

Review Of Long-Term Trends in the Ethel Gorge Stygobiont TEC Report



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PREPARED FOR:

Tanya Carroll
BHP WAIO

PREPARED BY:

Mathew Hourston, Erin Thomas, Richard de Lange

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1. Introduction

1.1 Project Background

The Ethel Gorge Aquifer contains a groundwater-dependent stygofauna community (stygobiont), located on the Fortescue River and Ophthalmia Floodplain, a Threatened Ecological Community (Ethel Gorge Stygobiont TEC) listed as Critically Endangered under the *Biodiversity Conservation Act 2016* (BC Act) and the *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act). This TEC is located within the Pilbara bioregion, approximately 15 km northeast of Newman, adjacent to Eastern Ridge, and downstream of the confluence of the Fortescue River with Homestead Creek (**Figure 1-1**)(RPS 2013).

Several BHP mining operations are located in the vicinity of the Ethel Gorge Stygobiont TEC including Eastern Ridge, Whaleback and Jimblebar, incorporating multiple orebodies (**Figure 1-1**). These operations have the potential to impact the TEC, most notably through dewatering of the Ethel Gorge Aquifer and the discharge of surplus water into Ophthalmia Dam; resulting in changes to the subterranean hydrological environment. For this reason, ongoing monitoring by BHP has been an integral part of the management of the TEC. To support this, BHP commissioned Stantec to undertake a desktop assessment (the Assessment), examining the long-term stygobiont data set, in line with recommendations from the 2019/2020 monitoring report (Stantec 2021).

1.2 Objectives and Scope

The key objectives of the Assessment were to review the existing long-term data set for the Ethel Gorge Stygobiont TEC to further understand the temporal and spatial trends in the fauna in response to environmental factors, and to assess the potential to develop tools to support management. To address the objectives, the following tasks were undertaken:

- review and analyse the Ethel Gorge Stygobiont TEC data set in relation to abiotic parameters;
- examine the suitability of trigger levels for species richness and abundance for management of the TEC;
- examine the potential of taxa for use as indicator species to support the management of the TEC; and
- investigate the trophic structure of the stygofauna of the Ethel Gorge Stygobiont TEC.

1.3 Guidance and Legislation

The Assessment was informed by regulatory guidelines and BHP documentation including:

- Environmental Factor Guideline – Subterranean Fauna (EPA 2016);
- Statement of environmental principles, factors, objectives and aims of EIA (EPA 2023);
- Technical Guidance - Subterranean Fauna Surveys for Environmental Impact Assessment (EPA 2021); and
- The Eastern Pilbara Water Resources Management Plan, 2014 (Douglas and Pickard 2014b).

1.4 Existing Environment

1.4.1 Location, Hydrology and Hydrogeology

The Ethel Gorge Stygobiont TEC is located on the Fortescue River and Ophthalmia floodplain, at the confluence of several Pilbara riverine systems including Whaleback, Shovellana, and Homestead creeks as they enter the Fortescue River. The TEC occurs within a shallow, alluvium aquifer, in association with a calcrete deposit up to 40 m thick. This unconfined alluvial and calcrete unit is separated from a deeper gravelly-alluvium aquifer by a low permeability clay sequence. The proximal Ophthalmia Dam provides substantial groundwater recharge and hydraulic loading to the alluvial aquifer, with direct infiltration from streamflow events also contributing (RPS 2013). As reported by RPS (2013), groundwater levels across the area range between 0 metres below ground level (mbgl) and 10 mbgl. While declines have been noted in response to pit dewatering within adjacent mining operations, and low rainfall periods (2018/2019), groundwater levels have remained within the range documented since Ophthalmia Dam was commissioned. Considerable recharge of the groundwaters was observed in January 2020, linked to substantial rainfall from tropical cyclone Blake (BHP 2022; EMM 2020).

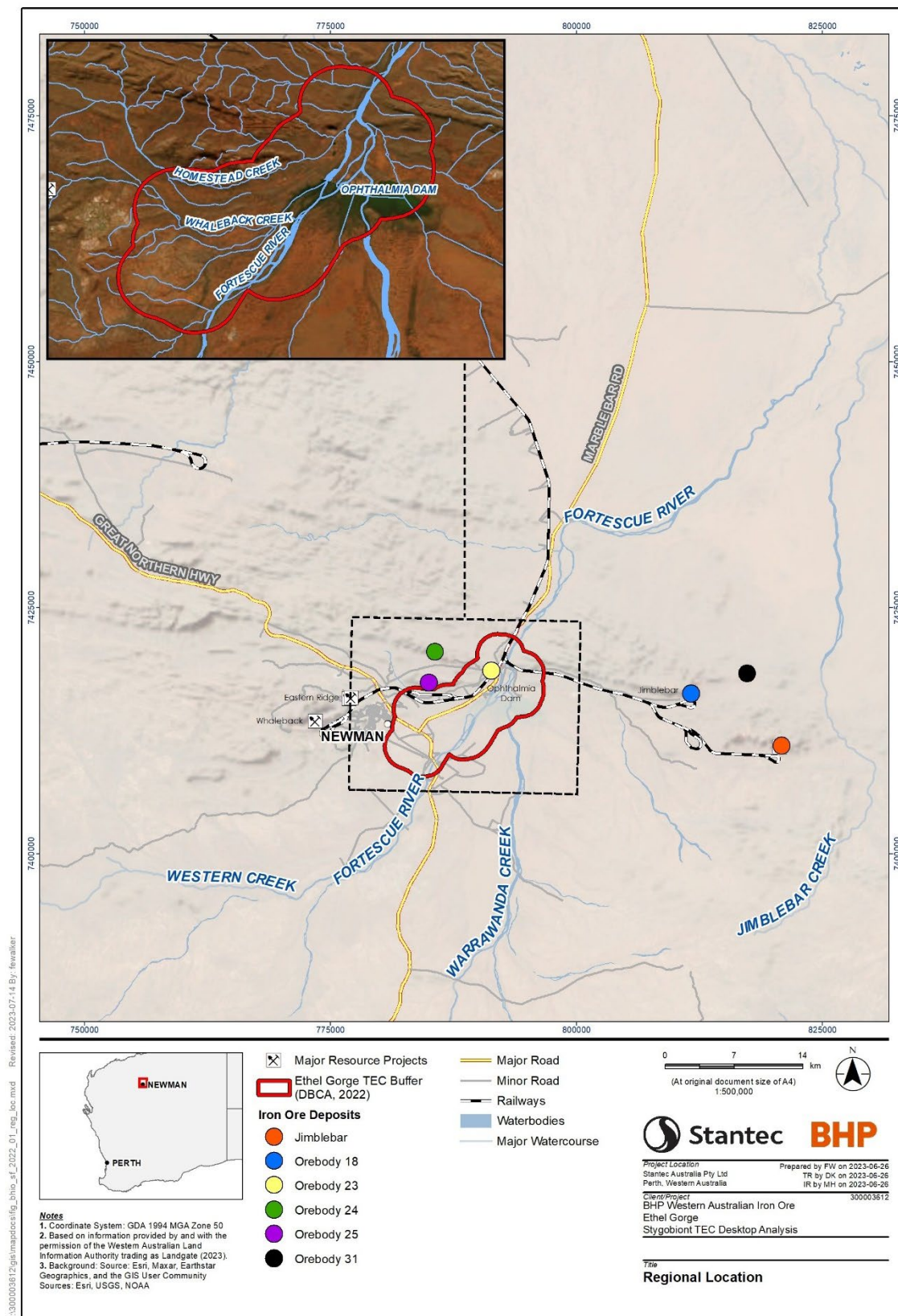


Figure 1-1: Regional location of the BHPIO WAIO deposits surrounding Ophthalmia Dam and the Ethel Gorge Stygobiont TEC buffer.

1.4.2 Climate

The climate of the Pilbara bioregion is classified as semi-arid with very hot summers and mild winters. Rainfall typically occurs during the wet season (December to April), in response to ex-tropical cyclones or isolated storm activity. Evaporation rates are high, with temperatures often exceeding 38°C in summer. The long-term average annual rainfall recorded at Newman Aero (weather station 007176; 1971 – 2022), the nearest Bureau of Meteorology (BOM) weather station to the Ethel Gorge Stygobiont TEC, is 323.8 mm (BoM 2023). Values have fluctuated considerably since stygofauna monitoring began at the Ethel Gorge Stygobiont TEC in 2003, ranging from 115 mm in 2019 to 545 mm in 2006 (**Figure 1-2**). Rainfall for five of the seven most recent years' has been below the long-term average (BoM 2023).

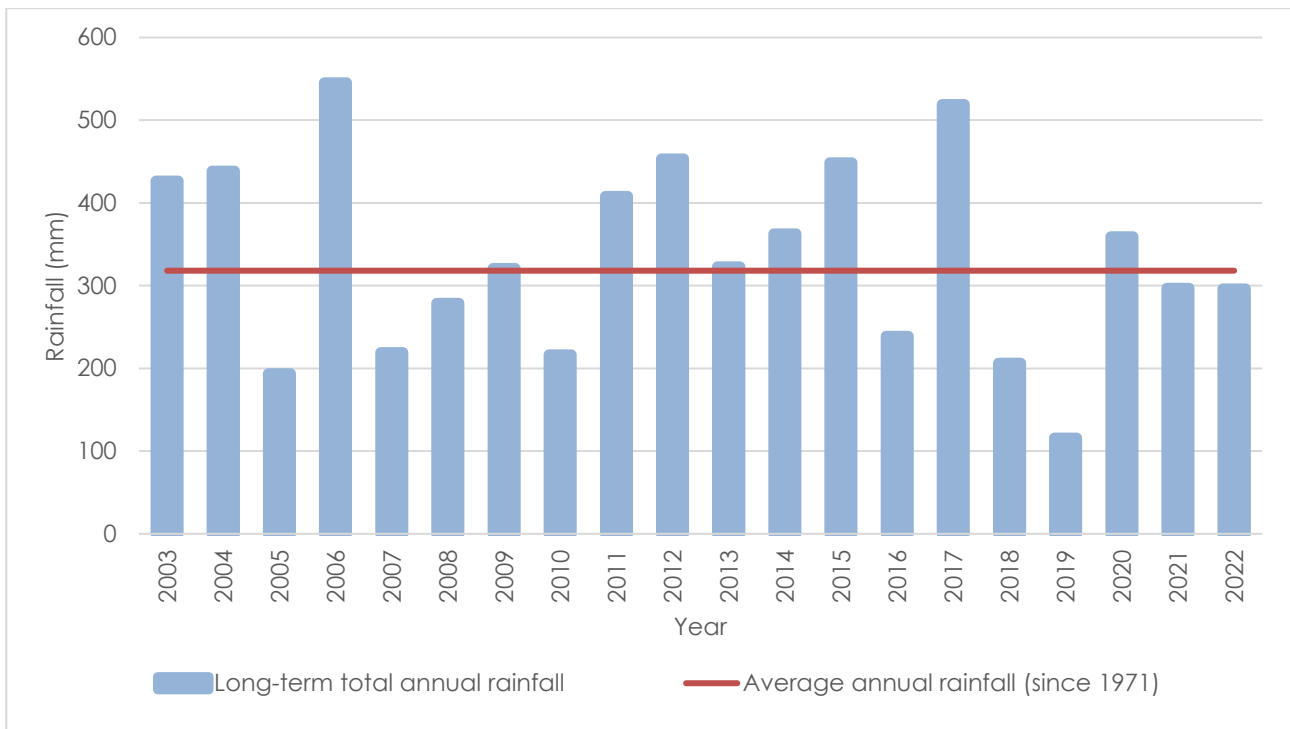


Figure 1-2: Long-term total annual rainfall (2003-2022) at Neman Aero (007176) compared to the long-term annual average rainfall since 1971 (red line) (BoM, 2023).

2. Ethel Gorge Stygobiont TEC Context and Data

The Ethel Gorge Stygobiont TEC was first identified and defined in 1997 during an environmental review of mining below the water table at Orebody 23. Those works determined that a diverse stygofauna assemblage was present in the local aquifer (Humphreys 1999). The Threatened Ecological Communities Scientific Committee (TECSC) recommended that the area be listed as a TEC and listed as Endangered. This reflected the limited known distributions of numerous stygofauna species at the time of discovery, and acknowledged the vulnerability of the community to known threatening processes (Bennelongia 2013). In 2013, the TEC was characterised and mapped by Bennelongia (2013), and the taxonomy of the community was reviewed and consolidated by Subterranean Ecology (2013), based on information available at the time. On the 26th of May 2023, the TEC was gazetted as Critically Endangered (Minister for Environment 2023).

The most up-to-date version of the Ethel Gorge Stygobiont TEC's geographical boundary is presented in **Figure 2-1** along with the current buffer provided by Department of Biodiversity, Conservation and Attractions (DBCA). Historically, the buffer constituted a 5 km radius around the TEC boundary but has since been reduced to 2 km.

The most recently reported survey of the Ethel Gorge Stygobiont TEC (Dry 2021 / Wet 2022), undertaken by Stantec (2022) determined that the stygofauna assemblage consists of 82 taxa within the Ethel Gorge Aquifer and/or adjacent local aquifers in the Newman area. Of the species within the Ethel Gorge Stygobiont TEC, 50 were recorded as "Core Species" by Bennelongia (2013), who defined "Core" as "those species known only from the wider Newman area", which approximately corresponds to the mapped area in **Figure 2-2**. As such, any species which are exclusively recorded from the samples in the consolidated data set can be considered as Core species. Those 50 species defined by Bennelongia (2013) primarily comprised microcrustaceans (copepods and ostracods), with isopods, oligochaetes, amphipods and bathynellids also prominent (Bennelongia, 2013). While copepods and ostracods have been numerically dominant, amphipods and bathynellids have been a diverse component of the assemblage over time (Stantec, 2017).

The consolidated Ethel Gorge Stygofauna data set contains records from 2003 to current. Records include samples from sporadic surveys between 2003 and 2007, annual surveys between 2007 and 2013, and biannual surveys since 2013. Surveys post-2013 include wet and dry seasons except for 2015 to 2017, which were conducted in the wet season only. Biannual monitoring recommenced in 2019. Samples from multiple bores are collected as part of a discrete survey period. These periods have been identified as survey rounds and are numbered chronologically (**Appendix A**). The Ethel Gorge Stygobiont consolidated database is held by BHP WAIO and includes data from designated monitoring rounds and any other relevant stygofauna data from the immediate area. Other relevant stygofauna data includes two rounds of targeted surveys in the East Ophthalmia area by Bennelongia in 2020 and 2021 (**Table 2-1**), designated as R26 and R29 in **Appendix A**.

Accompanying the stygofauna data collected from the TEC is a data set of abiotic parameters. The available, abiotic data suite commences in 2009, with data initially restricted to pH and salinity (as electrical conductivity or EC). Over time this was expanded to include additional parameters. Rounds R14 (Feb 2012) to Round R18 (March 2014) include records for ions for most samples (Cl, Ca, K, Mg, Na and SO₄) and since Round R19, nutrients (N, P) have also been measured. Metals were added in December 2019 (R24) however have not been considered in this Assessment due to the comparatively limited data set. The abiotic measurements since Round R19 have been consistently collected alongside the stygofauna samples in the survey rounds (except for some samples in Rounds R22 and R24, and the East Ophthalmia Rounds R26 and R29), so directly comparable environmental conditions are available for the majority of stygofauna samples since.

2.1 Sampling and Data Limitations

Since sampling commenced in 2003, there has been considerable variation in the survey intensity, rigour, and prior knowledge to inform survey planning. Several limitations are inherent in the data due to the above variability including:

- **Taxonomic accuracy and resolution** – the level and accuracy of taxonomic resolution used to classify the specimens was relatively coarse in the early surveys and in some cases is incompatible with the later years' data. This limitation was recognised by Subterranean Ecology (2013) where several morphospecies were designated as invalid, with subsequent exclusion from future data sets.

- **Species' abundances** – early surveys recorded abundances in log ranges rather enumerating individuals, making quantitative comparison between years difficult.
- **Taxonomic developments and synonyms** – there have been considerable taxonomic advances since the declaration of the TEC. In 2013, a consolidated species list was compiled to characterise the species of the Ethel Gorge Stygobiont TEC (Subterranean Ecology 2013). However, there has been substantial progress in relation to the taxonomy. While taxonomic synonymisation has been ongoing, taxa richness requires verification due to the long data set and multiple contributing projects.
- **Bore management** - over two decades of bore development and management has taken place since the stygofauna surveys commenced. This has resulted in multiple naming conventions for the bores, resulting in multiple bores with the same designation, and single bores having multiple names. Additionally, variation in recorded spatial coordinates for some bores has been noted.
- **Survey design** - the dynamic Pilbara surface environment under which the TEC is located has prompted changes in the survey designs over time. Many bores have been lost or collapsed, while new bores have been drilled. Access to parts of the survey area has been restricted over time, with high water levels in the dam and rivers blocking access tracks during wet seasons. This has resulted in a data set in which no two survey rounds have retrieved stygofauna from the same set of bores. The bores from which stygofauna were collected over time have been tabulated in **Appendix A**.
- **Sample method** - while the survey design and sampling intensity have varied between 2014 and 2022, the sampling methods to collect stygofauna from bores have been consistent, aligning with guidance as described in EPA (2016). A full description of sampling methods is included in Stantec (2022). For the years prior to 2014, the sampling methods are assumed to have been consistent with that of later years.

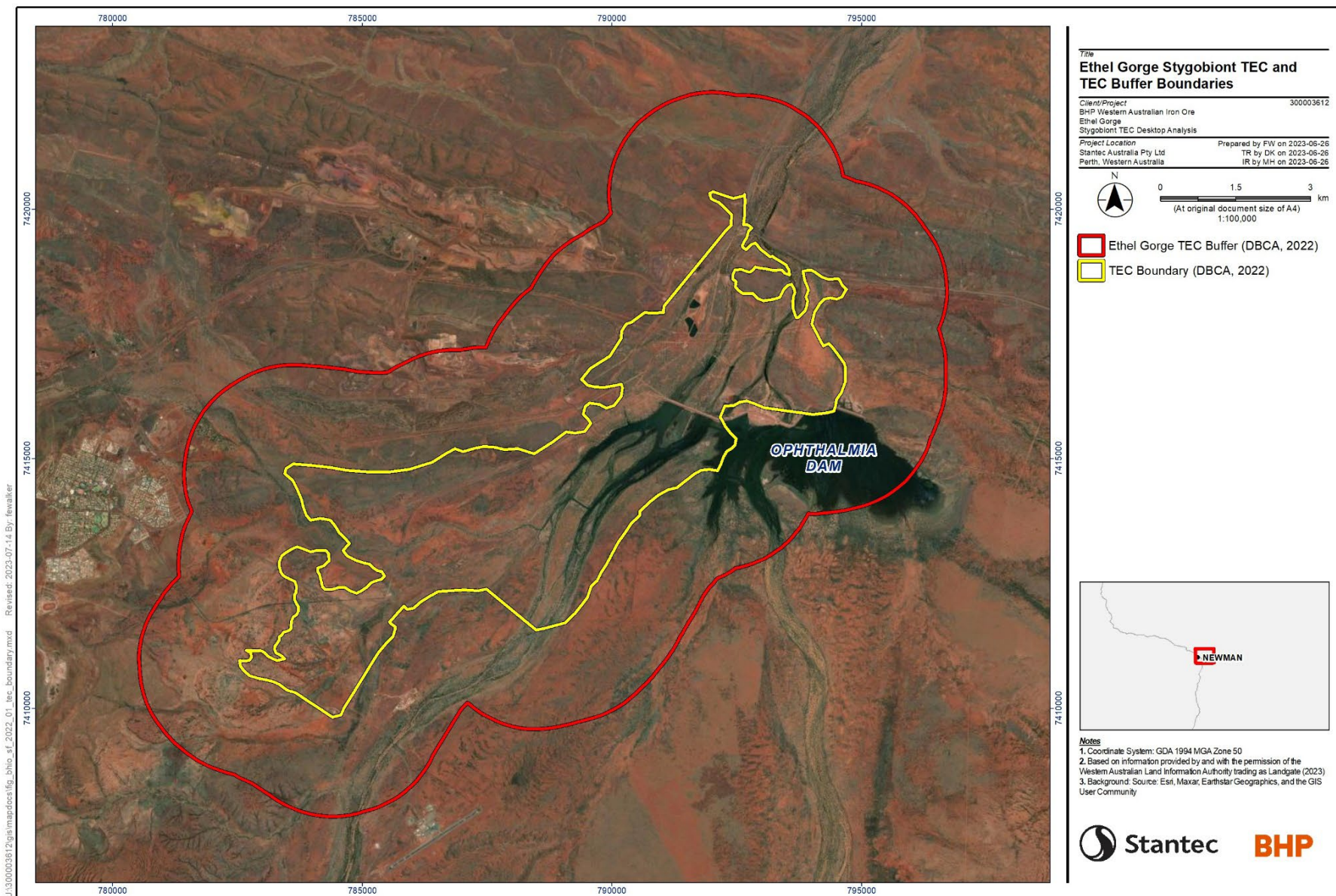


Figure 2-1: Current geographical boundary of the Ethel Gorge Stygobiont TEC and TEC buffer.

