

Ministers North:

Yandicoogina Creek Aquatic Ecosystem Surveys

Biologic Environmental Survey

Report to BHP Western Australia Iron Ore

November 2020

DOCUMENT STATUS				
Version No.	Author	Review / Approved for Issue	Approved for Issue to	
			Name	Date
1	J. Delaney K. Nguyen S. Rodman A. Riemer	J. Delaney M. Curran B. Durrant	S. Wild (BHP)	05/06/2020
2	J. Delaney	J. Delaney	S. Wild (BHP)	15/09/2020
3	J. Delaney	J. Delaney	S. Wild (BHP)	03/11/2020
Final	J. Delaney	B. Durrant	S. Wild (BHP)	27/11/2020

We have prepared this report for the sole purposes of BHP West Australia Iron Ore ("Client") for the specific purpose only for which it is supplied. This report is strictly limited to the Purpose and the facts and matters stated in it do not apply directly or indirectly and will not be used for any other application, purpose, use or matter.

In preparing this report we have made certain assumptions. We have assumed that all information and documents provided to us by the Client or as a result of a specific request or enquiry were complete, accurate and up to date. Where we have obtained information from a government register or database, we have assumed that the information is accurate. Where an assumption has been made, we have not made any independent investigations with respect to the matters the subject of that assumption. We are not aware of any reason why any of the assumptions are incorrect.

This report is presented without the assumption of a duty of care to any other person (other than the Client) ("Third Party"). The report may not contain sufficient information for the purposes of a Third Party or for other uses. Without the prior written consent of Biologic:

a) This report may not be relied on by a Third Party; and

b) Biologic will not be liable to a Third Party for any loss, damage, liability or claim arising out of or incidental to a Third Party publishing, using or relying on the facts, content, opinions or subject matter contained in this report.

If a Third Party uses or relies on the facts, content, opinions or subject matter contained in this report with or without the consent of Biologic, Biologic disclaims all risk and the Third Party assumes all risk and releases and indemnifies and agrees to keep indemnified Biologic from any loss, damage, claim or liability arising directly or indirectly from the use of or reliance on this report.

In this note, a reference to loss and damage includes past and prospective economic loss, loss of profits, damage to property, injury to any person (including death) costs and expenses incurred in taking measures to prevent, mitigate or rectify any harm, loss of opportunity, legal costs, compensation, interest and any other direct, indirect, consequential or financial or other loss.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

GLOSSARY

ALA	Atlas of Living Australia
BOM	Bureau of Meteorology
DBCA	Department of Biodiversity, Conservation and Attractions
DO	Dissolved oxygen
DoEE	Department of Environment and Energy
DPaW	Department of Parks and Wildlife
DPIRD	Department of Primary Industry and Regional Development
DRF	Declared Rare Flora
EC	Electrical conductivity
EPA	Western Australian Environmental Protection Authority
EPBC Act	*Environment Protection and Biodiversity Conservation Act 1999*
FFG	Functional feeding group
GDE	Groundwater dependent ecosystem
GDV	Groundwater dependent vegetation
GV	Guideline value
IUCN	International Union for the Conservation of Nature
PBS	Pilbara Biological Survey
PEC	Priority Ecological Communities
SRE	Short-range endemic
SSGV	Site-specific guideline value
WAM	Western Australian Museum

EXECUTIVE SUMMARY

Biologic Environmental Survey (Biologic) was commissioned by BHP Western Australia Iron Ore (WAIO) to undertake a two-season baseline aquatic ecosystem survey of Yandicoogina Creek (hereafter referred to as the Study Area), located within the Upper Fortescue River Catchment and the Weeli Wolli/Marillana sub-catchment. Perennial and semi-permanent pools were identified within a three km stretch of Yandicoogina Creek through a desktop assessment and reconnaissance survey. This three km reach became the focus of this study and is hereafter referred to as the Survey Area.

Aquatic ecosystem surveys were undertaken at eight sites, four within the Survey Area, and four reference sites located outside the Survey Area. Sampling was undertaken in October 2019 (dry-19 survey) and April 2020 (wet-20 survey). Ecosystem surveys included habitat assessments and sampling of water quality, wetland flora (submerged and emergent macrophytes), zooplankton, hyporheos, macroinvertebrates and fish. In the wet season, five additional hyporheic samples were collected within the Survey Area to gain a better understanding of the distribution of the stygal species collected during the dry-19.

The Survey Area occurs within an open to closed *Eucalyptus camaldulensis* and *Melaleuca argentea* woodland over *Acacia tumida* var. *pilbarensis* shrubland, with reeds and sedges (e.g. *Cyperus vaginatus*, *Schoenoplectus subulatus* and *Typha domingensis*) along the waterline (Biologic, 2020a). *Typha domingensis* was particularly dense in some parts of the Survey Area, including sites YC1, YC2 and YC3. Submerged macrophytes included *Chara* spp., *Vallisneria nana* and *Ruppia* spp. The presence of groundwater dependent vegetation (GDV) species (i.e. *Melaleuca argentea* and *Eucalyptus camaldulensis*) indicate the Survey Area qualifies as a Groundwater Dependent Ecosystem (GDE).

Water quality within Yandicoogina Creek was generally good and characterised by fresh, clear waters, with low dissolved oxygen saturation, neutral pH, and generally low nitrogen nutrient and dissolved metals concentrations. While water quality was generally within default ANZECC & ARMCANZ (2000) guidelines (DGVs) for the protection of lowland river systems of tropical north Australia, there were some exceedances. These included:

- DO – YC2 (both seasons) and YC4 (wet-20) recorded insufficient DO, below the lower default GV and below the limit of ecological stress (i.e. < 30%).
- N_NOx – YC1 and YC2 recorded nitrogen oxide concentrations in excess of the eutrophication DGV in the dry-19. Elevated N_NOx was also recorded from reference sites in the dry-19 (MUNJS) and wet-20 (WWS, MUNJS and SS).
- Total N – YC2 (dry-19) and YC3 (wet-20) recorded total N in excess of the eutrophication DGV. While total N also exceeded DGVs at reference sites (WM and MUNJS), the concentration recorded from YC2 in the dry-19 was particularly high, exceeding the eutrophication DGV by more than seven times.
- Total P – was high and exceeded the eutrophication DGV at all Yandicoogina Creek and reference sites. Concentrations from YC2 were notably high.

- dB – the 99% toxicity DGV was exceeded at all sites except WM. Elevated dB is commonly reported in surface waters of the Pilbara.
- dFe – YC2 (dry-19) exceeded the interim indicative working level provided in the ANECC/ARMCANZ (2000) guidelines.

Several water quality characteristics confirmed the connection with groundwater, including ionic composition dominated by calcium cations and hydrogen carbonate anions, as well as a lack of seasonal variation in electrical conductivity (EC). In contrast, ephemeral waters and creek pools generally display large seasonal variations in EC due to waters receding over the dry season and the evapo-concentration of ions.

A diverse range of aquatic fauna was recorded across the Survey Area, including 250 invertebrate taxa and three freshwater fish species. Two sites within Yandicoogina Creek (YC3 and YC4) are considered to be of high ecological value. These sites generally recorded high macroinvertebrate diversity, high richness of hyporheos fauna, and high Pilbara endemic taxa richness.

Seasonal variation in zooplankton richness was high, particularly at YC3 and reference site WWS. At YC3, zooplankton richness increased in the wet season, indicating that flooding may have prompted emergence and/or taxa had been flushed into the Survey Area from upstream. At WWS, flooding associated with wet season cyclonic rainfall in January 2020 likely flushed zooplankton taxa downstream, with the population yet to fully re-establish. All zooplankton taxa recorded are widely distributed and none are of conservation significance. The zooplankton taxa richness in the Survey Area was similar to previous studies of nearby creeklines.

Hyporheic samples were successfully collected from all sites except YC1 in the dry-19, due to the particularly dense *Typha* stands which obstructed access. Of the 108 invertebrate taxa recorded from hyporheic zones across the Survey Area, 13% are directly dependent on groundwater for their persistence (12% stygobites and 1% permanent hyporheos stygophiles). The percentage of stygobitic taxa recorded was considerably greater than that reported previously for Pilbara hyporheic zones (i.e. only 5% stygobitic fauna recorded in Halse *et al.* 2002), further highlighting the strong groundwater connection across the Survey Area.

Several Potential Short-Range Endemic (SRE) species were recorded from the hyporheos of Yandicoogina Creek (all stygal), including:

- The ostracod *Meridiescandona facies* (YC1 and YC9H) - known from Weeli Wolli Creek and the central and eastern Fortescue (and now Yandicoogina Creek).
- The ostracod *Gomphodella yandii* (YC7H) – known only from Weeli Wolli Creek, Marillana Creek, and now Yandicoogina Creek.
- The ostracod *Gomphodella alexanderi* (YC7H and YC8H) - known only from Marillana Creek, groundwater bores at Yandi, and now Yandicoogina Creek.
- The amphipod *Chydaekata* sp. \`E\` (YC3, YC5H, YC7H, YC9H, and reference site WWS) - previously known and appears to be restricted to Marillana Creek, Upper Weeli Wolli Creek, and now Yandicoogina Creek.

- The amphipod Paramelitidae `sp. Biologic-AMPH023` (YC1, YC3, YC5H, and YC9H) – previously known only from Marillana Creek.
- The isopod *Pygolabis weeliwolli* (YC3, YC4, YC5H, YC7H, and YC9H) – known only from Weeli Wolli Creek, Marillana Creek, Yandicoogina Creek (now), and bores within the Yandicoogina tenement.
- The syncarid Bathynellidae sp. BES7547 (YC9H) – new, undescribed genus, currently known only from the Survey Area.

Macroinvertebrate richness was generally high, especially at YC4 (72 taxa recorded in the dry-19) and some reference sites (i.e. SS and WWS). YC2 consistently recorded the lowest richness, which may have reflected difficulties associated with sampling. There was a high density of *Typha* throughout the site, with little open water and limited space with which to kick-sweep sample effectively.

Macroinvertebrate richness was compared statistically to previous aquatic surveys undertaken in the area. Overall, there was a significant difference in macroinvertebrate richness between creeks, but not between seasons. Richness recorded from Yandicoogina Creek was found to be statistically similar to Weeli Wolli Creek and Marillana Creek, but significantly lower than Weeli Wolli Spring. It is important to note that this analysis was influenced by the lower richness recorded from YC1 and YC2, which was likely a reflection of difficulties associated with sampling these sites. Individual site richness recorded from YC4 (67 taxa in the dry-19[1]) was actually similar to site richness recorded from Weeli Wolli Spring in this (64 taxa in the dry-19) and past surveys (i.e. 67 taxa recorded from BENS in the wet-14, and 69 taxa recorded from WWS in the wet-05). Multivariate analyses on the same dataset (current and previous surveys) found that Yandicoogina Creek macroinvertebrate assemblages were most similar to other spring sites, such as Munjina Spring, Weeli Wolli Spring and Skull Springs. This indicates a greater affinity with springs, rather than creek pools.

While most aquatic macroinvertebrates recorded from the Survey Area were common, ubiquitous species, several species were of conservation significance, including:

- the Pilbara pin damselfly *Eurysticta coolawanyah* (YC4 and a reference site BENS) - Vulnerable on the IUCN Redlist.
- the Pilbara emerald, *Hemicordulia koomina* (YC4 and a reference site BENS) - Vulnerable on the IUCN Redlist.
- the stygal Potential SRE amphipod *Chydaekata* sp. `E. (surface waters of YC3 and YC4, and reference site WWS) - known only from Yandicoogina Creek, Marillana Creek, and Upper Weeli Wolli Creek.

[1] The richness reported for the Survey Area in comparisons with previous studies was different to that reported for the study alone. This was the result of amalgamating datasets across years and between different samplers. Taxonomy has improved for several groups since some of the previous surveys were undertaken (i.e. the Pilbara Biological Survey), and some samplers did not identify macroinvertebrates to the same level of resolution as the current study. Therefore, taxonomy had to be aligned between the previous studies and this current project, prior to undertaking statistical analysis.

All freshwater fish species likely to populate the Survey Area were recorded, including the western rainbowfish *Melanotaenia australis* (Melanotaeniidae), Pilbara tandan *Neosilurus* sp. (Plotosidae), and spangled perch *Leiopotherapon unicolor* (Terapontidae). Although the Pilbara tandan is endemic to the Pilbara region, none of these species are of conservation significance and all are common and ubiquitous across the Pilbara. No introduced species were recorded. Greatest fish abundances were recorded in the wet season, following cyclonic rains and flooding throughout these creeklines. The presence of western rainbowfish new recruits and juveniles within Yandicoogina Creek suggests good levels of breeding and recruitment within the Survey Area. Spangled perch breeding and recruitment was also evident at YC1.

Overall, Yandicoogina Creek was found to support a GDE of high ecological value, characterised by mature stands of the obligate phreatophyte *Melaleuca argentea* and facultative phreatophyte *Eucalyptus camaldulensis*, with no obvious signs of canopy decline. A diversity of other mesic species also occur in close association with the creek, such as *Cyperus vaginatus*, *Schoenoplectus subulatus* and *Typha domingensis*. The presence of the aforementioned phreatophytic species suggests groundwater is persistently at or just below the surface. This is further supported by the presence of numerous permanent and semi-permanent pools and riffle sequences, which occur along the length of the Survey Area. These pools provide important habitat for aquatic fauna and a resource for terrestrial invertebrate and vertebrate species. The current study found that four of these pools support; aquatic invertebrates with restricted distributions that would be classified as potential SREs (i.e. *Chydaekata* sp. E, immature or damaged Paramelitidae sp.); a high diversity of Pilbara endemic aquatic invertebrate taxa; IUCN conservation listed species (*Eurysticta coolawanyah* and *Hemicordulia koomina*); and three species of freshwater fish. Additionally, hyporheic zones within the Survey Area supported Potential SREs, including *Chydaekata* sp. E, Paramelitidae `sp. Biologic-AMPH023`, *Pygolabis weeliwolli*, *Meridiescandona facies*, *Gomphodella yandii*, *Gomphodella alexanderi*, and Bathynellidae sp. BES7547. These important ecological values are supported by the high in-stream habitat diversity and heterogeneity characteristic of the system, as well as the strong connection to groundwater in this area.

Due to the aridity of the Pilbara, rivers of the region tend to be ephemeral. As such, permanent water sources in the region are relatively scarce and restricted to springs and permanent pools. This highlights the importance of the Survey Area in the broader Pilbara region. One permanent pool in the Survey Area (YC4), was found to support a notably high diversity of aquatic invertebrates, comparable to the Weeli Wolli Spring Priority Ecological Community (PEC) and Skull Springs. Permanent springs in shaded gorges and river beds support a suite of mesic-adapted species that are otherwise rare in the region.

For riverine pools to be termed GDEs they must have demonstrated long-term connectivity to the groundwater and be maintained by groundwater discharge during drought periods. GDEs are those parts of the environment, the species composition, and natural ecological processes that are dependent on the permanent or temporary presence or influence of groundwater (Murray *et al.*, 2003). A number of physical and ecological elements highlight the close connection to groundwaters within the Survey Area. These include:

- the presence of GDVs such as *Melaleuca argentea* and *Eucalyptus camaldulensis*;
- the relatively stable surface water levels between seasons (despite the dry-19 following on from a particularly dry period and the high flood event which occurred prior to the wet 2020 survey);
- stable electrical conductivity in surface waters between seasons, with little evidence of evapoconcentration effects associated with pool drying in the dry season;
- ionic composition dominated by calcium carbonate, similar to other spring systems of the Pilbara;
- the presence of stygofauna throughout the hyporheic zone and in surface water pools; and
- the macroinvertebrate assemblages having a greater affinity with other spring assemblages, rather than creek pools (determined through multivariate analysis with the PBS data).

As such, the stretch of Yandicoogina Creek encompassing the Survey Area should be considered an aquatic GDE that holds considerable importance in the region.

1. INTRODUCTION

1.1 Background and objectives

Biologic Environmental Survey (Biologic) was commissioned by BHP Western Australia Iron Ore (WAIO) to undertake a two-season baseline aquatic ecosystem survey of Yandicoogina Creek (hereafter referred to as the Study Area; Figure 2.1) located in the Ministers North area. Yandicoogina Creek is a major tributary of Marillana Creek, which flows into Weeli Wolli Creek, approximately 25 km upstream of the Fortescue Marsh. Yandicoogina Creek supports a potential groundwater dependent ecosystem (GDE), characterised by extensive closed *Melaleuca argentea* forest, with *Eucalyptus camaldulensis* over *Acacia tumida* var. *pilbarensis* shrubland, and reeds and sedges (e.g. *Cyperus vaginatus*, *Schoenoplectus subulatus* and *Typha domingensis*) along the waterline. The Study Area is located between the BHP WAIO Mining Area C (MAC) operation to the southwest and BHP WAIO Yandi operation to the north, within the Pilbara bioregion of Western Australia.

The overarching objective of the Level 2 (EPA, 2016a) survey was to identify the aquatic fauna found in perennial and semi-permanent pools along the creekline, and to determine the associated ecological values of aquatic fauna and habitats that may need to be considered during any future environmental approvals across the Study Area.

The specific scope of works included:

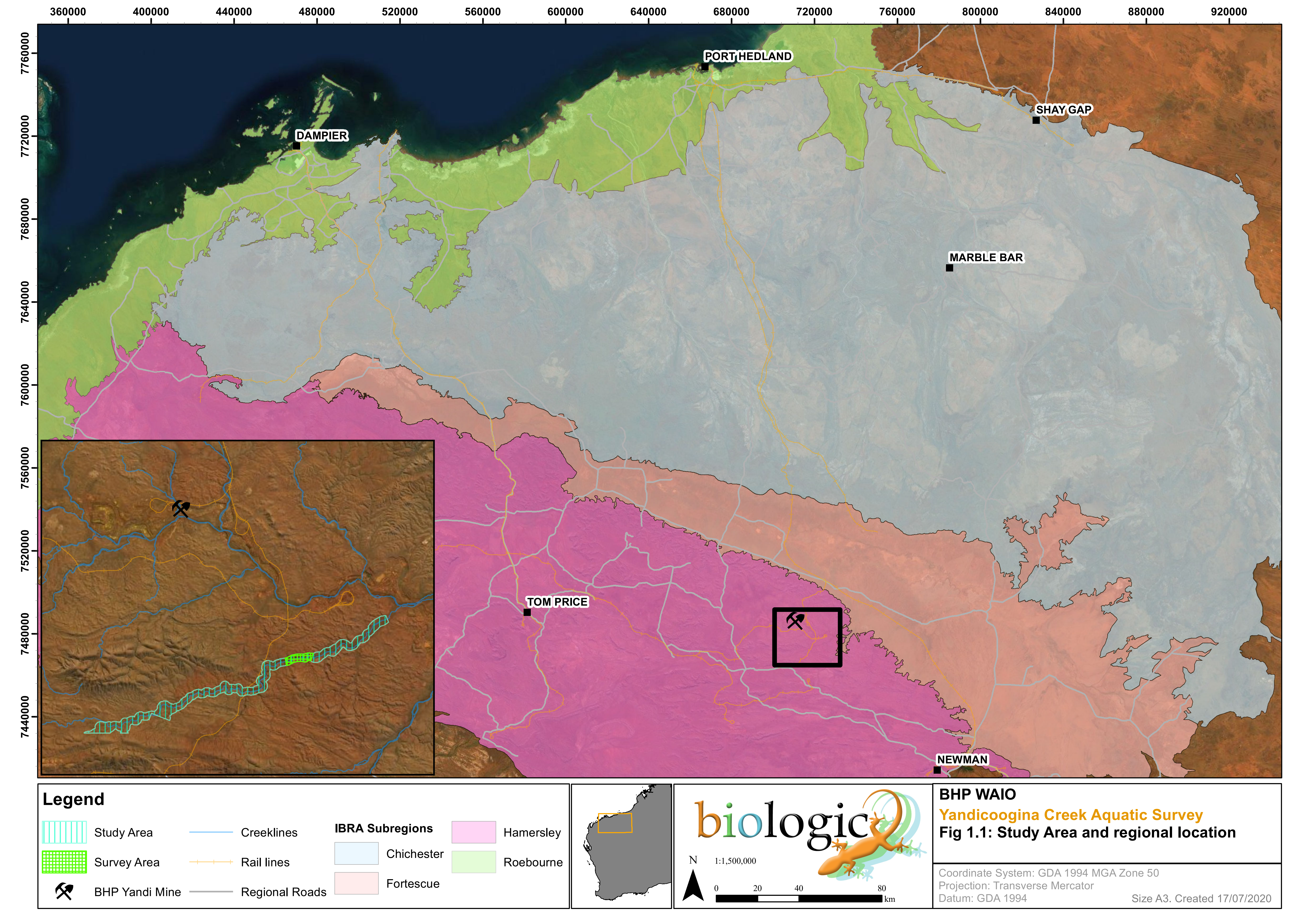
- A desktop assessment, including a review of previous biological surveys and government and non-government databases;
- Identification of perennial and semi-permanent pools along Yandicoogina Creek through a desktop assessment and reconnaissance survey;
- Undertaking a baseline aquatic survey, including identification and sampling of suitable reference sites; and
- Identification of any significant ecological values related to aquatic fauna and their habitats within the Study Area.

1.2 Legislation and guidance

The survey was carried out in accordance with the Western Australian Environmental Protection Authority (EPA) and BHP WAIO guidelines. There is currently (as at November 2020) no technical guidance applicable to the Inland Waters Environmental Factor; however, this survey was carried out in a manner consistent with the following:

- Environmental Factor Guideline, Inland Waters (EPA, 2018);
- Technical Guidance, Terrestrial Fauna Surveys (EPA, 2016a);
- Technical Guidance, Sampling of Short-Range Endemic Invertebrate Fauna (EPA, 2016b);
- Australian and New Zealand Guidelines for Fresh and Marine Water Quality (ANZECC & ARMCANZ, 2000; ANZG, 2018);

- Similar surveys, including the Pilbara Biological Survey (Pinder *et al.*, 2010) and National Monitoring River Health Initiative (MRHI; Choy & Thompson, 1995);
- BHP WAIO’s Biological Survey Spatial Data Requirements (SPR-IEN-EMS-015) (BHP, 2018); and
- BHP WAIO’s Aquatic Fauna Assessment Methods Procedure (0098594) (BHP, 2017).



