	23°25'35"S	119°46'37"E	10	2
HEOP0574M (W262)	23°18'22"S	119°51'42"E	89	29
HST0455R	23°18'31"S	119°45'35"E	0	
HEOP0798M				0
OB23REG1	23°19'37"S	119°50'59"E	75	101
T399	23°17'03"S	119°52'07"E	102	69
T411A	23°20'34"S	119°47'16"E	0	
W028	23°24'12"S	119°47'46"E	59	20
W116	23°14'48"S	119°54'26"E	372	7
W117	23°14'43"S	119°54'12"E	543	12
W152 (HEOP0462M)	23°15'54"S	119°53'12"E	121	58
W231	23°12'45"S	119°54'18"E	2	0
WP56	23°18'29"S	119°51'39"E	2	0

Previous reports referred also include:

- Characterisation and Mapping of Ethel Gorge Aquifer Stygobiont Threatened Ecological Community (December 2013) by Bennelongia Pty Ltd.
- Orebody 23/24/25 and Jimblebar Discharge Stygofauna Monitoring 2013 - 2014 (June 2014) by Subterranean Ecology

Those reports also highlighted locations with high number of stygofauna specimen, which is summarised in Table 3. As there is no mention of detailed bore ID in the report by Bennelongia, only a higher number of stygofauna bore IDs sampled by Subterranean Ecology was highlighted.

Table 3 Stygofauna identified in sampling events in 2013-2014

	2013	Bore_Label #	2013-2014
	Bennelongia Pty Ltd		Subterranean Ecology
Central Ophthalmia	7159	EEX917_11:1687	14
Orebody 23	550	EX895_LN:7491	1
Orebody 24	22	F3NR_10:0756	4
Orebody 25	226	NODDY_12:0488a	3
Lower Ophthalmia	9323	NODDY_12:0491b	46
Upper Ophthalmia	3930	OB23REG1_11:0046	23
Homestead	153	P13S_10:0728	3
Newman	1657	T399_10:0710a	11
Shovelanna/Sylvania Station	124	T401_10:0678	4
Orebody 29	5	T411A_10:0749a	10
Orebody 31	1106	UNK02_10:0670	6
Orebody 35	163	UNKN02_11:0009	79
Western Ridge	33	W013_10:0642	2
Orebody 18	9	W028_11:1589a	3
Orebody 19	1	W086_11:1678	4
Jimblebar West	230	W088_12:0447	25
Jimblebar South	614	W105_10:0686	26
Jimblebar East	0	W107_10:0681	4
Mesa Gap	3	W115_11:1585	7
Wheelarra Hill	1	W116_11:0071	49
		W152_LN8234	5
		W247_LN:7495	3
		W262_11:0082c	21
		W28_10:0645	3
		W78_10:0654	2
		W79D_11:1472a	11
		WP126NRE_LN:7510	3
		WP131_LN:7519b	40
		WP23-11i_12:0494	3
		WP56_11:0093	18

Propose sampling locations:

- For sample preserved for stygofauna speciation:
  W116, HEOP0504, HEA0126, HEOP0417, HEOP0524, T411A
- For sample preserved for live stygofauna (groundwater is required for acclimatising stygofauna in lab):
  W117, HEOP0462M, T399, HEOP0574M, HEA0121, OB23REG1, HEOP0388, HEOP0387, HEOP0425, HEOP0415, W028
- PFAS will be analysed for all the wells (5L).

APPENDIX B. Sampling locations

Figure B-1 Sampling locations for HHS0019M, HEC0448, HEOA0351, T411A, EQ112R, HEQ0006

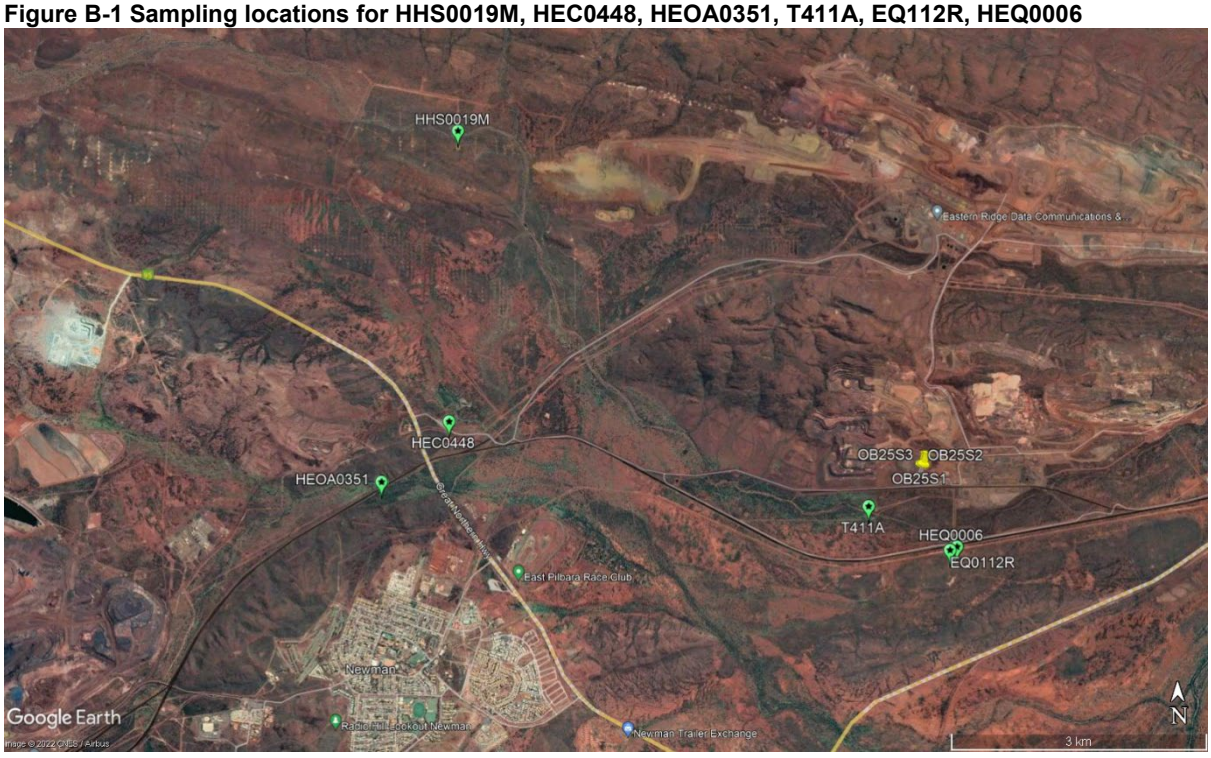
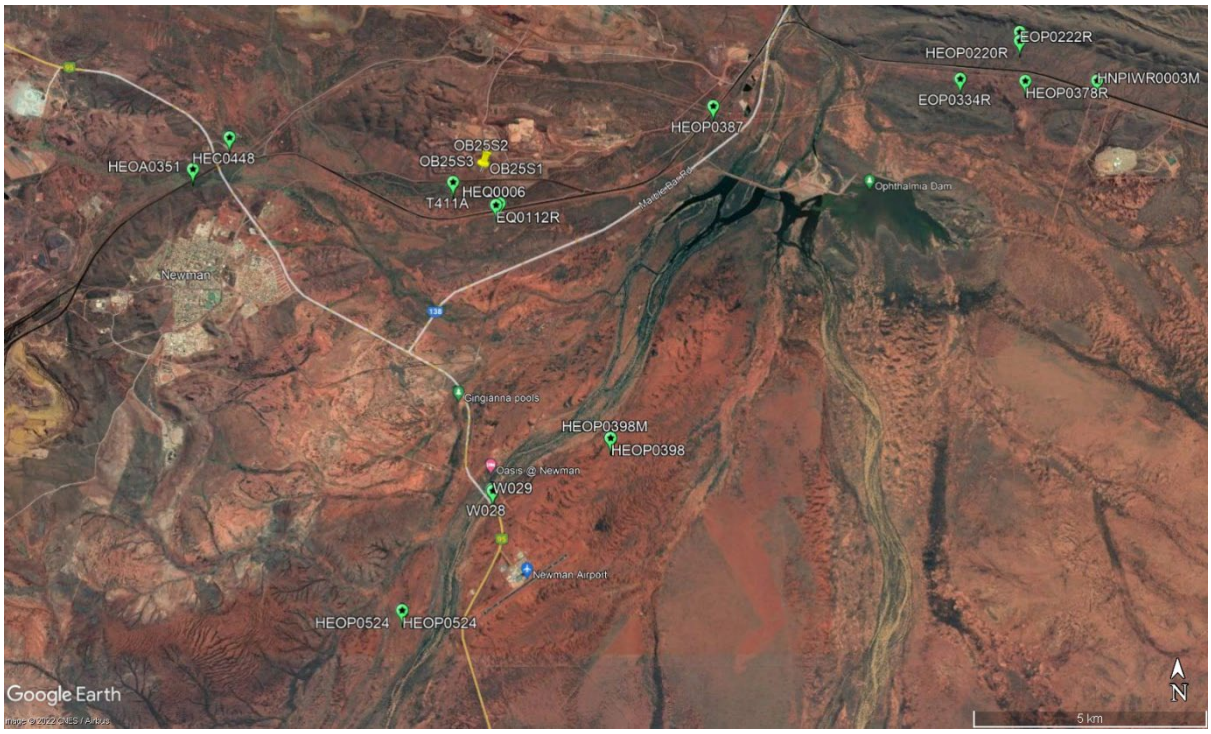