Table 2-1: Historic stygofauna surveys that contribute records to the consolidated Ethel Gorge Stygobiont TEC data set.

Year	Survey Timing	Sampler/Author	Reference
2003	Dry Season	Not Available	Not Available
2007	Dry Season	Biota Environmental Sciences	Not Available
2008	Dry Season	Ecowise Environmental	Not Available
2009	Wet and Dry Season	Ecowise Environmental, Subterranean Ecology	Not Available
2010	Wet and Dry Season	Subterranean Ecology	Not Available
2011	Wet Season	Subterranean Ecology	Not Available
2012	Wet Season	Subterranean Ecology	Subterranean Ecology (2012)
2013	Dry Season	Subterranean Ecology	Subterranean Ecology (2014)
2014	Wet and Dry Season	Subterranean Ecology, Stantec (MWH)	Subterranean Ecology (2014), MWH (2015)
2015	Wet Season	Stantec (MWH)	MWH (2015)
2016	Wet Season	Stantec (MWH)	MWH (2016)
2017	Wet Season	Stantec	Stantec (2017)
2019	Dry Season	Stantec	Stantec (2020)
2020	Wet and Dry Season	Stantec	Stantec (2020), Stantec (2021)
2020	Dry Season	Bennelongia	Unpublished (East Ophthalmia)
2021	Wet and Dry Season	Stantec	Stantec (2021), Stantec (2022)
2021	Wet Season	Bennelongia	Unpublished (East Ophthalmia)
2022	Wet and Dry Season	Stantec	Stantec (2022), Stantec (in prep)

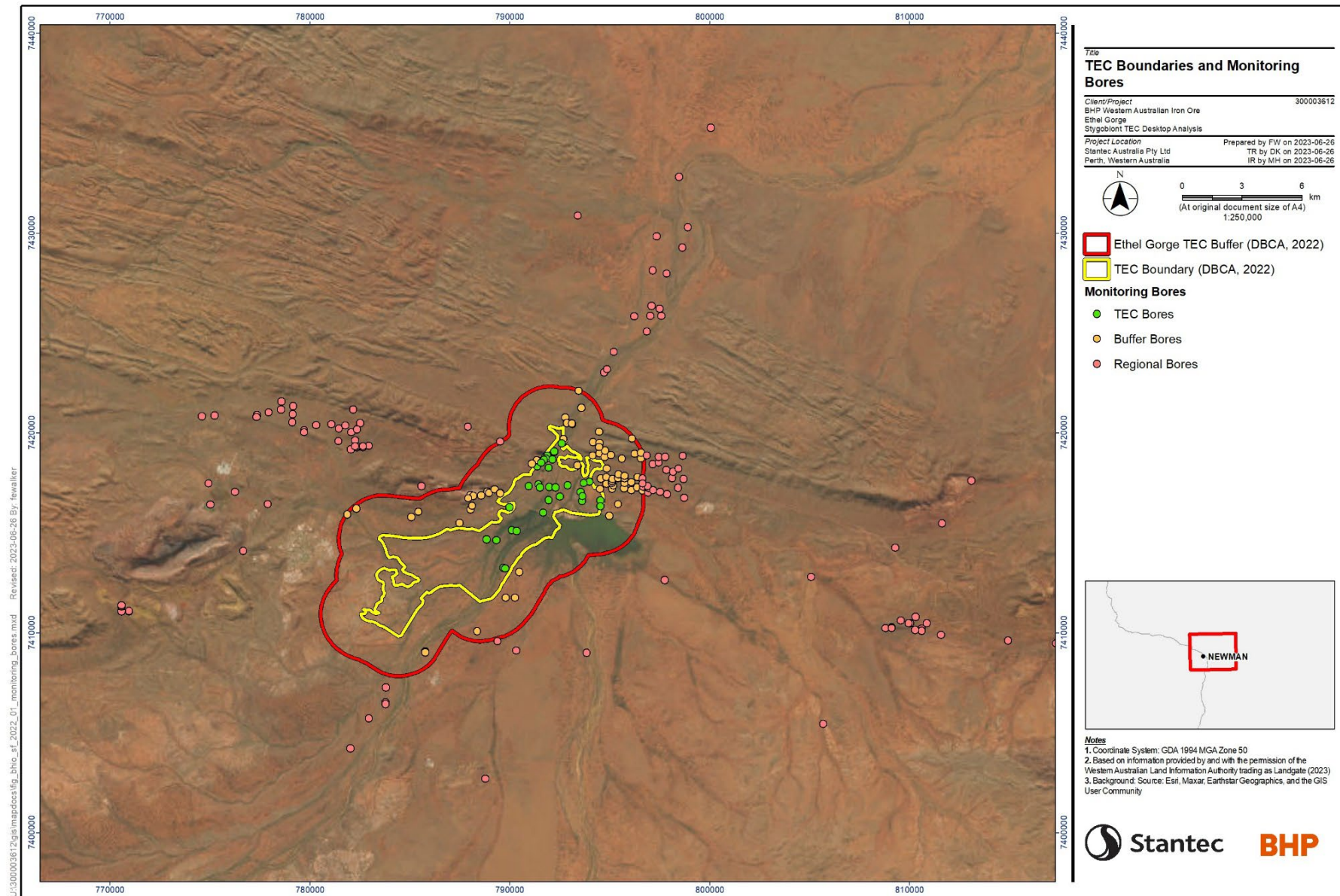


Figure 2-2: TEC boundary (yellow), buffer (red) and location of monitoring bores, classified by zone. TEC=green, BUF=yellow, REG=red.

2.2 Indicator Species

Indicator taxa are species or higher taxonomic groups whose temporal and spatial parameters (e.g., density or presence/absence) are used as proxy measures of ecosystem conditions. This represents an alternative method to monitor change in fauna communities rather than examining the entire community (Hilty and Merenlender 2000). The use of indicator taxa may assist in early detection of adverse changes in an ecosystem over large temporal and spatial scales. This concept can assist in outlining boundaries of an ecological community and assess the health of the ecosystem, by relieving the taxonomic burden and provide simpler sampling strategies and protocols. However, to be useful for this purpose, an indicator species must meet certain criteria. Foremost, it must be representative of the community. It must also occur in consistent and sufficiently high abundances across the range of the community to be captured in the chosen sampling effort (i.e., collected in a large enough number of samples). Ideally, indicator species will also be readily identifiable to relieve taxonomic burden. In addition, to be used as ecosystem health indicators, their preferences, sensitivities and tolerance levels should be well established, as well as their correlation to ecosystem changes (Hilty and Merenlender 2000; Holt and Miller 2010; Zettler *et al.* 2013). While the use of indicator species may be efficient in the long term, the level of knowledge required to identify and employ suitable indicators is relatively high.

Taxa that satisfy all the above criteria are the ideal candidates for indicators, however in a practical sense, it is rare that all criteria are met by a single taxon in any ecosystem. In stygal systems, this is likely to be exacerbated by the inherent variability and low abundance of stygofauna in bore samples. In this situation, species would need to be considered in a relative sense, focusing on species that have relatively low variability and relatively high abundance.

Defined sensitivities and tolerance levels are a recognised attribute of useful indicator species. An understanding of these limits may differentiate between taxa which characterise their community (i.e., consistently present in relatively high numbers) and those that can be effectively used to develop and support management systems. Knowledge of how stygal taxa respond to various environmental and anthropogenic changes is currently poor. As this applies across taxa, it can be considered a general limitation to using indicator species in stygal systems, rather than a discriminator to determine suitable candidate species.

Over time, several species have been suggested as potential indicator species for the Ethel Gorge Stygobiont TEC (Bennelongia 2013; Stantec 2021). These include the copepods *Nitocrella* OB, *Pilbaracyclops supersensus* and *Nitocrella karanovici*, and the amphipod *Chydaekata acuminata* (Bennelongia 2013). These taxa have been abundant and/or common within the TEC and investigations have been recommended to determine their suitability as indicator species. Their suitability as indicator species will be considered in subsequent sections, along with the rest of the assemblage.

3. Methods

3.1 Pre-treatment

The data set was derived from those studies detailed in **Table 2-1**. Pre-treatment of the consolidated Ethel Gorge TEC data set included:

- Consolidation and update of all taxonomic synonyms,
- Consolidation and update of all bore names, codes and geographical coordinates,
- Designating all taxa in the data set as meeting the criteria for “Defined” taxa or not,
- Mapping each bore into either TEC Boundary Zones (TEC, Buffer or Regional areas), and
- Mapping each bore into the monitoring zones as formally defined in the Eastern Pilbara Water Resource Management Plan (EPWRMP) (Douglas and Pickard 2014a).

3.2 Determination of Defined taxa

Defined taxa were considered to be taxa that were unlikely to comprise a mix of identified taxa or any ambiguously identified taxa. At a species level, Defined taxa included species that were:

- Formally named (e.g., *Chydaekata acuminata*),
- A distinct morphospecies even if it is not formally described (e.g., *Parastenocaris* ‘COP001’),
- A genetically circumscribed species (e.g., Phreodrilidae OB2).

An example of a taxon that did not meet the criteria at a species level was ‘Paramelitidae indeterminate’, which could represent one or a combination of several *Chydaekata*, *Maarrka* or undescribed species. All taxa that did not meet the aforementioned criteria were omitted from species-level analysis.

Further analyses aggregated records up to a family level. In this case all records were re-included prior to aggregation and then Defined taxa were evaluated again at that higher level. Any family-level taxa that did not meet the Defined taxon criteria were then omitted. This method allowed additional records to be included that had only been identified to a family or genus level, however it decreased taxonomic resolution. In the above example of ‘Paramelitidae indet.’, that taxon could reliably be included in the family-level Defined taxon ‘Paramelitidae’, allowing those previously omitted records to be included in analyses.

3.3 Spatial Frameworks

Analyses were performed around two spatial frameworks: 1) TEC Boundary Zone, and 2) Monitoring Zone. Designation of each of the bores under the two frameworks are specified in **Appendix A**.

3.3.1 TEC Boundary Zones

The TEC Boundary Zones comprised TEC and Buffer and Regional bores. The TEC and Buffer are defined as the spatial extent of the official Threatened Ecological Community (TEC) and the impact buffer zone as recorded by the Department of Biodiversity Conservation and Attractions (DBCA) (**Figure 2-1**). The Buffer is currently defined as a 2 km distance from the nearest boundary of the TEC outline (**Figure 2-1**), however, for the majority of the development of the monitoring program and the resultant data set, the buffer was recorded as 5 km from the TEC boundary. For the purposes of direct comparison of bores through time, bores have been classified according to the current extent of the buffer (2 km), but it should be noted that many bores that had been considered part of the buffer area throughout the monitoring program are now in the Regional category. Records in the data set have been allocated into three zones under this spatial framework (**Table 3-1**). Divided by this spatial framework, there have been 38 bores within the TEC, 72 in the Buffer, and a further 116 in the surrounding region, since 2003 (**Figure 2-2**).

Table 3-1: Three zones into which records have been sorted under the TEC Boundary Zone spatial framework.

Zone	Description
TEC	Threatened Ecological Community; records from the bores inside the TEC boundary
BUF	Buffer; records from bores within the 2km buffer boundary but outside the TEC boundary
REG	Regional bores; any records in the data set from bores outside the buffer boundary

3.3.2 Monitoring Zones

The Ethel Gorge Monitoring and Management Zone framework was formally defined and published in the Eastern Pilbara Water Resource Management Plan (EPWRMP) (Douglas and Pickard 2014b). The original spatial framework contained five areas, located to the north, east and west of Ophthalmia Dam (**Figure 3-1**).

Over time, bores were added to the monitoring program to expand the regional context of the surveys and help defined the extent of the assemblage. To accommodate these bores, monitoring Zones 2 and 3 were extrapolated outside the boundaries of the explicitly defined map of Douglas and Pickard (2014b). Similarly, MWH (2015) added two monitoring zones; Monitoring Zone 5: the Fortescue River to the north of Ophthalmia Dam and Monitoring Zone 6 Whaleback Creek to the west of the Dam to accommodate bores that could not be assigned to any of the existing Monitoring Zones. For the purposes of these current analyses, the original Monitoring Zones defined in Douglas and Pickard (2014b) have been used to allocate bores to Monitoring Zones, and any bores outside that area have been considered as an “Out of Scope” sample. It is noted that although considered Out of Scope for this spatial framework, this group still includes samples from Ethel Gorge Stygobiont, providing both regional and local context for the data set. The Monitoring Zones used in this analysis are described in **Table 3-2**. Split by Monitoring Zones, there are 14 bores in MZ1, six in MZ1B, 28 in MZ2, eight in MZ3, three in MZ4 and 167 were Out of Scope (**Figure 3-2**).

Table 3-2: Zones under the Monitoring Zone spatial framework. Monitoring Zones have been adapted from Douglas and Pickard (2014b).

Monitoring Zone	Description
Monitoring Zone 1 (MZ1)	This area is considered to be the core habitat area of the TEC. The subterranean habitat is comprised of the main calcrete body of the aquifer. It lies at the confluence of Shovellana, Homestead and Whaleback Creeks as they merge into the Fortescue River.
Monitoring Zone 1B/ Early Warning zone (MZ1B)	Initially termed the Early Warning Monitoring Zone, this area is located between the Monitoring Zones 1 and 4. It is intended to provide early warning of any water quality degradation associated with the dam prior to entering the Ethel Gorge receptor in Zone 1.
Monitoring Zone 2 (MZ2)	This area is the aquifer system associated with Shovellana Creek immediately prior to entering confluence zone with Fortescue River from the east.
Monitoring Zone 3 (MZ3)	This is the aquifer system associated with Homestead Creek immediately prior to entering the confluence zone with Fortescue River from the west.
Monitoring Zone 4 (MZ4)	Ophthalmia Dam.
Out of Scope (OOS)	This designation has been used as a catch-all for any bores that fall outside the original scope of the Monitoring Zone designation of Douglas and Pickard (2014b).

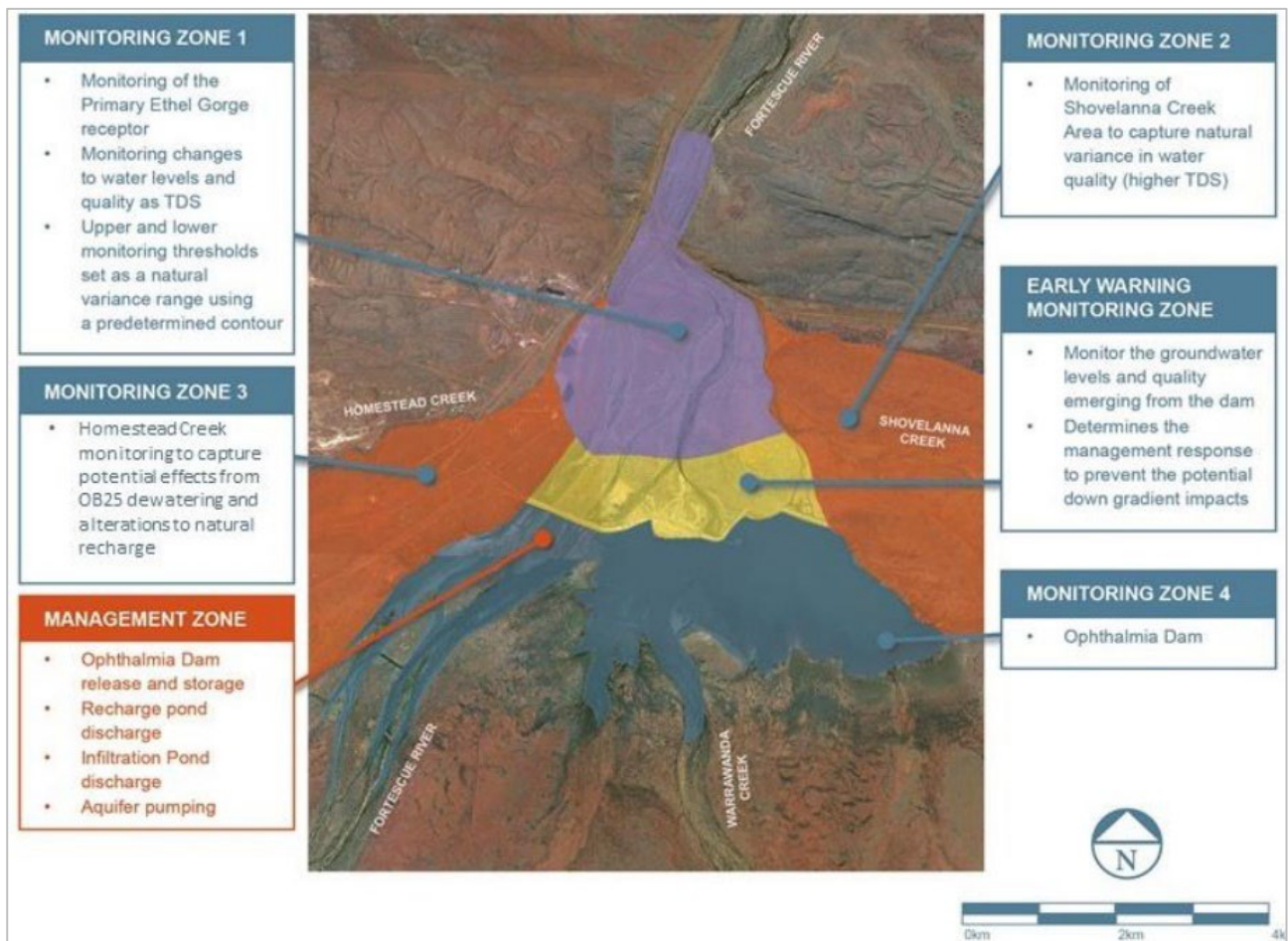


Figure 3-1: Monitoring Zones as originally defined. Figure reproduced from Douglas and Pickard (2014b).