BHP WAIO

Yandicoogina Creek Aquatic Survey

Fig 1.1: Study Area and regional location

Coordinate System: GDA 1994 MGA Zone 50
Projection: Transverse Mercator
Datum: GDA 1994
Size A3. Created 17/07/2020

2 ENVIRONMENT

2.1 Biogeography

The Study Area falls within the Pilbara biogeographical region as defined by the Interim Biogeographic Regionalisation of Australia (IBRA) (Thackway & Cresswell, 1995). The Pilbara bioregion is characterised by vast coastal plains and inland mountain ranges with cliffs and deep gorges (Thackway & Cresswell, 1995). Vegetation is predominantly mulga low woodlands or snappy gum over bunch and hummock grasses (Bastin, 2008). Within the Pilbara bioregion there are four subregions: Hamersley, Chichester, Roebourne and Fortescue Plains.

The Study Area lies within the Hamersley subregion which contains the southern section of the Pilbara Craton and comprises a mountainous area of Proterozoic sedimentary ranges and plateaux, dissected by basalt, shale and dolerite gorges (Kendrick, 2001). Vegetation in the valley floors is predominately characterised by low mulga woodland over bunch grasses, with *Eucalyptus leucophloia* over *Triodia brizoides* dominating the skeletal soils on the ranges. Drainage is into the Fortescue River to the north, the Ashburton River to the south, or the Robe River to the west (Kendrick, 2001).

2.2 Hydrology

The Study Area is located within the Upper Fortescue River Catchment and the Weeli Wolli/Marillana sub-catchment. Several ephemeral creeklines traverse the Ministers North area, including Marillana, Lamb, Herbert and Yandicoogina creeks. Yandicoogina Creek is approximately 42 km in length and flows north-east into Marillana Creek (Figure 2.1). The upper reaches of Yandicoogina Creek comprise a relatively broad, un-defined channel, however, in the mid to lower reaches, the creek flows through a gorge system and becomes well defined. It is through this section that the groundwater appears to intercept the surface, forming a series of seeps and pools that extend for approximately 3 km. Of note is one particularly deep pool (YC4). This pool is likely permanent and maintained partially by aspect and low evaporation (located against a cliff face), as well as groundwater inflow. Yandicoogina Creek meets Marillana Creek approximately 9 km downstream of this pool, where it flows eastwards for 7 km before draining into Weeli Wolli Creek.

Weeli Wolli Creek is approximately 70 km in length and has a catchment area of 4,100 km^2. It flows to the north, where it drains into the Fortescue River via the ecologically significant Fortescue Marsh (Figure 2.1). The two systems are only connected during flooding associated with intense cyclonic events (Kendrick, 2001). The Marsh is approximately 40 km downstream, and to the north, of Yandicoogina Creek (Figure 2.1). The Fortescue Marsh is a wetland system of national importance under the Directory of Important Wetlands in Australia (Environment Australia, 2001). It is a “good example of an extensive, inland floodplain system which is irregularly inundated”, and is a “unique wetland landform in Western Australia” (Environment Australia, 2001). The Fortescue Marsh extends east from Goodiaderrie Hills and comprises lakes, marshes, and pools along the floodplain in the middle reaches of the Fortescue River, and includes Powellinna Pool, Gnalka Gnoona Pool, Gidyea Pool, Chaddelinna Pool, Mungthannannie, Cook Pool and Moorimoordinia Pools (Environment Australia, 2001). Current and potential threats to the Fortescue Marsh include changes to hydrology, overgrazing by cattle, and pollution of surface inflow water from mine sites (Environment Australia, 2001).

2.3 Groundwater Dependent Ecosystems

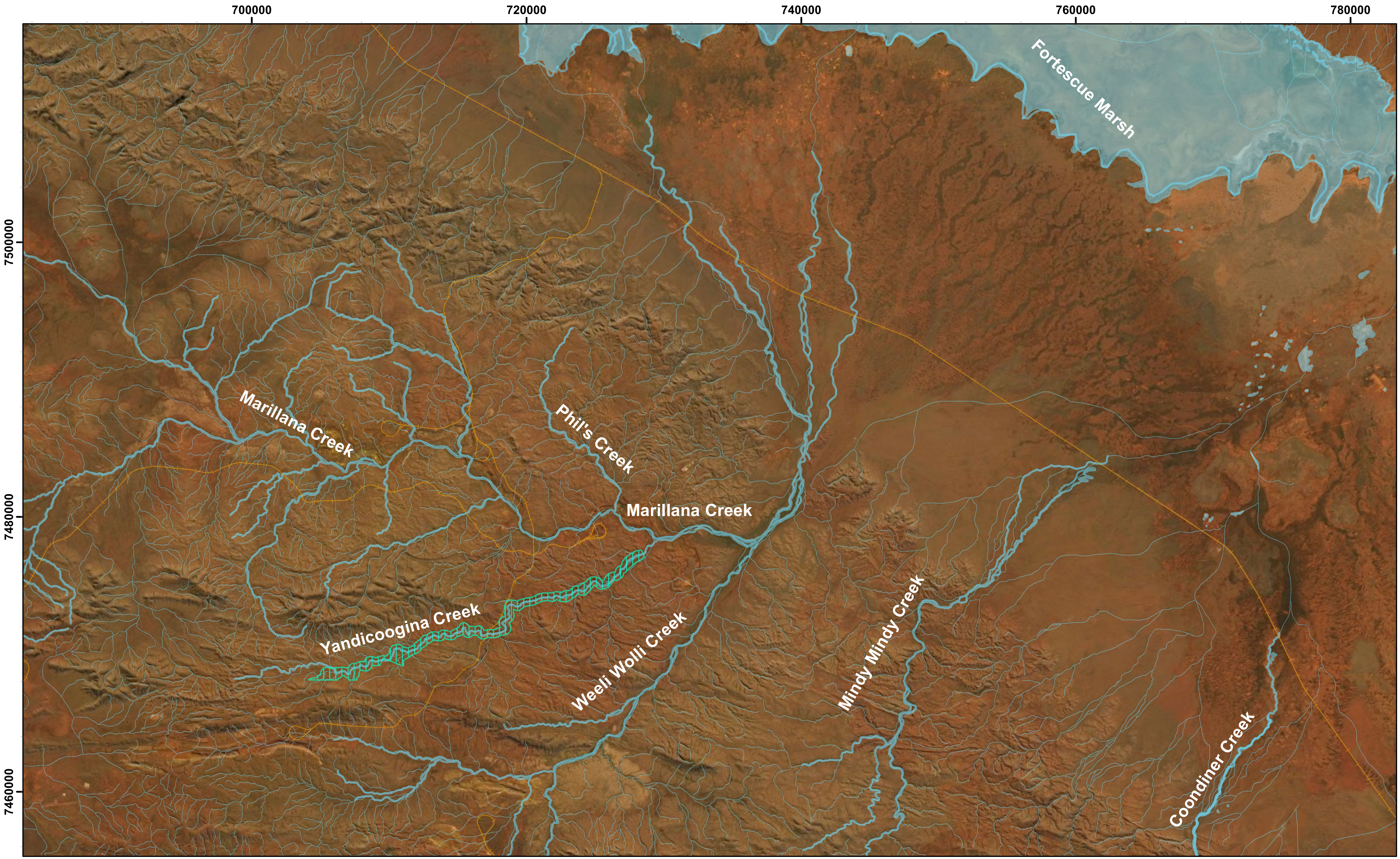
Groundwater-Dependent Ecosystems (or GDEs) are ecosystems that rely on groundwater for their continued existence (BoM, 2020). GDE's can be represented by many different assemblages of biota which rely on groundwater, and as a result come in many forms. For terrestrial ecosystems there are three key types of GDE;

1. Aquatic ecosystems; that rely on the surface expression of groundwater – this includes surface water ecosystems which may have a groundwater component, such as rivers, wetlands and springs.
2. Terrestrial ecosystems; that rely on the subsurface presence of groundwater–this includes all vegetation ecosystems or Groundwater Dependent Vegetation (GDV).
3. Subterranean ecosystems; this includes cave and aquifer ecosystems (BoM, 2020).

Above-ground terrestrial GDE's are typically characterised by the presence of flora species that rely on groundwater (i.e. phreatophytes). Phreatophytes may be classified as either obligate or facultative phreatophytes depending on their reliance on groundwater:

- Obligate phreatophytes are flora species confined to habitats with access to groundwater.
- Facultative phreatophytes are flora species that can utilise groundwater to satisfy a proportion of their ecological water requirement (EWR) when it is available. However, some individuals may also satisfy their EWR by relying solely on uptake from upper unsaturated soils layers where groundwater is inaccessible (Eamus *et al.*, 2016).

A national dataset of Australian GDEs was developed by the Bureau of Meteorology (BoM) to inform groundwater planning and management (BoM, 2020). This dataset is referred to as the Groundwater Dependent Ecosystems Atlas (GDE Atlas), and is the first and only national inventory of GDEs in Australia. The GDE Atlas contains information about the three key types of ecosystems described above (Aquatic; Terrestrial; and Subterranean). Importantly, the GDE Atlas also includes the national inflow-dependent landscapes layer which is derived from remotely sensed data. This layer indicates the likelihood that a landscape is accessing water in addition to rainfall (such as soil moisture, surface water or groundwater), and generally represents a potential GDE dataset for all areas not yet studied or investigated in any detail.



Legend

Study Area

Rail

Drainage Lines

biologic

N

1:250,000

0 4 8 16 km

BHP WAIO

Yandicoogina Creek Aquatic Survey

Fig 2.1: Surface drainage of the Study Area and surrounds

Coordinate System: GDA 1994 MGA Zone 50
Projection: Transverse Mercator
Datum: GDA 1994

Size A3. Created 17/07/2020

Mapping in the GDE Atlas comes from two broad sources:

- National assessment – national-scale analysis based on a set of rules that describe potential for groundwater/ ecosystem interaction and available GIS data.
- Regional studies – more detailed analysis undertaken by various State and regional agencies using a range of different approaches including field work, analysis of satellite imagery and application of rules/conceptual models.

The GDE Atlas indicates that the Study Area has potential to support GDEs based on the terrestrial and terrestrial inflow dependent ecosystem (IDE) assessment. One pool within the Study Area was identified as an unclassified potential aquatic GDE from regional studies, with a high IDE likelihood (BoM, 2020). Ground-truthing of this potential GDE pool was undertaken during the site reconnaissance in May 2019. It was considered unlikely to represent an aquatic GDE, with no GDVs and no surface water present. As such, this pool was not included in the Level 2 aquatic ecosystem survey.

Interestingly, Weeli Wolli Creek, which is a known terrestrial and aquatic GDE, is only classified as having a moderate potential to support GDEs. This may be a function of the national-scale analysis following a set of rules (Doody *et al.*, 2017). The national-scale GDE Atlas is an initial remotely-sensed task of the overall project, with follow-up surveys and investigations required to ground-truth the Atlas and identify the presence of any actual GDEs.

2.4 Climate

The Pilbara region has a semi-desert to tropical climate, with relatively dry winters and hot summers. Rainfall is highly variable and mostly occurs during the summer. It tends to be associated with convective thunderstorms, low pressure systems and tropical cyclones that generate ephemeral flows and occasional flooding in creeks and rivers. Due to the nature of cyclonic events and thunderstorms, total annual rainfall in the region is highly unpredictable and individual storms can contribute several hundred millimetres of rain at one time. The average annual rainfall over the broader Pilbara area ranges from around 200 – 350 millimetres (mm) (predominantly in January, February and March), although rainfall may vary widely from year to year (van Etten, 2009). Temperatures vary considerably throughout the year with average maximum summer temperatures reaching 35 °C to 40 °C and winter temperatures generally fluctuating between 22 °C and 30 °C.

Nearby rainfall gauging stations (GS) for the Study Area include the BoM Marillana Station (#5009; length of record 1936 to current), located approximately 30 km north- east of the Study Area, and the Department of Water and Environmental Regulation (DWER) Weeli Wolli Creek - Tarina Station (#505040; length of record 1985-current), located approximately 10 km south-east of the Study Area. Average annual rainfall recorded from Marillana is 324 mm (BoM, 2019), compared to 359 mm at Weeli Wolli Creek - Tarina (DWER, 2019). Annual rainfall at Weeli Wolli Creek - Tarina ranged from 159 mm (recorded in 2010) to 711 mm (in 2006), illustrating the high inter-annual variability in rainfall.

3 METHODS

3.1 Desktop assessment

A desktop assessment was undertaken comprising database searches and a literature review. The purpose of the desktop assessment was to determine the extent of any previous aquatic survey work in and around the Study Area, and the presence of aquatic fauna species known or likely to occur in the area, including conservation significant species.

3.1.1 Database searches

Five databases were searched for aquatic fauna records within and surrounding the Study Area (Table 3.1).

Table 3.1: Databases searched for the review of previous records

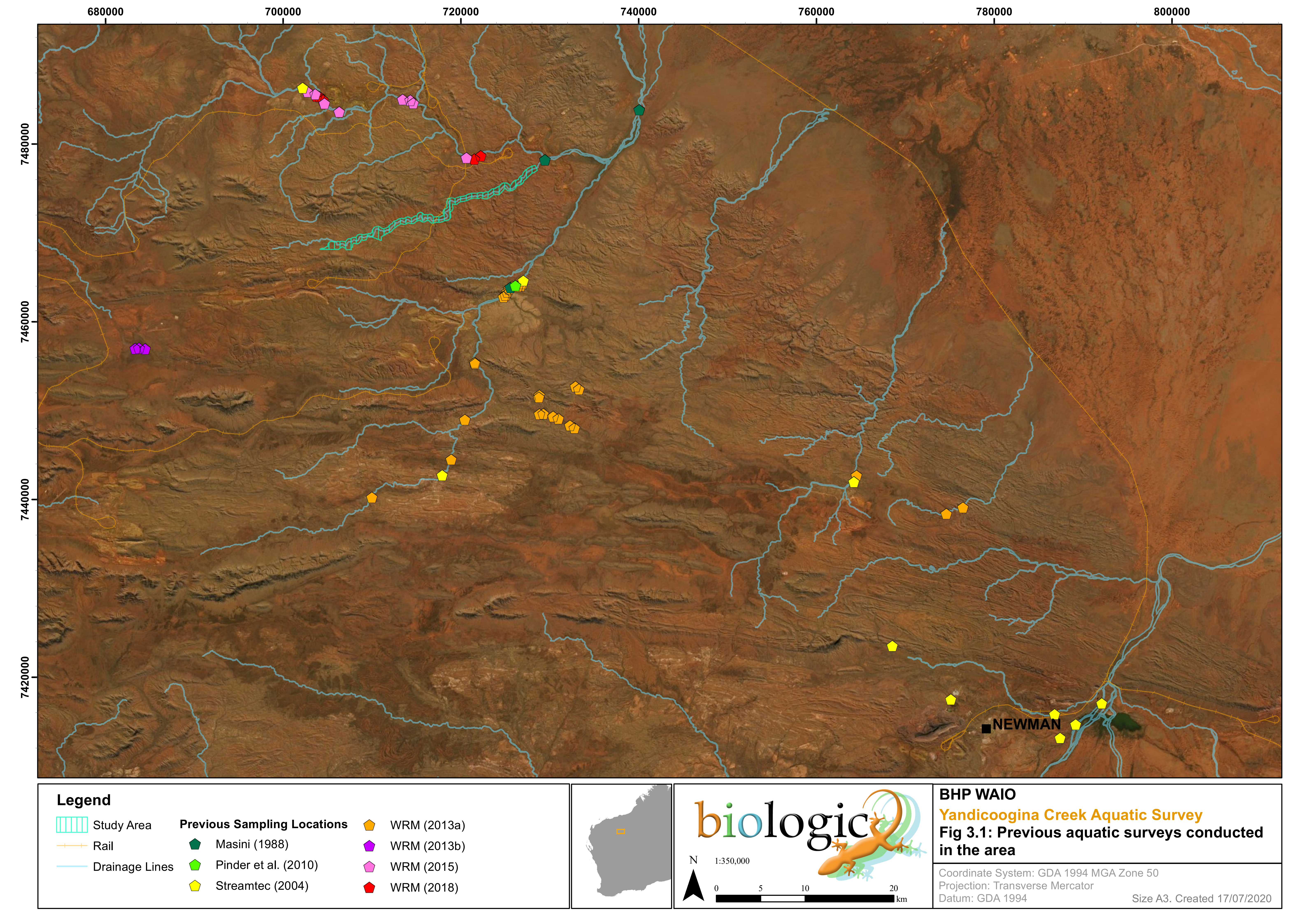
Provider	Database	Reference	Search parameters
DBCA	NatureMap	(DBCA, 2020)	50 km radius centred on the coordinates: -22.8257º, 119.1233º
ALA	Species Occurrence	(ALA, 2020)	
WAM	Arachnids and Myriapods	(WAM, 2019a)	
WAM	Crustaceans	(WAM, 2019b)	
WAM	Molluscs	(WAM, 2019c)	

Other data sources referenced for this desktop assessment included:

- The Australian Faunal Directory,
- The Australian National Insect Collection Database; and
- MRHI database.

3.1.2 Literature review

A review of available literature relevant to the Study Area was undertaken to compile a list of aquatic fauna species previously known to occur nearby, and therefore have the potential to occur within the Study Area. A number of surveys have included aquatic ecosystem sampling to varying degrees, with sites located as close as 4 km to the Yandicoogina Creek Study Area (MC9) (Table 3.2, Figure 3.1). None of these surveys included sites within the Study Area itself. Three of the reference sites utilised in the current survey were within the vicinity of previous survey sites (i.e. Bens Oasis, Weeli Wolli Spring and Wanna Munna).



BHP WAIO
Yandicoogina Creek Aquatic Survey
Fig 3.1: Previous aquatic surveys conducted in the area

Coordinate System: GDA 1994 MGA Zone 50
Projection: Transverse Mercator
Datum: GDA 1994
Size A3. Created 17/07/2020

Table 3.2: Literature sources used for the review.

Survey Title	Reference	Survey Type	Closest Site to Study Area (km)
Inland Waters of the Pilbara	Masini (1988)	Water Quality, Aquatic Flora, Waterbirds & Fish	8 km (Sites 24 & 25; Junction Marillana & Yandicoogina)
Aquatic Ecosystems of the Upper Fortescue River Catchment	Streamtec (2004)	Water Quality, Macroinvertebrates & Fish	11 km (Weeli Wolli Spring)
Pilbara Biological Survey	Pinder *et. al.* (2010)	Aquatic Flora, Zooplankton & Macroinvertebrates	11 km (PBS site PSW026 at Weeli Wolli Spring)
Jinidi: Baseline Aquatic Surveys at Weeli Wolli Creek	WRM (2013a)	Water Quality, Habitat, Zooplankton, Hyporheic Fauna, Macroinvertebrates & Fish	11 km (Weeli Wolli Spring, WWS5)
Lake Robinson Aquatic Invertebrate Fauna and Water Quality Surveys	WRM (2013b)	Water Quality, Zooplankton & Macroinvertebrates	23 km (WRM site LR3)
Yandi: Marillana Creek Aquatic Fauna Survey	WRM (2018)	Water Quality, Habitat, Zooplankton, Hyporheic Fauna, Macroinvertebrates & Fish	4.5 km (MC7)
Yandi Aquatic Fauna Survey	WRM (2015)	Water Quality, Habitat, Zooplankton, Hyporheic Fauna, Macroinvertebrates & Fish	4 km (MC9)

3.2 Field survey

3.2.1 Survey team

The field surveys were conducted by Biologic aquatic ecologists Jessica Delaney, Kim Nguyen, Syngeon Rodman and Alex Riemer; all with extensive experience undertaking aquatic ecosystem surveys the Pilbara. Assistance in the field was also provided by Suzi Wild (Principal Biodiversity, BHP) during the dry season 2019 survey.

Fauna sampling for this survey was conducted under DBCA Fauna Taking (Biological Assessment Regulation 27) Licence BA27000020 (dry season sampling) and BA27000223 (wet season sampling), and DPIRD Instrument of Exemption to the *Fish Resources Management Act 1994 Section 7 (2)* number: 3266, all issued to Jessica Delaney. Flora was collected under DBCA Flora Taking (Biological Assessment) Licence FB62000095, issued to Jessica Delaney.

Macroinvertebrate specimens were identified in-house by Alex Riemer, Kim Nguyen and Syngeon Rodman, with assistance from Jane McRae (Bennelongia Environmental Consultants) for specific groups, such as Cladocera, Copepoda and Ostracoda specimens from hyporheic samples. Flora samples (submerged and emergent macrophytes) were identified by Biologic's Flora Team, including Clinton van den Bergh, Samuel Coultas and Emily Eakin-Busher, in conjunction with Syngeon Rodman and Alex Riemer. Zooplankton samples were processed and identified by Dr Robert Walsh (Australian Water Life).

3.2.2 Survey timing and weather

The field survey comprised two phases. The dry season survey (Phase 1; hereafter referred to as dry-19) was undertaken between the 24th and 29th of October 2019, and the wet season survey (Phase 2; wet-20) between the 2nd and 6th of April 2020.

The dry-19 survey was undertaken at a time of above average ambient temperature. Maximum daytime temperatures over the dry season survey averaged 38.1 °C, in comparison to the long-term average for October of 35.3°C. The October 2019 survey followed a considerably dry period, with below average rainfall in all preceding months since May 2019 (Figure 3.2). This followed on from several consecutive wet seasons of below average rainfall. The dry conditions likely influenced the hydrology of local creeks in the vicinity of Yandicoogina Creek and led to reductions in surface water levels, with some pools receding more than would be typical for a Pilbara dry season by the time of the dry-19 survey.