Figure B-2 Sampling locations for HEOP0467, T399, HEOP0220R, EOP0222R, EOP0334R, HEOP0378R, HNPIWR0003M.

Figure B-3 Sampling locations for W028, W029, and HEOP0398M.



APPENDIX C. Groundwater analysis method – PFAS

Appendix C-1 Water sample treatment and analysis

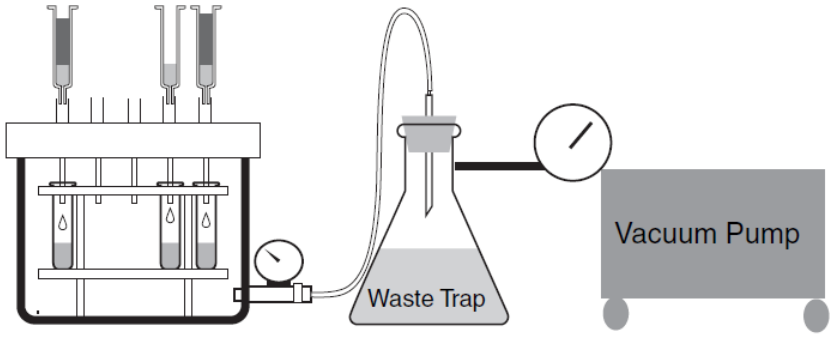
Solid Phase Extraction (SPE) of PFAS from Water Samples

Preparation of experiment

1. All chemicals, water and solvent used are LC-MS grade.
2. Prepare acetate buffer 0.025 M, pH 4:
   a) Mix 0.5 ml of acetic acid (LC-MS grade, >99.7%) with 349.5 ml of water.
   b) Dissolve 0.116g ammonium acetate in 60 ml of water.
   c) Mix 200ml of the diluted acetic acid (a) with 50 ml of the ammonium acetate solution (b) (ISO 25101).
3. Prepare 0.1% ammonium hydroxide in methanol: Mix 0.4 ml of 25% ammonia solution with 99.6 ml of methanol (ISO 25101).
4. Prepared water samples in 100 mL, 250 mL or 500 mL according to the targeted sample enrichment coefficient. Add a proper volume of isotope-labelled surrogate (23 PFAS mixture) to make the final surrogate concentration as 5 µg/L in 1 mL. The surrogate is to account for the total procedural losses, potential matrix effects and the systematic instrumental variation.
5. Waters SPE cartridge (WAX, 150mg), 24 -Port SPE manifold (Phenomenex)

Setup SPE Manifold

6. Wash all the parts (female luer fittings and male luer fittings of the manifold lid, stopcocks and the adaptor caps) thoroughly (last step of wash is by methanol). Without the SPE cartridge, wash each channel with syringes going to be used by LC-MS grade methanol.
7. Setup SPE manifold following Figure B1.



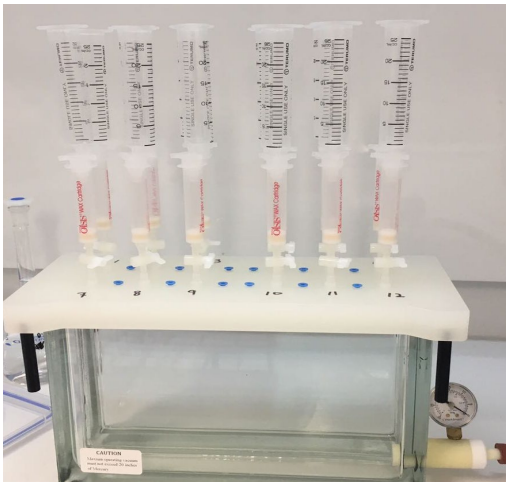


Figure C1. SPE setup

Conditioning of the SPE material

8. Prior to passing through water/liquid samples, condition the SPE cartridge with 4 mL of 0.1% ammonium hydroxide in methanol, followed by 4 mL methanol and 4 mL LC-MS grade water. Make sure the sorbent packed in the cartridge does not run dry.
9. Retain water in the cartridge (with the water level just above the cartridge) to keep the sorbent activated (ISO 25101; Silcock et al., 2016) if there is any interruption to the process.
   Adjust flow rate to 3-6 mL (approximately one drops per second (5 mL/min) (Ahrens et al., 2010; ISO 25101).

Sample extraction

1. After conditioning of SPE cartridge, transfer the prepared water samples to the syringe connected above to the SPE cartridge, turn on the vacuum and pass through the water samples immediately. Adjust the flow rate at one drop per second. Make sure no air bubbles are trapped in the sorbent bed when changing from conditioning to extraction.
2. Maintain the sorbent material in the cartridge in water at all times.
3. Entire sample plus bottle rinsate should be extracted.
4. After all water samples passing through the cartridge, remove residual water in the sorbent packing by applying a vacuum to the cartridge for 30 s. If the period of vacuum application is not enough to remove water, repeat the vacuum application several times, but not more than 2 min because overuse of vacuum may lead to loss of target compounds.