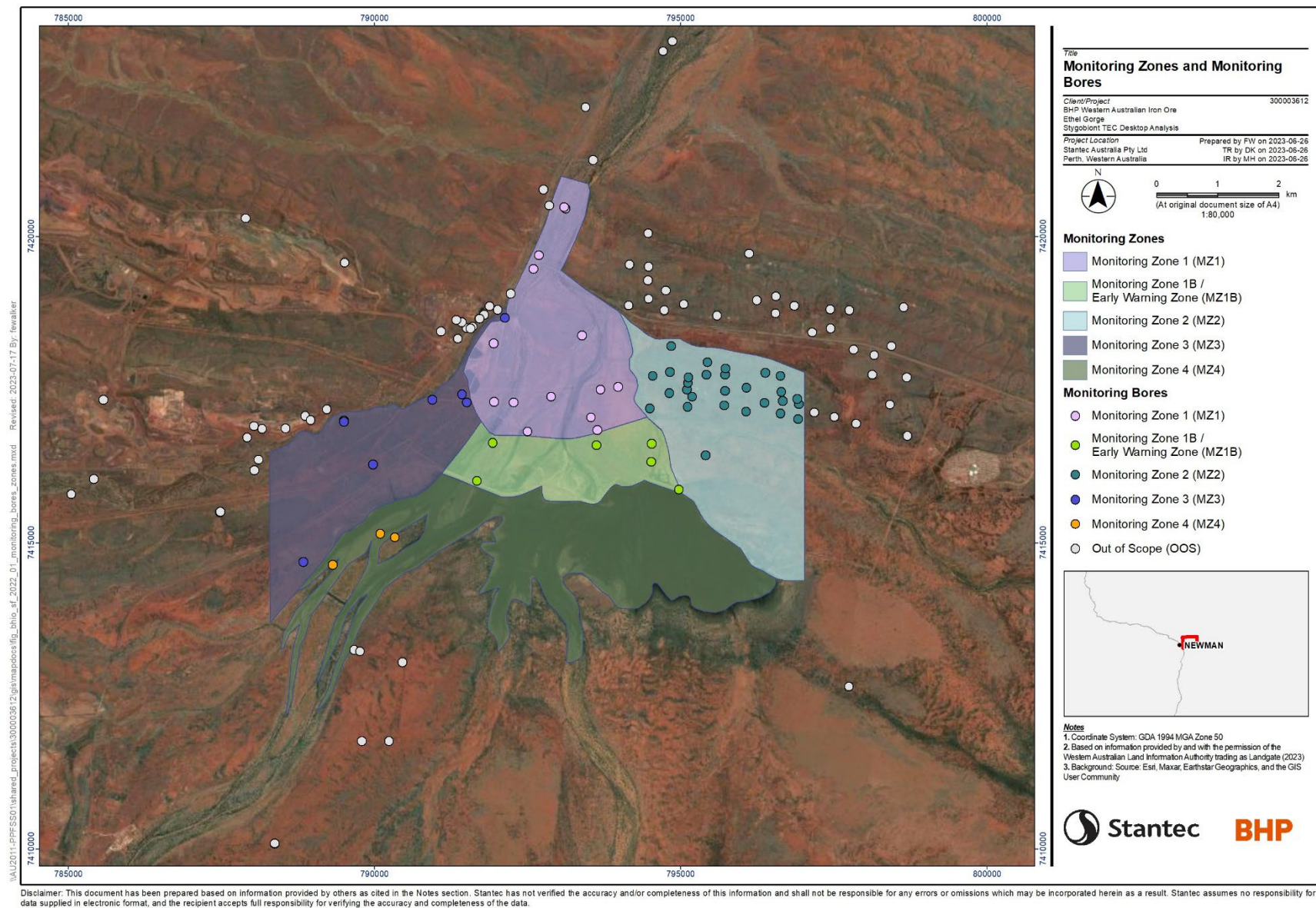


Figure 3-2: Monitoring zones and location of monitoring bores, classified by which MZ they belong to. Note that all bores outside the boundaries of the figure are classified as Out of Scope (OOS) as per Table 3-2 .

3.4 Temporal Framework

Analysis of temporal trends has focussed on detecting any changes sequentially throughout the time series of the monitoring data.

3.5 Indicator Species

Species contributing to the Ethel Gorge Stygobiont were evaluated for their suitability as an indicator of ecosystem health. The following attributes were investigated to determine suitability as an indicator species including:

- Representative of the community - this was determined by meeting the criteria of a Core Species as defined by Bennelongia (2013) *i.e.* being restricted to the greater Newman area;
- Abundant enough to reliably sample;
- Present throughout the area of interest or at least a large proportion of the samples - this was determined by the prevalence of specimens within samples from the TEC;
- Readily identifiable - this was determined by being a Defined taxon;
- Not characterised by naturally highly variable and patchy abundances.

Since the preferences and ecological responses to variations in environmental factors are poorly documented for stygofauna, this criterion has been relaxed for the purposes of the assessment.

3.6 Data Analyses

3.6.1 Abiotic Parameters

The pH and salinity (as EC) represent the only parameters with a time series from 2009 onwards throughout the Ethel Gorge TEC data set and were analysed as a separate subset. To analyse a broader suite of abiotic parameters, including pH, EC, calcium, sulphate, total nitrogen and total phosphorus, the data set was constrained to samples that contained all of those variables. The reduced data set comprised samples from survey rounds between November 2014 and May 2022. While these six are a subset of the parameters recorded, the omitted parameters were covariates of those that were included. To examine any trends though time in the six variables noted above, regressions were performed on the data set for all bores, and for several individual bores within Monitoring Zone 1. An r-squared adjusted (*r-sq adj*) value of greater than 50%, and a statistically significant p-value (<0.05) were considered an adequate explanatory relationship.

Multivariate statistical analysis was used to test for differences between groups in the two spatial frameworks and the among the different survey rounds. This analysis involves the analysis of more than one parameter at a time and was performed on the data sets using PRIMER, Version 7.0. Water quality (abiotic) data was pre-treated by normalising the data to remove the effect of differing units and scales between variables.

Analysis of Similarities (ANOSIM) were used as a formal test of significant differences among spatial and temporal groups. The null hypotheses, that significant differences were not present, was rejected at $p=0.05$. The ANOSIM R-statistic varies between -1 and 1, with values close to 1 indicating very strong differences between test groups, where all samples from within a group are more similar to each other than to samples from other groups.

Where appropriate (as determined by the previous analysis), samples from all survey rounds were averaged by bore. Data was then subjected to principal component analysis (PCA) to both visually represent the similarities of points to each other, and to determine which of the component variables was driving any visible trends in the data. The percentage variance is used to explain the strength of the PCA, presented over the first two axes of the plot. A value of more than 50% is considered a useful interpretation of the results (Clarke and Warwick 2001).

3.6.2 Stygofauna

The univariate indices of abundance and species richness were examined to determine if differences were apparent among groups over time and within each of the spatial frameworks. This was undertaken with histograms of mean and standard deviation.



Multivariate analyses were performed on the data set using Defined taxa only at a species-level and then again at family-level. This included ANOSIM to determine if significant differences existed among groups within the temporal and both spatial frameworks, and then SIMPER to determine which species were primarily responsible for those trends.

3.6.3 Indicator species

Two descriptive statistics were examined to understand the consistency of abundance, a trait recognised as a key characteristic of prospective indicator species. These included Coefficient of Variation (CoV) and percentage of samples present. CoV is a descriptive statistic that provides a measure of Standard Deviation that is relative to the magnitude of the mean. When interpreting CoV, low values indicate a consistent species, while a high value indicates a more variable species. Species with low mean abundances require a low Standard Deviation to score low CoV, while species with high mean abundances may have a higher Standard Deviation and still score low CoV. CoV of zero (0) indicates no variability, often identifying singleton species within the data. Percentage of samples present comprised the percentage of samples, which contained the target species, of the total number of samples that contained stygofauna. CoVs are only comparable within data sets and can only be compared in relative terms. As such, no threshold values for CoV or percentage occurrence were set. The species which showed the lowest (non-zero) CoVs and were present in a relatively high percentage of samples were identified as potential indicator species.

The multivariate BVstep matching routine attempts to find the subset of variables in one data set that creates a matrix that best matches the full data set of another model matrix. The BVstep routine was used as a final test on the fauna data set. In this implementation, the fauna matrix was used as both the model and matching matrices. This allowed the routine to select the individual species that were primarily responsible for the trends in the full fauna data, without requiring an a-priori spatial or temporal framework to attempt to explain any variance.

3.6.4 Relationships between fauna and environmental variables

To investigate the relationships between the stygofauna and abiotic data sets, the multivariate RELATE and BVstep routines were used. For both matching routines, the test statistic, Rho, varied between 0 (no correlation) and 1 (complete match), and the threshold for significance was $p=0.05$.

The RELATE routine is a matching procedure that determines the similarity between two data sets based on the rank similarity of the constituent samples. For this analysis both data sets were restricted to samples from December 2014 onwards to include the full suite of abiotic data. Tests were conducted on data sets containing individual samples as well as mean samples for bores. Means per bore were used following the results of prior analyses determining the relative strength of the temporal and spatial factors. As a multivariate technique, RELATE matches the full suite of environmental parameters in the abiotic data set, to the full suite of species in fauna samples.

BVstep is another matching routine that attempts to find the subset of variables in one data set that creates a matrix that best matches the full data set of another model matrix. In this case, the stygofauna data set was used as the model matrix, and the BVstep routine was used to find the best subset of abiotic variables that matched the patterns of species' abundance of the fauna.

4. Results and Discussion

4.1 Abiotic Parameters

4.1.1 Groundwater salinity and pH

Groundwater salinity and pH have been shown to influence stygofauna communities in calcrete aquifers (Sacco 2020). The data set for these parameters within the Ethel Gorge area extends back to 2009. Based on this long-term data set (2009 - 2022), pH values across the area have ranged from acidic (>6.5) to alkaline (>7.5) (Foged 1978). Mean values were between 7.4 to 8 when investigated in the context of Monitoring Zone or TEC Boundary Zone (**Table 4-1, Table 4-2**). While stygofauna often occur in waters with these moderate pH values, assemblages in other localities have been found in pH as low as 3.5 and as high as 10.3. This demonstrates that the values recorded in the Ethel Gorge TEC data set are well within the pH range known to support stygofauna (Glanville *et al.* 2016; Reeves *et al.* 2007; Schulz *et al.* 2013). The pH values typically showed greater variation within groups than between groups for both spatial frameworks, with small differences in the means between groups (**Table 4-1, Table 4-2**).

Salinity, as measured by electrical conductivity (EC), ranged from 185 $\mu\text{S/cm}$ to 8,613 $\mu\text{S/cm}$ across the Monitoring Zones between 2009 – 2022 (**Table 4-1**), being highest in Monitoring Zone 3. The individual measurements corresponded with fresh (<5,000 $\mu\text{S/cm}$) to hyposaline conditions (5,000 - 30,000 $\mu\text{S/cm}$) (Hammer 1986), with the means for each Monitoring Zone classified as fresh. Similar trends were also noted when interrogating the data set by TEC Boundary Zone (**Table 4-2**). Stygofauna have been documented over a broad range of salinities in Australian groundwaters, spanning from fresh to highly saline (Glanville *et al.* 2016; Outback Ecology 2012). In the Pilbara, records have typically been associated with salinities <13,000 $\mu\text{S/cm}$ (Halse 2018), corresponding with the fresh to hyposaline conditions reported for the Ethel Gorge area.

The majority of samples showed moderate pH values of around 7.6 (centre of plot; **Figure 4-1**), with relatively few outliers to the upper and lower limits. In contrast, salinity (as EC) values were skewed towards zero with fewer data points associated with comparatively higher values (top right of plot) (**Figure 4-1**) and the means of each zone classified as fresh (**Table 4-1, Table 4-2**).

Table 4-1: Summary of pH and salinity (as electrical conductivity ($\mu\text{S/cm}$) ranges for each Monitoring Zone, based on data from 2009 to 2022.

Monitoring Zone	pH values				EC ($\mu\text{S/cm}$)			
	Min.	Max.	Mean	#Records	Min.	Max.	Mean	#Records
MZ1	5.70	8.63	7.68	101	740	6,600	2,667	101
MZ1B	7.03	8.94	8.00	18	713	4,791	1,331	14
MZ2	-	-	-	-	-	-	-	-
MZ3	6.74	8.28	7.63	21	356	8,613	2,001	21
MZ4	7.28	7.55	7.42	3	1,343	1,540	1,471	4
OOS	4.89	9.75	7.56	326	185	5,035	1,881	313

Table 4-2: Summary of pH and salinity (as electrical conductivity) ($\mu\text{S/cm}$) ranges for the TEC Boundary Zones, based on data from 2009 to 2022.

TEC Boundary Zone	pH values				EC ($\mu\text{S/cm}$)			
	Min.	Max.	Mean	#Records	Min.	Max.	Mean	#Records
TEC	5.70	8.94	7.61	102	356	8,613	2,704	103
BUF	4.89	9.75	7.65	306	185	5,035	1,799	292
REG	5.85	8.62	7.41	61	436	4,200	2,085	58

Two-way crossed ANOSIM on the pH and salinity (as EC) identified significant differences ($p=0.001$) for both spatial and temporal factors, with differences among bores (spatial) being greater than among survey rounds (temporal) ($R=0.524$ and



R=0.192, respectively). Further two-way crossed ANOSIMs were performed to test the Monitoring Zone and TEC Boundary Zone spatial frameworks, using survey rounds as the crossed (temporal) factor in both cases. Significant but very small differences were present among TEC Boundary Zone groups (R=0.079, p=0.007), while differences among MZ groups were non-significant. This demonstrates that while there is a distinct spatial structuring in the data, it is at a bore-to-bore level, and by grouping bores together into TEC Boundary Zone or Monitoring Zone groups, there is variability within those groups that obscures the spatial structuring, but also the minor temporal structuring.

Bores were found to primarily vary in relation to pH and, to a lesser extent, salinity (as EC), with a limited number associated with hyposaline conditions (**Figure 4-1, Table 4-1**). Several of these hyposaline bores are within a small area within the TEC, specifically MZ 1 immediately north of the Ophthalmia Dam (HEOP0425, HEOP0415, EEX985 and EEX917). Examination of the time series indicates that these conditions are typical of that subset of bores and the salinity (as EC) has been decreasing slightly over time.

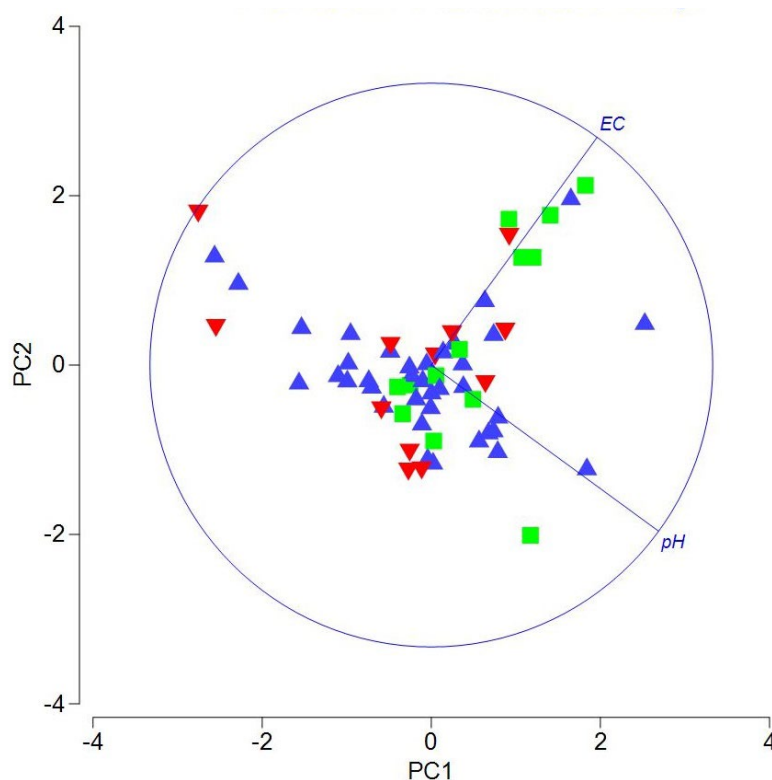


Figure 4-1: Principal Component Analysis of the pH and EC values at all bores in the Ethel Gorge Stygobiont data set (2009-2022), with 100% of variation explained in the first two axes. TEC=green, Buffer=blue, Regional=red.

4.1.2 Suite of Abiotic Parameters

From 2014 onwards, data were available for a suite of abiotic parameters. The pH ranges and means based on this time series (**Table 4-3, Table 4-4, Table 4-5**) were similar to the values calculated from the 2009 to 2022 data (**Table 4-1 and Table 4-2**). Conversely, the maximum salinity (as EC) of 5,510 $\mu\text{S}/\text{cm}$ was comparatively lower (**Table 4-3, Table 4-4, Table 4-5**), indicating that the highest value (8,616 $\mu\text{S}/\text{cm}$) was recorded prior to 2014 (**Table 4-1, Table 4-2**).

Calcium concentrations ranged from 2 to 126 mg/L and averaged 63 mg/L when assessing across all bores. Similar values were reported for Monitoring Zone 1 and TEC/BUF areas respectively. The means were comparatively higher than those recorded for bore groups during the regional Pilbara stygofauna survey (<45 mg/L) (Halse *et al.* 2014) and sampling of groundwaters in New South Wales (39 mg/L) (Halse *et al.* 2014; Korbel and Hose 2015), reflecting calcrite deposition (CaCO_3) within the Ethel Gorge area.

Sulphate ranged from 0.5 to 774 mg/L based on all bores, Monitoring Zone 1 (MZ1) and the TEC/BUF (**Table 4-3, Table 4-4, Table 4-5**). Sulphate concentrations may be naturally elevated in groundwaters, reflecting the composition of the host rock (Allen 1997). In the Ethel Gorge area, the highest values over time have been recorded from bore HEOP0425. This

bore, which is known to host stygal taxa, is primarily screened in shale (Stantec 2022), a unit which can be linked to elevated sulphate levels through oxidation of pyrite. Mean total nitrogen concentrations were typically below 4.5 mg/L (**Table 4-3**, **Table 4-4**, **Table 4-5**). The mean for total phosphorus was 0.112 mg/L when assessing across all bores (**Table 4-3**) however was considerably lower for MZ1 only (0.027 mg/L) (**Table 4-4**).