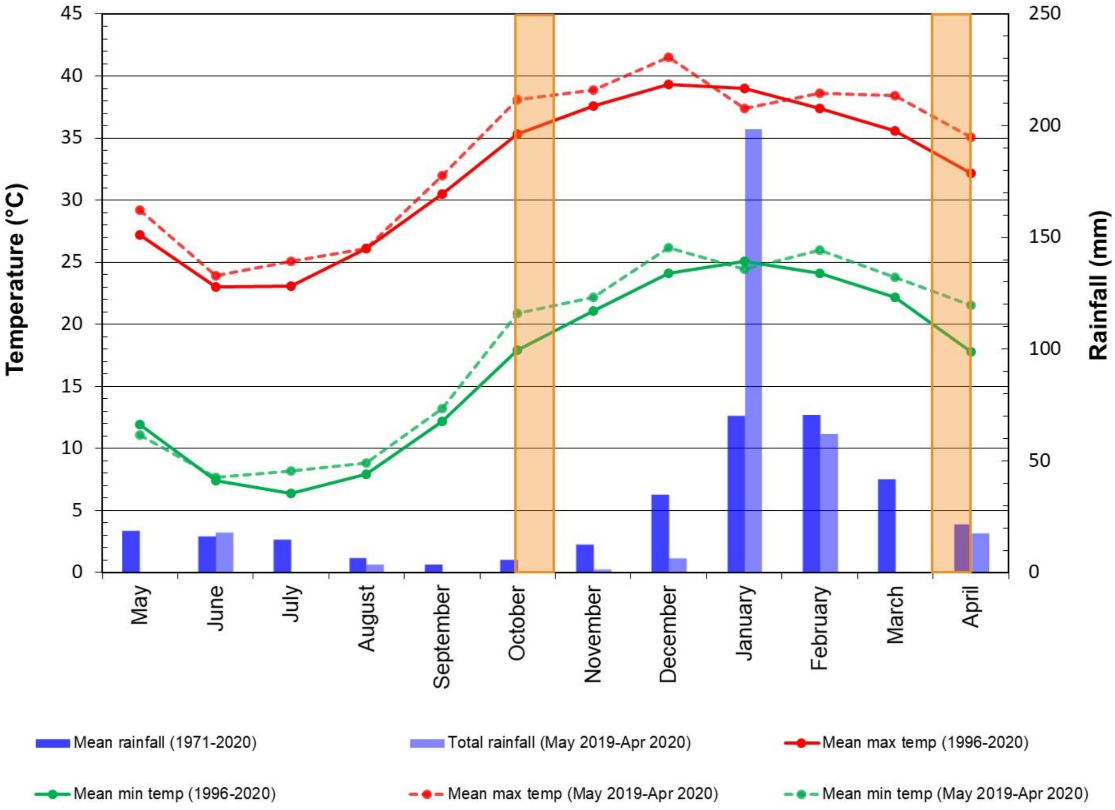


Figure 3.2: Total rainfall and long-term average monthly rainfall (mm) recorded from the Newman BoM gauging station in the year preceding the Yandicoogina Creek aquatic surveys. Orange bars indicate dry and wet season survey timing.

The wet season survey was also undertaken at a time of above average ambient temperature. Average maximum temperature was 2.9 °C hotter than the long-term average. However, the wet season survey came after a period of high rainfall (Figure 3.2). The 2019/20 wet season saw considerably high rainfall across the East Pilbara. Ex-Tropical Cyclone Blake produced heavy rainfall in early January 2020, with over 198 mm of rain recorded at the Newman GS that month (Figure 3.2). This compares to the long-term January average of 69.8 mm (Figure 3.2). Then in February, ex-Tropical Cyclone Damien brought more rainfall to the region. The high rainfall in January and February 2020 led to flooding in many creeks and river systems in the East Pilbara, including the nearby Marillana and Weeli Wolli creeks. Pools were flushed and filled up, and in many areas surface flows were still present at the time of the April 2020 survey.

No streamflow stations exist within the Study Area. The closest Department of Water and Environmental Regulation (DWER) streamflow gauging stations are located at Weeli Wolli Creek (Tarina, station number 708014, 8.5 km south-east of the Study Area), and Marillana Creek (Flat Rocks, station number 708001, 18 km north-west of the Study Area) (DWER, 2020). Average annual streamflow at Flat Rocks is 420 mm.

Streamflow in the Pilbara occurs as a direct response to rainfall. This is evident by the high flows experienced at the Flat Rocks gauging station in January and February 2020 (Figure 3.3), when cyclonic activity brought heavy rain to the area. The result was extensive flooding, with total streamflow for the month of January being 5,818 ML, in comparison to the long-term average of only 232 ML (Figure 3.3).

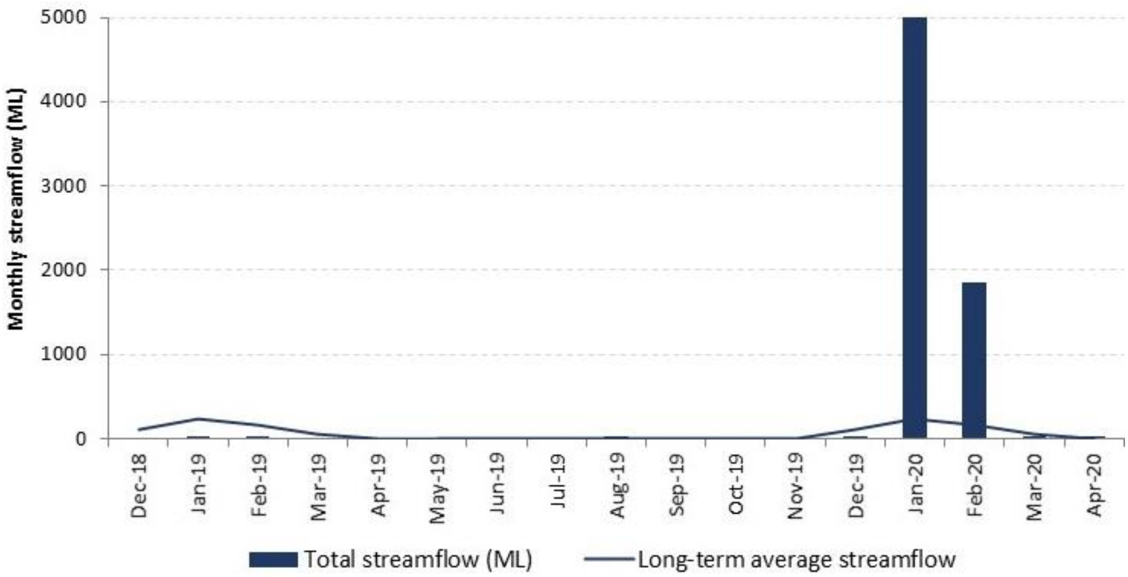


Figure 3.3: Monthly streamflow data for the DWER Flat Rocks Station on Marillana Creek, including monthly totals between Dec-18 and April-20 and long-term averages (1969-current).

Figure 3.4 further illustrates the relationship between rainfall and streamflow, with high flows occurring during high rainfall years. Rainfall and flows have been considerably lower since 2000, in comparison to the previous 20 year period (Figure 3.4).

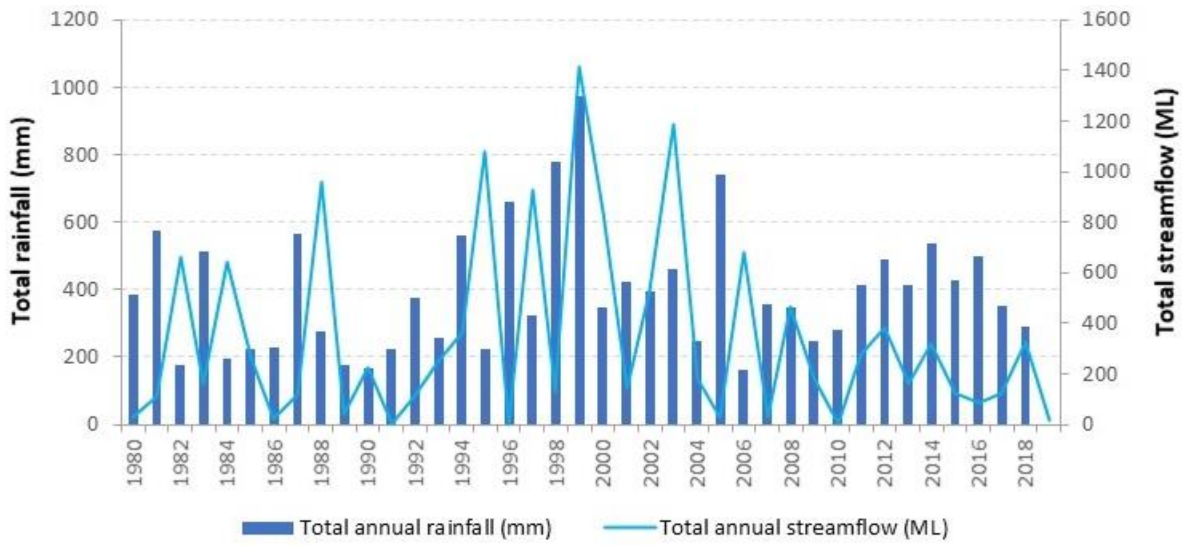


Figure 3.4: Annual rainfall (mm) and streamflow (ML) at the DWER Flat Rocks GS on Marillana Creek.

3.2.3 Sampling sites

The reconnaissance survey undertaken in May 2019 identified a series of perennial and semi-permanent pools within a three km stretch of Yandicoogina Creek. This section of creekline became the focus of this study and is hereafter referred to as the Survey Area. A total of eight sites were sampled in both seasons; four within the Survey Area, and four reference sites located outside the Study Area. Table 3.3 provides information on the sites sampled and their locations are shown in Figure 3.5.

The aim of reference site selection was to choose sites most similar to the potential GDE in Yandicoogina Creek, with respect to hydrology, persistence, morphology, and riparian vegetation, as well as being relatively close by and within the same climatic area. This is a difficult task in the Pilbara, a semi-arid region with few seeps and springs present, especially ones characterised by *Melaleuca argentea* and *Eucalyptus camaldulensis* riparian vegetation assemblages. As such, one reference site, Skull Springs, selected for inclusion, despite being located approximately 215 km to the north-east. While this site possibly experiences some differences in rainfall and streamflow to the Survey Area, it is more like Yandicoogina Creek in terms of morphology, hydrology, and vegetation than other sites located in closer proximity.

A brief description of Survey Area and reference sites is provided below:

<u>Within Survey Area</u>

- Yandicoogina Creek: Four sites (YC1, YC2, YC3 and YC4). YC1 through to YC3 are small seeps through *Typha domingensis* beds, and YC4 is the large pool located against the cliff face.

Table 3.3: Site locations, indicating site type and sampling effort. NB: D refers to dry season sampling (dry-19) and W refers to wet season sampling (wet-20). WQ = water quality, Zoop = zooplankton, Macro = macroinvertebrates and Hypo = hyporheic fauna.

					Sampling undertaken													
					Habitat		WQ		Flora		Zoop		Macro		Hypo		Fish	
Area	**Site**	**Latitude**	**Longitude**	**Type**	**D**	**W**	**D**	**W**	**D**	**W**	**D**	**W**	**D**	**W**	**D**	**W**	**D**	**W**
	YC1	-22.8282	119.1499		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	×	✓	-	-
	YC2	-22.8275	119.1510		✓	✓	✓	✓	✓	✓	×	×	✓	✓	✓	✓	-	-
	YC3	-22.8246	119.1637		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	-
	YC4	-22.8258	119.1628		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Yandicoogina Creek	YC5H	-22.8245	119.1638	Within Survey Area												✓		
	YC6H	-22.8257	119.1626													✓		
	YC7H	-22.8254	119.1602													✓		
	YC8H	-22.8255	119.1593													✓		
	YC9H	-22.8256	119.1582													✓		
Weeli Wolli Creek	WWS	-22.9181	119.1994		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	BENS*	-23.0558	119.1509	Reference	✓	*	✓	*	✓	*	✓	*	✓	*	✓	*	✓	*
	WM^	-23.1098	119.1278		^	✓	^	✓	^	✓	^	✓	^	✓	^	✓		✓
Munjina Spring	MUNJS	-22.5373	118.7046	Reference	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Davis River	SS	-21.8600	121.0114	Reference	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
			Total no. of samples		**8**	**8**	**8**	**8**	**8**	**8**	**7**	**7**	**8**	**8**	**7**	**13**	**5**	**5**

*sampled in the dry-19 only.

^sampled in the wet-20 only.

-Although fish sampling could not be undertaken at YC1, YC2 and YC3 (due to the small pool size, shallow depths and abundance of *Typha* instream) observations were made of the fish present at the time of sampling.

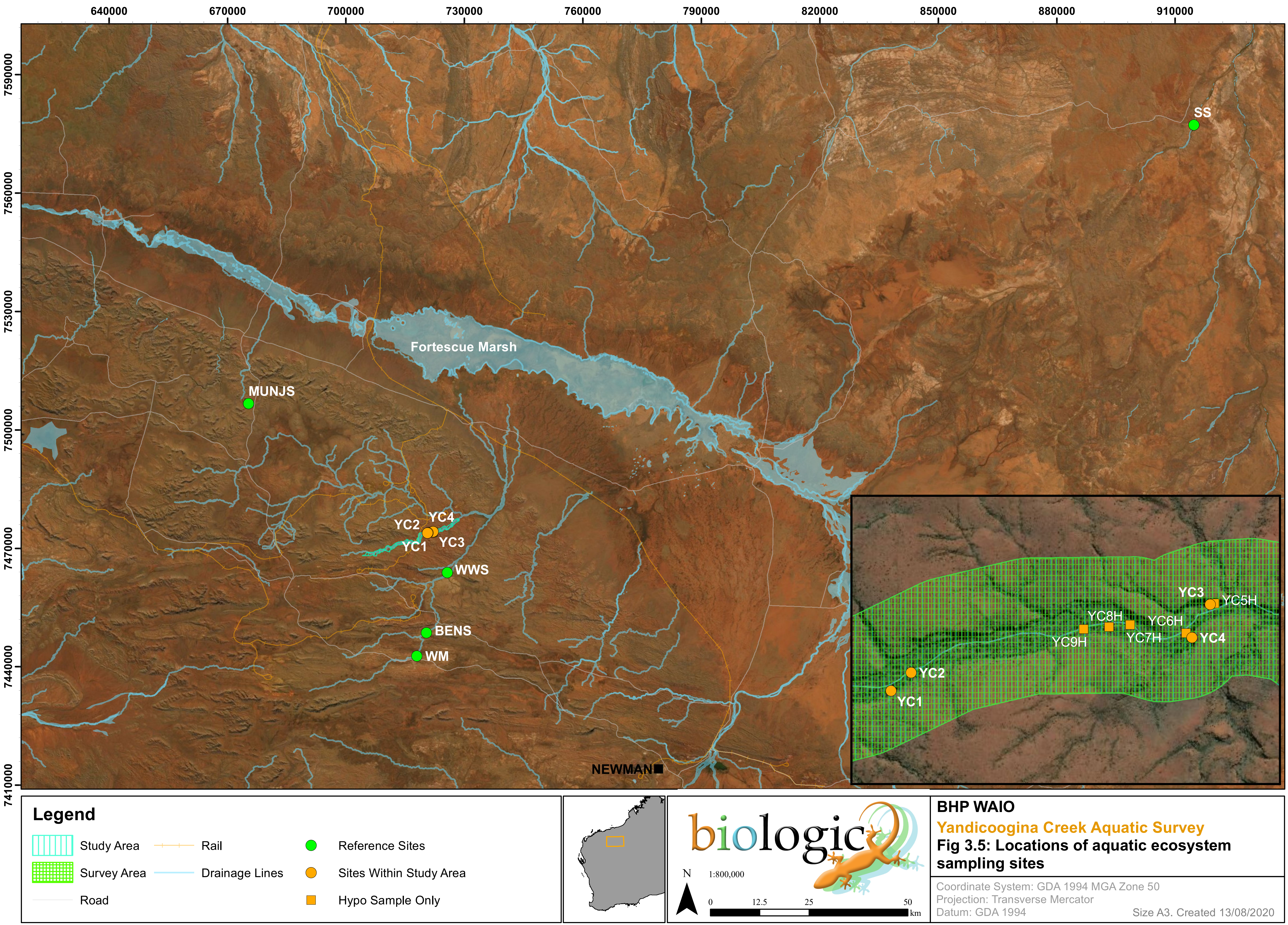


Fig 3.5: Locations of aquatic ecosystem sampling sites

Reference Sites

- Munjina Spring (MUNJS): a spring site located on Munjina Creek, within the Priority 2 Priority Ecological Community (PEC): *Riparian flora and plant communities of springs and river pools with high water permanence of the Pilbara*.
- Weeli Wolli Spring (WWS): a spring site on Weeli Wolli Creek, within the Weeli Wolli Spring Priority 1 PEC. While this site is currently impacted by dewatering and discharge from Rio Tinto's Hope Downs 1 mine, the aquatic fauna remains representative of the historic faunal community and occurs within a permanently flowing reach.
- Ben's Oasis (BENS): a spring site on Weeli Wolli Creek which represents a second occurrence of the Weeli Wolli Spring Priority 1 PEC. This site has been impacted in recent years by fire and cattle. BENS was sampled in the dry-19 only due to tenure access issues in the wet season.
- Wanna Munna (WM): semi-permanent creek pool on Weeli Wolli Creek, upstream of Ben's Oasis. This site was sampled in the wet-20 as a replacement for BENS which could not be accessed due to tenure restrictions.
- Skull Spring (SS): spring site on the Davis River (see Table 3.3 and Figure 3.5).

In the wet season, additional hyporheic samples were collected within the Survey Area to gain a better understanding of the distribution of some of the stygal species collected in the dry-19. These sites were YC5H, YC6H, YC7H, YC8H and YC9H (Figure 3.5).

3.2.4 Water quality

Water quality variables were recorded *in situ* from each site with a portable YSI Pro Plus multimeter. *In situ* variables included pH, redox potential (redox), electrical conductivity (EC), dissolved oxygen (DO), and water temperature. Undisturbed water samples were taken for laboratory analyses of ionic composition, nutrients, dissolved metals and turbidity. All water quality analyses were undertaken by ALS, a NATA accredited chemical analysis laboratory.

Water quality variables measured included:

- *In situ* – pH, DO (% and mg/L), EC (µS/cm), water temperature (°C) and redox (mV);
- Ionic composition - Ca, K, Mg, Na, HCO_3, Cl, SO_4, CO_3, alkalinity and hardness (mg/L);
- Water clarity – turbidity (NTU) and total suspended solids (TSS);
- Nutrients – N_NO_2, N_NO_3, N_NOx, N_NH_3, total N and total P (mg/L); and
- Dissolved metals – Al, As, B, Ba, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, S, Se, U, V and Zn (mg/L).

Samples collected for dissolved metals were filtered through 0.45 µm Millipore nitrocellulose filters in the field (Plate 3.1). Nutrient samples were not filtered as ALS filters all nutrient samples in the laboratory as part of their analytical methods. Following best practice and to minimise any potential for contamination, all water samples were collected using clean Nalgene sample bottles, and clean/new filters and syringes (Ahlers *et al.*, 1990; Batley, 1989; Madrid & Zayas, 2007). All water quality sampling equipment was stored in polyethylene bags, and samplers wore polyethylene gloves whilst sampling water quality.

Plate 3.1: Filtering dissolved metal samples at WWS (photo by Biologic ©).

All water samples were kept on ice in an esky whilst in the field, and either refrigerated (ions, dissolved metals, nutrients, general water), or frozen (total nutrients) as soon as possible for subsequent transport to the ALS laboratory.

3.2.5 Habitat

Habitat characteristics were recorded at each site to provide information on the variability of aquatic habitat present, and to assist in explaining patterns in aquatic faunal assemblages. Details of in-stream habitat and sediment characteristics were recorded by the same team member for all sites to reduce the potential for habitat differences related to subjective recordings by different personnel. Habitat characteristics recorded included percent cover by inorganic sediment, submerged macrophyte, floating macrophyte, emergent macrophyte, algae, large woody debris (LWD), detritus, roots, and trailing vegetation. Details of substrate composition included percent cover by bedrock, boulders, cobbles, pebbles, gravel, sand, silt, and clay.

3.2.6 Wetland flora

Macrophytes are important structural and biological components of lowland streams, providing aquatic fauna with habitat, breeding sites, food and cover from predators. Therefore, submerged macrophytes and emergent riparian vegetation from the families Cyperaceae (sedges) and Restionaceae (rushes) were collected from each site, where present. Submerged macrophytes were hand collected and placed in sample containers and assigned a unique number. Sufficient water from the waterbody was included in the sample container to ensure the collected material did not dry out or degrade. Roots, stem and flowering/fruiting bodies from emergent and riparian sedges and rushes were hand collected, ensuring sufficient material to allow confident identification. The emergent samples were assigned a unique number and pressed in the field. All specimens collected were processed as per WA Herbarium guidelines and identified in the Biologic laboratory.

3.2.7 Zooplankton (microinvertebrate fauna)

Zooplankton are microscopic invertebrates living near the surface of a water body, and include micro-crustacea (ostracods, copepods and cladocera) and rotifers. They form a vital component of aquatic food webs, feeding upon phytoplankton, bacteria and detritus, and provide an important food source for higher invertebrate consumers and fish. They are generally poor swimmers, instead relying on surface flows for dispersal.

Zooplankton can be useful bioindicators of water quality, eutrophication, productivity and disturbance because their development and distribution are subject to both abiotic (temperature, salinity, stratification, presence of pollutants, water flow) and biotic parameters (limitation of food, predation and competition) (Ramchandra *et al.*, 2006). Many zooplankton species are known to be highly sensitive to a wide range of pollutants. The use of zooplankton assemblages as bioindicators is most effective in lentic and slow-flowing rivers, where they occur in abundance (ANZG, 2018). In fast-flowing river systems, densities may be greatly reduced due to dilution, or absent where high flows prevent populations from establishing.