Sample elution

5. Add 4 ml of 0.025M acetate buffer solution to the dried cartridge and discard the eluates. Apply a vacuum to remove completely the residual solution form the cartridge. This step is to remove the impurities held on the SPE sorbent.
6. Elute the target analyte into 10 mL PP tubes with 4 mL of methanol followed with 0.1% NH_4OH in methanol at a rate of one drop per second.

Sample preparation

7. Evaporate the eluate with a gentle stream of N_2 gas to below 1 mL. Before N_2 blowing, add a proper amount of acetic acid to make the final acetic acid concentration around 0.1% in 1 mL.
8. Top up the concentrated eluate to 1mL by 0.1% acetic acid.
9. Filter the final extracts by PP syringe filter.
10. Load samples to LC-MSMS instrument.

Notes: Method Blank (Mill-Q water) will be performed the same way as environmental samples with each batch. Acceptable surrogate recovery was >30%.

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Appendix C-2 Water sample treatment for TOPA analysis

Method description

In aqueous solutions, PFAA precursor molecules containing C8-perfluorinated chain have shown to undergo hydroxyl radical mediated oxidative reaction and partially transformed to PFAAs. These precursor molecules include C8 sulfonamide compounds and 8:2 fluorotelomer alcohol as well [86, 87]. The hydroxyl radicals (·OH) have not shown to oxidize the transformed PFAA molecules at an appreciable amount.

The TOP assay (or TOPA) is a simple method that generates an excess amount of hydroxyl radicals which facilitated the oxidative reaction to convert PFAA precursors to PFCAs (perfluorinated carboxylic acids). In the TOPA method, potassium persulfate is used to generate to hydroxyl radicals by thermolysis at basic conditions (pH >12). During thermolysis, persulfate is converted to sulfate radicals ($SO_4^{-}\cdot$), which then quickly convert into hydroxyl radicals [88]. The excess amount of hydroxyl radicals formed by this reaction converts all precursor compounds to PFCAs. While the sulfate radical can react directly with PFOA [89], its conversion to ·OH is much faster than its reaction with PFOA at elevated pH values.

$S_2O_8^{2-} + Heat \longrightarrow 2SO_4^{-}\cdot$ Reaction (1)

$SO4^{-} + OH \longrightarrow SO_4^{2-} + \cdot OH$ Reaction (2)

Method procedure

Sample treatment

1) Water samples were collected into methanol-rinsed PP bottles (125 mL) and kept on ice until transferred to the laboratory (for <12h). Then the samples were stored at 4 ^{0}C up to 3 months before analysis. Reagent blank is prepared from HPLC-grade water which is transferred to a clean HDPE sampling bottle. Two grams (60 mM) of potassium persulfate and 1.9 mL of 10 N NaOH (150 mM) was added to the sample.
2) One sample from each site was subsampled in duplicate bottles amended with potassium persulfate and NaOH.
3) Fill the tube completely with MQ water to avoid headspace. The bottles were then placed in a temperature-controlled water bath at 85 °C for 6 h, which results in a reduction in concentration of persulfate of approximately 95% [90].
4) Then the samples were cooled to room temperature in an ice bath prior to analysis. Using concentrated H_2SO_4, neutralize the samples to a pH value of 4.
5) Add the surrogate

SPE using: Oasis SPE-WAX, Waters Corporation

1) Condition the column by 4 ml NH4OH (0.1% in methanol), then 4 ml of methanol, then 4 ml of HPLC grade water [91]
2) The samples were loaded to the column, then rinse the tubes with 5 ml HPLC-grade water
3) Adding 4 ml of acetate buffer
4) Elute the sample with adding 4 ml MeOH and then 4 ml NH4OH (0.1% in MeOH)
5) Concentrate the sample to below 1 ml under N_2
6) Add a proper amount of acetic acid to make the final acetic acid concentration around 0.1% in 1 mL.
7) Top up the concentrated eluate to 1mL by 0.1% acetic acid
8) All samples were analyzed on an Agilent HPLC coupled with an Agilent 6410 triple quadrupole mass spectrometer operating in negative electrospray ionization mode

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Appendix C-3 LC-MS-MS method

Instrument used

- LC Model - Agilent 1260 Infinity
- MS Model - Agilent Triple Quad 6470
- Analytical column - Agilent C18 RRHD 2.1x50mm, 1.8 Micron
- Delay column – Agilent C18 RR 4.6x50mm, 3.5 Micron

Instrument Parameters

- Flow rate – 400µl
- Injection volume – 5µl

Table C3a. LC-MSMS instrument parameters

LC Gradient parameters			**MS parameters**	**Value on (-) Mode**
Time	A% (10 mM ammonium acetate)	B% (MeOH)	Gas Temp (°C)	340
0.5	90	10	Gas Flow (l/min)	8
2	70	30	Nebulizer (psi)	25
16	5	95	SheathGasHeater	350
19	1	99	SheathGasFlow	11
20	90	10	Capillary (V)	4500

Sample QA/QC

USEPA QA/QC protocols were strictly adhered to. In summary, the following has been included in analysis:

a) Calibration of the instrument using calibration standards ensuring strongly significant relationship ($R^2 > 0.99$) between dose and instrument response (Table below);

b) Every batch of samples included initial blank runs;

c) Following every 10-samples run, there was a blank run and calibration verification standards (CCV). Recovery for CCV 0.2 µg/L 80.8 ± 9.2 %, 1 µg/L: 115.7 ± 16.1 %.

d) Detection limit: 0.05-0.1 µg/L

Table C3a The MRM (Multiple Reaction Monitoring) information for compounds analysed

Compound	Type	Retention time	Precursor ion	Quantifier (Product ion 1)	Qualifier (if any) (Product ion 2)	Mode
PFBA	Target	4.230	213	168.9	N/A	Negative
PFBS	Target	5.109	299	79.9	98.9	Negative
PFDA	Target	6.973	513	468.8	268.9	Negative
PFDoA	Target	7.707	613	568.8	318.9	Negative
PFDS	Target	7.301	599	79.9	98.9	Negative
PFHpA	Target	5.805	363	318.8	168.9	Negative
PFHpS	Target	6.169	448.9	79.9	98.9	Negative
PFHxA	Target	5.454	313	268.9	N/A	Negative
PFHxS	Target	5.804	399	79.9	98.9	Negative
PFNA	Target	6.578	463	418.8	218.9	Negative
PFNS	Target	6.940	548.9	79.9	98.9	Negative
PFOA	Target	6.180	413	368.8	168.9	Negative
PFOS	Target	6.561	499	79.9	98.9	Negative
PFPeA	Target	5.044	262.9	218.9	N/A	Negative
PFPeS	Target	5.469	348.9	79.9	N/A	Negative
PFTeDA	Target	8.283	712.9	668.8	368.9	Negative
PFTrDA	Target	8.072	663	618.8	368.9	Negative
PFUdA	Target	7.344	563	518.8	268.9	Negative
102FTS	Target	7.723	627	607.0	80.1	Negative
42FTS	Target	5.404	327	80.9	286.8	Negative
62FTS	Target	6.179	427	406.8	80.9	Negative
82FTS	Target	7.005	527	506.8	80.9	Negative
EtFOSA	Target	8.723	526	218.9	168.9	Negative
EtFOSAA	Target	7.392	584	418.9	525.9	Negative
EtFOSE	Target	8.712	630	59.0	N/A	Negative
FOSA	Target	7.656	498	77.9	N/A	Negative
FOSAA	Target	6.964	556	497.8	N/A	Negative
Me-FOSA	Target	8.502	512	168.9	218.9	Negative
Me-FOSAA	Target	7.166	569.9	418.9	N/A	Negative
MeFOSE	Target	8.516	616	59.0	N/A	Negative
42FTS-13C2	ISTD	5.412	329.1	308.8	N/A	Negative
62FTS-13C2	ISTD	6.170	429	80.9	409	Negative
82FTS-13C2	ISTD	7.013	529.2	508.8	N/A	Negative
ET-FOSAA-D5	ISTD	7.375	589	530.9	418.8	Negative
Et-FOSa-D5	ISTD	8.714	531	168.9	N/A	Negative
Et-FOSE-D9	ISTD	8.695	639	59.0	N/A	Negative
FOSA-13C8	ISTD	7.656	506.1	77.9	N/A	Negative
MeFOSAA-D3	ISTD	7.165	573	418.8	N/A	Negative
MeFOSA-D3	ISTD	7.375	589.2	530.9	N/A	Negative
MeFOSE-D7	ISTD	8.499	623	59.0	N/A	Negative
PFBA13C4	ISTD	4.228	216.9	171.9	N/A	Negative