Table 4-3: Summary of ranges for a suite of abiotic parameters across all bores, based on data from 2014 to 2022.

	pH	EC (µS/cm)	Calcium (mg/L)	Sulphate (as SO ₄ - Turbidimetric) (mg/L)	Nitrogen (Total) (mg/L)	Phosphorus (Total) (mg/L)
Min	4.89	185	2	0.5	0.05	0.005
Max	9.75	5,510	126	774	63.7	2.44
Mean	7.73	1,851	63	148	3.96	0.112
Median	7.94	1,572	67	133	1.2	0.0225

Table 4-4: Summary of ranges for a suite of abiotic parameters, based on data from 2014 to 2022 within MZ1.

	pH	EC (µS/cm)	Calcium (mg/L)	Sulphate (as SO ₄ - Turbidimetric) (mg/L)	Nitrogen (Total) (mg/L)	Phosphorus (Total) (mg/L)
Min	5.7	740	6	0.5	0.1	0.005
Max	8.63	5,510	108	774	18.5	0.12
Mean	7.88	2,353	61	239.4	3.41	0.027
Median	7.95	1,670	64	134	1.1	0.02

Table 4-5: Summary of ranges for a suite of abiotic parameters, based on data from 2014 to 2022 within the TEC/BUF area.

	pH	EC (µS/cm)	Calcium (mg/L)	Sulphate (as SO ₄ - Turbidimetric) (mg/L)	Nitrogen (Total) (mg/L)	Phosphorus (Total) (mg/L)
Min	4.89	185	2	0.5	0.05	0.005
Max	9.75	5,510	126	774	63.7	2.44
Mean	7.76	1,821	62	150.4	4.22	0.12
Median	7.95	1,520	67	131	1.1	0.025

ANOSIMs using the full suite of abiotic parameters including nutrients, ions, salinity and pH, but restricted to 2014-2022, showed similar trends to the tests using only pH and EC. In two-way crossed tests, spatial differences were significant ($p < 0.005$) when samples were grouped by bore ($R = 0.745$) and were greater than temporal differences ($R = 0.232$). No tests were significant using TEC Boundary Zone or Monitoring Zone spatial frameworks. To visually represent the data, the samples were averaged over the factor with the lower R-statistic (temporal) and then plotted by the dominant factor (spatial). However, since no significant differences were detected among the TEC Boundary Zone or Monitoring Zone groups, no coding has been overlaid (**Figure 4-2**). The constituent eigenvectors of the axes showed that salinity (as EC) and sulphate displayed the greatest variation among bores along PC1, while pH was the dominant parameter along PC2 (**Figure 4-2**). It should be noted that the highest values of EC and pH remained within the range considered favourable for stygofauna habitation (Glanville *et al.* 2016; Schulz *et al.* 2013).

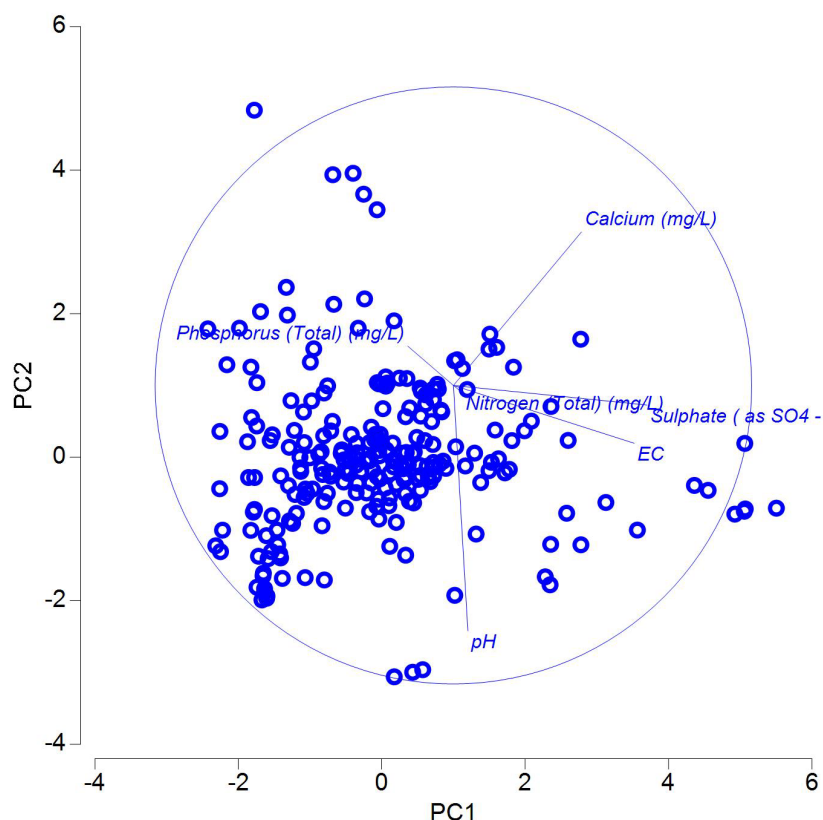


Figure 4-2: Principal Component Analysis of the six abiotic parameters set for all bores in the Ethel Gorge Stygobiont data set between Nov 2014 and May 2022). As neither spatial framework showed significant differences, no coding has been applied, with 65.9% of variation explained in the first two axes.

Linear regression did not reveal any strong trends in abiotic factors over time as related to all bores, Monitoring Zone 1 only or TEC/BUF (**Table 4-6**). While some temporal patterns were observed at an individual bore level, with salinity (as EC) decreasing over time at bore HEOP0417 and a similar trend noted for total nitrogen at bore HEOP0425 (**Table 4-7**), these patterns were not reflected in the broader data sets.

Table 4-6: Regression analysis of the suite of abiotic parameters based on data from November 2014 and May 2022, analysed by all bores, MZ1 only and TEC/BUF.

Parameter	All bores		MZ1 Only		TEC/BUF	
	R-Sq(adj) %	p-value	R-Sq(adj)	p-value	R-Sq(adj)	p-value
pH	12.17	0.00	19.57	0.001	11.27	0.000
EC	0.00	0.316	2.94	0.129	0.00	0.335
Calcium	0.00	0.775	2.99	0.143	0.00	0.683
Sulphate (as SO4 - Turbidimetric)	0.00	0.717	0.00	0.324	0.00	0.950
Nitrogen (Total)	0.00	0.383	9.63	0.027	0.00	0.361
Phosphorus (Total)	0.25	0.215	0.00	0.868	0.21	0.240

Table 4-7: Regression analysis of pH, EC and total nitrogen for select bores, based on data from November 2014 and May 2022, within MZ1.

Bore	pH value (pH)		Electrical Conductivity (EC)		Nitrogen (Total)	
	R-Sq(adj) %	p-value	R-Sq(adj) %	p-value	R-Sq(adj) %	p-value
HEOP0417	0.00	0.365	64.55	0.006	5.4	0.282
HEOP0425	28.41	0.1	0.00	0.769	74.26	0.008
HEOP0504	0.00	0.75	0.00	0.414	66.04	0.059
HEOP0574M	28.76	0.079	4.10	0.285	0.00	0.405
W056	0.00	0.409	0.00	0.384	34.95	0.072

Note: Only bores with sufficient data to complete regression analysis were included. Shading indicates a value which has both a correlation (R-Sq (adj) %) and is statistically significant ($p < 0.05$).

4.2 Stygofauna Distribution

Initial examination of the compiled stygofauna data set determined that of the 141 taxa, 76 were considered Defined taxa (**Appendix B**). The majority of Core Species according to the classification criteria in Bennelongia (2013) are represented in the Defined taxa list, although some have changed name or been synonymised. A few are no longer categorised as Core Species, with their known range expanding outside the Newman area since the 2013 publication.

When compared to the Bennelongia (2013) Core Species list, several new species have been encountered within the Newman area, and several species previously considered Core, have been found in the greater Pilbara area. Due to this, as well as taxonomic changes within the last decade, there have been several changes to what is considered the Core Species assemblage. **Appendix B** details species that were considered Core in 2013, and which species are currently considered to be Core Species based on the current taxonomy and known distribution of the species in the consolidated Ethel Gorge TEC data set.

The Defined taxa belonged to nine high-level taxonomic groups including Haplotaxida, Polychaeta, Acarina, Amphipoda, Isopoda, Cyclopoida, Harpacticoida, Syncarida and Ostracoda. Haplotaxids (worms) were the most diverse, represented by 16 taxa, followed by the crustacean group Cyclopoida (13 taxa). In terms of abundance, crustacean taxa have been numerically dominant, with Copepoda, Amphipoda, and Ostracoda all having instances of high abundance.

Of the 76 defined taxa present in the full Ethel Gorge Stygobiont TEC data set (**Appendix B**), 49 of those taxa were identified as occurring inside the TEC boundary. Of those 49, only 7 were recorded as occurring exclusively inside the TEC: *Phreodrilidae* sp. OB4, *Enchytraeidae* sp. OB4, *Coxicerberus* sp. OB2, *Anzycyclops* sp. OB, *Dussartcyclops uniarticulatus*, *Pilbaracyclops* sp. OB and *Parastenocaris* 'COP002'. When extended to include the 2 km radius buffer, the number of taxa recorded from inside the TEC plus Buffer (TEC+BUF) area increases to 66, with 28 taxa occurring exclusively in samples from inside the area. All of the taxa occurring in each of the zones above are specified in **Appendix B**.

Many of the 66 taxa in the TEC+BUF area are from samples that predate the structured monitoring program beginning in 2013 and have not been recorded since. As such, it is difficult to verify their identity to confirm that they are genuinely restricted to the Ethel Gorge TEC+BUF area. This group includes: *Anzycyclops* sp. OB, *Coxicerberus* sp. OB2, *Enchytraeidae* sp. OB3, *Enchytraeidae* sp. OB4, *Ilyodromus* indet., *Notacandona* sp. OB1, *Phreodrilidae* sp. OB4 and *Pilbaracyclops* sp. OB.

Several more species, while restricted to the TEC+BUF area in the data set, are found elsewhere in the Pilbara. This includes *Pilbaranella ethelensis*, *Maarka etheli*, *Billibathynella cassidis*, *Thermocyclops aberrans*, *Phreodrilus peniculus*, *Dussartcyclops uniarticulatus* and *Sarscypridopsis ochracea*, which are all more widely distributed throughout the Eastern Pilbara. Note that the first three of these species were previously considered core species but no longer meet the criteria for that designation because their range is now known to extend outside the Newman area.

4.3 Stygofauna Species Richness and Abundance

Stygofauna monitoring data was analysed to examine the suitability of trigger values for species richness and abundance for management of the TEC. The data set was constrained to between 2014 to 2022, and spatial frameworks for TEC boundary (TEC, buffer, regional) and Monitoring Zone, and also over time, were examined.

Prior to applying the TEC Boundary and Monitoring Zone spatial frameworks to assess species richness and abundance, the stygofauna data was analysed among the bores from the broader Ethel Gorge area, to determine whether temporally consecutive samples from the same bore were more similar to each other than neighbouring bores sampled at the same time. Multivariate analyses (2-way ANOSIM) determined that there were moderately strong and significant differences in the assemblages found at the different bores, that were consistent through the duration of the data set ($R=0.369$, $p=0.003$). However, there were no significant differences among the survey rounds, indicating the assemblages in individual bores had not shown any consistent, measurable changes through time.

4.3.1 TEC Boundary Zone Trends

Mean species richness (**Figure 4-3a**) and mean abundance (**Figure 4-3b**) in bores that contain stygofauna, were similar across the TEC, Buffer and Regional zones, with the variability throughout the time period within each group, being far greater than the mean. The width of the error bars (a measure of variability, as standard deviation) is greater than the mean value in each of the groups, and is also far greater than the differences between the means.

With such large temporal and spatial variability within test groups, any measurable trends are obscured. This spatial and temporal variability in species richness and abundance may be influenced by a variety of factors including limitations with sampling design and effort, constrained access to certain bore holes and seasonal conditions. It also becomes difficult to demonstrate with any certainty that new values represent a departure from the historical state. For this reason, the simple indices of species richness or abundance are considered unsuitable for use as management tools for the Ethel Gorge Stygobiont TEC.

Multivariate analyses were performed on the data set using Defined taxa only, at a species-level and then again at family-level. Overall, ANOSIMs determined that significant but small differences existed between samples in the TEC+BUF area versus the REG area at both species-level ($R=0.092$, $p=0.001$) and family-level ($R=0.093$, $p=0.001$) taxonomic resolution. This is considerably lower than the differences determined among individual bores (**Section 4.3**), indicating that while there were spatial differences in the stygofauna assemblage across the data set, they were not necessarily defined by the TEC boundaries.

SIMPER analysis determined that both TEC+BUF and REG groups were characterised by *Diacyclops humphreysi* and *Chydaekata acuminata* at a species-level but were discriminated from each other by a higher mean abundance of *Diacyclops humphreysi* in the TEC+BUF, and *Chydaekata acuminata* in the REG bores. Additional discrimination between the groups was contributed by *Archinitocrella newmanensis* and *Pygolabis humphreysi*, which had a higher average abundance in the TEC+BUF bores. SIMPER at the family-level reflects the species-level results, with higher abundance of Cyclopidae in the TEC+BUF group and Paramelitidae in the REG group.

The presence of the TEC has been well documented and defined, however the poor discrimination of the TEC+BUF from the REG samples indicates that while the TEC+BUF area may encompass a representative area of the TEC, the current 2 km Buffer boundary separating it from the REG samples may not fully encompass the diversity of the community. In particular, the characterisation of the REG bores by *Chydaekata acuminata* indicates there is an appreciable amphipod assemblage that occurs outside of the 2 km buffer boundary.

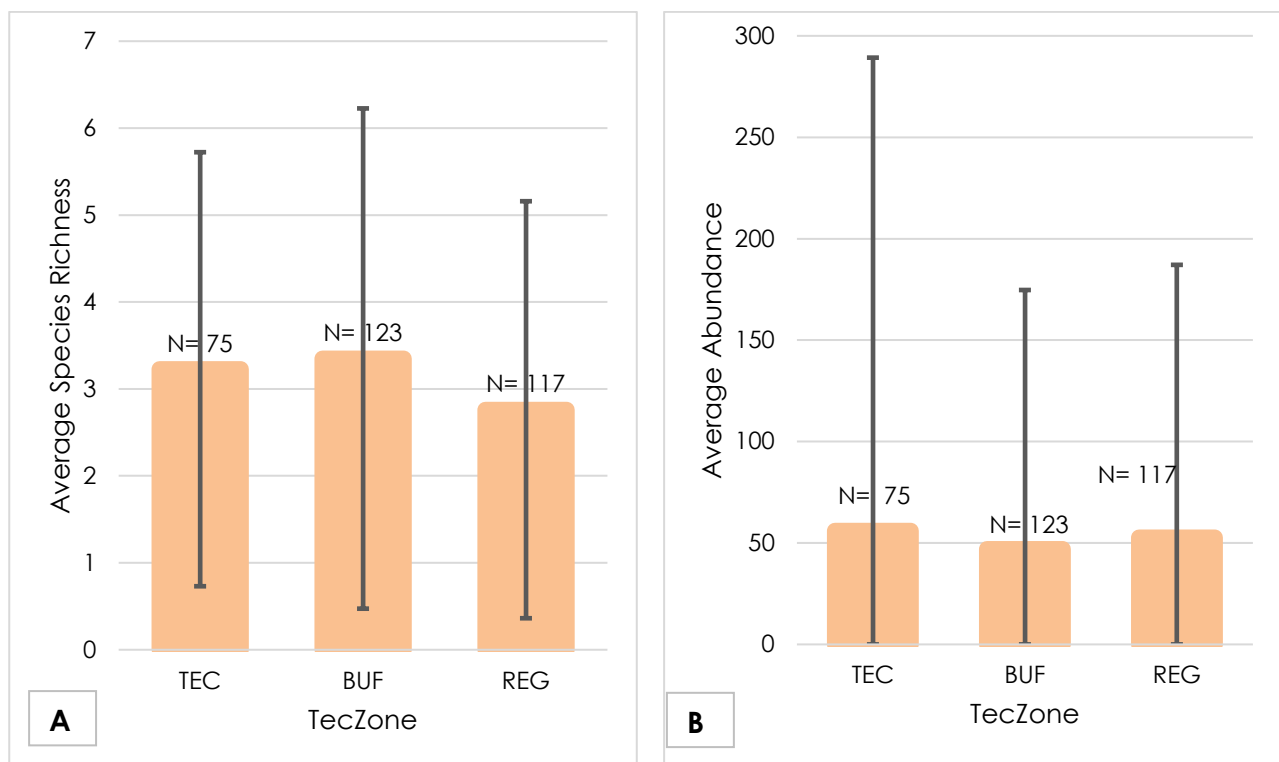


Figure 4-3: Mean species richness (and standard deviation) (A) and average abundance (and standard deviation) (B) across the TEC Boundary Zones including TEC, Buffer (BUF), and Regional Zones (REG) in bores that contained stygofauna.

4.3.2 Monitoring Zone Trends

Of the 76 Defined taxa in the stygofauna data set, 39 have been present in samples from MZ1. When all Monitoring Zones are considered together (including MZ1 - 4 as defined in the EPWRMP) a further ten species are accounted for, bringing the total to 49.

MZ1 had higher mean species richness and abundance per bore than the other Monitoring Zones, apart from MZ4, which had only a single sample (**Figure 4-4**). However, species richness and abundance across Monitoring Zone groups showed greater variability within groups than between groups. As discussed for the TEC boundaries, the high inherent variability among bores and survey rounds within each Monitoring Zone group obscures any measurable or statistically testable differences that may be present. Again, this indicates that using these simple data metrics of species richness and abundance for the development of reference or trigger points is likely to be unsuitable.

Multivariate analysis (2-way crossed ANOSIM) indicated that the spatial structuring noted when analysing across individual bores was not apparent at the Monitoring Zone level and there were no significant differences among the assemblages in the Monitoring Zones. This indicates that the Monitoring Zones do not align with the spatial structure of the assemblage.

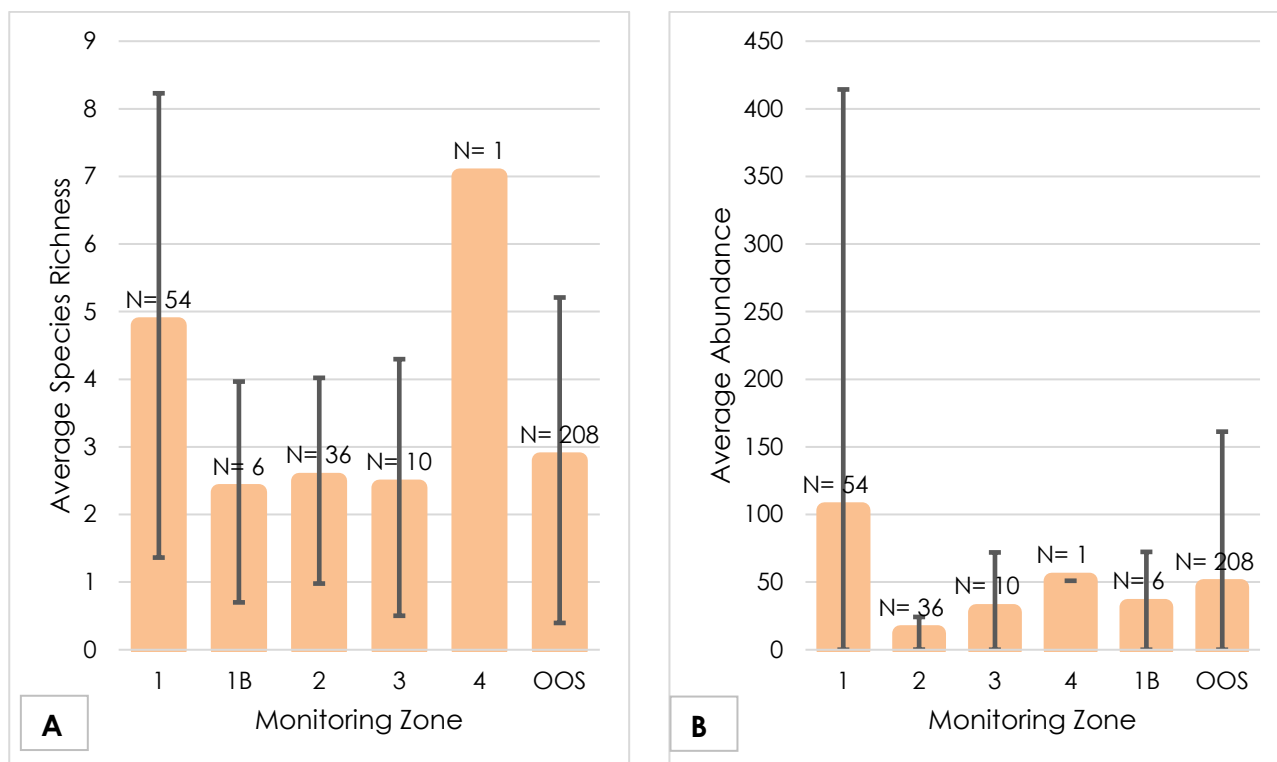


Figure 4-4: Mean species richness (and standard deviation) (A) and average abundance (and standard deviation) (B) across Monitoring Zones 1-4, 1B, and OOS in bores that contained stygofauna.