Zooplankton samples were collected by gentle sweeping over an approximate 15 m distance with a 53 μm mesh pond net. Samples were preserved in 100% ethanol in the field and sent to Dr Robert Walsh (Zooplankton taxonomist; Australian Waterlife).

In the laboratory, microinvertebrate samples were sorted using a Greiner tray under a low power dissecting microscope. All micro-crustacea were removed from samples and identification made under a compound microscope, to the lowest possible level of taxonomy (genus or species). Rotifera were identified from a 1 ml aliquot taken from the sample, using a Sedgwick rafter counting tray on a compound microscope.

3.2.8 Hyporheos fauna

The hyporheic zone is an ecotone between the surface and groundwater, and provides a number of ecosystem services to both habitats, including mediating exchange processes, regulating water flows and transfer of nutrients, carbon, oxygen and nitrates, as well as the maintenance of biodiversity (Boulton, 2001; Dole-Olivier & Marmonier, 1992a; Edwards, 1998). Fauna utilising this habitat are also an ecotone between surface and groundwater, with representatives of both benthic epigean species and stygofauna. Benthic macroinvertebrates migrate vertically to exploit hyporheic habitats as a nursery to protect juveniles from predation (Bruno *et al.*, 2012; Jacobi & Cary, 1996), and during times of floods (Dole-Olivier & Marmonier, 1992b; Edwards, 1998; Palmer *et al.*, 1992), drought (Coe, 2001; Cooling & Boulton, 1993; Hose *et al.*, 2005), and disturbance in food supplies (Edwards, 1998). The hyporheic zone serves to enhance the resilience of the benthic community to disturbance and influence river recovery following perturbations. Hyporheos[2] fauna have been used worldwide as an indicator of ecosystem health, especially in ephemeral creeks, with reported responses to disturbances such as metal pollution and eutrophication (Boulton, 2014; Leigh *et al.*, 2013; Moldovan *et al.*, 2013; Pacioglu & Moldovan, 2016).

At each site, the hyporheic zone was sampled using the Karaman-Chappuis (karaman) method (Chappuis, 1942; Karaman, 1935). This involved digging a hole (approximately 20 cm deep, 40 cm diameter) in alluvial sediments adjacent to the water's edge. The hole was swept with a modified 110 µm mesh plankton net immediately once it had filled with water, after approximately 30 minutes, and then again at the completion of sampling at that site. Although Bou-Rouch (Bou, 1974) sampling has widely been used to sample the hyporheic zone, the karaman method has been found to be more effective, with a greater diversity of taxa collected (Canton & Chadwick, 2000; Strayer & Bannon-O'Donnell, 1988).

Hyporheic samples were preserved in 100% ethanol in the field and returned to the Biologic laboratory for processing. Hyporheos fauna present were removed by sorting under a low power dissecting microscope. Specimens were identified in-house, or sent to appropriate taxonomic experts for identification, where necessary (i.e. Jane McRae for micro-crustacea).

3.2.9 Macroinvertebrates

Aquatic macroinvertebrates are invertebrates (animals without a backbone) that can be seen with the naked eye. They are used worldwide as indicators of ecosystem health for a number of reasons: they are ubiquitous; relatively easy to collect; have high species diversity and varying sensitivity to environmental disturbances; have relatively long life cycles; and are continuously exposed to environmental conditions and constituents of the surface water they inhabit (Bressler *et al.*, 2006; Cain *et al.*, 1992; Carew *et al.*, 2007; Hodkinson & Jackson, 2005). In Australia, the inherent value in using aquatic macroinvertebrates as key biological indicators is evidenced by their inclusion in river health initiatives across the country, including the Monitoring River Health Initiative, the Australian River Assessment System (AusRivAS), and the Framework for the Assessment of River and Wetland Health, to name a few.

Macroinvertebrate sampling was conducted with a 250 µm mesh D-net to selectively collect the macroinvertebrate fauna. At each site, sampling was undertaken across as many habitats as possible, including open water, macrophyte beds, large woody debris (LWD), leaf litter and edge habitat. The kick-sweep method was used in open areas, riffles and along edge habitat, whereby the sediments were disturbed (kicked) and the water column immediately swept with the dip net throughout the disturbed area. Each sample was washed through a 250 µm sieve to remove fine sediment, with leaf litter and other coarse debris being removed by (Plate 3.2). The net was thoroughly cleaned between sites to avoid cross contamination. Samples were preserved in 100% ethanol in the field (equivalent to 70% ethanol including the

[2] Fauna residing in the hyporheic zone with intent. Surface water species utilising the zone for protection against perturbations in the river environment and obligate groundwater species, are collectively known as hyporheos fauna (Brunke & Gonser, 1997).

macroinvertebrate sample) and transported to the Biologic laboratory for processing. Sorting was conducted under a low power dissecting microscope. Specimens were identified to the lowest possible level (genus or species level) and enumerated to log_{10} scale abundance classes (*i.e.* 1 = 1 individual, 2 = 2 - 10 individuals, 3 = 11 - 100 individuals, 4 = 101-1000 individuals, 5 = >1000). All macroinvertebrate groups were identified using in-house expertise; however, external taxonomists were used when required (i.e. some Chironominae, *Oecetis*, *Hydrochus*, and Paramelitids by Jane McRae, Bennelongia).

Plate 3.2: Sieving a macroinvertebrate sample at MUNJS.

3.2.10 Fish

Fish sampling included a variety of methods to collect as many species and individuals as possible. Methods included light-weight fine mesh gill nets (10 m net, with a 2 m drop, using 10 mm, 13 mm, 19 mm and 25 mm stretched mesh) set across the creek/pool (Plate 3.3), seine netting (10 m net, with a 2 m drop and 6 mm mesh) and direct observation. The seine was deployed in shallow areas with little vegetation or large woody debris, and up to three seine hauls were undertaken per site.

Fish were identified in the field and standard length (SL[3]) measured. All fish were released alive to the site where they were collected.

It was anticipated that electrofishing would also be conducted but conditions within Yandicoogina Creek precluded its use, including thick vegetation, high cover of emergent macrophytes (*Typha domingensis*), submerged macrophytes/algae, and difficult bank access at some sites. Due to this, access within Yandicoogina Creek was via helicopter, which precluded use of the electrofisher due to aircraft weight restrictions. As the electrofisher was

[3] Standard length (SL) - measured from the tip of the snout to the posterior end of the last vertebra or to the posterior end of the midlateral portion of the hypural plate (i.e. this measurement excludes the length of the caudal fin).

not able to be used within Yandicoogina Creek, it was also dropped from the sampling program for reference sites to ensure comparable methods across the study.

Plate 3.3: Light-weight fine mesh gill nets set across the channel at SS.

3.2.11 Other aquatic fauna

Any other vertebrate fauna observed or caught during aquatic surveys were also recorded for each site. In the case of crayfish, the carapace length (CL) of each individual was measured.

3.3 Data analysis

3.3.1 Water quality

Water quality data were compared against the default ANZECC & ARMCANZ (2000) water quality guideline values (GVs) for the protection of aquatic ecosystems in the tropical north-west of Western Australia (see Appendix B for default values). For this purpose, sites sampled in the current study were classified as lowland rivers.

The primary objective of the guidelines is to "*provide authoritative guidance on the management of water quality in Australia and New Zealand …. and includes setting water quality and sediment quality objectives designed to sustain current, or likely future, community values for natural and semi-natural water resources*" (ANZG, 2018). Default GVs (DGVs) are provided for a range of parameters designed to protect aquatic systems at a low level of risk but are not designed as pass or fail compliance criteria. Rather, exceedances of DGVs are triggers which can be used to inform managers and regulators that changes in water quality are occurring and may need to be investigated.

Differing levels of protection are provided within the guidelines, depending on the condition of the ecosystem in question;

- High conservation/ecological value systems - where the goal is to maintain biodiversity with no (or little) change to ambient condition. 99% species protection GVs for toxicants apply[4].
- Slightly to moderately disturbed systems - where aquatic biodiversity has already been adversely impacted to a small but measurable degree by human activity. The aquatic ecosystem remains in a healthy condition and ecological integrity is largely retained. The aim is to maintain current biodiversity and ecological function. 95% species protection GVs for toxicants apply.
- Highly disturbed systems - are measurably degraded and of lower ecological value. Guideline aims for these systems may be varied and more flexible, ranging from maintenance of the current yet modified ecosystem that supports management goals, to continual improvement in ecosystem condition. For toxicant, the 90% or 80% species protection GVs may be applied.

All sites sampled in the current study show evidence of varying levels of impact from pastoral use or mining activity and were classified as slightly to moderately disturbed systems and the 95% GVs applied.

Two GVs relating to nutrient concentrations are provided for within the default ANZECC & ARMCANZ (2000) guidelines:

- a toxicity GV above which direct toxic effects to aquatic biota can be expected (ammonia, nitrate); and
- a eutrophication GV, above which nutrient concentrations are such that algal blooms and eutrophic conditions can be expected (nitrogen oxides, total nitrogen, total phosphorus).

The guidelines have recently been updated to reflect a better understanding of physical and chemical stressors, the availability of additional monitoring data, the addition of recent toxicity data in GVs for a number of toxicants, a weight of evidence approach, and the fact that water quality varies greatly across ecosystem types and regions (ANZG, 2018). The guidelines are now presented via an interactive online platform to improve usability and facilitate updates as new information becomes available. While information relating to management frameworks, background to derivation of GVs, and approaches for sampling design and monitoring programs are available online, GVs are not currently presented for all ecoregions. The Study Area falls within the Indian Ocean Inland Waters region, data for which is not currently available online. As such, data from the current study were compared against the default ANZECC & ARMCANZ (2000) GVs for systems within the tropical north-west of Western Australia.

[4] For toxicants, default GVs were derived using the species sensitivity distribution (SSD) approach; methods are described in ANZECC & ARMCANZ (2000). Refer to (Warne *et al.*, 2018) for updated GVs. Where the SSD approach could not be used, the less preferred 'assessment-factor approach' was used, following methods detailed in ANZECC & ARCMANZ (2000). For toxicants, GVs relate to differing levels of species protection, i.e. the 99% GVs protect 99% of species, the 95% GVs protect 95% of species present, and so on.

3.3.2 Invertebrates

All taxa recorded from hyporheic samples were classified based on categories of Boulton (2001):

- stygobite – obligate groundwater species, with special adaptations to survive such conditions;
- permanent hyporheos stygophiles - epigean species (living on or near the surface of the ground) which can occur in both surface- and groundwaters, but is a permanent inhabitant of the hyporheos;
- occasional hyporheos stygophiles – use the hyporheic zone seasonally or during early life history stages; and
- stygoxene (species that appear rarely and apparently at random in groundwater habitats, there by accident or seeking refuge during spates or drought; not specialised for groundwater habitat).

Additionally, Biologic propose one further hyporheic classification:

- possible hyporheos stygophile – likely to be hyporheos fauna, but due to taxonomic resolution or a lack of ecological information we are unable to say this with certainty.

All invertebrates collected were compared against appropriate threatened and priority species lists including the *Biodiversity Conservation Act 2016* (BC Act), the *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act), the International Union for Conservation of Nature (IUCN), Australian Society for Fish Biology Conservation List 2016, and Priority Fauna recognised by the DBCA (see Appendix A). In addition, species were assigned to one of the following conservation categories based on species distributions:

- Cosmopolitan – species is found widely across the world;
- Australasian – species is found across Australia, New Guinea and neighbouring islands, including those of Indonesia;
- Australian endemic – species is only found in Australia;
- Northern Australia – species with distributions across the northern, tropical regions of the Australian continent;
- North Western Australia – found across northern W.A., including the Pilbara and Kimberley regions;
- Western Australian endemic – only known from W.A. (is restricted to, but is widely distributed across the state);
- Pilbara endemic - restricted to the Pilbara region of Western Australia;
- Short range endemic (SRE) – an SRE is a species occupying an area of less than 10,000 km^2 (Harvey, 2002). Such species have traits which make them vulnerable to disturbance and changes in habitat, and affords them high conservation value; and
- Indeterminate distribution – taxa could not be assigned to one of the above, as there is currently insufficient knowledge on either its distribution or taxonomy to assess its level of endemism.

Two-way analysis of variance (ANOVA) was undertaken in SPSS v21 to compare richness (zooplankton and macroinvertebrate richness) between creeks (the Survey Area vs nearby creeks, as sampled during previous surveys outlined in Section 4.1) and season (dry vs wet). A Levene's test was undertaken prior to analysis to analysis to ensure assumptions of the ANOVA test were met (i.e. to test for equality of variances).

Macroinvertebrate data were also compared against previous surveys, including the PBS and WRM studies outlined in section 3.1.2. To undertake this comparison, the dataset had to be amalgamated, and taxonomy aligned, to ensure any differences in taxonomic knowledge between samplers and years was appropriately accounted for. This meant that the level of resolution was brought back to align with WRM (i.e. specifically Chironominae, *Hydrochus* and *Oecetis*). Assemblage structure was analysed using multivariate techniques in PRIMER v7 (Clarke & Gorley, 2015), including cluster analysis and ordination. Ordination was by non-metric Multi-Dimensional Scaling (nMDS), which, unlike other ordination techniques uses rank orders, and therefore can accommodate a variety of different types of data. Ordination was based on the Bray-Curtis similarity matrix (Bray & Curtis, 1957). To test for significant differences in *a priori* groups (i.e. creeks and seasons), two-way ANOSIM was undertaken.

3.3.3 Fish

Length-frequency analysis was undertaken for each fish species recorded, whereby each species was classified into four age classes based on body size (SL mm). Age classes were determined from the literature (Allen *et al.*, 2002a; Puckridge & Walker, 1990) (Table 3.4).

Table 3.4: Standard lengths used for each age class for each species recorded.

	Standard Length (mm)		
Age class	**Western rainbowfish**	**Spangled perch**	**Pilbara tandan**
New recruit	< 30	< 30	< 30
Juvenile	31-40	31-50	31-70
Sub-adult	41-50	51-70	71-90
Adult	>50	>70	>90

4 RESULTS

4.1 Database searches

The database searches identified 322 records of aquatic fauna taxa and waterbirds (Table 4.1). The total included 279 species of invertebrate and 36 species of vertebrate. Insects and crustaceans accounted for over 75% of all taxa previously recorded. These records provide context for the aquatic ecosystems of the Yandicoogina Creek Study Area.

Table 4.1: Aquatic fauna recorded within 50 km of the Study Area.

Type	Taxonomic Group	Common Name	Number of Taxa
Invertebrate	Annelida	Segmented Worms	15
Invertebrate	Nematoda	Roundworms	5
Invertebrate	Platyhelminthes	Flatworms	1
Invertebrate	Arachnida	Mites	21
Invertebrate	Insecta	Insects	118
Invertebrate	Crustacea	Crustaceans	90
Invertebrate	Mollusca	Molluscs	5
Invertebrate	Cnidaria	Hydras	1
Invertebrate	Rotifera	Rotifers	22
Vertebrate	Actinopterygii	Fish	4
Vertebrate	Amphibia	Frogs	9
Vertebrate	Aves	Waterbirds	31
		Total	**322**

Of the taxa recorded within 50 km of the Study Area, nine species of waterbird are considered to be of conservation significance (Table 4.2). The waterbirds *Calidris ferruginea* (curlew sandpiper) and *Rostratula australis* (Australian painted snipe), are listed as Critically Endangered (CR) and Endangered (EN) on the IUCN Redlist of Threatened Species, respectively. These waterbirds are known to occur in temporary freshwater wetlands. An additional seven waterbirds are listed as migratory under both the State BC Act and Federal EPBC Act. These include *Charadrius veredus* (Oriental plover), *Onychoprion anaethetus* (bridled tern), *Tringa glareola* (wood sandpiper), *Tringa nebularia* (common greenshank), *Actisis hypoleucos* (common sandpiper), *Calidris acuminata* (sharp-tailed sandpiper) and *Calidris melanotos* (pectoral sandpiper). All migratory species are matters of national environmental significance (MNES) under the EPBC Act.

Table 4.2: Endemic and conservation significant aquatic fauna recorded within 50 km of the Study Area

Taxonomic Group	Family	Taxa	Conservation Status			Endemic
			WA	EPBC	IUCN	
Crustacea	Paramelitidae	*Maarrka weeliwolli*				Y
Crustacea	Paramelitidae	Paramelitidae cf. sp. 9 (PSS)				Y
Annelida	Naididae	*Ainudrilus* sp. WA26 (PSS)				Y

Taxonomic Group	Family	Taxa	Conservation Status			Endemic
			WA	EPBC	IUCN	
Crustacea	Candonidae	*Candonopsis williami* (PSS)				Y
Crustacea	Candonidae	*Deminutiocandona mica*				Y
Crustacea	Candonidae	*Meridiescandona* cf. *facies* (PSS)				Y
Crustacea	Candonidae	*Meridiescandona facies* (PSS)				Y
Crustacea	Candonidae	*Meridiescandona 'marillaneae'* (PSS)				Y
Crustacea	Candonidae	*Meridiescandona* sp. 1 (PSS)				Y
Crustacea	Candonidae	*Neocandona* sp. 1 (PSS)				Y
Crustacea	Candonidae	*Notacandona boultoni*				Y
Crustacea	Candonidae	*Notacandona* cf. *modesta* (PSS)				Y
Crustacea	Candonidae	*Notacandona modesta*				Y
Aves	Charadriidae	*Charadrius veredus* (Oriental plover)	MI	MI		
Aves	Laridae	*Onychoprion anaethetus* (bridled tern)	MI	MI		
Aves	Rostratulidae	*Rostratula australis* (Australian painted-snipe)	EN	EN	EN	
Aves	Scolopacidae	*Tringa glareola* (wood sandpiper)	MI	MI		
Aves	Scolopacidae	*Tringa nebularia* (common greenshank)	MI	MI		
Aves	Scolopacidae	*Actisis hypoleucos* (common sandpiper)	MI	MI		
Aves	Scolopacidae	*Calidris acuminata* (sharp-tailed sandpiper)	MI	MI		
Aves	Scolopacidae	*Calidris ferruginea* (curlew sandpiper)	CR	CR & MI	NT	
Aves	Scolopacidae	*Calidris melanotos* (pectoral sandpiper)	MI	MI		

NT= Near Threatened, EN = Endangered, CR = Critically Endangered, MI = Migratory species (see Appendix A for descriptions of conservation codes).

The database search also identified thirteen invertebrates that were endemic to the search area (Table 4.2). These included the stygal amphipods *Maarrka weeliwolli* and Paramelitidae cf. sp. 9. The former is an SRE known only from groundwater and hyporheic zones along Marillana and Weeli Wolli creeks. The latter is a similar morphotype to a species first recorded during the Pilbara Stygofauna Survey. This may relate to one of the Paramelitid species now known from Weeli Wolli and Marillana creeks (i.e. Paramelitidae Genus 2 sp. B02 or Paramelitidae Genus 2 sp. B03); however, without access to the specimens this remains unclear. Other invertebrates restricted to the search area included several stygal ostracods which appear to be restricted to the Weeli Wolli Creek and Marillana area (i.e. *Meridiescandona* cf. *facies Meridiescandona 'marillaneae'*, *Meridiescandona* sp. 1, *Notacandona boultoni*, *Notacandona modesta*, *Notacandona* cf. *modesta*). *Meridiescandona facies* is also known from the central and eastern Fortescue.

Table 4.3: Information relating to sites sampled, sampling occasions and ecological components surveyed during previous aquatic surveys undertaken within 50 km of the Study Area.

Reference	Sites sampled	Sampling occasions	Components sampled
Masini (1988)	23 (WW Creek d/s Marillana Ck confluence) 24 & 25 (Yandicoogina Ck @ confluence with Marillana Ck)	Wet-1983	Water quality Habitat assessment Fringing vegetation Aquatic macrophytes Phytoplankton Fish Waterbirds
Streamtec (2004)	Weeli Wolli Spring (d/s of the 1st crossing) Flat Rocks Wanna Munna Eagle Rock	Dry-2001 Wet-2002 Wet-2003 Dry-2003	Water quality Macroinvertebrates Fish
Pinder et al. (2010)	Weeli Wolli Spring (whirlwind pool, u/s of the 1st crossing)	Dry-2003 Wet-2005	Water quality Zooplankton Macroinvertebrates
WRM (2013a)	JIN1, JIN1A, JIN2, JIN3 Six sites on a tributary of Weeli Wolli Creek Bens Oasis WWUS, WWUS1 Eight sites within Weeli Wolli Spring (inc. the Streamtec site and DBCA site) Three Weeli Wolli Creek pools upstream of all mining (WM, WMC, WMU) Coondiner Creek (ER and ERP)	Wet-2011 Dry-2011 Wet-2012	Water quality Habitat assessment Zooplankton Hyporheos fauna Macroinvertebrates Fish
WRM (2013b)	Three sites within Lake Robinson (LR1, LR2 and LR3	Wet-2011 Wet-2012	Water quality Rehydrates Zooplankton Macroinvertebrates
WRM (2015)	Nine sites on Marillana Creek (MC1 to MC7, Flat Rocks and Flat Rock d/s) Bens Oasis Wunna Munna (WM) and Wunna Munna Up (WMU)	Wet-2014 Dry-2014	Water quality Habitat assessment Zooplankton Hyporheos fauna Macroinvertebrates

Reference	Sites sampled	Sampling occasions	Components sampled
			Fish
WRM (2018)	12 sites on Marillana Creek Bens Oasis Wunna Munna (WM) and Wunna Munna Up (WMU)	Wet-2017 Dry-2017	Water quality Habitat assessment Zooplankton Hyporheos fauna Macroinvertebrates Fish

4.2 Literature review

No previous aquatic sampling has been conducted within the Yandicoogina Creek Study Area; however, several studies have sampled sites nearby to varying degrees (i.e. Pinder *et al*., 2010; WRM, 2013a; WRM, 2013b; WRM, 2015; WRM 2018). Information relating to sites sampled and ecological components surveyed is provided in Table 4.3.