Compound	Type	Retention time	Precursor ion	Quantifier	Qualifier (if any)	Mode
				(Product ion 1)	(Product ion 2)	
PFBS-13C3	ISTD	5.116	302	79.9	N/A	Negative
PFDA-13C2	ISTD	6.973	515	469.9	N/A	Negative
PFDoDA-13C2	ISTD	7.707	615.1	569.8	N/A	Negative
PFHpA-13C4	ISTD	5.804	367	321.9	N/A	Negative
PFHxA-13C2	ISTD	5.446	315	269.9	N/A	Negative
PFHxS-18O2	ISTD	5.803	402.9	84.0	N/A	Negative
PFNA-13C5	ISTD	6.569	468	422.8	N/A	Negative
PFOA-13C4	ISTD	6.188	417	371.9	N/A	Negative
PFOS13C4	ISTD	6.560	503	79.9	98.9	Negative
PFPeA-13C5	ISTD	5.051	268	222.9	N/A	Negative
PFTeDA-13C2	ISTD	8.283	715.1	669.8	N/A	Negative
PFUdA-13C7	ISTD	7.343	570	525.0	N/A	Negative

Table C3b. The calibration curves

Compound	LOR	Y=ax+b	R^2
PFBA	0.01	Y=0.6355*x-0.0012	0.998
PFBS	0.002	y=1.1438*x+0.0446	0.9983
PFDA	0.002	y=1.1168*x+0.0427	0.9974
PFDoA	0.002	y=0.5099*x+0.0067	0.9977
PFDS	0.002	y=0.6334*x-0.00257	0.998
PFHpA	0.002	y=1.155*x-0.0019	0.9986
PFHpS	0.005	y=1.0586*x-0.0011	0.9982
PFHxA	0.002	y=0.8587*x+0.0111	0.9979
PFHxS	0.002	y=3.0951*x+0.0014	0.9983
PFNA	0.002	y=347.3053*x-0.7783	0.9967
PFNS	0.002	y=1.1357*x-0.0036	0.9975
PFOA	0.002	y=1.0034*x+0.0011	0.9985
PFOS	0.005	y=1.3426*x+0.0328	0.9905
PFPeA	0.002	y=1.1819*x+0.0044	0.9982
PFPeS	0.002	y=1.7484*x-0.0041	0.9983
PFTeDA	0.002	y=0.7169*x+0.0113	0.9967
PFTrDA	0.002	y=0.8965*x-7.7E-004	0.9975
PFUdA	0.002	y=1.6373*x-0.0015	0.9984
10:2FTS	0.005	y=1.1998*x-0.0099	0.9926
4:2FTS	0.005	y=0.6047*x+0.0057	0.9983
6:2FTS	0.005	y=2.843*x+0.0231	0.9981
8:2FTS	0.005	y=1.2624*x+0.0080	0.9985
EtFOSA	0.005	y=1.0794*x-0.0031	0.9985
EtFOSAA	0.002	y=1.3542*x-5.8E-004	0.9978
EtFOSE	0.005	y=1.2700*x-0.0031	0.9983
FOSA	0.002	y=1.8721*x-0.0042	0.9985
FOSAA	0.005	y=1.3972*x-0.0038	0.9978
Me-FOSA	0.005	y=1.0686*x-0.0039	0.9963
Me-FOSAA	0.002	y=1.9115*x-0.0039	0.9983
MeFOSE	0.005	y=2.1413*x-0.0069	0.9985

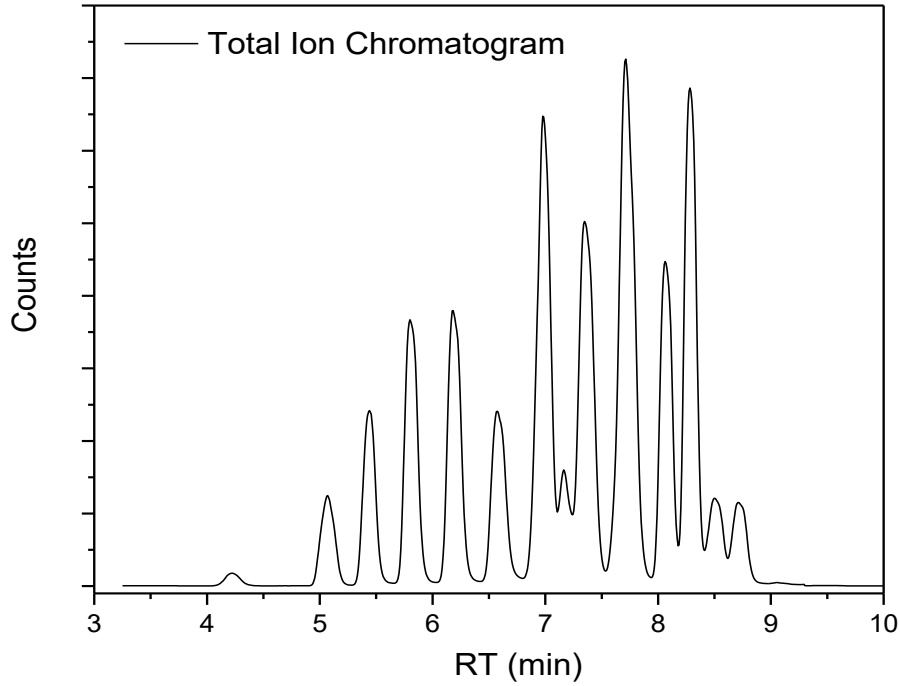

Figure C1. The TIC curve for calibration samples

APPENDIX D. Geochemical properties of groundwater samples

Table D-1 General geochemical properties of groundwater samples (Field data provided by Stantec)