4.3.3 Temporal Trends

Mean species richness between seasons ranged from one taxon (2016-W) to 4.5 taxa (2013-D) per bore (**Figure 4-5**). Between years, the mean species richness per bore has fluctuated from one (2016-W) to six (2014-W), and mean abundance per bore from 10 (2014-D) to 114 (2013-D) (**Figure 4-6**). The average abundance per bore was greater during dry seasons compared to wet seasons (58 taxa compared to 47 taxa) (**Figure 4-6**). This result was influenced by the dry season in 2013, which recorded the highest average abundance per bore (114). This primarily reflected the high abundance recorded from bore HEOP0425 (1,995 specimens) in this survey round. However, these numbers represented the highest abundance of stygal specimens in a single sample, predominantly comprising cyclopoid copepods, and emphasise the variable nature of stygofauna in the system.

Over time there has typically been greater variation within survey rounds than between survey rounds (**Figure 4-5** and **Figure 4-6**). As for the spatial frameworks discussed above, the level of variability (shown as standard deviation) in species richness and abundance within each survey round is greater than the mean, effectively obscures any statistically measurable trends throughout the time series.

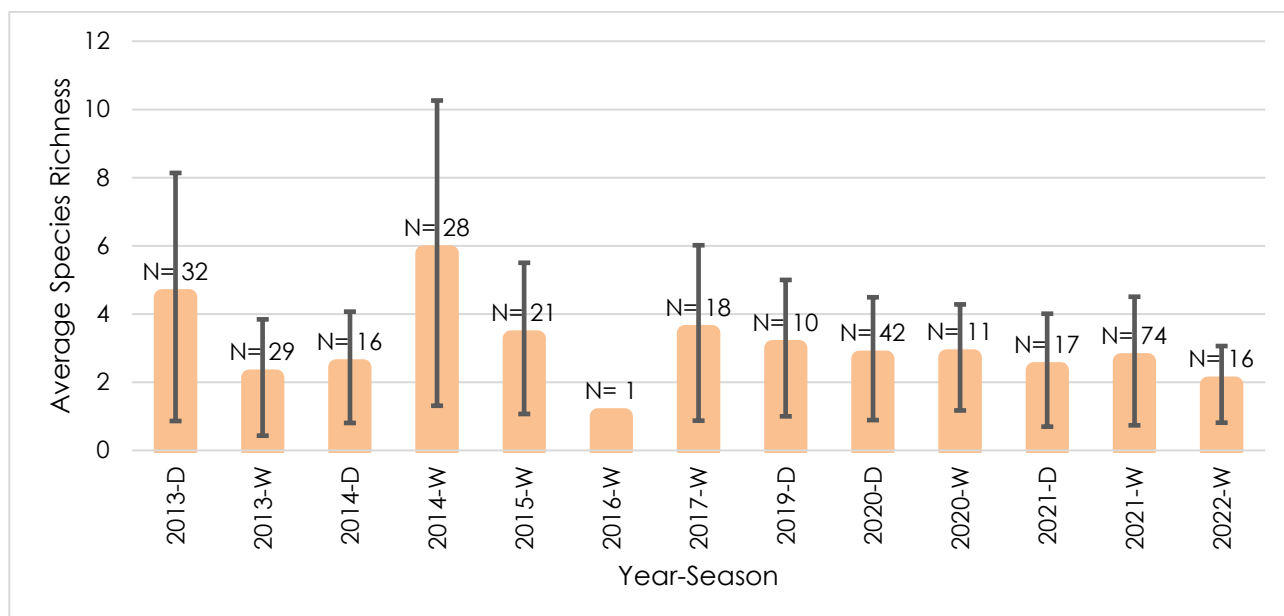


Figure 4-5: Average species richness (and standard deviation) per bore between 2013 – 2022 across wet (W) and dry (D) season survey rounds during the Assessment.

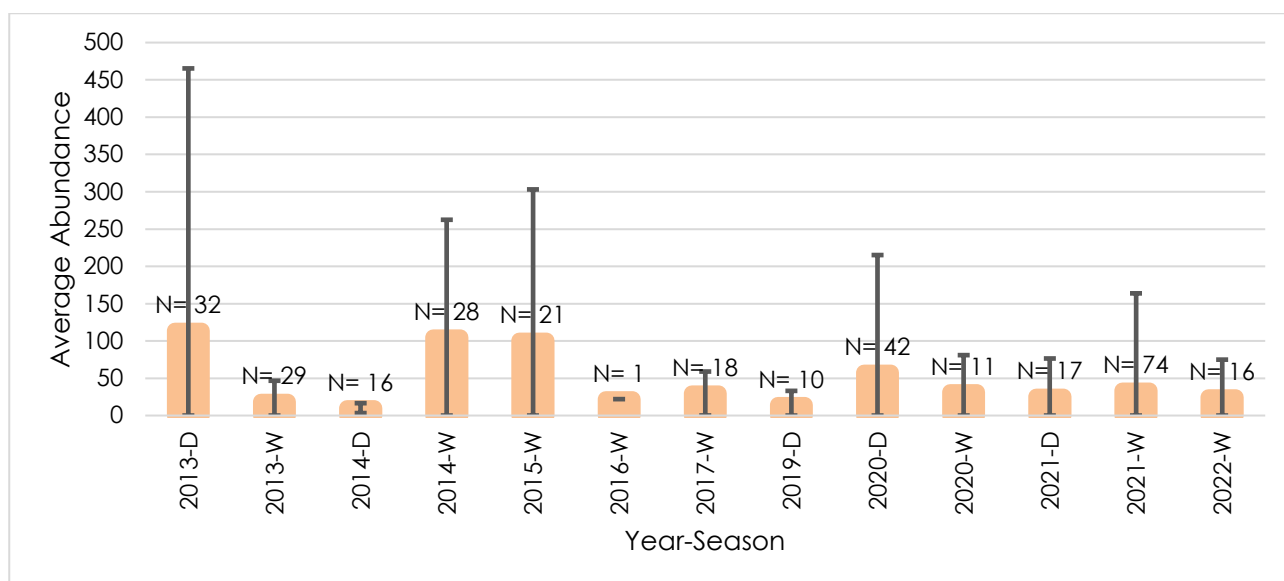


Figure 4-6: Average abundance (and standard deviation) per bore (2013 – 2022) across wet (W) and dry (D) season survey rounds during the Assessment.

4.4 Indicator Species

The stygofauna data set was examined using Coefficient of Variation (CoV) values to investigate potential indicator species. Initially, only MZ1 samples were considered, and then all samples were considered. As noted previously, CoVs were only comparable within data sets and could only be compared in relative terms. Based on this, the species which showed the lowest (non-zero) CoVs were identified as potential indicator species, where present in a relatively high percentage of samples.

In the samples from MZ1, species' CoV values ranged between 2.6 and 9.3.

- The cyclopoid copepod *Diacyclops humphreysi*¹ occurred in 55.6% of MZ1 samples and had a CoV of 3.35. MZ1 bores that consistently produced this species throughout the time series included: EEX917, HEOP0387, HEOP0425, HEOP574M and W056
- The harpacticoid copepod, *Archinitocrella newmanensis*, occurred in 37% of MZ1 samples and had a CoV of 4.82. MZ1 bores that consistently produced this species throughout the time series included: EEX917, HEOP0387 and HEOP0425.
- The isopod, *Pygolabis humphreysi*, occurred in 31% of MZ1 samples and had a CoV of 3.75. MZ1 bores that consistently produced this species throughout the time series included HEOP0415 and HEOP0387.
- The paramelitid amphipod, *Chydaekata acuminata*, occurred in 29.6% of MZ1 samples with a CoV of 5.3. MZ1 bores that produced this species, albeit with greater variability, throughout the time series included: HEOP0387, HEOP074M and W056. It should be noted that this species was found by previous analyses to also be characteristic of areas outside the TEC and MZ1 (**Section 4.3.1** SIMPER analyses).

The same consideration of prevalence and CoV was made on the data set for all bores, where CoV values ranged between 4.08 and 12.31.

- *Diacyclops humphreysi* had the highest prevalence, occurring in 46.7% of samples that contained stygofauna, with a relatively low CoV of 4.43.
- *Pygolabis humphreysi* occurred in 21.6% of samples that contained stygofauna, with a CoV of 4.78.

All four of the species above meet the majority of characteristics for indicator species. They are present in a relatively large proportion of the samples, have relatively consistent abundance (low CoV), are abundant enough to reliably sample and readily identifiable. Only *Chydaekata acuminata* and *Pygolabis humphreysi* are considered Core Species restricted to the greater Newman area, which was a further ideal characteristic of an indicator species. This is not the case for *Archinitocrella newmanensis* and *Diacyclops humphreysi*, which are more broadly distributed in the Pilbara.

Bennelongia (2013) and Stantec (2022) suggested that *Nitocrella* OB, *Pilbaracyclops supersensus*, *Nitocrella karanovici*, and *Chydaekata acuminata* warranted further investigation into their suitability as indicator species. These species were examined, along with the rest of the assemblage in the consolidated data set, to understand the consistency of abundance, a trait recognised as a key characteristic of prospective indicator species. The naturally low abundances and patchy distribution of most stygofauna taxa in the bore samples eliminated the majority as potential indicator species. *Nitocrella* OB, *Pilbaracyclops supersensus* and *Nitocrella karanovici*, were considered unsuitable as indicator species as they occurred in less than 10% of MZ1 samples and less than 7% of samples overall. These taxa also had comparatively higher CoV values than the four species identified above (CoV=7.4).

For both Monitoring Zone and TEC Boundary Zone based analyses, *Diacyclops humphreysi*, *Archinitocrella newmanensis*, *Pygolabis humphreysi* and *Chydaekata acuminata* occurred in the highest proportion of samples. Several species had similarly low CoV values, but were present in far fewer samples, decreasing their effectiveness as indicators. This latter group includes the ostracods *Pilbaracandona eberhardi*, *Pilbaracandona colonia*, *Origocandona inanitas* and *Gomphodella hirsuta*, the copepod *Diacyclops sobeprolatus*, and the amphipod *Maarrka etheli*.

¹ 1. While *Diacyclops humphreysi* may form a species complex within the broader Pilbara region, it represents a discrete morphological unit relative to the other taxa within the Ethel Gorge TEC

A final BVstep matching routine used the stygofauna data as both model and match data, to determine the species that were most responsible for the observable trends in the data. This analysis provides a method of finding the patterns in where and when species are present and abundant, and then selecting a small subset of those species that collectively match that same pattern. It is designed to find which species are actually responsible for the patterns and to separate them from species that only contribute noise to the data or vary in the same way (are covariates).

This test determined that 95% of the patterns in the stygofauna data could be matched by using the abundances of just three species; *Chydaekata acuminata*, *Diacyclops humphreysi* and *Pygolabis humphreysi*. This analysis reiterates the potential suitability of these species to act as indicators of the rest of the Stygobiont assemblage, in relation to TEC ecosystem health. Their abundances give 95% of the pattern of the whole assemblage, without having to quantify all of the other species.

Based on the CoV and BVstep analysis, the four species identified in this section may prove useful as indicators of ecosystem health within the TEC. However, not one of these species possess all the characteristics of a suitable indicator species. *Diacyclops humphreysi*, while commonly recorded, is not restricted to the Ethel Gorge/Newman area. Whereas *Chydaekata acuminata* and *Pygolabis humphreysi* are Core Species for the area but are more variable in distribution and abundance.

Relying on abundance or presence of any single species alone may produce indicators that are overly prone to variability. This would make it difficult to determine whether a change to the species presence and/or abundance over time is an artefact of the limitations discussed in **Section 2.1** or represents an actual indicator of change to the community structure. A more robust approach than relying on a single species' abundance may be to use the abundances of a limited suite of species within the TEC, such as the four identified during analyses.

As identified in **Section 2.2**, one of the key elements of developing useful indicator species to support the management of the system is determining the relationship between stygofauna community and the abiotic environment. The development of this link requires an understanding of how the taxa are influenced by environmental and anthropogenic changes. Without that link, it is unclear what management action is required to address any observed changes in the stygofauna. Having identified the species that are likely to be most suitable as indicators (*C. acuminata*, *P. humphreysi*, *A. newmanensis* and *D. humphreysi*) Potential relationships between these species' presence and abundance and key abiotic variables within the TEC habitat is investigated in **Section 4.5**.

4.5 Relationships Between Fauna and Environmental Variables

One of the primary aims of this Assessment was to determine how the stygofauna of the TEC varied in relation to the abiotic parameters. As discussed in **Section 4.3**, spatial structuring was present in the stygofauna data set, at the level of individual bores. However, the TEC Boundary and Monitoring Zone spatial frameworks did not correspond with the distribution pattern of stygofauna. On this basis, matching procedures were run at the level of individual samples (individual bores during an individual survey round), and then again using mean values for individual bores (average for all monitoring rounds).

RELATE demonstrated that although a significant ($p=0.005$) correlation was present between the abiotic and stygofauna data sets at the individual sample levels, the match was low ($Rho=0.121$). This indicated that while bores with similar environmental characteristics yielded similar species, there was considerable variability that was not explained by the measured variables. The match was improved by averaging all years' data at individual bores ($Rho=0.248$, $p=0.045$). This indicated that when the variability in both environmental characteristics and species composition through the years was removed, the relationship between the environment and the species composition was clearer. Nevertheless, the Rho value for the second, averaged, analysis was still low and indicated that there was variability in the species composition that was not explained by the measured abiotic parameters.

The BVstep matching analysis performed on the stygofauna and abiotic data worked in the same way as the species matching routine in **Section 4.4**, however, instead of finding the individual species which best explained the patterns in the stygofauna data set, this process focused on the abiotic parameter or set of parameters which most closely matched the stygofauna. In all solutions, the analysis identified pH within the suite of parameters that best matched the patterns in the stygofauna data set. At the level of individual samples, the best match was produced with a combination of calcium, pH and Alkalinity ($Rho=0.209$, $p=0.02$). When averaged over bores, correlation to pH alone was $Rho=0.383$ ($p=0.05$). In conjunction, these results indicated that pH was most closely correlated with the patterns observed in the stygofauna.

To further examine the relationships between the abiotic parameters and the stygofauna, the species richness and total abundance in individual samples were plotted against several of the relevant abiotic parameters analysed in **Section 4.1**.



This was also undertaken for the abundances of *Diacyclops humphreysi*, *Archinitocrella newmanensis*, *Pygolabis humphreysi* and *Chydaekata acuminata*. These four species were selected for analysis based on the investigations into potential indicator species for TEC health outlined in **Section 4.4**. The abiotic parameters of pH and EC were included as primary parameters, with nitrogen investigated as a measure of the nutrients, and calcium as a measure of the ions. Note that the trends described below should be treated as correlative only, and do not imply causative relationships.

Plotted against pH, the majority of samples, both containing organisms and nil samples were distributed between pH 7 and 8.5 (**Figure 4-7**). This corresponds with the mean pH values across the various spatial framework groups (**Table 4-1** and **Table 4-2**). Higher stygofauna abundances and richness were commonly associated with a pH range between 7.2 and 8.2, reflecting the broader trend observed for calcrete-hosted groundwaters (Humphreys 2008). Samples containing stygofauna had pH values as low as 5.5 (**Figure 4-7a-b**), however, most samples yielding specimens of *Diacyclops humphreysi*, *Archinitocrella newmanensis*, *Pygolabis humphreysi* and *Chydaekata acuminata* occurred within the pH range 7 to 8.5 (**Figure 4-7 c- f**). Few samples outside of this pH range contained these taxa.

Plotted against salinity (as electrical conductivity, EC), samples containing stygofauna and nil samples ranged between 16 $\mu\text{S}/\text{cm}$ and 5,500 $\mu\text{S}/\text{cm}$ (**Figure 4-8**). This aligned with broader patterns in the Pilbara, with most stygal species occurring at salinities below 16,000 $\mu\text{S}/\text{cm}$ (Halse 2018). Samples containing stygofauna tended to have higher EC than nil samples, with the greatest abundances generally ranging between 1,000 and 2,000 $\mu\text{S}/\text{cm}$ (**Figure 4-8a**). While peak abundance of the four species were mostly recorded between 1,000 and 2,000 $\mu\text{S}/\text{cm}$ (**Figure 4-8**) and abundance appeared to decrease above that value, all four species were present at values above 5,000 $\mu\text{S}/\text{cm}$. Species richness remained largely consistent up to at least 5,000 $\mu\text{S}/\text{cm}$ (**Figure 4-8b**). This supports earlier investigations on the salinity tolerance of stygofauna in the Ethel Gorge area, which found little discernible pattern in species richness under fresh conditions (MWH 2016). As identified above, the ranges of salinity measured in the Ethel Gorge Area are well within the tolerances for Stygofauna in other Australian groundwater systems, and within those experiences in other parts of the Pilbara (Glanville *et al.* 2016; Halse 2018; Outback Ecology 2012,).

Plots of nitrogen and phosphorus showed that bores tended to have low to moderate nutrient loads, with no clear trends in relation to stygofauna (**Figure 4-9**, **Figure 4-10**). Phosphorus was generally below <0.25 mg/L, which is the upper boundary of a mesotrophic state in epigeal lotic systems (Carlson 1996). Total nitrogen concentrations in most samples were below 3 mg/L, although outlier values exceeding 30 mg/L were documented. Comparing the distribution of the samples containing stygofauna with the nil samples, most plots appear to be distributed similarly, indicating no appreciable effect. However, several of the samples with higher nitrogen values (~20 mg/L) had moderate to elevated total abundances (**Figure 4-9a**), mostly due to the presence of copepods.