In the wet season of 1983, Masini (1988) sampled a total of 76 sites across the Pilbara, with the objective of producing an inventory of permanent and ephemeral inland surface waters in the region, and a means for establishing priorities for management and/or reservation. The survey included water quality (with a particular focus on nutrient status), habitat assessments, fringing vegetation, emergent and submerged macrophytes, phytoplankton, benthic microalgae, waterbirds and fish. Zooplankton samples were also collected from some sites, but not those in the vicinity of the Study Area (Masini, 1988). Three genera of Chlorophyta were recorded (green algae; *Desmidium* sp., *Oedogonium* sp. and *Spirogyra* sp.) from the three sites near the Study Area. Charophyta (stoneworts; Charales spp.) and Diatomaceae (diatoms) were also present (Masini, 1988). Riparian vegetation included *Eucalyptus camaldulensis*, *Melaleuca leucandendra* and *Melaleuca glomerata* overstorey, with sedges along the creeklines (*Cyperus ixiocarpus* and *Schoenoplectus litoralis*). Two species of native freshwater fish were recorded from the three sites, including western rainbowfish (*Melanotaenia australis*) and spangled perch (*Leiopotherapon unicolor*) (Masini, 1988). Birds observed at the three sites included the pacific black duck (*Anas superciliosa*), sacred kingfisher (*Halcyon sancta*) and little corella (*Cacatua sanguinea*). None of these are migratory birds or are listed for conservation significance.

As part of the PBS (Pinder *et al.* 2010), water quality and aquatic fauna (zooplankton and macroinvertebrates) were sampled at 100 sites across the Pilbara, between 2003 and 2006. Aquatic macrophytes and riparian flora were also sampled in conjunction with the aquatic fauna (Gibson *et al.*, 2015)[5]. The PBS included most wetland types from the region, such as wetlands, river pools, claypans, rock pools and springs. Overall, invertebrate community composition (relative richness of different species assemblages) was found to be associated with flow, estimated permanence, water chemistry, macrophytes and sediments (Pinder *et al*., 2010). Only one PBS site was sampled within 50 km of the Study Area; Weeli Wolli Spring (at whirlwind pool, upstream of the first crossing). A total of 47 zooplankton and 121 macroinvertebrate taxa were recorded from Weeli Wolli Spring, over two seasons. Of these taxa, two invertebrates are listed (the damselflies *Eurysticta coolawanyah* and *Austroagrion pindrina*) and two are of further scientific and conservation interest (the stygal amphipod *Chydaekata* sp. and the water mite *Gondwanabates* nr *bodivus*; Table 4.4).

The Pilbara pin damselfly, *Eurysticta coolawanyah* is currently listed as Vulnerable (IUCN 2020; see Appendix A for IUCN classification definitions). This listing was based on its collection from less than five locations. Although the listing was revised recently (2016), the revision did not

[5] Biologic have requested these data from the DBCA but have not yet received them. It is hoped they will be available for inclusion in the final report.

consider grey literature records (baseline surveys and impact assessments associated with mining and development in the region). Its extent of occurrence, based on a polygon around the known occupied areas (four locations listed in the IUCN listing), is 7,937 km² (Dow, 2019a); however, Bush *et al.* (2014, Table S2) provide an estimate of the current extent of suitable habitat as 298,177 km². Including the PBS and grey literature records (sampling programs undertaken by the authors and others), the species has now been recorded from numerous locations in the Pilbara, albeit in low numbers and with a disjunct distribution (Pinder *et al.* 2010, Jess Delaney, unpub. data).

The Pilbara billabong fly *Austroagrion pindrina* is also currently listed on the IUCN Redlist (2020) as Vulnerable. Little is known about this damselfly species, though it appears to inhabit inland waters such as permanent rivers and streams, including waterfalls, as well as freshwater marshes and pools. *A. pindrina* is endemic to the Pilbara region with an estimated extent of occurrence of 10, 755 km^2, which attributes to its Vulnerable status (Dow, 2017a). The IUCN assessment of this species in 2016 found less than ten records of this species; however, much like *E. coolawanyah*, this assessment has not included grey literature records or records from baseline surveys for developments. Pinder *et al*., (2010) recorded 67 occurrences of this species in the PBS. The IUCN assessment of *A. pindrina* was based on a precautionary approach (Dow, 2017a).

The stygal amphipod, *Chydaekata* sp. is an SRE known from a small number of systems in close proximity, including Marillana Creek, Weeli Wolli Creek, Coondiner Creek and Mindy Mindy Creek. In these systems it has been recorded from groundwater, the hyporheic zone, and surface waters (where there is vertical connection between the groundwater and surface water). Although other species of *Chydaekata* are known from the Pilbara, this particular undescribed species is known to be genetically distinct. The water mite *Gondwanabates* nr *bodivus* is known only from Weeli Wolli Spring and Skull Springs (Pinder *et al*., 2010).

Several aquatic survey reports undertaken for various BHP Projects have also been provided to Biologic for this review, including Streamtec (2004) and WRM (2013a, 2013b, 2015, 2018). Sampling by Streamtec (2004) included water quality, macroinvertebrates, and fish. A total of 12 sites were sampled, including four located within 50 km of the Yandicoogina Creek Study Area. In November 2003 (the only sampling occasion for which data was included in Streamtec 2004), 80 invertebrate taxa were recorded; however, a large number of invertebrates were only identified to a high level, i.e. water mites to Acarina spp., segmented worms to Oligochaeta spp., non-biting midges to Chironomidae, etc. In addition, the invertebrate list included some zooplankton taxa (micro-crustacea, although identified to Class only). No invertebrate taxa were considered to be of conservation significance (Streamtec, 2004). Three species of freshwater fish were recorded from the four sites near Yandicoogina Creek, including western rainbowfish, spangled perch and Hyrtl's tandan catfish (*Neosilurus hyrtlii*) (Streamtec, 2004). The latter has since been re-named as genetic analyses discovered Pilbara specimens are genetically distinct from other northern Australian populations of *N. hyrtlii* (Unmack, 2013).

WRM (2013a) undertook aquatic surveys within, and adjacent to, the Jinidi Project area on behalf of BHP on three occasions, the wet and dry seasons of 2011, and the wet of 2012. Up to 26 sites were sampled on each occasion. Aquatic surveys included water quality, habitat, zooplankton, hyporheos fauna, macroinvertebrates, and fish. A total of 141 zooplankton taxa and 281 macroinvertebrate taxa were recorded over the course of the study. Of these, a number were of conservation significance, including:

- Two rare rotifer species - *Lindia torulosa* and *Proales similis.*
- *Anthalona* n. sp. – a new species of Cladocera.
- Stygal SRE amphipods - *Chydaekata* sp., *Maarka weeliwolli*, Paramelitidae sp. B[6], and Paramelitidae sp. D[7].
- Stygal SRE isopod - *Pygolabis weeliwolli*.
- Stygal SRE mite - *Hesperomomonia humphreysi* (known only from Weeli Wolli and Marillana creeks).
- *Gondwanabates* sp. nov. – new species of water mite recorded from Weeli Wolli Spring
- A stygal water mite whose only known record appears to be from Weeli Wolli Spring – *Limnesia* sp.
- *Eurysticta coolawanyah* – Vulnerable IUCN Redlist.
- *Hemicordulia koomina* - Vulnerable IUCN Redlist (Table 4.4).

During the Jinidi baseline aquatic surveys, the same three freshwater fish species reported by Streamtec (2004) were also recorded from sites in the vicinity of the Yandicoogina Creek Study Area (WRM, 2013a).

Aquatic invertebrate surveys were undertaken in Lake Robinson, approximately 40 km south-west of the Study Area in the wet 2011 and wet 2012 seasons (WRM, 2013b). The lake was dry in 2011, and as such, sediments were collected and rehydrate/emergence trials conducted (WRM, 2013b). In the wet of 2012, aquatic surveys included water quality, habitat, zooplankton, and macroinvertebrates. Overall, a total of 32 zooplankton taxa and 28 macroinvertebrate taxa were recorded from Lake Robinson (WRM, 2013b). The taxa list included two species of rotifer which were new to science, *Centropyxis* n. sp. and *Difflugia* n. sp. (Table 4.4).

Aquatic fauna surveys have also been previously completed within BHP's Yandi tenement (WRM, 2015; WRM, 2018). In 2014, up to nine sites were sampled in both the wet and dry seasons (WRM, 2015). During these surveys, 113 zooplankton taxa and 212 macroinvertebrate taxa were recorded (WRM, 2015). A number of conservation significant invertebrate fauna were recorded, including; the stygal SRE amphipods *Chydaekata* sp., Paramelitidae sp. B and Paramelitidae sp. D; a locally restricted stygal ostracod known only from bores within the Yandi area and hyporheic zones along Marillana and Weeli Wolli Creeks *Gomphodella* n. sp. (BOS334); the Vulnerable (IUCN Redlist) Pilbara emerald dragonfly *Hemicordulia koomina*; the

---

[6] also referred to as Paramelitidae Genus 2 sp. B02 (Bennelongia morphotype).
[7] also referred to as Paramelitidae Genus 2 sp. B03 (Bennelongia morphotype).

Vulnerable (IUCN Redlist) Pilbara pin damselfly *Eurysticta coolawanyah*; and a new species of water mite *Stygolimnochares* nr *australica* (Table 4.4).

BHP's Yandi tenement was again sampled in the wet and dry seasons of 2017 (WRM, 2018). Up to 13 sites were sampled on each occasion and a total of 92 zooplankton taxa and 222 macroinvertebrate taxa were recorded (WRM, 2018). Conservation significant aquatic fauna recorded by WRM (2018) included:

- An undescribed species of rotifer *Lecane* 'bulloid' n. sp. – known only from Marillana Creek, Weeli Wolli Creek Kalgan Creek and Mindy Mindy Creek.
- Stygal SRE amphipods - *Chydaekata* sp., Paramelitidae sp. B, and Paramelitidae sp. D.
- Stygal SRE isopod - *Pygolabis weeliwolli*
- *Stygolimnochares* nr *australica* – known only from Marillana Creek and Koodaideri Spring.
- *Eurysticta coolawanyah* – Vulnerable IUCN Redlist.
- An uncommonly recorded Pilbara endemic beetle – *Laccobius billi* (Table 4.4).

Table 4.4: Results of previous aquatic surveys conducted in the vicinity of the Study Area.

Report Reference	Conservation Significant Aquatic Fauna Recorded	Sites Recorded
Pinder *et al.* (2010)	*Eurysticta coolawanyah* (IUCN Vulnerable) *Austroagrion pindrina* (IUCN Vulnerable) *Chydaekata* sp. (SRE) *Gondwanabates* nr *bodivus*	Weeli Wolli Spring
WRM (2013a)	*Anthalona* n. sp. (new species)	WWS1, WWUS1
	Chydaekata sp. (SRE)	Several Weeli Wolli Spring sites
	Maarka weeliwolli (SRE)	Several Weeli Wolli Spring sites
	Paramelitidae sp. B (SRE)	Several Weeli Wolli Spring sites
	Paramelitidae sp. D (SRE)	Several Weeli Wolli Spring sites
	Limnesia sp. (SRE)	JIN1A, JIN2, JIN3, WWTR3, WWUS1, BENS, several WW Spring sites
	Hesperomomonia humphreysi (SRE)	WWDEC
	Gondwanabates sp. nov. (new species)	WWS1
	Eurysticta coolawanyah (IUCN Vulnerable)	WWS5, WWST, WWDEC, ER
	Hemicordulia koomina (IUCN Vulnerable)	BENS, WWS1
WRM (2013b)	*Centropyxis* n. sp. (new species) *Difflugia* n. sp. (new species)	Lake Robinson
WRM (2015)	*Chydaekata* sp. (SRE)	MC5, MC6
	Paramelitidae sp. B (SRE)	MC5, MC6
	Paramelitidae sp. D (SRE)	MC5, MC6
	Gomphodella n. sp. (BOS334)	MC1, MC6
	Hemicordulia koomina (IUCN Vulnerable)	FR
	Eurysticta coolawanyah (IUCN Vulnerable)	FR, MC5, MC7
	Stygolimnochares nr *australica* (stygal, SRE)	MC6
WRM (2018)	*Lecane* 'bulloid' n. sp. (undescribed, locally restricted rotifer)	MC1-B, MC6, WM, WMC
	Chydaekata sp. (SRE)	MC1-B, MC7

Report Reference	Conservation Significant Aquatic Fauna Recorded	Sites Recorded
	Paramelitidae sp. B (SRE)	MC1, MC1-B, MC5, MC7,
	Paramelitidae sp. D (SRE)	MC7, MC8, MC9
	Pygolabis weeliwolli (SRE)	MC7
	Eurysticta coolawanyah (IUCN Vulnerable)	MC7, MC8
	Laccobius billi (uncommon)	MC2

It is noted here that many invertebrate taxa reported in these surveys are unable to be classified due to taxonomic limitations or impediments (such as damaged or immature specimens), as well as a general lack of reliable information regarding distributions. Records of morphotypes which could not be identified to species-level due to a lack of suitable keys or lack of taxonomy in the Pilbara for that group limits knowledge of occurrences outside of the PBS. This is the case for many taxa, in particular Diptera (true flies), water mites, some Trichoptera (caddisfly) genera (i.e. *Oecetis*) and Hydrochidae beetles (*Hydrochus*). It is likely that several endemic species exist within these groups, but remain undescribed, and subsequently lack distribution information.

4.3 Habitat Assessment

A summary of the overall habitat assessment is provided in (Table 4.5) and all raw data in Appendix C. Sites within the Survey Area occurred within vegetation comprising open to closed *Eucalyptus camaldulensis* and *Melaleuca argentea* woodland over *Acacia tumida* var. *pilbarensis* shrubland . *Cyperus vaginatus* and *Typha domingensis* sedgeland occurred along the waterline (Biologic, 2020a). Weeds were present throughout the Survey Area, though no other disturbances were apparent. There are currently no mining related impacts within the Survey Area. Riparian vegetation was in good condition, with a number of groundwater dependant flora taxa recorded.

While most sites were dominated by transmissive sediments such as gravel, pebbles and sand, clay dominated the substrate at YC4. Composition by bedrock was greatest at reference site MUNJS (Munjina Spring) and YC1.

In-stream habitat diversity was generally high throughout the Survey Area and included complex heterogenous substrates, such as submerged and emergent macrophytes, large woody debris (LWD), root mats, detritus, and trailing vegetation. In-stream habitat showed little seasonal change between the dry-19 and wet-20 sampling events.

Reference sites also recorded high in-stream habitat diversity, with the exception of WM (Wanna Munna) in the wet-20. This site generally lacked complex habitat types and instead was dominated by open sediment, with some detritus, LWD and algae. At SS, algae cover increased from 9% in dry-19 to 20% in wet-20. Water depth also increased considerably at SS, from 1.6 m in the dry to 3 m in the wet.

Algal cover was variable across sites and between sampling events. At sites where algal cover was present, percentage cover ranged from 5% at YC1 (dry-19) and YC4 (wet-20) to 20% at

YC3 (dry-19) and SS (wet-20). Algae cover was consistently high at Yandicoogina Creek site YC3 (20% in the dry and 18% in the wet). YC2 had no obvious algal growth in either season.

Table 4.5: Summary of aquatic habitats sampled, including site photos.

Site	Habitat	Description	Site photo
YC1	**Small pool**	Small shallow seep. Groundwater dependant vegetation present (*Melaleuca argentea* and *Eucalyptus camaldulensis*). Emergent vegetation dominated by *Typha domingensis*. *Chara* spp. submerged macrophyte in-stream. Bedrock as the dominant substrate, followed by silt and pebbles, with some gravel, cobbles, sand and clay. Maximum water depth of 0.4 m in the dry and 0.6 m in the wet.	
YC2	**Small pool**	Small seep area with highly abundant *Typha domingensis*. Riparian vegetation comprising *Eucalyptus camaldulensis* and *Melaleuca argentea* open woodland over patches of *Typha domingensis* and *Cyperus vaginatus* sedgeland. Weeds present (i.e. Natal grass; (Biologic, 2020a)). Mineral substrate comprising gravel, sand, silt and clay. Detritus and LWD present. Maximum water depth of 0.6 m in the dry and in the wet.	

Site	Habitat	Description	Site photo
YC3	**Small pool**	Small, shallow seep area. *Melaleuca argentea* with scattered *Eucalyptus camaldulensis* subsp. *obtusa* as the dominant overstorey. Emergent vegetation comprising *Typha domingensis* and *Cyperus vaginatus* sedgeland, with *Lobelia arnhemiaca* present on the banks in the dry. No submerged macrophyte present. Algae bloom on water surface in both seasons. Weeds present, including buffel grass, common sowthistle, flaxleaf fleabane and natal grass (Biologic, 2020a) Mineral substrate comprising gravel, pebbles, cobbles, sand and silt. Maximum water depth 0.3 m in the dry and 0.6 m in the wet.	
YC4	**Permanent, spring-fed creek pool**	Large permanent pool against a cliff face. *Melaleuca argentea* and scattered *Eucalyptus camaldulensis* open woodland over *Typha domingensis* sedgeland. Emergent macrophyte also included *Cyperus vaginatus* and *Schoenoplectus subulatus*. *Lobelia arnhemiaca* also present in the dry. Low abundances of scattered weeds were present, especially close to the cliff face (i.e. natal grass and flaxleaf fleabane; (Biologic, 2020a) Submerged macrophyte comprising *Chara* spp., *Vallisneria nana* and *Ruppia* sp. present. Clay as the dominant mineral substrate, followed by silt and sand. Maximum water depth of 5 m in the dry and 5.5 m in the wet.	
WWS	**Spring**	Permanent spring comprising a number of pools and interconnecting riffles. Located within Rio Tinto's HD1 discharge area – surface flows maintained by discharge from spurs. Overstorey vegetation comprising *Melaleuca argentea* and *Eucalyptus camaldulensis* subsp. *obtusa* over trees of *E. victrix* and a dense shrub layer. Emergent macrophyte comprising *Typha domingensis*, *Cyperus vaginatus, Schoenoplectus subulatus* and cf. *Schoenoplectus subulatus* Fringing *Lobelia arnhemiaca* throughout. WWS is a PEC. Maximum water depth of 1.1 m in the dry and 1.4 m in the wet.	

Site	Habitat	Description	Site photo
BENS	**Spring**	Second occurrence of the WWS PEC (often referred to as Ben's Oasis). Permanent spring located upstream of the main WWS. Riparian vegetation consisting of *Eucalyptus camaldulensis* and *Melaleuca argentea* woodland over *Acacia* spp. shrubland, and sparse sedges (*Cyperus vaginatus*). Detritus and LWD present. Mineral substrate dominated by gravel and pebbles, with some sand, silt, bedrock and boulders. Obvious impacts by cattle, with sedges grazed, and erosion of banks. Maximum water depth of 1.2 m in the dry. BENS was not sampled in the wet-20 survey due to logistical issues with access.	
MUNJS	**Permanent creek pools**	A series of long permanent pools, with numerous riffle sections. Mineral substrate almost exclusively bedrock. Riparian vegetation comprising *Eucalyptus camaldulensis Melaleuca argentea* and *M. bracteata*. Emergent macrophyte comprising *Typha domingensis, Cyperus vaginatus*, *Schenoplectus subulatus*, and *Schoenus falcatus*. *Vallisneria annua* and *Potamogeton tepperi* submerged macrophytes present in-stream. No fish. No obvious signs of disturbance. *Stylidium fluminense* present throughout in the dry. Maximum water depth of 2.0 m in the dry and 2.2 m in the wet.	
SS	**Spring**	Permanent spring flowing into a series of pools via a braided channel. Riparian vegetation comprising *Melaleuca argentea* and sedges (*Cyperus vaginatus* and *Eleocharis geliculata*). Submerged macrophyte comprising *Nitella* spp., *Najas marina*, *Vallisneria annua*, *Potamogeton tepperi* and *Ruppia* sp. P2 Priority flora (ground creeper *Ipomoea racemigera*) present. Mineral substrate heterogenous, dominated by gravel, pebbles and sand. Disturbances included cattle impacts and introduced vegetation (such as Mexican poppy *Argemone ochroleuca* subsp. *ochroleuca*). Maximum water depth of 1.6 m in the dry and 3 m in the wet.	

Site	Habitat	Description	Site photo
WM	**Semi-permanent creek pools**	A series of semi-permanent creek pools located on Weeli Wolli Creek, upstream of Ben's Oasis and upstream of any mine impacts. *Eucalyptus* sp. present over *Acacia ?elacantha* and *A. ?coriacea* var. *pendens*. Low in-stream habitat diversity, with no submerged or emergent macrophytes recorded. Mineral substrate as the dominant habitat type, followed by LWD and detritus. Mineral substrate was heterogenous, comprising sand, gravel, pebbles and silt. Small amounts of bedrock and boulders also recorded. Maximum water depth of 1.5 m recorded in the wet. WM was not sampled in the dry-19. It was sampled in the wet-20 as a replacement for BENS.	

4.4 Water quality

All raw water quality data are provided in Appendix D.

4.4.1 In situ

Electrical conductivity (EC) within Yandicoogina Creek was fresh in both seasons and ranged from 571 µS/cm (YC2) to 664 µS/cm (YC3) in the dry-19, and 554 µS/cm (YC2) to 641 µS/cm (YC4) in the wet-20. While all Yandicoogina Creek and reference sites recorded EC in excess of the ANZECC & ARMCANZ (2000) default guideline value (DGV), none were considered to pose a threat to aquatic life. Generally, sites with EC less than 1,500 µS/cm experience little ecological stress, but a considerable shift in aquatic fauna assemblages is known to occur above this threshold. All sites sampled in the current study generally had low levels of seasonal variation in EC, likely reflecting their connection to groundwaters (Figure 4.1).