ObjectID	Site Code	Standing Water Level (mbSWL) Metres	Depth Below SWL	End Of Hole (m)	Temperature C	Dissolved Oxygen %	EC (uS/cm)	pH (unit)	Notes
1	W028	4.91		12	30.3	29.2	1730	7.39	
2	W029	4.84		20	29.9	14.1	2120	7.6	
3	HHS0019M	35.63		42	28.9	12.4	1174	7.11	Frog in bore hole
4	HEC0448	19.62		118	27	4.1	2190	7.65	REDOX: -297.7
5	HEA0351	18.66		50	26.2	41.1	1247	7.01	Redox: -48.1
6	T0399	5.65		12	27.5	9	2189	7.26	REDOX: -217.0
7	HEQ0006	16.55		52	29.6	29.5	1122	7.57	Redox: -104.2
8	HNPIWR0003M	30.3			28.7	40.1	2491	8.69	Redox: -119, rust in water due to bore casing
9	EOP0378R	12		17	28.5	46.5	2808	7.4	Redox: -39.8
10	EOP0378R	0							No sample
11	EOP0334R	14		51	29.4	27.8	2002	7.04	Redox: 18.9
12	EOP0222R	24.92		117	29.2	51.2	2441	7.14	Redox: -5.8
13	HEOP0398	5.85		22	27.8	21	1357	7.43	Redox: -20.6
14	HEOP0524 (unknown3)	6.25			29.2	40.7	1658	8.28	Redox: -63.3
15	EOP0220R	20.5		61	28.5	45.8	6454	7.07	Redox: 31.4
16	T0411A	21.61		51	27.9	19.6	927	7.2	Redox: 16.5
17	EQ0112R	31	21.09	38	29.8	43.8	1350	7.14	Redox -36.9
18	HEOP0467M	5.3		12	29.7	31.9	1995	7.02	-27.1

Note: highlighted data indicating no water samples obtained.

APPENDIX E. Groundwater analysis results

Table E-1 Dissolved organic and inorganic carbon analysis (mg/L)

mg/L	Sample name	TOC	TC	IC
LOR		1	1	1
1	W028-2	6.5	68.2	61.6
2	W029-1	2.2	68.7	66.5
3	HHS0019M	5.9	85.8	79.8
4	HEC0448	174.9	306.3	131.4
5	HEA0351	6.3	107.6	101.3
6	T0399	3.7	107.1	103.4
7	HEQ0006	3.9	85.0	81.1
8	HNPIWR0003M	3.0	15.9	12.9
9	EOP0378R	5.9	75.6	69.7
11	EOP0334R	5.6	94.1	88.5
12	EOP0222R	3.7	53.5	49.8
13	HEOP0398M	1.7	56.2	54.5
14	HEOP0524	4.4	55.2	50.8
15	EOP0220R	7.4	134.3	126.9
16	T0411A	7.4	105.9	98.6
17	EQ0112R	0.5	93.0	92.5
18	HEOP0467M	3.5	89.3	85.8
mean		14.5	94.2	79.7
Median		4.4	85.8	81.1
min		0.5	15.9	12.9
max		174.9	306.3	131.4

Table E-2 Cation analysis in groundwater samples (mg/L)

	mg/L	Ca	K	Na	P	S	Si	Mg
LOR		**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**	**0.25**
1	W028	69.19	9.35	127.54	<LOR	52.66	40.31	68.65
2	W029	77.54	14.53	196.72	<LOR	84.76	25.44	76.91
3	HHS0019M	46.84	9.52	76.95	<LOR	23.47	25.81	61.85
4	HEC0448	56.38	11.93	258.53	<LOR	51.76	25.82	71.87
5	HEA0351	61.48	8.05	81.21	<LOR	26.97	29.67	80.75
6	T0399	56.80	20.19	289.15	<LOR	56.05	17.62	73.90
7	HEQ0006	40.37	9.96	81.03	<LOR	26.03	10.77	84.15
8	HNPIWR0003M	19.56	22.34	344.84	<LOR	0.58	0.15	65.83
9	EOP0378R	71.80	23.19	388.97	<LOR	101.97	22.55	79.68
11	EOP0334R	62.73	15.39	210.29	<LOR	66.34	28.31	64.14
12	EOP0222R	82.88	31.54	243.97	<LOR	58.98	7.41	82.34
13	HEOP0398M	53.78	10.97	100.22	<LOR	32.25	34.28	45.77
14	HEOP0524	72.15	12.00	200.37	<LOR	80.18	15.23	69.06
15	EOP0220R	110.02	71.90	1024.34	<LOR	301.19	25.59	79.80
16	T0411A	49.71	9.04	24.98	<LOR	4.16	19.10	62.40
17	EQ0112R	62.83	11.22	95.21	<LOR	41.10	21.20	177.10
18	HEOP0467M	63.45	16.19	244.37	<LOR	67.30	27.58	87.25
	mean	62.21	18.08	234.63		63.28	22.17	78.32
	median	62.73	12.00	200.37		52.66	25.44	73.90
	min	19.56	8.05	24.98		0.58	0.15	45.77
	max	110.02	71.90	1024.34		301.19	40.31	177.10

APPENDIX F. Groundwater PFAS analysis results

Table F-1 PFSAs concentration of water samples (μg/L)

ID	Sample	PFBS	PFPeS	PFHxS	PFHpS	PFNS	PFOS	PFDS	Sum
1	W028	<0.002	<0.002	<0.002	<0.005	<0.002	<0.005	<0.002	0.000
2	W029	<0.002	<0.002	0.002	<0.002	<0.002	<0.002	<0.002	0.002
3	HHS0019M	<0.002	<0.002	0.003	<0.002	<0.002	<0.002	<0.002	0.003
4	HEC0448	<0.005	<0.002	0.005	<0.005	<0.002	0.015	<0.002	0.021
5	HEA0351	<0.005	<0.002	0.005	<0.005	<0.002	<0.005	<0.002	0.005
6	T0399	<0.002	<0.002	0.006	<0.002	<0.002	<0.002	<0.002	0.006
7	HEQ0006	<0.002	<0.002	<0.002	<0.005	<0.002	<0.005	<0.002	0.000
8	HNPIWR0003M	<0.005	<0.002	<0.002	<0.005	<0.002	<0.005	<0.002	0.000
9	EOP0378R	<0.005	<0.002	<0.002	<0.005	<0.002	<0.005	<0.002	0.000
11	EOP0334R	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	0.000
12	EOP0222R	<0.002	<0.002	<0.002	<0.005	<0.002	<0.005	<0.002	0.000
13	HEOP0398	<0.002	<0.002	<0.002	<0.005	<0.002	<0.005	<0.002	0.000
14	HEOP0524	<0.002	<0.002	<0.002	<0.005	<0.002	<0.005	<0.002	0.000
15	EOP0220R	<0.002	<0.002	<0.002	<0.002	<0.002	0.006	<0.002	0.006
16	T0411A	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	0.000
17	EQ0112R	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	0.000
18	HEOP0467M	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	0.000

Table F-2 PFCAs concentration of water samples (μg/L)

ID	Sample	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUdA	PFDoA	PFTrDA	PFTeDA	Sum
1	W028	<0.01	<0.002	<0.002	<0.002	0.003	<0.002	<0.002	<0.002	<0.002	<0.002	<0.005	0.003
2	W029	<0.01	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.005	0.000
3	HHS0019M	<0.01	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.005	0.000
4	HEC0448	0.026	0.005	0.007	0.003	0.025	<0.002	<0.002	<0.002	<0.002	<0.002	<0.005	0.025
5	HEA0351	<0.01	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.005	0.000
6	T0399	<0.01	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.005	0.000
7	HEQ0006	<0.01	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.005	0.000
8	HNPIWR0003M	<0.01	<0.002	0.002	<0.002	0.005	<0.002	<0.002	<0.002	<0.002	<0.002	<0.005	0.005
9	EOP0378R	<0.01	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.005	0.000
11	EOP0334R	<0.01	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.005	0.000
12	EOP0222R	<0.01	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.005	0.000
13	HEOP0398	<0.01	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.005	0.000
14	HEOP0524	<0.01	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.005	0.000
15	EOP0220R	<0.01	<0.002	<0.002	<0.002	0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.005	0.002
16	T0411A	<0.01	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.005	0.000
17	EQ0112R	<0.01	<0.002	<0.002	<0.002	0.004	<0.002	<0.002	<0.002	<0.002	<0.002	<0.005	0.004
18	HEOP0467M	<0.01	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.005	0.000