Species richness remained consistent across nitrogen levels but was lower for the few samples with higher phosphate concentrations (**Figure 4-9b**, **Figure 4-10b**). Plots of the four species largely showed similar trends to the nil samples, indicating no effect with increasing nitrogen and phosphorus levels (**Figure 4-9 c-f**, **Figure 4-10 c-f**). However, the copepods *Archinitocrella newmanensis* and *Diacyclops humphreysi* did have higher abundances at the few samples in which they occurred with nitrogen concentrations above 18 mg/L, and *Diacyclops humphreysi* was present in the sample with the highest nitrogen values (63.7 mg/L, **Figure 4-9e**). This may be indicative of a positive correlation of nutrient enrichment with copepod abundances as documented in epigeal marine and freshwater systems where elevated phytoplankton productivity supports a higher population of copepods as primary consumers (Marcus 2004) (Li *et al.* 2016). Stygal systems, being light deprived, do not have the same trophodynamics as epigeal aquatic systems. It is therefore unclear if the trophic links are direct, through enhanced productivity, or indirect, with nitrogen correlating with the presence of detrital matter on which the copepods feed.

While calcium levels were only partially correlated with pH (**Figure 4-2**) they showed much the same trend in terms of stygofauna distribution. Most samples containing the four species identified in **Section 4.4** (**Figure 4-11c-f**) had moderate calcium concentrations, between 60-120 mg/L (**Figure 4-11**). Overall abundance and species richness was greatest within that same range, although there were stygofauna in samples with low calcium concentrations, albeit with generally low abundance and richness (**Figure 4-11a-b**). While this trend may indicate a biological preference for this water chemistry, it may also reflect the presence of suitable voids for diverse and abundant stygofauna communities within the calcium-rich calcrete habitat.

For sulphate, the highest species richness and abundance was typically associated with values of ≤ 300 mg/L. While this was also reflected in the abundance of *Archinitocrella newmanensis*, *Diacyclops humphreysi*, *Pygolabis humphreysi* and *Chydaekata acuminata*, each taxon was also recorded at concentrations exceeding 600 mg/L (**Figure 4-12**).

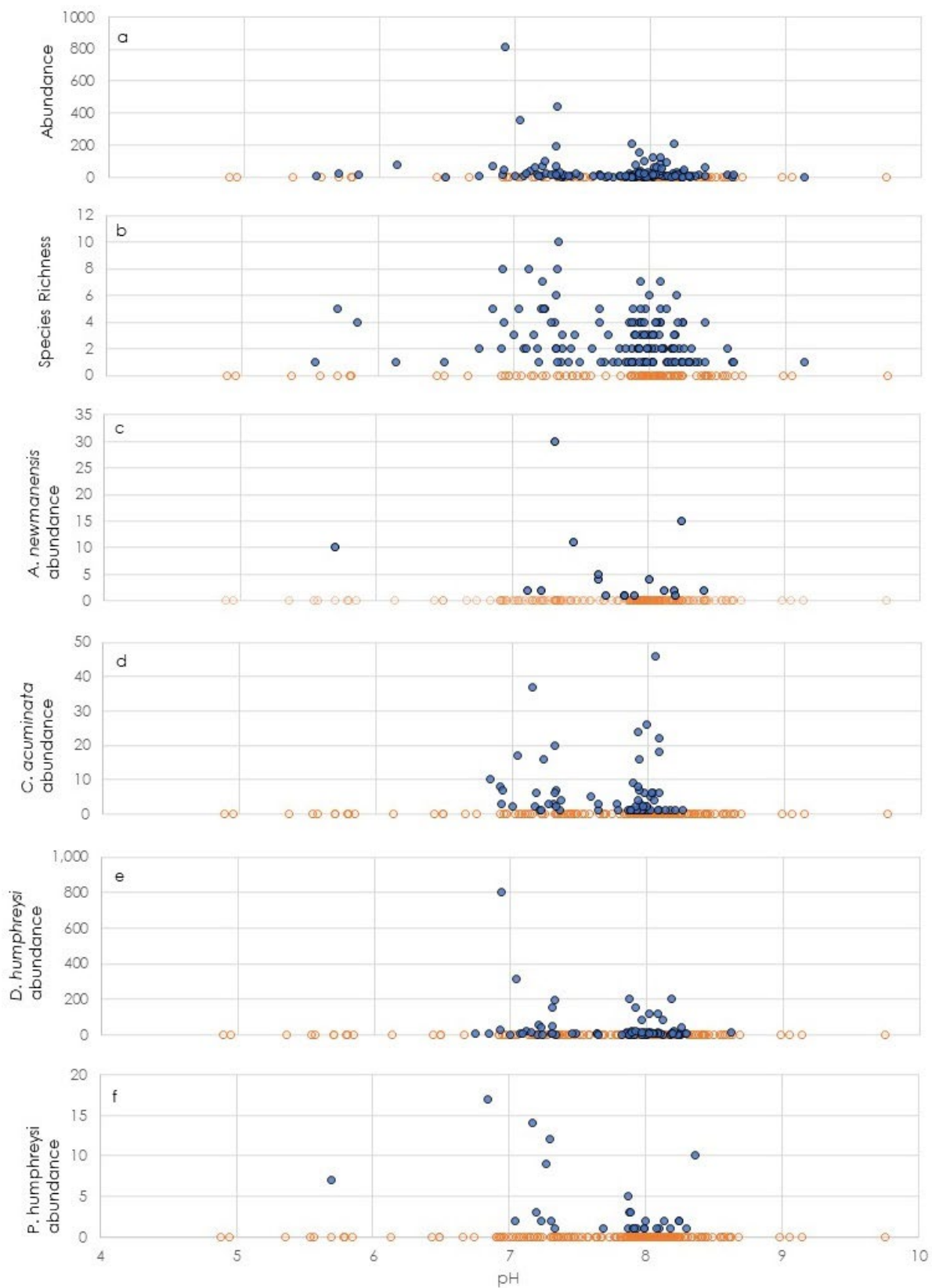


Figure 4-7: Scatterplots of pH vs a) total abundance, b) species richness, c) abundance of *Archinitocrella newmanensis*, d) *Chydaekata acuminata*, d) *Diacyclops humphreysi* and e) *Pygolabis humphreysi*. Data set includes samples between December 2014 to May 2022. Orange circles = zero on the y-axis, blue circles = values greater than zero on the y-axis.

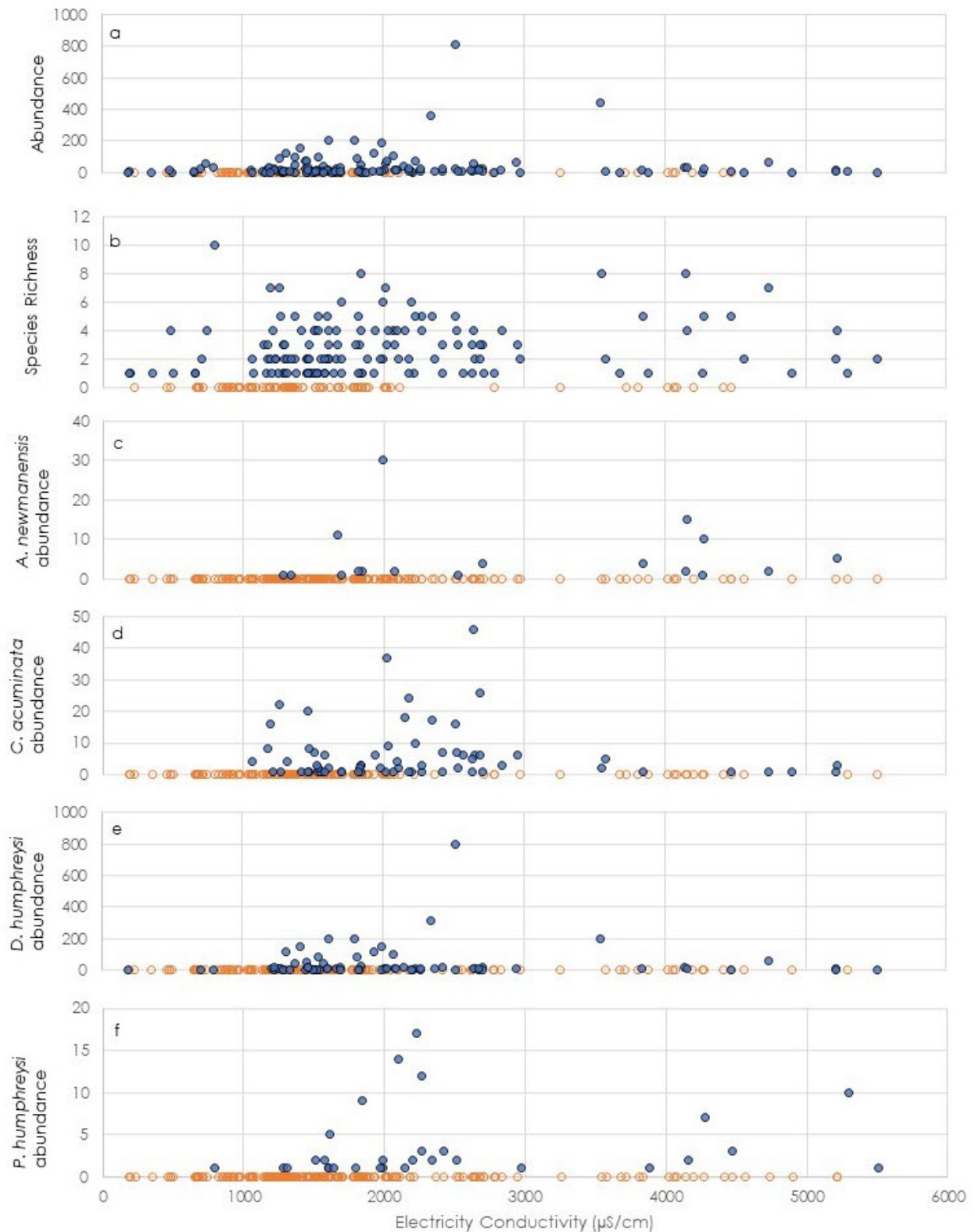


Figure 4-8: Scatterplots of electrical conductivity as a measure of salinity (EC) vs a) total abundance, b) species richness, c) abundance of *Archinitocrella newmanensis*, d) *Chydaekata acuminata*, d) *Diacyclops humphreysi* and e) *Pygolabis humphreysi*. Data set includes samples between December 2014 to May 2022. Orange circles = zero on the y-axis, blue circles = values greater than zero on the y-axis.

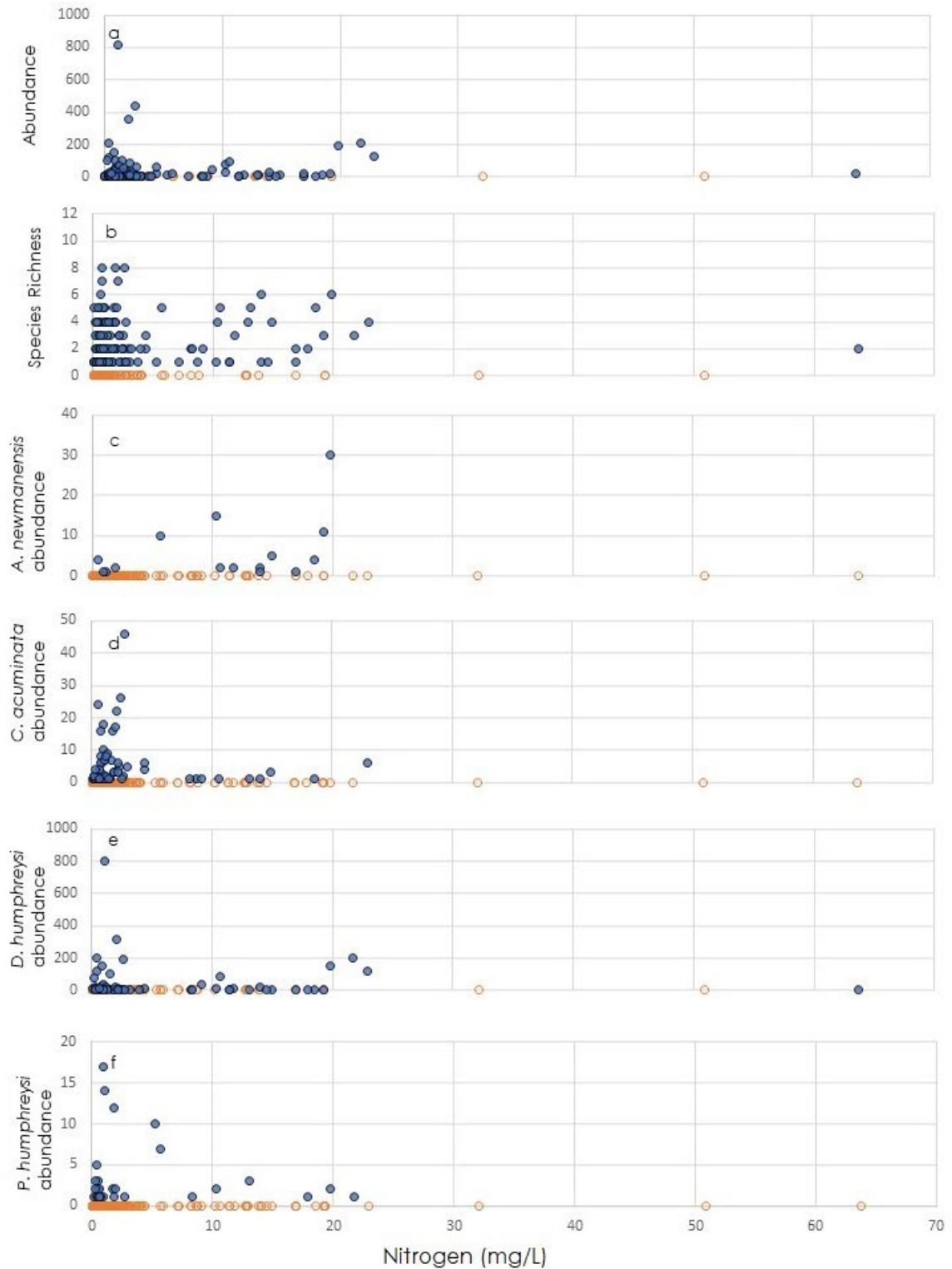


Figure 4-9: Scatterplots of total nitrogen vs a) total abundance, b) species richness, c) abundance of *Archinitocrella newmanensis*, d) *Chydaekata acuminata*, e) *Diacyclops humphreysi* and f) *Pygolabis humphreysi*. Data set includes samples between December 2014 to May 2022. Orange circles = zero on the y-axis, blue circles = values greater than zero on the y-axis

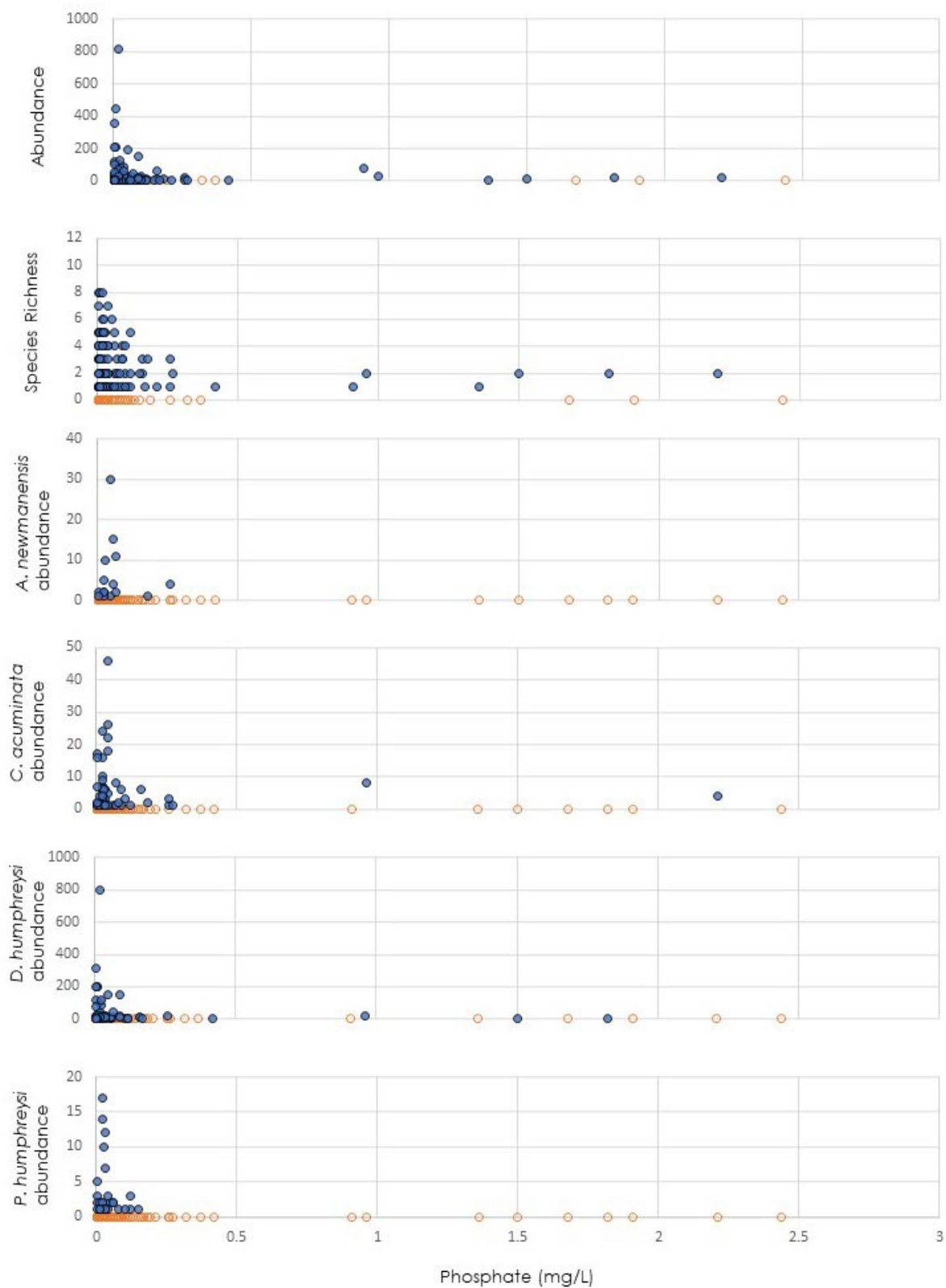


Figure 4-10: Scatterplots of total phosphorus vs a) total abundance, b) species richness, c) abundance of *Archinitocrella newmanensis*, d) *Chydaekata acuminata*, d) *Diacyclops humphreysi* and e) *Pygolabis humphreysi*. Data set includes samples between December 2014 to May 2022. Orange circles = zero on the y-axis, blue circles = values greater than zero on the y-axis