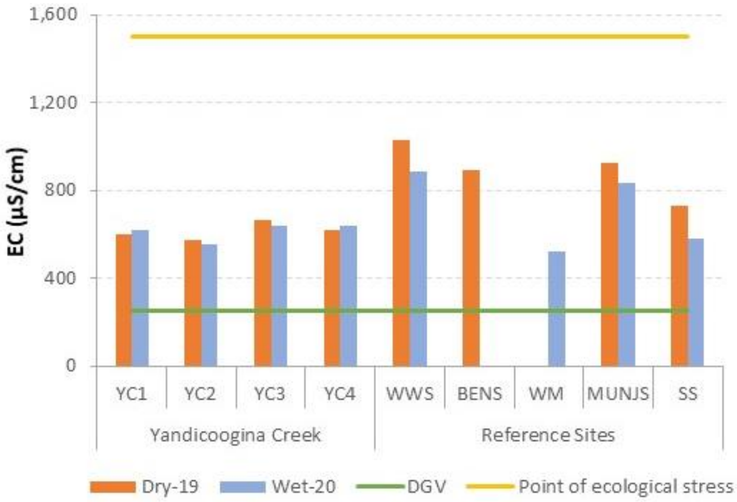


Figure 4.1: Electrical conductivity (EC; µS/cm) recorded from all sites in comparison to the ANZECC & ARMCANZ (2000) DGV and point of ecological stress.

Dissolved oxygen (DO) concentrations within Yandicoogina Creek were generally in the lower range, with all sites recording saturation levels below the lower DGV, in both seasons. Reference sites recorded similarly low DO saturation, except for Munjina Spring in the dry season and Wanna Munna in the wet. In the dry-19, DO ranged from 27.0% (at YC2) to 108.8% (at MUNJS). The range in DO during the wet-20 was from 23.3% (YC4) to 92.4% (at WM).

Surface waters within the Survey Area were slightly basic to circum-neutral, and similar to reference sites. Lowest pH was recorded from YC2 in the dry-19 (6.8) and highest from WM in the wet-20 (8.22). Most sites were within the default ANZECC GVs for pH, with the exception of reference sites MUNJS (in the dry-19), WM (wet-20) and SS (wet-20). None of these sites recorded pH considered to be of ecological concern. Pilbara waters, especially those of springs, often record slightly basic pH (Jess Delaney, unpub. data).

Turbidity was low at all Yandicoogina Creek sites, indicating high water clarity and light penetration in both seasons. In the dry-19, turbidity ranged from 0.3 NTU (at YC3) to 3.8 (at YC2), while in the wet turbidity ranged from 1.5 NTU (at YC4) to 10.9 (YC2). Turbidity was similarly low at reference sites. Although all levels were low, and well within the DGV, turbidity did increase slightly between seasons. This is likely a reflection of catchment runoff associated with wet season rains and flooding.

4.4.2 Ionic composition

Cation composition at all Yandicoogina Creek sites was dominated by calcium (Ca) cations and hydrogen carbonate (HCO_3) anions. There was no seasonal change in ionic dominance at any of the Yandicoogina Creek sites, likely indicating inflow from groundwater and minimal evapo-concentration. While reference site Bens Oasis displayed this same signature dominance, Weeli Wolli Spring was dominated by sodium (Na) cations in the dry-19. There was seasonal variation at this site, however, with cation dominance switching to Ca in the wet-20. Of the remaining reference sites, Munjina Spring was dominated by Ca cations and HCO_3 anions in the dry season, and Na cations and HCO_3 anions in the wet, and vice versa at Skull Springs. The dominance of Ca and HCO_3 in surface waters often indicates connection to groundwater, while Na dominance tends to indicate contribution by rainfall and evapo-concentration effects.

Alkalinity measures the capacity of the water to resist sudden changes in pH, i.e. it is the buffering capacity of the water. Alkalinity of less than 20 mg/L is considered low and the system would have limited ability to buffer against rapid changes in pH. Alkalinity recorded in the current study was generally high, with most sites recording values greater than 250 mg/L. Only two sites recorded alkalinity below 200 mg/L, both of which were reference sites (WM and MUNJS in the wet-20), however, alkalinity recorded from both these sites was well above the threshold of 20 mg/L.

4.4.3 Nutrients

Nitrogen ammonia (N_NH_3) concentrations were low at all sites sampled, in both seasons (Figure 4.2). All values were below the limit of detection (LOD; i.e. < 0.01 mg/L), except for

Munjina Spring in the dry-19 (0.02 mg/L N_NH_3). All concentrations were well below ANZG (2018) default guideline value (DGVs) for the protection of 99% of species.

Nitrogen nitrate (N_NO_3) concentrations were also low and ranged from values below the LOD (< 0.01 mg/L; at YC3, WWS and Bens Oasis in the dry-19, and YC2 and Wanna Munna in the wet-20) to 0.08 mg/L (at Skull Springs in the wet-20) (Figure 4.2). No N_NO_3 concentrations were in excess of toxicity DGVs[8] in either season (Figure 4.2).

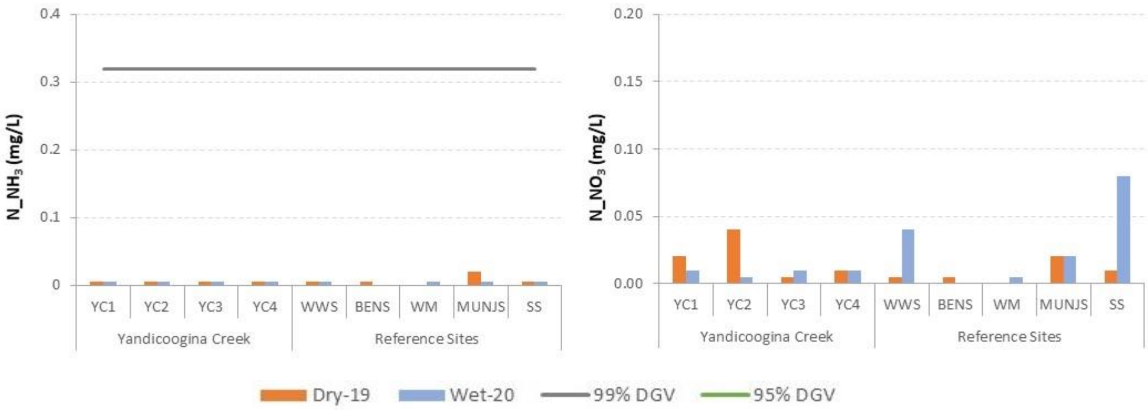


Figure 4.2: Ammonia (N_NH_3; left) and nitrate (N_NO_3; right) concentrations recorded from each site (mg/L), in comparison to default ANZECC & ARMCANZ (2000) 99% toxicity GVs. NB: y-axis scales are different for each analyte.

As nitrate generally comprises the largest portion of nitrogen oxide (N_NOx) concentrations, with negligible contribution by nitrite, N_NOx concentrations were similarly variable, i.e. ranged from below LODs to 0.08 mg/L (Figure 4.3). Concentrations of N_NOx exceeded the default eutrophication GV at two Yandicoogina Creek sites in the dry-19 (YC1 and YC2). Reference sites also recorded exceedances of the eutrophication DGV, with N_NOx concentrations being elevated at Munjina Spring in the dry-19, and Weeli Wolli Spring, Munjina Spring and Skull Spring in the wet-20 (Figure 4.3).

Concentrations of total nitrogen (TN) at Yandicoogina Creek were variable, ranging from 0.06 mg/L at YC3 in the dry-19 to 2.2 mg/L at YC2, also in the dry-19 (Figure 4.3). Concentrations recorded from several sites exceeded the DGV for protection against eutrophication, including YC2 (in the dry-19), YC3 (wet-20), WM (wet-20) and MUNJS (dry-19) (Figure 4.3). YC2 recorded particularly high TN in the dry-19, with the concentration being notably higher than all other sites, including reference sites, and exceeding the eutrophication DGV by more than seven times.

[8] There is no current, available toxicity DGV for N_NO_3. Historic ANZECC & ARMCANZ (2000) GVs were found to be erroneous and notably low/conservative (ANZG, 2018). It was anticipated that values would be updated in the recent online, interactive version of the ANZECC guidelines (ANZG, 2018), however this has not been the case. In the absence of updated ANZECC DGVs for N_NO_3, ANZG (2018) suggest referring to the current New Zealand nitrate toxicity guidelines, specifically the 'Grading' GVs published in the '*Updating Nitrate Toxicity Effects on Freshwater Aquatic Species*' report (NIWA, 2013).

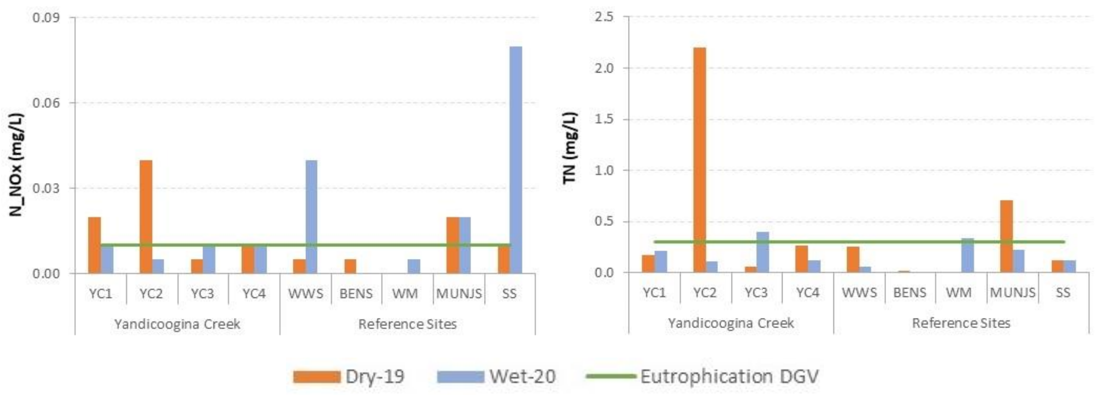


Figure 4.3: Nitrogen oxide (N_NOx; left) and total nitrogen (TN; right) concentrations recorded from each site (mg/L), in comparison to ANZECC & ARMCANZ (2000) eutrophication DGVs. NB: y-axis scales are different for each analyte.

Total phosphorus (TP) was high across all Yandicoogina Creek and reference sites (Figure 4.4). Within Yandicoogina Creek, concentrations ranged from 0.03 mg/L (at YC3 in the dry-19 and YC4 in the wet-20) to 0.089 mg/L (at YC2 in the dry-19; Figure 4.4). All sites, including reference sites, recorded elevated TP concentrations in excess of the eutrophication DGV. Concentrations from YC2 and MUNJS were notably high, with both sites recording TP more than eight times the DGV (Figure 4.4).

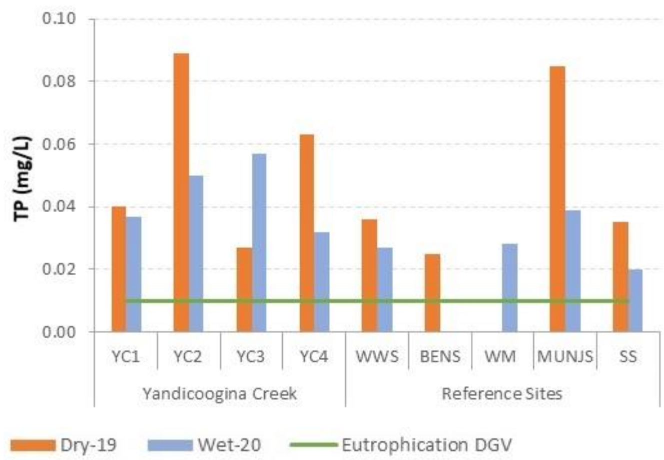


Figure 4.4: Total phosphorus (TP) concentrations recorded from each site (mg/L), in comparison to the eutrophication DGV.

4.4.4 Dissolved metals

Dissolved metal concentrations within Yandicoogina Creek were generally low, with many analytes recording concentrations below LODs at most, if not all sites (i.e. aluminium, cadmium, cobalt, nickel, lead, selenium, and zinc). However, several dissolved metals recorded concentrations greater than DGVs at some sites (Figure 4.5). Elevated dissolved metals from Yandicoogina Creek included:

- Dissolved boron (dB) concentrations exceeded the 99% DGV at all sites, except reference site WM (wet-20). The 95% DGV was also exceeded at reference site BENS (dry-19).

- The dissolved iron (dFe) concentration recorded from YC2 in the dry-19 was greater than the interim indicative working level[9] provided in the ANZECC & ARMCANZ (2000) guidelines. dFe recorded from YC2 was notably higher than all other sites sampled, being almost six times higher than the next greatest dFe concentration; 0.08 mg/L recorded from MUNJs in the dry-19 (Figure 4.5).

There was also an exceedance of the 99% and 95% DGVs for dissolved copper (dCu) at reference site WM in the wet-20 (Figure 4.5). dCu concentrations recorded from Yandicoogina Creek were all within DGVs.

Dissolved chromium (dCr) could not be compared against the 99% DGV as the LOD (< 0.0002 mg/L) was higher than the DGV (0.00001 mg/L). No dCr concentrations were in excess of the 95% toxicity DGV (Figure 4.5).

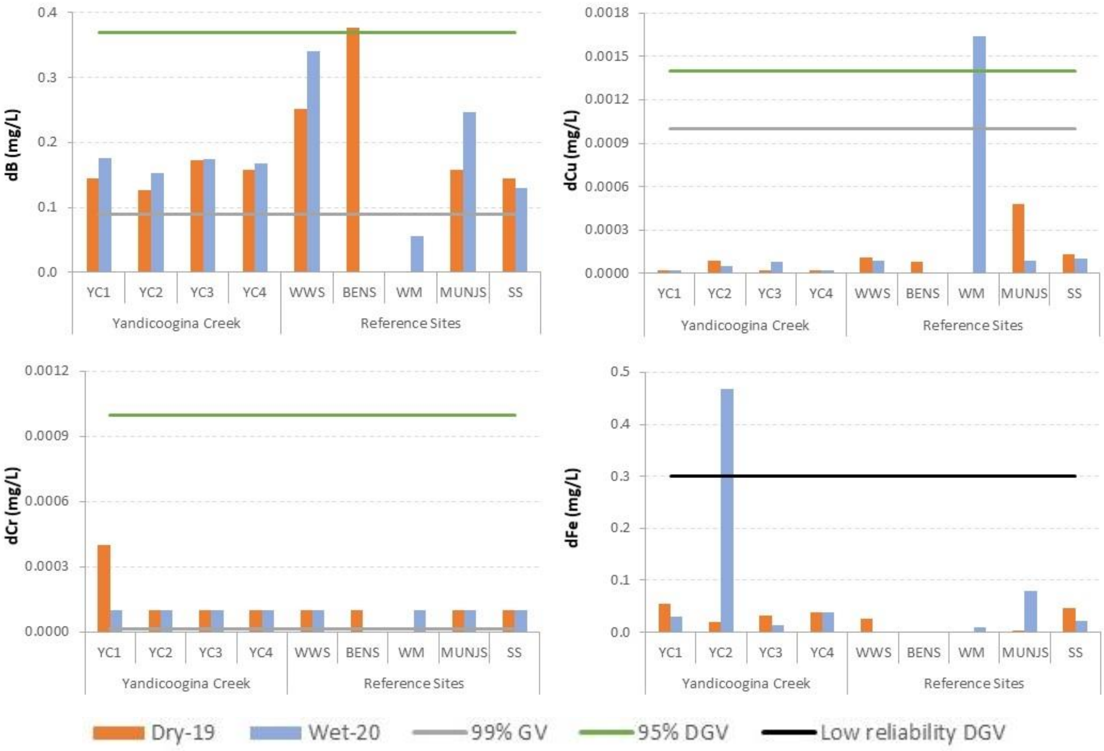


Figure 4.5: Concentrations of selected dissolved metals recorded from each site, in comparison to ANZECC DGVs, including dB, dCu, dCr and dFe. NB: y-axis scales are different for each analyte.

4.5 Wetland Flora

4.5.1 Taxa composition and richness

A total of six macrophyte taxa were recorded from Yandicoogina Creek (Table 4.6). This included three emergent macrophyte taxa and three submerged macrophytes (Table 4.6).

[9] ANZECC & ARMCANZ (2000) had insufficient toxicity data with which to derive a reliable GV for dFe, and instead deferred to the current Canadian guideline of 0.30 mg/L. This was provided as an interim indicative working level, with further work required to establish a concentration appropriate for Australian waters.

Other riparian vegetation taxa were also recorded within the Survey Area, including various *Eucalyptus*, *Melaleuca*, and *Acacia* species, as well as herbs, shrubs and grasses (Table 4.6).

Emergent macrophytes included *Cyperus vaginatus*, *Schoenoplectus subulatus* and *Typha domingensis* (Plate 4.1). There was little seasonal variation in emergent macrophyte taxa composition, reflecting the presence of permanent water. Emergent macrophytes were recorded from all sites except the semi-permanent reference pool WM. The greatest diversity was recorded from reference sites MUNJS and WWS (four taxa), followed by YC4 within the Survey Area (three taxa) (Table 4.6).

Plate 4.1: *Typha domingensis* at YC1.

Submerged macrophytes recorded from Yandicoogina Creek comprised *Vallisneria nana*, *Ruppia* sp., and *Chara* spp. (Table 4.6). Taxonomic limitations for Pilbara species of *Chara* and *Nitella* precluded identification to species. Like the emergent macrophytes, there was little seasonal variation in submerged macrophyte composition, with the exception of YC3. This site recorded *Chara* spp. in the wet season only, following inundation. The low seasonal variation in submerged macrophytes is unsurprising and relates to water permanence. Submerged macrophytes were recorded from just over half of all sites sampled (Table 4.6). Skull Springs recorded the greatest diversity of submerged macrophytes (five taxa), followed by YC4 and MUNJS (both with four taxa).

Table 4.6: Flora taxa recorded during the current study.

NB: D refers to dry season flora records, and W refers to wet season records.

			Yandicoogina Creek								Munjina Spring		Weeli Wolli Creek				Davis River	
Class/Order	**Family**	**Lowest taxon**	YC1		YC2		YC3		YC4		MUNJS		WWS		BENS	WM	SS	
			D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W
CHLOROPHYTA																		
CHAROPHYCEAE																		
Charales	**Characeae**	*Chara* spp.↓	✓	✓				✓	✓	✓	✓	✓						
		Nitella spp.↓															✓	✓
PLANTAE																		
MAGNOLIOPSIDA																		
Asterales	**Asteraceae**	*? *Sigesbeckia orientalis*							✓									
	Campanulaceae	*Lobelia arnhemiaca*^					✓		✓				✓				✓	
	Stylidiaceae	*Stylidium fluminense*^									✓	✓						
		Stylidium weeliwolli^ (P3)											✓	✓				
Caryophyllales	**Amaranthaceae**	*Ptilotus auriculifolius*														✓		
		Ptilotus calostachyus										✓						
Fabales	**Fabaceae**	*Acacia ? coriacea* var. *pendens*^										✓						
		Acacia ? elachantha														✓		
		Senna glutinosa subsp. *chatelainiana*														✓		
		Vigna lanceolata var. *lanceolata*															✓	✓
Lamiales	**Plantaginaceae**	*Stemodia ? viscosa*^																
Malvales	**Malvaceae**	*Triumfetta ? leptacantha*				✓												
Malpighiales	**Euphorbiaceae**	*Euphorbia biconvexa*														✓		
Myrtales	**Myrtaceae**	*Eucalyptus* sp.														✓		
		Eucalyptus camaldulensis	✓	✓	✓	✓	✓	✓	✓	✓			✓	✓	✓			
		Eucalyptus ? camaldulensis									✓	✓						

			Yandicoogina Creek								Munjina Spring		Weeli Wolli Creek				Davis River	
Class/Order	**Family**	**Lowest taxon**	**YC1**		**YC2**		**YC3**		**YC4**		**MUNJS**		**WWS**		**BENS**	**WM**	**SS**	
			D	**W**	**D**	**W**	**D**	**W**	**D**	**W**	**D**	**W**	**D**	**W**	**D**	**W**	**D**	**W**
		Melaleuca argentea^	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓
		Melaleuca bracteata^^									✓	✓						
Ranunculales	**Papaveraceae**	**Argemone ochroleuca* subsp. *ochroleuca*															✓	
Solanales	**Convolvulaceae**	*Ipomoea racemigera* (P2)															✓	
LILIOPSIDA																		
Alismatales	**Hydrocharitaceae**	*Najas marina*↓															✓	✓
		Vallisneria annua↓									✓	✓					✓	✓
		Vallisneria nana↓							✓	✓								
	Potamogetonaceae	*Potamogeton tepperi*↓									✓	✓					✓	✓
	Ruppiaceae	*Ruppia* sp.↓							✓	✓							✓	✓
Poales	**Cyperaceae**	*Cyperus vaginatus*^	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓
		Eleocharis geniculata^										✓					✓	✓
		Schoenoplectus subulatus^							✓	✓	✓	✓	✓	✓				
		cf. *Schoenoplectus subulatus*^											✓	✓				
		Schoenus falcatus^									✓	✓						
	Poaceae	*Eulalia aurea*						✓								✓		
		Enneapogon robustissimus														✓		
		**Setaria verticillata*								✓								
	Typhaceae	*Typha domingensis*^	✓	✓	✓	✓	✓	✓	✓	✓			✓	✓				
		Taxa richness	**5**	**5**	**4**	**5**	**5**	**7**	**10**	**10**	**10**	**13**	**8**	**8**	**3**	**7**	**12**	**10**

* Introduced species

(P2) Declared rare flora

^ Associated with creeks

^^ Seasonal wet areas, claypans and rivers

↓ submerged macrophyte

4.5.2 Conservation significant flora

None of the wetland flora species recorded from Yandicoogina Creek were of significance; however, a DBCA Priority flora species, *Ipomoea racemigera*, was recorded from Skull Spring during the dry-19 survey (Table 4.6). This creeping annual is a Priority Two (P2, poorly known) species, known only from a few locations (generally less than five) (DBCA, 2019; Western Australian Herbarium, 1998–). *Ipomoea racemigera* is known to occur on sandy soils along watercourses.