Table F-3 Perfluoroalkyl Sulfonamides and (n:2) Fluorotelomer Sulfonic Acids (FTS) concentration of water samples (μg/L)

ID	Sample	FOSA	Me-FOSA	Et FOSA	ET-FOSE	FOSAA	Me-FOSAA	EtFOSAA	42FTS	62FTS	82FTS	Sum
1	W028	<0.002	<0.005	<0.005	<0.005	<0.005	<0.002	<0.002	<0.005	<0.005	<0.005	0.000
2	W029	<0.002	<0.005	<0.005	<0.005	<0.005	<0.002	<0.002	<0.005	<0.005	<0.005	0.000
3	HHS0019M	<0.002	<0.005	<0.005	<0.005	<0.005	<0.002	<0.002	<0.005	<0.005	<0.005	0.000
4	HEC0448	<0.002	<0.005	<0.005	<0.005	<0.005	<0.002	<0.002	<0.005	0.006	<0.005	0.006
5	HEA0351	0.008	<0.005	<0.005	<0.005	<0.005	<0.002	<0.002	<0.005	<0.005	<0.005	0.000
6	T0399	<0.002	<0.005	<0.005	<0.005	<0.005	<0.002	<0.002	<0.005	<0.005	<0.005	0.000
7	HEQ0006	<0.002	<0.005	<0.005	<0.005	<0.005	<0.002	<0.002	<0.005	<0.005	<0.005	0.000
8	HNPIWR0003M	<0.002	<0.005	<0.005	<0.005	<0.005	<0.002	<0.002	<0.005	<0.005	<0.005	0.000
9	EOP0378R	<0.002	<0.005	<0.005	<0.005	<0.005	<0.002	<0.002	<0.005	<0.005	<0.005	0.000
11	EOP0334R	<0.002	<0.005	<0.005	<0.005	<0.005	<0.002	<0.002	<0.005	<0.005	<0.005	0.000
12	EOP0222R	<0.002	<0.005	<0.005	<0.005	<0.005	<0.002	<0.002	<0.005	<0.005	<0.005	0.000
13	HEOP0398	<0.002	<0.005	<0.005	<0.005	<0.005	<0.002	<0.002	<0.005	<0.005	<0.005	0.000
14	HEOP0524	<0.002	<0.005	<0.005	<0.005	<0.005	<0.002	<0.002	<0.005	<0.005	<0.005	0.000
15	EOP0220R	0.010	<0.005	<0.005	<0.005	<0.005	<0.002	<0.002	<0.005	<0.005	<0.005	0.000
16	T0411A	<0.002	<0.005	<0.005	<0.005	<0.005	<0.002	<0.002	<0.005	<0.005	<0.005	0.000
17	EQ0112R	<0.002	<0.005	<0.005	<0.005	<0.005	<0.002	<0.002	<0.005	<0.005	<0.005	0.000
18	HEOP0467M	<0.002	<0.005	<0.005	<0.005	<0.005	<0.002	<0.002	<0.005	<0.005	<0.005	0.000

Table F-4 PFSAs concentration of water samples after TOPA (μg/L)

ID	Sample	PFBS	PFPeS	PFHxS	PFHpS	PFNS	PFOS	PFDS	Sum
1	W028	<0.002	<0.002	0.004	<0.005	<0.002	0.025	<0.002	0.029
2	W029	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	0.000
3	HHS0019M	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	0.000
4	HEC0448	<0.005	<0.002	0.004	<0.005	<0.002	0.017	<0.002	0.022
5	HEA0351	<0.005	<0.002	0.008	<0.005	<0.002	<0.005	<0.002	0.008
6	T0399	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	0.000
7	HEQ0006	<0.002	<0.002	<0.002	<0.005	<0.002	0.008	<0.002	0.008
8	HNPIWR0003M	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	0.000
9	EOP0378R	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	0.000
11	EOP0334R	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	0.000
12	EOP0222R	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	0.000
13	HEOP0398	<0.002	<0.002	0.008	<0.002	<0.002	<0.002	<0.002	0.008
14	HEOP0524	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	0.000
15	EOP0220R	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	0.000
16	T0411A	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	0.000
17	EQ0112R	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	0.000
18	HEOP0467M	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	0.000

Table F-5 PFCAs concentration of water samples after TOPA (μg/L)

ID	Sample	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUdA	PFDoA	PFTrDA	PFTeDA	Sum
1	W028	0.023	<0.002	0.010	<0.002	0.019	<0.002	<0.002	<0.002	<0.002	<0.002	<0.005	0.021
2	W029	<0.01	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.005	0.000
3	HHS0019M	<0.01	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.005	0.000
4	HEC0448	0.050	0.010	0.011	0.004	0.029	<0.002	<0.002	<0.002	<0.002	<0.002	<0.005	0.029
5	HEA0351	<0.01	<0.002	<0.002	<0.002	0.005	<0.002	<0.002	<0.002	<0.002	<0.002	<0.005	0.005
6	T0399	<0.01	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.005	0.000
7	HEQ0006	<0.01	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.005	0.000
8	HNPIWR0003M	<0.01	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.005	0.000
9	EOP0378R	<0.01	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.005	0.000
11	EOP0334R	<0.01	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.005	0.000
12	EOP0222R	<0.01	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.005	0.000
13	HEOP0398	<0.01	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.005	0.000
14	HEOP0524	<0.01	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.005	0.000
15	EOP0220R	<0.01	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.005	0.000
16	T0411A	<0.01	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.005	0.000
17	EQ0112R	<0.01	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.005	0.000
18	HEOP0467M	<0.01	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.005	0.000

Table F-6 Perfluoroalkyl Sulfonamides and (n:2) Fluorotelomer Sulfonic Acids (FTS) concentration of water samples after TOPA (μg/L)