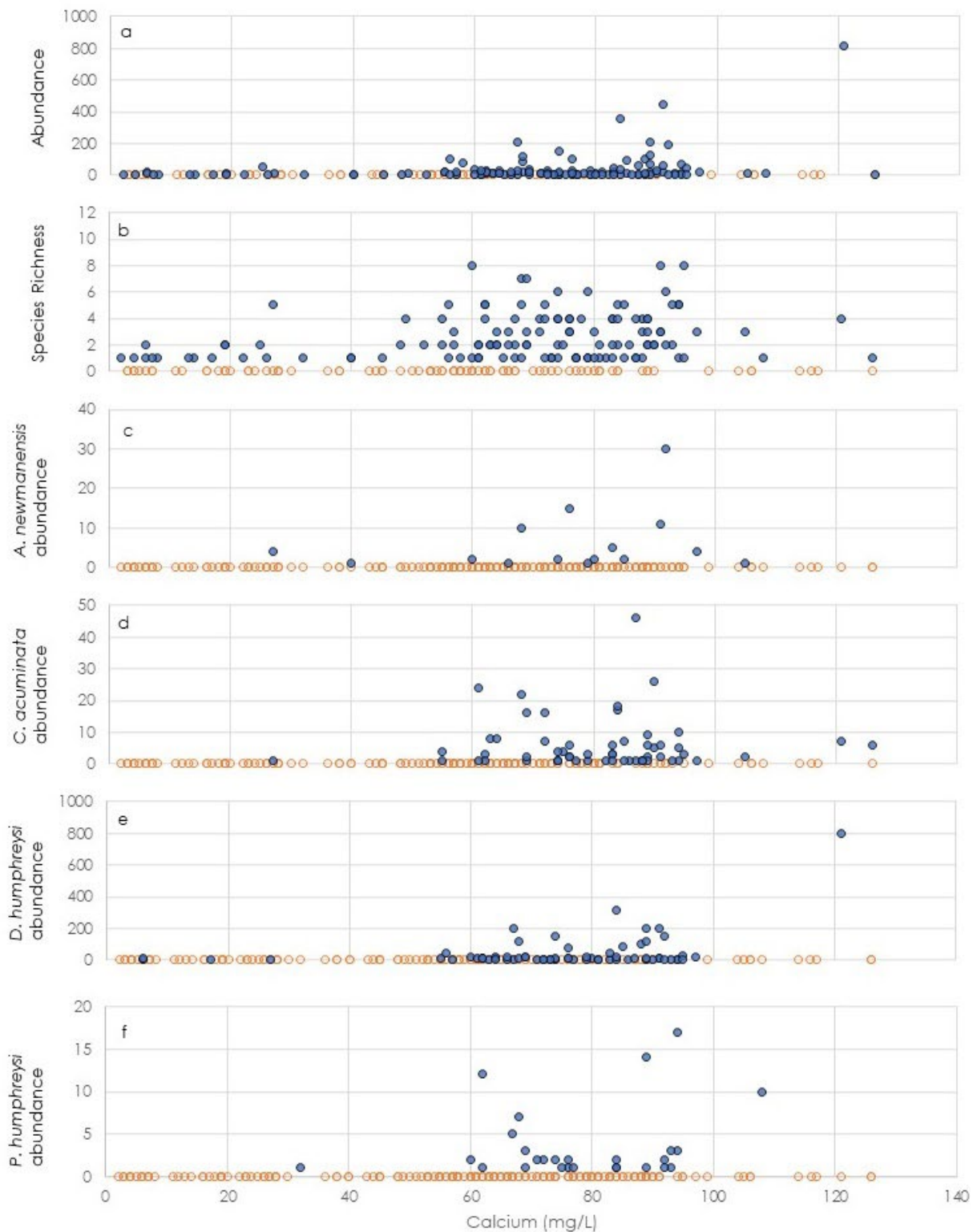


Figure 4-11: Scatterplots of calcium vs a) total abundance, b) species richness, c) abundance of *Archinitocrella newmanensis*, d) *Chydaekata acuminata*, e) *Diacyclops humphreysi* and f) *Pygolabis humphreysi*. Data set includes samples between December 2014 to May 2022. Orange circles = zero on the y-axis, blue circles = values greater than zero on the y-axis

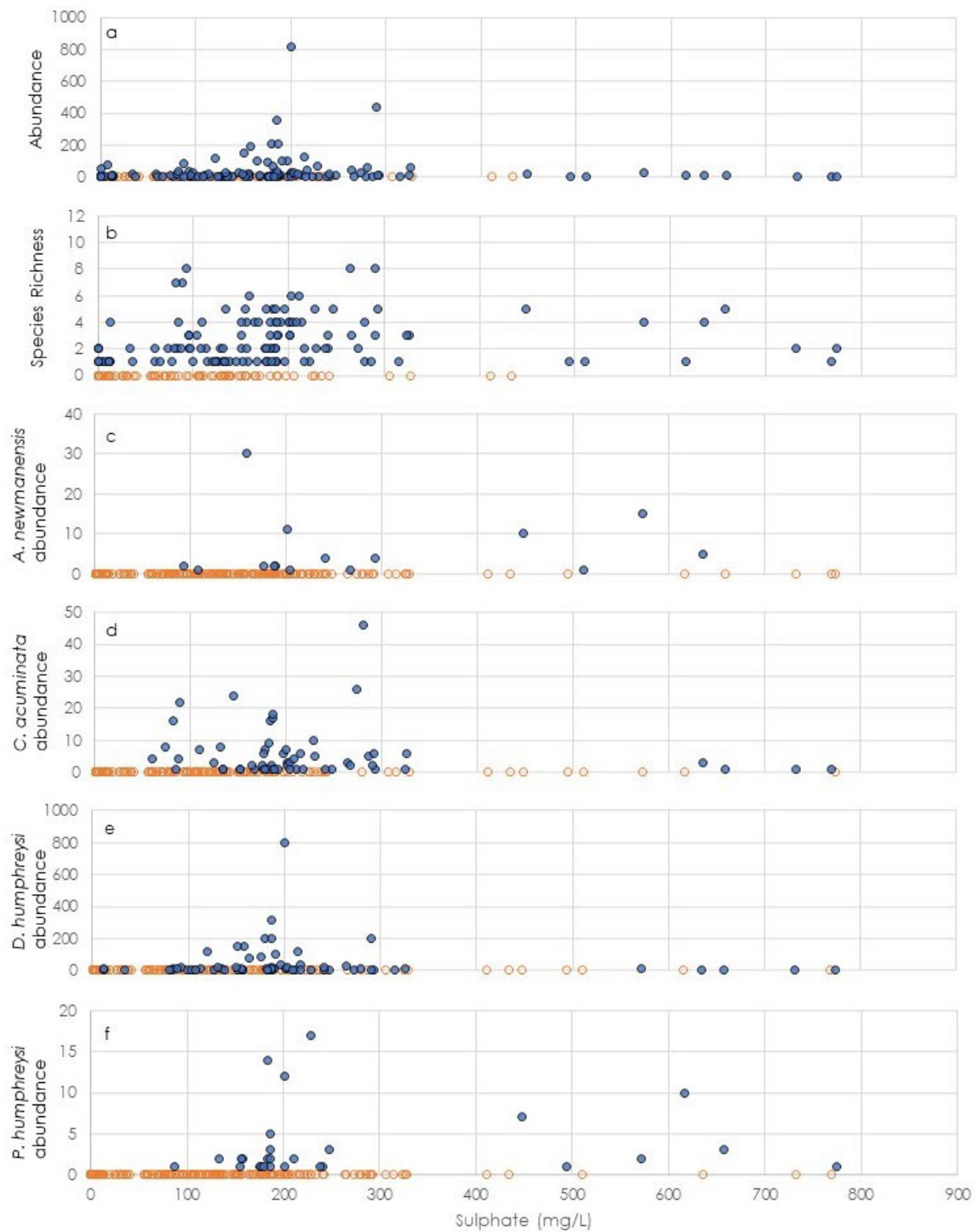


Figure 4-12: Scatterplots of sulphate vs a) total abundance, b) species richness, c) abundance of *Archinitocrella newmanensis*, d) *Chydaekata acuminata*, d) *Diacyclops humphreysi* and e) *Pygolabis humphreysi*. Data set includes samples between December 2014 to May 2022. Orange circles = zero on the y-axis, blue circles = values greater than zero on the y-axis

4.6 Trophic Structure

Trophic structures, the tiered structure of organisms within an ecosystem, which demonstrates which organisms share a similar function and food source, provide important information about ecological functions within ecosystems (Lindeman 1942; Polis and Winemiller 2013; Start 2018). Subterranean habitats impose major constraints on trophic interactions, and stygofauna have been displayed to have low degrees of specialisation driven by the lack of resources in groundwaters (Culver 1994; Gibert and Deharveng 2002; Hancock *et al.* 2005). They have also been shown to have truncated food webs due to the lack of autochthonous primary producers (Saccò *et al.* 2022).

The growth and productivity of stygofauna within subterranean environments is thought to be largely dependent on external inputs of organic matter from primary producers at or near the surface (Kløve *et al.* 2011; Venarsky *et al.* 2022) via water flow, percolation or animal or plant material falling directly into the aquifer (Humphreys 2006). Due to these constraints, there is a strong selection for omnivory and a broadening of diet as a result of reduced resource levels, which may explain the success of taxa that are scavengers, omnivores and detritivores (Culver 1994).

Studies (Saccò *et al.* 2020; Saccò *et al.* 2022) conducted within calcrete aquifers in the Yilgarn region of Western Australia support this, as Amphipoda taxa were found to consume organic material from plant roots, sediment, particulate organic carbon, and two other stygofauna taxa; Copopoda and Harpacticoida. Copepods were identified as primary consumers, with particulate organic carbon (a mixture of leaves, dead wood, guano, and animal carrion) being the majority their diets (Saccò *et al.* 2022; Venarsky *et al.* 2022). Ostracods and oligochaetes are also known to feed on organic material (Culver 1994). Other taxa including bathynellaceans and isopods can range from primary to secondary/tertiary consumers (Cho *et al.* 2006; Coineau and Camacho 2013; Culver 1994).

The only suggestion of trophic interactions in the Ethel Gorge data set examined above is the possibility that higher abundances of copepod species correlate with higher nitrogen concentrations. As discussed in **Section 4.5**, the mechanism behind this apparent link is unclear but may help direct further research into the trophic interactions of stygal systems.

It is likely that the Ethel Gorge TEC system has a truncated food web, in line with various groundwater systems. It is also probable that at least some of the taxa are omnivorous. However, the specific trophic levels are difficult to discern, based on current information. It is recommended that trophic pathways be investigated further through DNA-based molecular methods, stable isotope or radiocarbon analyses.

5. Summary and Conclusions

In order to investigate trends in Ethel Gorge Stygobiont TEC, data was assessed in relation to spatial frameworks including TEC Boundary Zones; TEC, BUF (2 km TEC buffer) and REF (regional), and Monitoring Zones. A review of long-term data (2009 to 2022) for pH and salinity (as measured by electrical conductivity, EC) indicated that groundwater in the broader Ethel Gorge area ranged from acidic to alkaline with salinities categorised as fresh to hyposaline. Values for both pH and salinity (as EC) were within the ranges known to support stygofauna. Differences in these parameters were identified spatially (among bores) and, to a lesser extent, temporally (among survey rounds). Variation in pH and salinity (as EC) between TEC Boundary Zones were very minor, while no significant trends were evident in relation to Monitoring Zone. This reiterated that spatial structuring was primarily at a bore-to-bore level.

A data set with a broader suite of parameters was available from 2014 onwards. Calcium concentrations were comparatively high for groundwaters in the Pilbara, attributable to calcrete deposition within the Ethel Gorge area. A wide range of sulphate levels were recorded however were likely linked to local geology and did not preclude stygofauna. Nitrogen and phosphorus concentrations were also variable. As for pH and salinity alone, analyses incorporating the additional parameters identified spatial differences (among bores) which exceeded temporal differences. There were no strong trends in abiotic factors over time when assessed for all bores, or in the context of selected Monitoring Zone or TEC Boundary Zone. While some temporal patterns were observed at an individual bore level, based on testing of bores from a MZ1 subset, these trends were not reflected in the broader data.

To elucidate patterns within the TEC stygofauna data set and determine the suitability of trigger values for species richness and abundance, a total of 76 Defined taxa were identified from across nine high-level taxonomic groups, predominantly crustaceans. Of these, 49 were identified as occurring within the TEC Boundary, with several recorded from the TEC only. The data set was tested across individual samples, individual bores, survey rounds and against TEC Boundary and Monitoring Zone spatial frameworks. Analyses indicated that stygofauna varied more spatially than temporally at a bore-to-bore level, at a finer scale than could be represented by the monitoring zones or TEC boundaries.

When assessed against TEC Boundary Zones, species richness and abundance were highly variable, as indicated by the standard deviations exceeding the means for each group. Given the large temporal and spatial variability within groups, any measurable trends would be obscured. Factors potentially contributing to the variability included limitations with sampling design and effort, constrained access to certain bore holes and seasonal conditions. High variability in species richness and abundance among bores and survey rounds was also apparent for Monitoring Zone groups while the greatest variation was noted within survey rounds, when assessing for temporal trends. Such variability makes it difficult to discern whether a new value diverges from the historic state. Given this inherent variability, trigger levels for species richness or abundance would not be recommended for use in the management of the Ethel Gorge Stygobiont TEC.

The potential use of taxa as indicator species to support the management of the TEC was also considered as part of the Assessment. Coefficient of Variation (CoV) values and percentage occurrence across samples were calculated to understand which taxa were most consistent in abundance, focusing on Monitoring Zone 1 and then all bores. This process identified four taxa including the copepods *Diacyclops humphreysi*, *Archinitocrella newmanensis*, the isopod *Pygolabis humphreysi* and the paramelitid amphipod *Chydaekata acuminata* as consistently representative of the stygofauna community within the TEC area. BVstep matching analysis identified three of the four taxa as drivers of the spatial trends in the Ethel Gorge Stygobiont. However, none of these taxa met all the criteria of an ideal indicator species. While *Diacyclops humphreysi* is common and abundant, it is not restricted to the greater Newman area, *Chydaekata acuminata* and *Pygolabis humphreysi* are categorised as Core Species yet have been more variable in presence and abundance.

Relying on single univariate measures (e.g., richness and abundance), or the abundance or presence of a single species is considered likely to produce indicators prone to variability. This creates difficulties when assessing whether changes over time represent a shift in community structure or instead reflect sampling or data limitations. A more robust approach may be to employ a limited suite of species within the TEC. Based on this Assessment, *Diacyclops humphreysi*, *Archinitocrella newmanensis*, *Chydaekata acuminata*, and *Pygolabis humphreysi* are taxa that potentially warrant further investigation.

However, one of the key elements of developing useful indicator species to support the management of the system is determining the relationship between stygofauna community and the abiotic environment. The development of this link requires an understanding of how the taxa are influenced by environmental and anthropogenic changes. Without that link, it is unclear what management action is required to address any observed changes in the stygofauna. The causal responses



between environmental factors and stygofauna are poorly understood and documented, and this lack of knowledge represents a clear impediment to the development of stygofauna indicators for the Ethel Gorge Stygobiont TEC.

Analyses undertaken to better understand the relationship between the stygofauna and abiotic parameters revealed low correlations between the abiotic factors and the distribution of stygofauna assemblages across the broader TEC area. Stygofauna species richness and total abundance were investigated in relation to key abiotic parameters (pH, salinity as EC, calcium, nitrogen, phosphorus and sulphate) to further examine the relationships between abiotic parameters and the stygofauna. This line of investigation was also extended to the four taxa identified as a potential indicator suite for TEC health, *Diacyclops humphreysi*, *Archinitocrella newmanensis*, *Pygolabis humphreysi* and *Chydaekata acuminata*.

The results indicated that higher stygofauna abundances and richness were commonly associated with a pH range between 7.2 and 8.2, reflecting broader trends in calcareous groundwater systems. The minimum pH for stygofauna records was 5.5, with *Diacyclops humphreysi*, *Archinitocrella newmanensis*, *Pygolabis humphreysi* and *Chydaekata acuminata* mostly collected within the pH range 7 to 8.5. Stygofauna were generally most abundant at salinities (as electrical conductivity, EC) ranging between 1,000 and 2,000 $\mu\text{S}/\text{cm}$. However, stygal specimens, including representatives of *Diacyclops humphreysi*, *Archinitocrella newmanensis*, *Pygolabis humphreysi* and *Chydaekata acuminata*, were recorded at values above 5,000 $\mu\text{S}/\text{cm}$. Species richness was largely consistent up to at least 5,000 $\mu\text{S}/\text{cm}$.

In general, there were no clear trends in stygofauna species richness or abundance relative to nitrogen and phosphorus concentrations. However, moderate to elevated abundances, primarily comprising copepods, were noted for several of the samples with higher nitrogen values ($\sim 20 \text{ mg/L}$). This may be indicative of a positive correlation of nutrient enrichment with copepod abundances but would require additional investigation to understand trophic links. In relation to calcium, stygofauna abundance and species richness was generally highest at moderate calcium levels. Whether this reflects a biological preference for this water chemistry or the presence of suitable voids within the calcium-rich calcrete habitat is uncertain. Sulphate concentrations were elevated in some instances however were considered to reflect local geology and did not preclude stygofauna.

A review of available information on trophic levels suggests that the Ethel Gorge TEC system has a truncated food web, in line with various groundwater systems. It is also probable that at least some of the taxa are omnivorous, however, the specific trophic levels are difficult to discern, based on current information. Current analyses indicated that copepods may be more abundant with higher nutrient concentration, however it is not clear if the relationship is causative, correlative or spurious. It is recommended that trophic pathways be investigated further through studies employing more direct techniques such as DNA-based molecular, stable isotope or radiocarbon analyses.

6. References

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Appendices



Appendix A Historical Samples

Table A1: Bores from which stygofauna were collected during the Ethel Gorge Program (denoted by “X”) between 2003 and 2022, indicating TEC Boundary Zone and Monitoring Zone for each bore and season for each survey round.