4.5.3 Introduced flora

A specimen potentially representing the introduced *Sigesbeckia orientalis* (Indian weed) was collected from Yandicoogina Creek (YC4) in the dry-19. While the identification of this species could not be definitively determined due to the degraded condition of the specimen, it is likely to be Indian weed, given this species was also recorded during the flora survey (Biologic, 2020a). Other weeds recorded by the flora team, and in locations close to the aquatic ecosystem sampling sites, included; mimosa bush (*Vachellia farnesiana*), natal grass (*Melinis repens*), buffel grass (*Cenchrus ciliaris*), common sowthistle (*Sonchus oleraceus*), and flaxleaf fleabane (*Conzya bonariensis*) (Biologic, 2020a).

One other confirmed introduced flora species was collected during the current study, the Mexican poppy *Argemone ochroleuca* subsp*. ochroleuca*. This species was only recorded from a reference site (Skull Springs in the dry-19), and not within the Survey Area.

4.5.4 Flora comparison with previous studies

Data on wetland vegetation of the Pilbara is limited, with varied sampling effort and taxonomic resolution across studies. However, wetland flora was sampled as part of the PBS, with a paper discussing conservation significance and distribution information due for publication in 2021 (Mike Lyons, DBCA, unpub. data). In order to compare species lists with the current study, the DBCA kindly provided Biologic with data from Weeli Wolli Spring, near the Study Area.

Macrophyte richness recorded from Yandicoogina Creek was considerably lower than that recorded during the PBS from Weeli Wolli Spring (Figure 4.6). The greatest richness recorded from the Survey Area was six taxa (from the permanent pool, YC4), in comparison to the nine taxa recorded from Weeli Wolli Spring during the PBS, in September 2003 and May 2005 (Figure 4.6). Interestingly, five additional taxa were recorded from Weeli Wolli Spring during the PBS, in comparison to WWS as sampled in the dry-19 and wet-20 (Figure 4.6). It must be noted that site locations differed between surveys, with the PBS being located approximately 660 m downstream of the WWS site sampled in the current study.

Seven taxa recorded during the PBS from Weeli Wolli Spring were not recorded from Yandicoogina Creek during the current study. These included the charophyte taxa *Chara fibrosa* var. *benthamii*, *Nitella heterophylla*, *Nitella* sp. nov. *'verrucate'* (M.T. Casanova PBS7), submerged macrophyte *Najas marina*, herb *Lobelia quadrangularis*, sedges *Eleocharis geniculata*, and *Cladium procerum* (Priority 2). Of these, *Chara* spp. was recorded from YC1, YC3 and YC4, however, due to taxonomic limitations it is not possible to determine whether it represents the species identified during the PBS (*Chara fibrosa* var. *benthamii*). While *Nitella* spp., *Najas marina*, and *Eleocharis geniculata* were all recorded from reference sites during the current study, they were not recorded from Yandicoogina Creek.

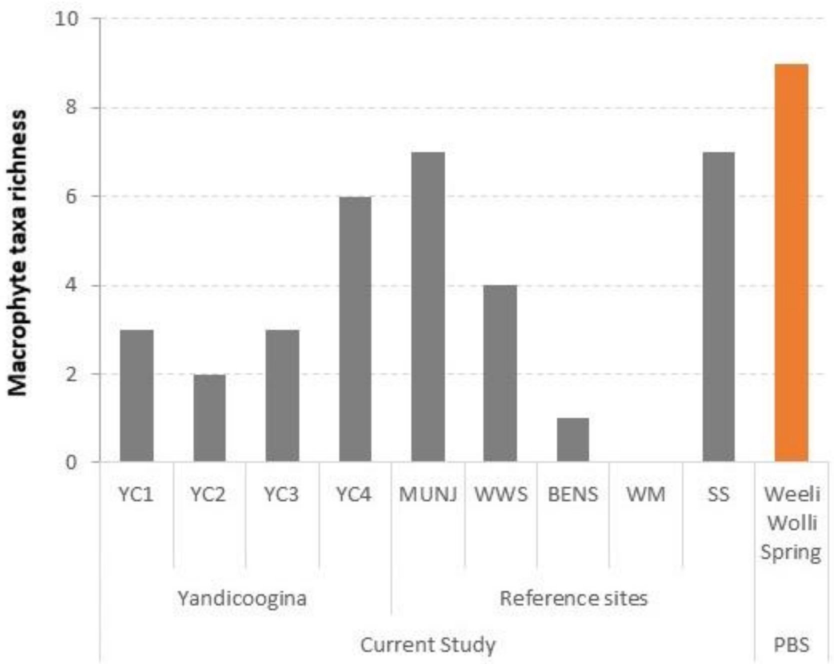


Figure 4.6: Macrophyte (emergent and submerged) richness recorded during in the current study (dry and wet seasons combined), in comparison to the PBS from Weeli Wolli Spring (September 2003 and May 2005; Mike Lyons, unpub. data).

4.6 Zooplankton

4.6.1 Taxa composition and richness

A total of 35 zooplankton taxa[10] were recorded from Yandicoogina Creek, comprising four Protista, 23 Rotifera, five Maxillopoda (Copepoda), and three Cladocera (water fleas) (see Appendix E for a full taxonomic list).

In the dry season, zooplankton richness ranged from five (at YC3) to 18 (at WWS), and in the wet season ranged from two (at WWS) to 14 (at YC1; Figure 4.7). In general, richness recorded from Yandicoogina Creek was comparable to, if not slightly higher than, reference sites. YC3 recorded a large seasonal variation in zooplankton taxa richness, with considerably greater richness recorded in the wet season, following flooding (Figure 4.7). Interestingly, reference site WWS recorded both the lowest richness (in the wet-20) and the highest richness (in the dry-19).

[10] As not all specimens could be identified to species due to immaturity, damage, unknown or unresolved taxonomy and/or a lack of suitable keys, taxa refers to the lowest level of identification possible (generally genus).

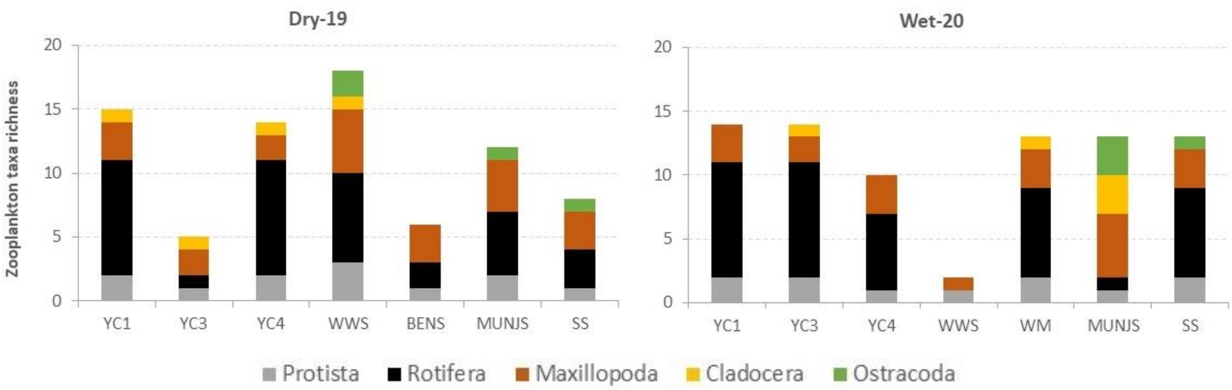


Figure 4.7: Zooplankton taxa richness recorded from each site in the dry-19 (left) and wet-20 (right).

Zooplankton composition was dominated by rotifers at most sites, in both seasons, generally followed by Maxillopoda (copepods; Figure 4.7). Diversity of Cladocera and Ostracoda was low across all sites, with some sites recording no individuals from these groups. Ostracods were not recorded from any sites within the Yandicoogina Creek Survey Area (Figure 4.7).

4.6.2 Conservation significant zooplankton taxa

All zooplankton taxa recorded are widely distributed across northern Australia or the world (cosmopolitan species). No zooplankton taxa recorded from Yandicoogina Creek are considered to have restricted ranges, and none are listed for conservation significance.

4.6.3 Zooplankton comparison with previous studies

Yandicoogina Creek zooplankton data was compared with previous studies detailed in section 4.2 above, for those studies which sampled more than one replicate site within a creek system (Figure 4.8). Weeli Wolli Creek sites were split into Weeli Wolli Spring (recorded from the historic spring area, including Bens Oasis) and Weeli Wolli Creek (upper Weeli Wolli Creek river pools), to reflect differences in water permanence and hydrology between these two areas; factors which would influence zooplankton assemblages. Weeli Wolli Spring sampling undertaken as part of the PBS could not be included here, as only one site was sampled in each season.

Yandicoogina Creek generally recorded average richness comparable to nearby creek systems (Figure 4.8). Average wet season richness was slightly lower than that recorded from Marillana and Weeli Wolli creeks, but average dry season richness was greater (Figure 4.8). The semi-permanent and ephemeral river pools in Upper Weeli Wolli Creek recorded high average zooplankton richness in the wet season, but low in the dry. Generally, zooplankton richness was greatest in the wet season, across all creeks (Figure 4.8). The large standard error bars reflect the high within-system variability in zooplankton richness. Interestingly, variability within Yandicoogina Creek was noticeably lower than other creek systems, in both seasons (Figure 4.8).

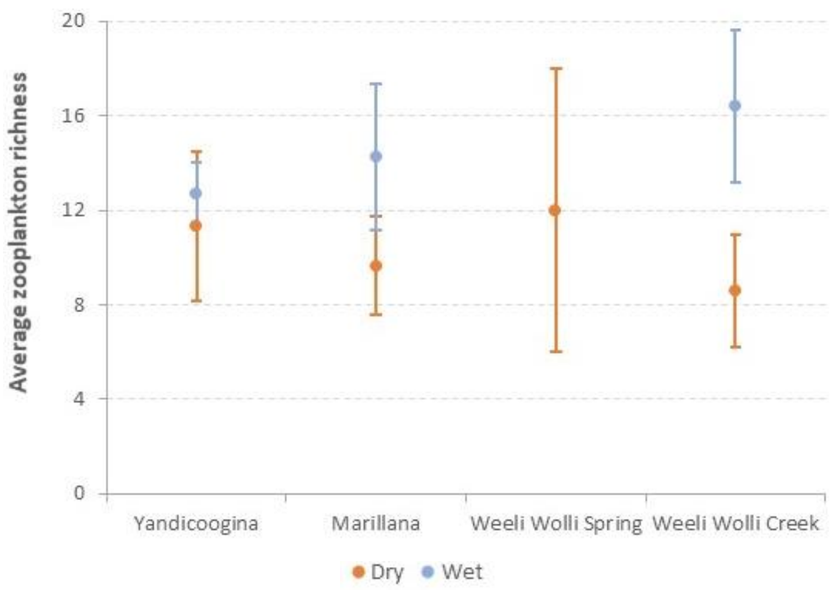


Figure 4.8: Average zooplankton taxa richness (± se) recorded from Yandicoogina Creek, in comparison to other studies and nearby creek systems, in both seasons.

Overall, any differences in zooplankton richness between creek (Two-way ANOVA; df = 3, F = 0.046, p = 0.987) and season (df = 1, F = 1.955, p = 0.170) were not significant. There was also no significant interaction between creek and season (df = 2, F = 0.256, p = 0.775).

4.7 Hyporheos fauna

Hyporheic samples were successfully collected from all sites except YC1 in the dry-19. Conditions at the time precluded hyporheic sampling at this site, particularly the dense *Typha* stands along banks impeding access to the hyporheos. During the wet-20, five additional hyporheic samples were collected from the Survey Area (YC5H to YC9H).

4.7.1 Taxa composition and richness

A total of 108 invertebrate taxa was recorded from hyporheic zones within Yandicoogina Creek (see Appendix F for a full taxonomic list). The taxonomic list included Oligochaeta (aquatic segmented worm; 13 taxa), Mollusca (freshwater snails; two taxa), Ostracoda (seed shrimp; 12 taxa), Copepoda (eight taxa), Syncarida (one taxa), Amphipoda (side swimmer; three), Isopoda (one), Acarina (water mites; 12), Collembolla (springtails; three), Coleoptera (beetles; 14), Diptera (two-winged fly larvae; 30), Trichoptera (caddisfly larvae; one), Ephemeroptera (mayfly larvae; three), Hemiptera (true bugs; three), Lepidoptera (moth larvae; one), and Odonata (dragonflies; one). Just over half of these taxa were stygoxenes (59%) and do not have specialised adaptations for life in groundwater habitats. These taxa were recorded from the hyporheic zone ‘by chance’ but can actively seek out this habitat as a refuge during times of drought or flood. Hyporheos fauna, comprising styogbites, permanent hyporheos stygophiles, occasional hyporheos stygophiles and possible hyporheic taxa, made up the remaining 41% of taxa collected. Of these, a total of 13% are directly dependent on groundwater for their persistence. This result is consistent with other Pilbara studies, where generally less than 20%

of invertebrate taxa recorded from hyporheic samples are totally reliant on groundwater (Halse *et al.*, 2002). Despite this similarity, the percentage of stygobitic fauna recorded from hyporheic samples within the Survey Area was considerably greater (12%) than that reported by Halse *et al.* (2002) (only 5% stygobitic fauna). This further highlights the strong groundwater connection within the Yandicoogina Creek Survey Area.

Possible hyporheic taxa included higher-level identifications for which taxa may have belonged to a stygal or hyporheos species. These include the oligochaetes Oligochaeta sp. imm./dam., *Pristina* sp., Naidinae spp. and Phreodrilidae spp., and juvenile ostracods (Cyprinopsinae sp. imm.), *Cypridopsis* sp., the copepod *Thermocyclops* sp., immature or damaged water mites (Acari sp. imm./dam.), and immature Baetid mayflies (Baetidae sp.). One hydrophilid taxa (in larval form) was unable to be identified using available keys and current taxonomic knowledge; Hydrophilidae sp. (L) (hypo?). This is likely to belong to a known taxon which is common in hyporheic habitats of the Pilbara, including those in the vicinity of the Survey Area. It was considered a possible hyporheic taxa due to its pale colouration and reduced eyes.

Hyporheos taxa recorded from the Survey Area included:

<u>Occasional hyporheos stygophiles:</u>

- oligochaetes *Allonais pectinata*, *Allonais ranauna*, *Nais variabilis*, *Pristina aequiseta*, *Pristina longiseta*, *Pristina jenkinae* and *Pristina sima*
- ostracods *Riocypris fitzroyi* and *Penthesilenula brasiliensis*
- copepods *Microcyclops varicans*, *Mesocyclops notius* and *Paracyclops intermedius*
- collembola Entomobryoidea spp., Poduroidea spp. and Symphypleona sp.
- beetles *Limbodessus occidentalis*, *Austrolimnius* sp. (L), *Hydraena* sp. and Scirtidae spp. (L).
- true bug *Nerthra* sp.

<u>Permanent hyporheos stygophiles:</u>

- the ostracod *Limnocythere dorsosicula*

<u>Stygobites:</u>

- ostracods *Meridiescandona facies*, *Cypridopsis* sp. `BOS1401`, *Vestalenula marmonieri*, *Gomphodella alexanderi* and *Gomphodella yandii*
- copepods *Diacyclops humphreysi* and Harpacticoida sp.
- amphipod *Chydaekata* sp. `E`, Paramelitidae `sp. Biologic-AMPH023`, and Paramelitidae spp. (imm./dam.)
- syncarid Bathynellidae sp. BES7547
- isopod *Pygolabis weeliwolli*
- water mite *Wandesia* sp.

Overall, site invertebrate richness ranged from five (at YC2 in the dry-19) to 44 (at YC5H in the wet-20; Figure 4.9). Stygoxenes dominated taxa richness at most sites except those with low overall richness such as YC2 (in the dry-19) and MUNJS (wet-20). A number of sites recorded no groundwater dependent taxa (stygobites or permanent hyporheos stygophiles). These included YC2 (both seasons), WM (wet-20), MUNJS (wet-20) and SS (wet-20); although all four of these sites supported occasional hyporheos stygophiles (Figure 4.9), taxa which take advantage of the protection afforded by the hyporheic zone seasonally, or during early life history stages. The lack of stygobitic taxa recorded from YC2 was likely influenced by the dense *Typha* stands present at this site, which impeded access to the hyporheos. The greatest richness of hyporheos taxa (including occasional stygophiles and possible hyporheic taxa) was recorded from SS in the dry-19 (20 taxa), closely followed by YC5H (18 taxa). YC7H, YC8H and YC9H all recorded the third highest richness of hyporheos fauna, with 14 taxa recorded in the wet-20 (Figure 4.9). The high hyporheos richness at these sites suggests a strong connection to groundwaters.

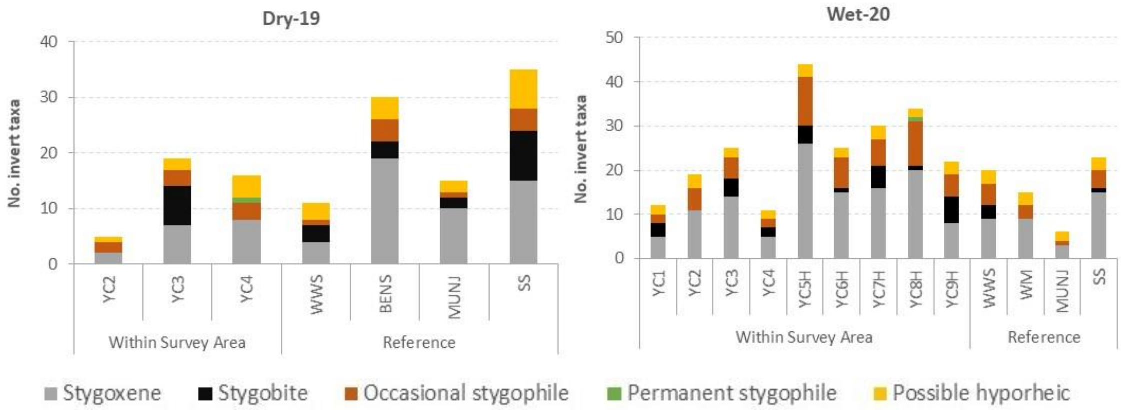


Figure 4.9: Classification of invertebrate taxa recorded from the hyporheic zone, in each season.

4.7.2 Conservation significant hyporheos taxa

While most of these taxa are generally common and ubiquitous across the Pilbara, a number are of conservation significance and are either locally restricted or rarely collected.

Ostracoda

Data recorded during the PBS indicated that stygobitic ostracod species are generally confined to single sub-catchments, except for *Areacandona scanlonii* and *Gomphodella hirsuta*, which are more widespread. In contrast, surface water ostracods were found to occur across several sub-regions (Halse *et al.*, 2014). Of the stygal ostracods recorded in the current study, a number are restricted to relatively short ranges, including *Meridiescandona facies*, *Gomphodella yandii*, *Gomphodella alexanderi*, *Notacandona boultoni* and *Vestalenula matildae*. *Meridiescandona facies* was recorded from the hyporheos of YC1 and YC9H in the wet-20. This species is known from Weeli Wolli Creek and the central and eastern Fortescue (Karanovic, 2007). *Gomphodella yandii* is known to be restricted to Weeli Wolli Creek and Marillana Creek. Its collection from YC7H in the wet-20 appears to constitute the first record from Yandicoogina Creek. *Gomphodella alexanderi* was previously known only from interstices of Marillana Creek and groundwater bores at Rio Tinto's Yandi Mine, and was recently described from borehole specimens (Karanovic & Humphreys, 2014). During the current study, it was recorded from the hyporheos of YC7H and YC8H. Based on the WAM classification system, the two *Gomphodella* ostracod species recorded from Yandicoogina Creek are considered potential SREs, sub-category data deficient. While their known range is < 10,000 km^2 (or linear range < 100 km), there is insufficient taxonomic and distribution information to confirm SRE status.

The remaining ostracod species with fairly restricted ranges were recorded from reference sites (i.e. *Notacandona boultoni* from the hyporheos of Weeli Wolli Spring, and *Vestalenula matildae* from Skull Springs).

The cypridid ostracod *Cypridopsis* sp. `BOS1401` is moderately common in the Pilbara and was considered likely to be the same species as that recorded from Gabanitha and Mulga East (Stuart Halse, Bennelongia, pers. comm.). During the dry-19, *Cypridopsis* sp. `BOS1401` was recorded from the hyporheic zone of YC3, as well as reference sites BENS and MUNJS.

<u>Amphipoda</u>

Molecular analysis was undertaken on 20 stygal amphipod specimens collected from the Survey Area and nearby Weeli Wolli Creek and aligned with morphological identifications to determine species' distributions (Appendix G). Outcomes from this work indicated that the Survey Area supports two species with restricted ranges: *Chydaekata* sp. `E` and Paramelitidae `sp. Biologic-AMPH023`. *Chydaekata* sp. `E` was recorded from the hyporheos of YC3, YC5H, YC7H, and YC9H, as well as reference site WWS. Although an undescribed morphotype, this species is previously known and appears to be restricted to Marillana Creek and Upper Weeli Wolli Creek. The *Chydaekata* sp. recorded from Skull Springs belongs to a different undescribed species.

Paramelitidae `sp. Biologic-AMPH023` was recorded from the hyporheic zone of YC1, YC3, YC5H, and YC9H within the Survey Area. This species was given a unique Biologic morphotype following molecular analysis, with results indicating it is previously known from Marillana Creek. Its current known distribution therefore includes Yandicoogina Creek and Marillana Creek. These creek systems are all in close proximity, with Yandicoogina Creek being a tributary of Marillana Creek, and lying a mere 9 km away. Genetic analysis undertaken by others have indicated that most paramelitid species have ranges in the tributary-scale (Finston *et al.,* 2007, 2008, 2011). Based on the WAM classification system, the stygal amphipods recorded from Yandicoogina Creek would be considered potential SREs (sub-category data deficient; insufficient taxonomic and distribution information).

Immature or damaged stygal amphipods were also recorded from hyporheic zone sampled within the Survey Area in the wet-20, including YC1, YC3, YC4, YC5H and YC9H. These specimens likely belong to either *Chydaekata* sp. `E` or Paramelitidae `sp. Biologic-AMPH023`.