ID	Sample	FOSA	Me-FOSA	Et FOSA	ET-FOSE	FOSAA	Me-FOSAA	EtFOSAA	42FTS	62FTS	82FTS	Sum
1	W028	<0.002	<0.005	<0.005	<0.005	<0.005	<0.002	<0.002	<0.005	0.006	0.009	0.015
2	W029	<0.002	<0.005	<0.005	<0.005	<0.005	<0.002	<0.002	<0.005	<0.005	<0.005	0.000
3	HHS0019M	<0.002	<0.005	<0.005	<0.005	<0.005	<0.002	<0.002	<0.005	<0.005	<0.005	0.000
4	HEC0448	<0.002	<0.005	<0.005	<0.005	<0.005	<0.002	<0.002	<0.005	0.011	<0.005	0.011
5	HEA0351	<0.002	<0.005	<0.005	<0.005	<0.005	<0.002	<0.002	<0.005	<0.005	<0.005	0.000
6	T0399	<0.002	<0.005	<0.005	<0.005	<0.005	<0.002	<0.002	<0.005	<0.005	<0.005	0.000
7	HEQ0006	<0.002	<0.005	<0.005	<0.005	<0.005	<0.002	<0.002	<0.005	<0.005	<0.005	0.000
8	HNPIWR0003M	<0.002	<0.005	<0.005	<0.005	<0.005	<0.002	<0.002	<0.005	<0.005	<0.005	0.000
9	EOP0378R	<0.002	<0.005	<0.005	<0.005	<0.005	<0.002	<0.002	<0.005	<0.005	<0.005	0.000
11	EOP0334R	<0.002	<0.005	<0.005	<0.005	<0.005	<0.002	<0.002	<0.005	<0.005	<0.005	0.000
12	EOP0222R	<0.002	<0.005	<0.005	<0.005	<0.005	<0.002	<0.002	<0.005	<0.005	<0.005	0.000
13	HEOP0398	<0.002	<0.005	<0.005	<0.005	<0.005	<0.002	<0.002	<0.005	<0.005	<0.005	0.000
14	HEOP0524	<0.002	<0.005	<0.005	<0.005	<0.005	<0.002	<0.002	<0.005	<0.005	<0.005	0.000
15	EOP0220R	<0.002	<0.005	<0.005	<0.005	<0.005	<0.002	<0.002	<0.005	<0.005	<0.005	0.000
16	T0411A	<0.002	<0.005	<0.005	<0.005	<0.005	<0.002	<0.002	<0.005	<0.005	<0.005	0.000
17	EQ0112R	<0.002	<0.005	<0.005	<0.005	**<0.005**	<0.002	<0.002	<0.005	<0.005	<0.005	0.000
18	HEOP0467M	<0.002	<0.005	<0.005	<0.005	<0.005	<0.002	<0.002	<0.005	<0.005	<0.005	0.000

APPENDIX G. Species analysis report

Table G-1 Species identified for groundwater samples

Locality	Phylum	Subphylum	Class	SuperOrder	Order	Family	Species	Habitus	No. of animals
HNPJWR003M									0
HEA0351									0
EOPO378R									0
SN11119, EOPO220R, 1/4/2021	Arthropoda	Chelicerata	Arachnida	Parasitiformes	Acariformes		*Not determined Mite*	*Edaphobite*	1
	Arthropoda	Chelicerata	Arachnida				*Not determined spider*	*Edaphobite*	1
	Arthropoda	Hexapoda	Insecta		Trichoptera	Leptoceridae	*Oecetis sp.*	*Stygoexene*	1
EOPO334R									0
EOPO334R Others	Arthropoda	Crustacea	Malacostraca	Pericarida	Amphipoda	Neoniphargidae	*Neoniphargus sp.*	*Phreatobite*	1
EOPO334R Copepoda	Arthropoda	Crustacea	Maxillopoda		Cyclopoida	Cyclopidae	*Mesocyclops australiensis*	*Phreatobite*	2
HEC0448									0
EOPO222R									0
EQO112R, 1/4/2021	Arthropoda	Crustacea	Malacostraca	Pericarida	Amphipoda	Neoniphargidae	*Neoniphargus sp.*	*Phreatobite*	2
	Arthropoda		Collembola			Hypogastruridae	*Hypogastrura sp.*	*Phreatobite*	1
T0411A, 1/4/2021	Annelida	Clitellata	Oligochaeta	Tubificata	Haplotaxida	Naididae	*Pristinella sp.*		1
HEOPO524, 31/3/2021	Annelida	Clitellata	Oligochaeta	Tubificata	Haplotaxida	Naididae	*Pristinella sp.*	*Phreatobite*	4
	Arthropoda	Chelicerata	Arachnida					*Edaphobite*	1
HEOPO467M									0
HEOPO467M	Annelida	Clitellata	Oligochaeta	Tubificata	Haplotaxida	Naididae	*Pristinella sp.*	*Phreatobite*	1
	Arthropoda	Crustacea	Maxillopoda	Podoplea	Harpacticoida	Canthocampidae	*Canthocamptus australicus c.f.*	*Phreatobite*	1
							Egg capsule?		1

HEQ006	Arthropoda	Hexapoda	Insecta		Thysanoptera	Thripidae	*Thrips australia*	*Stygoexene*	1
HEOPO398M							*Ant leg*		1
W028	Annelida	Clitellata	Oligochaeta	Tubificata	Haplotaxida	Naididae	*Pristinella sp.*	*Phreatobite*	2
W028 toxicity testing	Arthropoda	Crustacea	Maxillopoda	Copepoda	Cyclopoida	Cyclopidae	*Diacyclops humphreysi*	*Phreatobite*	136
W029	Arthropoda	Crustacea	Malacostraca	Pericarida	Amphipoda	Neoniphargidae	*Neoniphargus sp.*	*Phreatobite*	2
W029 toxicity testing	Arthropoda	Crustacea	Maxillopoda	Copepoda	Cyclopoida	Cyclopidae	*Diacyclops humphreysi*	*Phreatobite*	63
T0399	Arthropoda	Crustacea	Malacostraca	Pericarida	Isopoda	Tainisopidae	*Pygolabis humphreysi*	*Phreatobite*	3
T0399	Arthropoda	Crustacea	Malacostraca	Pericarida	Amphipoda	Neoniphargidae	*Wesiphargus nichollsi*	*Phreatobite*	3
T0399 sediment									0
HHS0019M	Arthropoda	Crustacea	Ostracoda		Myodocopida	Candonidae	*Hancockcandonopsis sp.*	*Phreatobite*	2
HHS0019M	Annelida	Clitellata	Oligochaeta		Haplotaxida	Naididae	*Pristinella sp.*		1
HHS0019M							*Metacyclops cf.*		1

APPENDIX H. The counting of copepods during toxicity study

Table H-1 The record for number of alive copepods during toxicity testing study

Concentration (µg/L)	Days						
	D4	D7	D14	D21	D28	D42	D56
0	20	20	20	20	19	10	10
0.05	20	20	20	18	18	14	11
0.1	20	20	20	19	17	14	10
1	20	20	20	19	16	14	9
5	20	20	20	19	18	12	11
25	20	20	19	19	18	14	12
50	20	20	20	18	16	12	10
100	20	20	20	19	18	15	14
200	20	20	20	16	14	11	10
500	20	19	13	10	8	5	4
1000	20	20	16	11	7	3	1

APPENDIX I. Scoring for the toxicity study

Table I1 Scoring system for assessing the quality of toxicity data for non-metals to freshwater non-plants to be used in the derivation of guideline values for toxicants