Date	Aug-03	Dec-07	Mar-08	Sep-08	Nov-08	Mar-09	May-09	Nov-09	Jan-10	Apr-10	Jul-10	Nov-10	Feb-11	Feb-12	Apr-12	Apr-13	Dec-13	Mar-14	Dec-14	Mar-15	Mar-16	Apr-17	Dec-19	Mar-20	Oct-20	Nov-20	May-21	May-21	Dec-21	May-22	Survey Rounds	TEC	MZ	Survey Type
Round	R01	R02	R03	R04	R05	R06	R07	R08	R09	R10	R11	R12	R13	R14	R15	R16	R17	R18	R19	R20	R21	R22	R24	R25	R26	R27	R28	R29	R30	R31				
Season	W	D	W	D	D	W	W	D	W	W	W	D	W	W	W	W	D	W	D	W	W	W	D	W	D	D	W	W	D	W				
# Bores with Stygofauna	4	1	4	54	14	11	63	17	4	14	2	27	23	30	32	22	32	28	16	21	1	18	10	11	30	12	18	56	17	16				
OB23REG1				X								X	X	X	X		X	X		X		X	X	X			X		X	X	14	TEC	3	Mon
W056								X		X		X	X	X	X		X	X		X						X	X		X	X	13	TEC	1	Mon
HEOP0387				X			X					X	X	X	X		X	X	X	X		X									12	TEC	1	Mon
HEOP0417				X		X	X					X	X	X			X			X		X		X			X		X		12	TEC	1	Mon
HEA0133	X			X						X				X	X		X	X	X							X	X		X	X	12	TEC	OOS	Mon
HEA0126				X								X	X	X	X		X	X				X					X		X	X	11	TEC	OOS	Mon
HEOP0415				X		X	X					X	X	X	X				X	X											10	TEC	1	Mon
HEA0121												X	X	X	X		X	X				X				X	X		X		10	TEC	OOS	Mon
EEX917				X			X					X	X	X	X		X	X	X												9	TEC	1	Mon
EEX931				X								X	X	X	X		X	X	X								X				9	TEC	1B	Mon
W079				X			X	X		X		X	X																		8	TEC	3	Mon
EEX849				X			X										X	X													4	TEC	1B	Mon
HEOP0308								X		X		X					X														4	TEC	4	Mon
HEA0119				X								X			X			X													4	TEC	OOS	Mon
HEA0134								X				X	X									X									3	TEC	OOS	Mon
EEX985								X												X											2	TEC	1	Mon
EOP0245RE																									X			X			2	TEC	1	Add
EOP0253R																									X			X			2	TEC	1	Add
W236				X			X																								2	TEC	1	Mon
HEA0138				X			X																								2	TEC	OOS	Mon
HEA0139							X																								2	TEC	OOS	Mon
A Wall Composite							X																								1	TEC	1B	Mon
EAS0049				X																											1	TEC	1B	Mon
HEOP0798M							X																								1	TEC	1B	Mon
HEOP0524 (NEW)																													X		1	TEC	3	Mon
HEOP0314						X																									1	TEC	3	Mon
HEOP0430M							X																								1	TEC	3	Mon
W077							X																								1	TEC	3	Mon
HEOP0799				X																											1	TEC	4	Mon
HEOP0801							X																								1	TEC	4	Mon
HEA0114				X																											1	TEC	OOS	Mon
HEA0117				X																											1	TEC	OOS	Mon
HEA0140				X																											1	TEC	OOS	Mon
HEA0143				X																											1	TEC	OOS	Mon
HEA0224				X																											1	TEC	OOS	Mon
P14-S	X																														1	TEC	OOS	Mon
HEOP0574M				X		X	X	X				X	X	X	X		X	X	X	X		X	X	X		X	X		X	X	19	BUF	1	Mon
W028				X		X	X	X		X		X	X	X	X		X	X	X	X		X	X			X	X		X	X	19	BUF	OOS	Mon
T399								X		X		X	X	X	X		X	X	X	X		X	X	X		X	X		X	X	18	BUF	OOS	Mon
HEOP0388												X	X	X	X		X	X	X			X	X	X		X	X		X	X	14	BUF	OOS	Mon

[illegible]

Date																																Survey Rounds	TEC	MZ	Survey Type
	Aug-03	Dec-07	Mar-08	Sep-08	Nov-08	Mar-09	May-09	Nov-09	Jan-10	Apr-10	Jul-10	Nov-10	Feb-11	Feb-12	Apr-12	Apr-13	Dec-13	Mar-14	Dec-14	Mar-15	Mar-16	Apr-17	Dec-19	Mar-20	Oct-20	Nov-20	May-21	May-21	Dec-21	May-22					
Round	R01	R02	R03	R04	R05	R06	R07	R08	R09	R10	R11	R12	R13	R14	R15	R16	R17	R18	R19	R20	R21	R22	R24	R25	R26	R27	R28	R29	R30	R31					
Season	W	D	W	D	D	W	W	D	W	W	W	D	W	W	W	W	D	W	D	W	W	W	D	W	D	D	W	W	D	W					
# Bores with Stygofauna	4	1	4	54	14	11	63	17	4	14	2	27	23	30	32	22	32	28	16	21	1	18	10	11	30	12	18	56	17	16					
EOP0320R																											X			1	BUF	2	Add		
EOP0326R																									X					1	BUF	2	Add		
EOP0017R																									X					1	BUF	OOS	Add		
EOP0104R																											X			1	BUF	OOS	Add		
EOP0118R																											X			1	BUF	OOS	Add		
EOP0162R																											X			1	BUF	OOS	Add		
EOP0173R																											X			1	BUF	OOS	Add		
EOP0179R																											X			1	BUF	OOS	Add		
EXR0528																											X			1	BUF	OOS	Add		
HEC0105													X																	1	BUF	OOS	Mon		
D14/7				X																										1	BUF	OOS	Mon		
EES0501	X																													1	BUF	OOS	Mon		
HEA0144				X																										1	BUF	OOS	Mon		
HEC0117				X																										1	BUF	OOS	Mon		
HEOP0374						X																								1	BUF	OOS	Mon		
HEOP0585				X																										1	BUF	OOS	Mon		
WPP3-4S	X																													1	BUF	OOS	Mon		
EOP0284R																											X			1	BUF	2	Add		
HEOP0462M				X			X	X		X		X	X	X	X		X	X	X	X		X	X	X		X	X		X	X	19	REG	OOS	Mon	
W116							X	X		X		X	X	X	X		X	X	X	X		X	X	X		X	X		X	X	18	REG	OOS	Mon	
HEOP0425				X				X		X		X	X	X	X		X	X	X			X	X	X		X	X		X	X	17	TEC	1	Mon	
HEOP0524 (UNK3)				X										X	X		X	X	X	X		X	X	X					X	X	12	REG	OOS	Mon	
W117							X												X	X	X	X	X			X	X		X	X	11	REG	OOS	Mon	
WP126				X			X	X				X	X	X	X																8	REG	OOS	Mon	
EA0285R				X											X		X			X		X									6	REG	OOS	Mon	
HEOP0543				X			X								X		X	X													5	REG	OOS	Mon	
W081				X			X								X		X			X											5	REG	OOS	Mon	
HEOP0489				X			X											X		X											4	REG	OOS	Mon	
NODDY							X							X	X		X														4	REG	OOS	Mon	
W251				X			X										X	X													4	REG	OOS	Mon	
EA0284R			X	X																											3	REG	OOS	Mon	
HEOP0490							X							X	X																3	REG	OOS	Mon	
W229				X											X			X													3	REG	OOS	Mon	
EOP0343R																									X			X			2	REG	2	Add	
EOP0344R																									X			X			2	REG	2	Add	
EEX0572																X															2	REG	OOS	Mon	
EKP0271							X		X																						2	REG	OOS	Mon	
EMP0070																X															2	REG	OOS	Mon	
EMP0139																X															2	REG	OOS	Mon	
EOP0195R																									X			X			2	REG	OOS	Add	
EOP0198R																									X			X			2	REG	OOS	Add	
EOP0215R																									X			X			2	REG	OOS	Add	
EOP0393R																									X			X			2	REG	OOS	Add	
EOP0417R																									X			X			2	REG	OOS	Add	

Date																																			
Round	R01	R02	R03	R04	R05	R06	R07	R08	R09	R10	R11	R12	R13	R14	R15	R16	R17	R18	R19	R20	R21	R22	R24	R25	R26	R27	R28	R29	R30	R31	Survey Rounds	TEC	MZ	Survey Type	
Season	W	D	W	D	D	W	W	D	W	W	W	D	W	W	W	W	D	W	D	W	W	W	D	W	D	D	W	W	D	W					
# Bores with Stygofauna	4	1	4	54	14	11	63	17	4	14	2	27	23	30	32	22	32	28	16	21	1	18	10	11	30	12	18	56	17	16					
EOP0418R																									X			X			2	REG	OOS	Add	
EOP0426R																									X			X			2	REG	OOS	Add	
EXR0979					X	X																									2	REG	OOS	Mon	
EXR0983					X	X																									2	REG	OOS	Mon	
EXR1010					X	X																									2	REG	OOS	Mon	
EXR1677R					X	X																									2	REG	OOS	Mon	
HEOP0222				X			X																								2	REG	OOS	Mon	
HEOP0538				X			X																								2	REG	OOS	Mon	
HHS0019M																X															2	REG	OOS	Mon	
HST0130R																X															2	REG	OOS	Mon	
HST0186R																X															2	REG	OOS	Mon	
HST0216D																X															2	REG	OOS	Mon	
W190				X			X																								2	REG	OOS	Mon	
WBGW007							X		X																						2	REG	OOS	Mon	
WBGW010							X		X																						2	REG	OOS	Mon	
WBGW045D							X		X																						2	REG	OOS	Mon	
WP131		X	X					X		X																					2	REG	OOS	Mon	
HMG0064		X	X																												2	REG	OOS	Mon	
OB25P1UNK				X																											2	REG	OOS	Mon	
EOP0338R																												X			1	REG	2	Add	
EOP0348R																												X			1	REG	2	Add	
EOP0349R																												X			1	REG	2	Add	
OB42UNK1																												X			1	REG	2	Add	
EA0170R																X															1	REG	OOS	Mon	
EEX0560																X															1	REG	OOS	Mon	
EEX0561																X															1	REG	OOS	Mon	
EEX0573																X															1	REG	OOS	Mon	
EKP0056							X																								1	REG	OOS	Mon	
EKP0186							X																								1	REG	OOS	Mon	
EMP0054																X															1	REG	OOS	Mon	
EOP0212R																												X			1	REG	OOS	Add	
EOP0222R																												X			1	REG	OOS	Add	
EOP0238R																												X			1	REG	OOS	Add	
EOP0356R																												X			1	REG	OOS	Add	
EOP0365R																												X			1	REG	OOS	Add	
EOP0373R																												X			1	REG	OOS	Add	
EOP0380R																									X						1	REG	OOS	Add	
EOP0390R																												X			1	REG	OOS	Add	
EOP0403R																												X			1	REG	OOS	Add	
EXR1659R											X																				1	REG	OOS	Mon	
EXR1660R											X																				1	REG	OOS	Mon	
HEOP0540													X																		1	REG	OOS	Mon	
HHS0032																X															1	REG	OOS	Mon	
HHS0035M																X															1	REG	OOS	Mon	
HHS0037M																X															1	REG	OOS	Mon	

Date	Aug-03	Dec-07	Mar-08	Sep-08	Nov-08	Mar-09	May-09	Nov-09	Jan-10	Apr-10	Jul-10	Nov-10	Feb-11	Feb-12	Apr-12	Apr-13	Dec-13	Mar-14	Dec-14	Mar-15	Mar-16	Apr-17	Dec-19	Mar-20	Oct-20	Nov-20	May-21	May-21	Dec-21	May-22	Survey Rounds	TEC	MZ	Survey Type
Round	R01	R02	R03	R04	R05	R06	R07	R08	R09	R10	R11	R12	R13	R14	R15	R16	R17	R18	R19	R20	R21	R22	R24	R25	R26	R27	R28	R29	R30	R31				
Season	W	D	W	D	D	W	W	D	W	W	W	D	W	W	W	W	D	W	D	W	W	W	D	W	D	D	W	W	D	W				
# Bores with Stygofauna	4	1	4	54	14	11	63	17	4	14	2	27	23	30	32	22	32	28	16	21	1	18	10	11	30	12	18	56	17	16				
HIST0723R																				X											1	REG	OOS	Mon
HST0032																X															1	REG	OOS	Mon
HST0071R																X															1	REG	OOS	Mon
HST0098R																X															1	REG	OOS	Mon
HST0133R																X															1	REG	OOS	Mon
HST0205D																X															1	REG	OOS	Mon
HST0212D																X															1	REG	OOS	Mon
HST0217D																X															1	REG	OOS	Mon
HST0907R														X						X											1	REG	OOS	Mon
JUMP UP														X																	1	REG	OOS	Mon
EAP0176			X																												1	REG	OOS	Mon
EJ0434R			X																												1	REG	OOS	Mon
EMR0041				X																											1	REG	OOS	Mon
EXR0644					X																										1	REG	OOS	Mon
EXR0789						X																									1	REG	OOS	Mon
EXR0984					X																										1	REG	OOS	Mon
EXR1542R					X																										1	REG	OOS	Mon
EXR1544R					X																										1	REG	OOS	Mon
EXR1678					X																										1	REG	OOS	Mon
EXR639					X																										1	REG	OOS	Mon
FG2201R					X																										1	REG	OOS	Mon
FG2214R					X																										1	REG	OOS	Mon
HEOP0525							X																								1	REG	OOS	Mon
HEOP0526							X																								1	REG	OOS	Mon
HEOP0528							X																								1	REG	OOS	Mon
HEOP0556							X																								1	REG	OOS	Mon
Ninga							X																								1	REG	OOS	Mon
OB35SEPT095							X																								1	REG	OOS	Mon
OB35SEPT096							X																								1	REG	OOS	Mon
Ophthalmia							X																								1	REG	OOS	Mon
Shovelanna							X																								1	REG	OOS	Mon
W135							X																								1	REG	OOS	Mon
W157							X																								1	REG	OOS	Mon
W196							X																								1	REG	OOS	Mon
WBGW0050D							X																								1	REG	OOS	Mon
WBGW019D							X																								1	REG	OOS	Mon
WJR001					X																										1	REG	OOS	Mon
YOB-010					X																										1	REG	OOS	Mon

Appendix B Defined Taxa

Table B1 Stygofauna taxa from the Ethel Gorge Program that were considered to represent Defined taxa at the species-level, in relation to TEC class. The criteria for core taxa are as per Bennelongia (2013).

Phylum	Class	Order	Family	Taxon	Core in 2013	Core Current	TEC	TEC Only	BUF	TEC + BUF	TEC + BUF Only	REG	REG only
Annelida													
	Clitellata												
		Haplotaxida											
			Phreodrilidae										
				<i>Insulodrilus</i> OB1	Y	X						X	X
				<i>Insulodrilus</i> WA31	N				X	X		X	
				Phreodrilidae sp. 1	N							X	X
				Phreodrilidae sp. 2		X						X	X
				Phreodrilidae sp. OB2	Y	X	X		X	X		X	
				Phreodrilidae sp. OB3	Y	X						X	X
				Phreodrilidae sp. OB4	Y	X	X	X		X	X		
				Phreodrilidae sp. OP1	Y	X			X	X		X	
				<i>Phreodrilus peniculus</i>	N				X	X	X		
		Tubificidae											
			Enchytraeidae										
				<i>Achaeta</i> sp.	N				X	X	X		
				Enchytraeidae sp. OB_MC	N		X		X	X		X	
				Enchytraeidae sp. OB2	Y	X	X			X		X	
				Enchytraeidae sp. OB3	Y	X			X	X	X		
				Enchytraeidae sp. OB4	Y	X	X	X		X	X		
				Enchytraeus sp. Ench1	N							X	X
			Naididae										
				<i>Pristina</i> sp. OB	Y	X	X		X	X		X	
	Polychaeta												
			Aeolosomatidae										
				<i>Aeolosoma</i> sp. OB	N		X			X		X	
Arthropoda													
	Arachnida												
		Trombidiformes											
			Pezidae										
				<i>Peza</i> sp. OB			X		X	X		X	
	Malacostraca												
		Amphipoda											
			Paramelitidae										
				<i>Chydaekata</i> `AMP005`	Y	X			X	X	X		
				<i>Chydaekata acuminata</i>	Y	X	X		X	X		X	
				<i>Kruptus</i> sp. JB1	Y	X						X	X
				<i>Maarrka etheli</i>	Y		X		X	X	X		
				<i>Maarrka</i> sp. nov.		X			X	X	X		
				Paramelitidae gen. nov. 1 `AMP001`	Y	X	X		X	X		X	
				Paramelitidae gen. nov. 1 `AMP002`	Y	X	X		X	X		X	
				Paramelitidae gen. nov. 1 `AMP003`	Y	X	X		X	X		X	
		Isopoda											
			Microcerberidae										
				<i>Coxicerberus</i> `ISO019`	N		X		X	X	X		
				<i>Coxicerberus</i> sp. OB2	Y	X	X	X		X	X		
			Stenoniscidae										
				Stenoniscidae sp. OB		X	X			X		X	
			Tainisopidae										
				<i>Pygolabis humphreysi</i>	Y	X	X		X	X		X	
				<i>Pygolabis weeliwolli</i>			X		X	X		X	
		Syncarida											
			Bathynellidae										
				<i>Bathynella</i> sp. B11		X						X	X
				<i>Bathynella</i> sp. B12		X						X	X
				<i>Billibathynella</i> sp. OB1	Y	X	X		X	X		X	
				<i>Pilbaranella</i> `A`		X			X	X		X	
				<i>Pilbaranella</i> `B`	Y	X	X		X	X	X		
				<i>Pilbaranella ethelensis</i>	Y		X		X	X	X		
			Parabathynellidae										
				<i>Atopobathynella</i> indet.	Y	X	X			X			
				<i>Billibathynella cassidis</i>	Y		X		X	X	X		
				<i>Brevisomabathynella pilbaraensis</i>	Y	X	X		X	X		X	
	Maxillopoda												
		Cyclopoida											
			Cyclopidae										
				<i>Anzycyclops</i> sp. OB	Y	X	X	X		X	X		

Phylum	Class	Order	Family	Taxon	Core in 2013	Core Current	TEC	TEC Only	BUF	TEC + BUF	TEC + BUF Only	REG	REG only			
				<i>Diacyclops cockingi</i>	N				X	X		X				
				<i>Diacyclops humphreysi</i>	N		X		X	X		X				
				<i>Diacyclops sobeprolatus</i>	N		X		X	X		X				
				<i>Dussartcyclops uniarticulatus</i>	N		X	X		X	X					
				<i>Mesocyclops brooksi</i>	N				X	X		X				
				<i>Metacyclops pilbaricus</i>	N							X	X			
				<i>Microcyclops varicans</i>	N		X		X	X		X				
				<i>Orbuscyclops westaustraliensis</i>	N		X		X	X		X				
				<i>Pilbaracyclops</i> sp. OB			X	X		X	X					
				<i>Pilbaracyclops supersensus</i>		X	X		X	X	X					
				<i>Thermocyclops aberrans</i>	N				X	X	X					
		Harpacticoida														
				Ameiridae												
					<i>Archinitocrella newmanensis</i>	N		X		X	X		X			
					<i>Nitocrella`ophthalmia`</i>				X	X	X					
					<i>Nitocrella karanovici</i>		X		X	X		X				
					<i>Stygonitocrella bispinosa</i>							X	X			
				Parastenocarididae												
					<i>Dussartstenocaris`BHA303`</i>		X			X	X	X				
					<i>Parastenocaris`COP001`</i>	Y	X	X		X	X		X			
					<i>Parastenocaris jane</i>	N		X		X	X		X			
					<i>Parastenocaris`COP002`</i>	Y	X	X	X		X	X				
				Ostracoda												
				Podocopida												
					Candonidae											
						<i>Candonopsis tenuis</i>	N		X		X	X		X		
						<i>Notacandona gratia</i>	Y	X	X		X		X			
						<i>Notacandona modesta</i>				X	X	X				
						<i>Notacandona</i> sp. OB1				X	X	X				
						<i>Origocandona`BOS099`</i>	Y	X	X		X	X	X			
						<i>Origocandona inanitas</i>	N		X		X	X		X		
						<i>Pilbaracandona colonia</i>	N		X		X	X		X		
						<i>Pilbaracandona eberhardi</i>	Y	X	X		X	X		X		
						<i>Pilbaracandona kosmos</i>	Y	X	X		X	X		X		
						<i>Pilbaracandona</i> OST001	Y	X	X		X	X		X		
						<i>Pilbaracandona</i> OST002	Y	X	X		X	X	X			
						<i>Pilbaracandona temporaria</i>	Y	X			X	X	X			
					Cyprididae											
						<i>Cypretta</i> spp.	N		X		X	X				
						<i>Ilyodromus</i> spp.	N		X		X					
						<i>Sarscypridopsis ochracea</i>	N			X	X	X				
				<i>Stenocypris malcolmsi</i>		N							X	X		
				Limnocytheridae												
						<i>Gomphodella hirsuta</i>	N		X		X	X		X		

Stantec Australia Pty Ltd
Ground Floor
226 Adelaide Terrace
Perth WA 6000
Tel +61 8 6222 7000



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