QUESTION		MARK	This study
1	Was the duration of the exposure stated (for example 48 or 96 h)?	Yes (10), No (0)	**10**
2	Was the biological endpoint (for example immobilisation or population growth) stated and defined?	Yes (10), Stated only (5), Neither (0)	**10**
3	Was the biological effect stated (for example LC or NOEC)?	Yes (5), No (0)	**5**
4	Was the biological effect quantified (for example 50% effect, 25% effect)? Note: The effect for NOEC and LOEC data must be quantified.	Yes (5), No (0)	**5**
5	Were appropriate controls (for example a no-toxicant control and/or solvent control) used?	Yes (5), No (0)	**5**
6	Was each control and chemical concentration at least duplicated?	Yes (5), No (0)	**5**
7	Were test acceptability criteria stated (for example mortality in controls must not exceed a certain percentage) or were test acceptability criteria inferred (for example test methods used were USEPA or OECD? Note: Data that fail the acceptability criteria are automatically deemed to be of unacceptable quality and must not be used.	Stated (5), Inferred (2), Neither (0)	**5**
8	Were the characteristics of the test organism (for example length, mass, age) stated?	Yes (5), No (0)	**0**
9	Was the type of test media used stated?	Yes (5), No (0)	**5**
10	Was the type of exposure (for example static, flow-through) stated?	Yes (4), No (0)	**4**
11	Were the contaminant concentrations measured at the beginning and end of the exposure? Note: Normally, toxicity data calculated using nominal concentration data would not be used to derive GVs; however, professional judgement can be used to include such data, provided a justification for their use is provided.	Yes (4), Measured once (2), Not measured or stated (0)	**2**
12	Were parallel reference toxicant toxicity tests conducted?	Yes (4), No (0)	**0**
13	Was there a concentration-response relationship either observable or stated?	Yes (4), No (0)	**4**
14	Was an appropriate statistical method or model used to determine the toxicity? Note: They should be accepted by a recognised national or international regulatory body (for example USEPA, OECD or ASTM)	Yes (4), No (0)	**4**

QUESTION		MARK	This study
15	For LC/EC/NEC/BEC data, was an estimate of variability provided? OR For NOEC/LOEC/MDEC/MATC data, was the significance level 0.05 or less?	Yes (4), No (0)	**4**
16	Were the following parameters measured and stated?		
16.1	pH - pH should be measured at least at the beginning and end of the toxicity test	Measured at the beginning and end of the test and stated (3), Measured once (1), Not measured or stated (0)	**1**
16.2	Dissolved oxygen	Measured and stated (3), Measured only (1), Neither (0)	**1**
16.3	Conductivity	Measured and stated (3), Measured only (1), Neither (0)	**1**
17	Was the temperature measured and stated?	Measured and stated (3), Measured but not stated or temperature of the room or chamber was stated (1), Neither (0)	**3**
18	Were test solutions, blanks and/or controls tested for contamination or were analytical reagent grade chemicals or the highest possible purity chemicals used for the experiment?	Yes (3), No (0)	**3**
	Total score **Total possible score for FW/non-metal/non-plant data = 94**		
	Quality score: [Total score/Total possible score] x 100		**77**
	Quality class: **high quality = when quality score ≥ 80%** **acceptable quality = when quality score ≥50–<80%** **unacceptable quality = when quality score <50%**		**81.9%**

Source: modified from Zhang et al. 2015 (note the modifications only affect the appearance of the table)

APPENDIX J. PFAS guideline values

Table J1 Current PFAS guideline values (µg/L) for drinking water, recreation water, surface water, wastewater effluents in Australia and other countries and organisations (ITRC)

ITRC – Interstate Technology Regulatory Council, USA
ITRC https://pfas-1.itrcweb.org/fact-sheets/

µg/L	Agency / Dept	Year Last Updated	Standard / Guidance	Type*	Promulgated Rule (Y/N/O)	Footnote	PFOA	PFOS	PFNA	PFBA	PFBS	PFHxS	PFHxA	PFPeA	PFHpA	PFOSA	PFDA	6:2 FTS	8:2 FTS
CAS number							335-67-1	1763-23-1	375-95-1	375-22-4	375-73-5	355-46-4	307-24-4	2706-90-3	375-85-9	754-91-6	335-76-2	39108-34-4	39108-34-4
USEPA	Office of Water	2016	HA	DW	N	a	0.070	0.070											
	Regions	2021	RSL	GW	N	b					6								
	Regions	2021	RSL Calculation	GW	N	c	0.400	0.400			6.01								
	OLEM	2019	Interim Recommend-ation	GW	N	d	0.040	0.040											
Australia	DOH	2017	health-based	DW		e	0.560	0.070				0.070							
		2017	health-based	RW		e	5.6	0.700				0.700							
British Columbia, Canada		2018	water standard	DW/GW			0.200	0.300			80								

Canada	HC	2016	DWSV	DW			0.200	0.600	0.020	30	15	0.600	0.200	0.200	0.200				
	HC	2019	DWSV	DW														0.200	0.200
	HC	2018	MAC	DW	Y		0.200	0.600											
European Union	EU	2013	EQS AAC	SW-inland				0.00065											
	EU	2013	EQS AAC	SW-other				0.00013											
	EU	2013	EQS MAC	SW-inland				36											
	EU	2013	EQS MAC	SW-other				7.2											
Denmark	EPA	2015	health-based	DW/GW		f	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	
Germany	GMH	2006	health-based	DW			0.300	0.300											
			administrative	DW		g	0.100	0.100											
		2018	GFS	GW			0.100	0.100	0.060	10	6	0.100	6						
Italy		2017	health-based	DW			0.500	0.030		7	3		1	3					
		2017	screening value	FW		h	0.100			7	3		1	3					
Netherlands	EPA	2020	INEV	DW			0.39	0.00065											
		2020	INEV	GW			170	0.00013											
Norway		2014	EQS	SW			9.1	0.00065											
		2014	EQS	CW			9.1	0.00013											
Sweden		2014	health-based	DW				0.090											
		2014	administrative	DW		i	0.090	0.090			0.090	0.090	0.090	0.090	0.090				
UK	DWI	2009	health-based	DW			10	0.300											
		2021	Tier 2, Regulation 10	DW			0.010	0.010											
		2021	Tier 3, Regulation 4(2)	DW			0.100	0.100											
		2021	Tier 4, Water Industry	DW			1.0	1.0											

Notes:

a Applies to the individual results for PFOA and PFOS, as well as the sum of PFOA + PFOS.

b Regional Screening Level (RSL) as presented in the USEPA Regional Screening Level (RSL) Summary Table (TR=1E-06, HQ=1) November 2014 through May 2018.

c As of June 2018, calculated by the USEPA RSL calculator using USEPA OW RfDs, HQ of 1, and residential exposure assumptions. Note: RSL users screening sites with multiple contaminants should consult the USEPA (2018) RSL User's Guide and USEPA (1989) Risk Assessment Guidance.

d Interim screening level for groundwater at sites contaminated with PFOS and PFOA, based on target hazard quotient of 0.1

e The Australian Government Department of Health values for PFOS/PFHxS are combined value when both are present.

f Applies to the individual results for PFOA, PFOS, PFNA, PFBA, PFBS, PFHxS, PFHxA, PFPeA, PFHpA, PFOSA, PFDA, AND 6:2 FTS as well as the sum of concentrations of these 12 PFAS.

g The GMH administrative guidance value of 0.1 µg/L is a composite precautionary value for both PFOA and PFOS for long term exposure in drinking water.

h Annual Average - Environmental Quality Standards. PFOA AA-EQS based on secondary poisoning of wildlife.

i Administrative value is for the sum of seven PFAS found in drinking water: PFOS, PFOA, PFHxS, PFBS, PFHpA, PFHxA, and PFPeA. PFOS is considered to be the most toxic. Water can still be used at up to 0.09 µg/L.

* DW-drinking water; GW-groundwater; FW-fresh water; CW-coastal water; SW-surface water and/or effluent; RW-recreational water