<u>Isopoda</u>

The stygobitic isopod *Pygolabis weeliwolli* was recorded from the hyporheic zone of YC3, YC4, YC5H, YC7H, and YC9H. This species is a known SRE, with its range restricted to the groundwater and hyporheos of Weeli Wolli Creek and Marillana Creek, and groundwater bores within the Yandicoogina tenement (Biota, 2010).

Syncarida

Two individual stygal syncarids were recorded from YC9H in the wet-20; Bathynellidae sp. BES7547. These specimens belong to a new, undescribed genus of Bathynellidae (G. Perena, Biologic, pers. comm.). They are morphologically distinct to other stygal bathynellids from nearby, including Ethel Gorge, the lower Fortescue, and the DeGrey River catchment (G. Perena, Biologic, pers. comm.), and do not appear to match any known Bathynellid taxa. This appears to be the first record of this new genus. As such, Bathynellidae sp. BES7547 represents a potential SRE, sub-category data deficient.

Acarina

The water mite *Wandesia* sp. is a stygal species which could not be identified to species because the taxonomy of this genus in Western Australia is poorly known and the geographic ranges of the various species have not been determined. All described species of *Wandesia* are known from river interstices in eastern Australia. One known but undescribed species, *Wandesia* sp. P1 (nr *glareosa*), was recorded during the PBS from river pools and springs. It is not known whether the *Wandesia* sp. recorded from the hyporheic zone of YC3 and SS is the same as this known morphotype.

Other species of interest

Other species of interest were recorded from reference sites only and were not found to be present within the Survey Area (i.e. Canthocamptidae sp. B02, *Kinnecaris* sp., Paramelitidae `sp. Biologic-AMPH024`, and *Atopobathynella* sp.). Canthocamptidae sp. B02 is currently undescribed and known previously from Christmas Creek. Species of *Kinnecaris* are rarely collected in the Pilbara and are generally known to be highly locally restricted, i.e. at the scale of a single tributary. *Kinnecaris* sp. was recorded from the hyporheos of reference site Skull Spring in the dry-19. Paramelitidae `sp. Biologic-AMPH024` was identified through molecular analysis (Appendix G). This stygal amphipod species appears to be restricted to Weeli Wolli Creek. While molecular analysis distinguished this species from Paramelitidae `sp. Biologic-AMPH023`, morphological characters are relatively similar and difficult to distinguish currently. *Atopobathynella* sp. recorded from the hyporheos of Skull Springs could not be identified further due to taxonomic limitations within the group but did not appear to match any known Pilbara morphotypes. Many parabathynellid species have been found to be restricted to a single calcrete (Guzik *et al.*, 2008), with more than two-thirds of species having a known range less than 10 km (Bennelongia, 2008).

4.8 Macroinvertebrates

4.8.1 Taxa composition and richness

A total of 151 macroinvertebrate taxa was recorded within the Survey Area, comprising Nematoda (round worms), three gastropod taxa (freshwater snails), seven oligochaete taxa (aquatic segmented worms), two Crustacea (amphipods), 14 Arachnida (water mites), Collembolla (springtails), 36 Coleoptera (beetles), 37 Diptera (two winged flies), seven

Ephemeroptera (mayflies), 21 Hemiptera (true bugs), Lepidoptera (moth larvae), 13 Odonata (dragonflies and damselflies) and eight Trichoptera (caddisflies). See Appendix H for the full taxonomic list.

Of the 151 taxa recorded from Yandicoogina Creek, 76 were singletons and recorded from one site only. More common taxa, recorded from 75% of samples (six or more samples), included the gastropods *Ferrissia petterdi* and *Gyraulus* sp., water mite *Limnesia* sp. 4, beetles *Hydraena* sp., *Anacaena horni* and *Sternolophus marginicollis*, biting midges Ceratopogoninae spp. and *Dasyhelea* spp., non-biting midge larvae *Chironomus* aff. *alternans*, *Larsia* ?*albiceps* and *Paramerina* sp. 1, Stratiomyidae spp., true bugs *Diplonychus eques*, and immature damselfly larvae Zygoptera spp. (imm./dam.).

Macroinvertebrate diversity recorded from each site was generally high. Greatest richness was recorded from YC4 and reference site SS in the dry-19 (72 taxa), and SS and WWS in the wet-20 (66 taxa; Figure 4.10). YC2 consistently recorded the lowest richness, with 33 taxa recorded in the dry-19 and 27 in the wet-20. The low richness at YC2 was influenced by a low diversity of Coleoptera, Hemiptera and Odonata compared to other Yandicoogina Creek sites, as well as a complete lack of *Hydra*, Nematoda, Platyhelminthes, Crustacea, Ephemeroptera and Trichoptera (Figure 4.10). The low richness at this site may have reflected difficulties associated with sampling, due to the high abundance of *Typha* throughout the site, with little open water and limited space with which to kick-sweep sample effectively.

Most sites were dominated by slow flow and relatively tolerant taxa, i.e. Coleoptera and Diptera. Dominance of Diptera within aquatic macroinvertebrate assemblages of the Pilbara is common (see Pinder *et al.,* 2010). Taxa which require faster flows, such as Lepidoptera, leptophlebiid mayflies, Simulidae (Diptera) and *Cheumatopysche* caddisflies (Trichoptera) were generally restricted to the flowing reference sites, including Weeli Wolli Spring, Munjina Spring and Skull Spring (Figure 4.10). Yandicoogina Creek generally recorded a low richness of Trichoptera in comparison to reference sites.

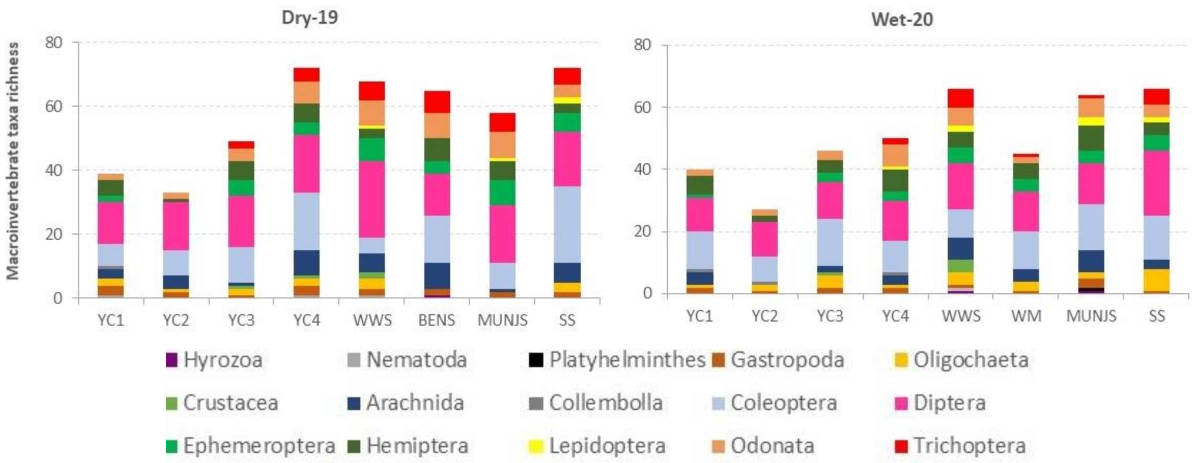


Figure 4.10: Macroinvertebrate taxa richness recorded from each site, in each season.

Macroinvertebrate richness was higher in the dry-19 than wet-20 at all sites except YC1 where one additional taxon was recorded in the wet season. YC4 recorded a considerable decrease in richness between seasons, with 22 fewer taxa recorded in the wet-20 (Figure 4.10).

4.8.2 Conservation significant macroinvertebrate taxa

The vast majority of aquatic macroinvertebrates recorded from the Survey Area were common, ubiquitous species with distributions extending across north Western Australia (3%), Western Australia (4%), Northern Australia (12%), Australia (18%), Australasia (10%) or the world (cosmopolitan species; 4%); however, 10% were endemic to the Pilbara. Pilbara endemic taxa were recorded from all sites, with the greatest number being recorded from WWS in the wet-20 (eight taxa; Figure 4.11). This was closely followed by YC4, which recorded seven Pilbara endemic taxa in the dry-19 (Figure 4.11).

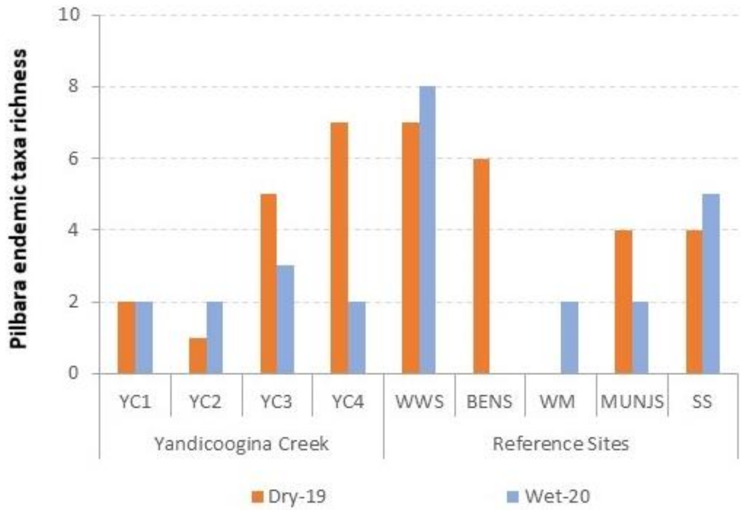


Figure 4.11: Number of Pilbara endemic macroinvertebrate taxa recorded from each site in each season.

Within the Pilbara endemic fauna were five taxa of further interest; two conservation significant species currently listed on the IUCN Redlist of Threatened Species, two short-range endemics and an uncommon species.

Odonata

As mentioned previously, the Pilbara pin damselfly *Eurysticta coolawanyah* is currently listed on the IUCN Redlist as Vulnerable. During the current study, it was recorded from YC4 and a reference site (BENS).

The Pilbara emerald, *Hemicordulia koomina*, is also currently listed on the IUCN Redlist (2020) as Vulnerable. Its listing was based on it being known from only five sites in the Pilbara (Millstream station, Koomina Pools on Tanberry Creek, Palm Pool south of Karratha, Fortescue Crossing, and Millstream Spring). Lowering water levels from groundwater abstraction and climate change were highlighted as a considerable threat to this species. The listing also reported that its distribution is severely fragmented (IUCN, 2020). Like *E. coolawaynah*, the IUCN listing for *H. koomina* was updated fairly recently (2016), but the update did not appear to take into account grey literature records or those recorded during baseline surveys for developments. Including known locations reported in Pinder *et al.,* (2010) and sites known by the authors, *H. koomina* likely occurs at more than 15 sites across the Pilbara. The IUCN listing did indicate that its maximum known extent of occurrence based on was 6,504 km^2 (Dow, 2019b); however, Bush *et al.,* (2014, Table S2) provide an estimate of the current extent of suitable habitat as 119,416 km^2. This species is still considered rare and is infrequently collected and rarely recorded. It was recorded from YC4 and BENS during the current study.

Amphipoda

The amphipods recorded from Yandicoogina Creek surface waters during the current study were stygal and belonged to the same species recorded from the hyporheic zone, *Chydaekata* sp. `E. This species was recorded from surface waters at YC3 and YC4, as well as reference site WWS. It is a potential SRE (sub-category data deficient) known only from Yandicoogina Creek, Marillana Creek, and Upper Weeli Wolli Creek. Immature or damaged paramelitids were also recorded from surface waters within the Survey Area (YC3), which may belong to either *Chydaekata* sp. `E' or Paramelitidae `sp. Biologic-AMPH023` (recorded from hyporheic samples within the Survey Area). The collection of stygal amphipods from surface waters of YC3 and YC4 highlight the strong connection with groundwater through the profile at these sites. An additional species of stygal amphipod was recorded from the WWS reference site; *Maarrka weeliwolli*. This species is a confirmed SRE known only from Marillana and Weeli Wolli Creeks.

Acarina

The *Stygolimnochares* water mite recorded from WWS in the wet-20 is a currently undescribed species from the Pilbara which mostly closely resembles the Queensland species *Stygolimnochares australica*. *Stygolimnochares* nr *australica* is the only known species within this genus in the Pilbara. It appears to be rare and currently known only from Koodaideri Spring and Marillana Creek.

4.8.3 Introduced macroinvertebrate taxa

Only one introduced macroinvertebrate taxon was recorded during the current study, from reference site WWS. The redclaw (*Cherax quadricarinatus*), a species of freshwater crayfish, was recorded during the wet season survey and is discussed further below (section 4.9).

4.8.4 Macroinvertebrate comparison with other studies

Macroinvertebrate richness was compared to other the other aquatic studies undertaken in the area detailed in section 4.2 above (for those studies which sampled more than one replicate site within a creek system). As with the zooplankton data, Weeli Wolli Creek sites were split into Weeli Wolli Spring (recorded from the historic spring area, including Bens Oasis) and Weeli Wolli Creek (upper Weeli Wolli Creek river pools), to reflect differences in water permanence and hydrology between these two areas; factors which would influence aquatic macroinvertebrate assemblages. Weeli Wolli Spring data recorded during the PBS could not be included in the univariate analysis, as only one site was sampled in each season. This was also the case for reference sites MUNJS and SS from the current study. As detailed in the methods, the dataset was amalgamated, and taxonomy aligned, prior to analysis to ensure any differences in taxonomic knowledge between samplers and years was accounted for.

Yandicoogina Creek generally recorded similar average richness to nearby Marillana Creek and Weeli Wolli Creek (Figure 4.12). However, average richness was slightly lower than the highly diverse Weeli Wolli Spring PEC (Figure 4.12). Overall, differences in macroinvertebrate richness were significant between creek (Two-way ANOVA; df = 3, F = 6.35, p = 0.001), but not between season (df = 1, F = 0.13, p = 0.717). There was no significant interaction between creek and season (df = 2, F = 0.76, p = 0.476). The Tukey's post-hoc test indicated that richness recorded from Yandicoogina Creek was statistically similar to Weeli Wolli Creek and Marillana Creek, but significantly lower than Weeli Wolli Spring.

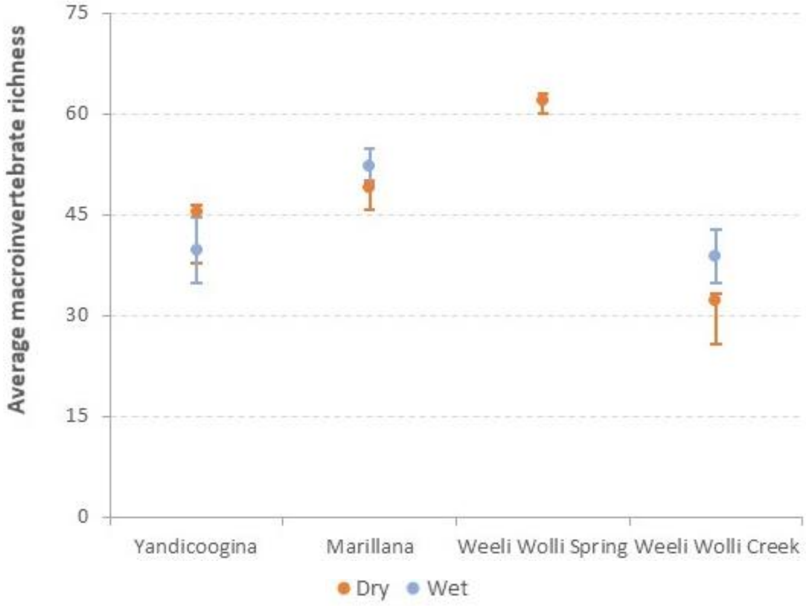


Figure 4.12: Average macroinvertebrate taxa richness (± se) recorded from Yandicoogina Creek, in comparison to other studies and nearby creek systems, in both seasons.

It is important to note that this analysis was influenced by the slightly lower richness recorded from YC1 and YC2, which was likely a reflection of difficulties associated with sampling these sites which were choked with *Typha*. Individual site richness recorded from YC4 (67 taxa in the dry-19) was actually similar to site richness recorded from Weeli Wolli Spring in this (64 taxa in the dry-19) and past surveys (i.e. 67 taxa recorded from BENS in the wet-14, and 69 taxa recorded from WWS in the wet-05).

For multivariate analyses, all data were included, i.e. PBS and current Biologic reference sites at Munjina Creek and Skull Springs were also incorporated into the dataset. Macroinvertebrate assemblages of Yandicoogina Creek formed a relatively tight cluster, and generally separated from other creeks in ordination space (Figure 4.13). The non-spring sites (i.e. Upper Weeli Wolli Creek and Marillana Creek) showed greater within-creek variability that springs/seeps, such as Yandicoogina Creek and Weeli Wolli Springs which displayed high within-creek fidelity. Yandicoogina Creek samples fell closest in ordination space to other springs, such as Munjina

Spring, Weeli Wolli Spring and Skull Springs (Figure 4.13). This indicates a greater similarity with springs, rather than creek pools. Overall, there was a significant difference in macroinvertebrate assemblages between creek (Two-way ANOSIM; R = 0.4, $p < 0.001$), but not between season (R = 0.05, $p = 0.137$).

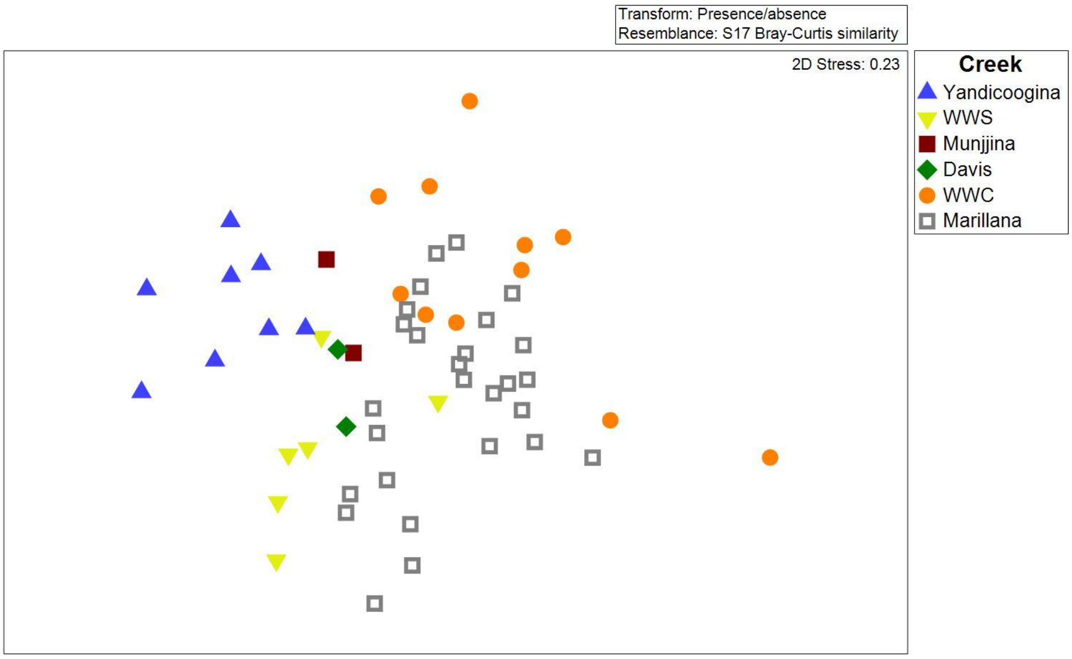


Figure 4.13: nMDS of macroinvertebrate assemblages recorded during the current study, with data from previous studies included. Samples are identified by creek.

4.9 Crayfish

During the wet-20 survey, the invasive redclaw, *Cherax quadricarinatus* (Plate 4.2), was recorded from reference site WWS. Two juveniles (18 mm carapace length; CL) were recorded in the macroinvertebrate sample and a further eight adults approximately 60 mm CL were observed inhabiting the main pools of the WWS sampling site. This suggests a healthy breeding population is present. The fact that no redclaw were recorded from this site in the dry-19 suggests the introduction is fairly recent, and that the population has established rapidly.

Plate 4.2: Introduced redclaw, *Cherax quadricarinatus*, recorded at WWS in the wet-20 (photo by Biologic ©).

4.10 Fish

4.10.1 Species composition and richness

Three freshwater fish species were recorded from the Survey Area; the western rainbowfish *Melanotaenia australis* (Melanotaeniidae), Pilbara tandan *Neosilurus* sp.[11] (Plotosidae) and spangled perch *Leiopotherapon unicolor* (Terapontidae). Two additional species were recorded from the Skull Springs reference site; Pilbara bony bream *Nematalosa* sp. (Clupeidae) and oxeye herring/tarpon *Megalops cyprinoides* (Megalopidae).

All species considered likely to populate the Survey Area were successfully recorded. No introduced species were recorded or are currently known from the Survey Area.

4.10.2 Abundance

A total of 204 individual freshwater fish were recorded from the Survey Area; 49 in the dry-19 and 155 in the wet-20. During the wet season, reference site WWS recorded the greatest abundance (246 individual fish), followed by Skull Springs (217) and YC4 (99; Figure 4.14). No fish were recorded from YC2 or reference site Munjina Spring MUNJS. Of the sites which recorded fish, the lowest abundance was recorded from YC1 in the dry-19 (one individual rainbowfish observed). Diversity was greatest at SS in the wet-20, with five species recorded.

Western rainbowfish was the most widespread and abundant species recorded within the Survey Area, and in fact across the entire study. A total of 143 individuals recorded in the dry-19 and 337 individuals in the wet-20 (across all sites). Spangled perch were the next most common species within the Survey Area, with 69 individuals recorded in the wet-20. Across the entire study, a total of 156 spangled perch individuals were recorded in the dry-19 and 299 individuals in the wet-20 (Figure 4.14). Pilbara tandan were the least abundant and widespread

[11] The *Neosilurus* catfish known from the Pilbara is genetically distinct to the described species *Neosilurus hyrtlii* (Unmack 2013). The Pilbara species is currently known as *Neosilurus* sp. until further taxonomic work has been undertaken and descriptions have been made.

species recorded in the current study. In the dry-19, they were recorded in relatively low abundance from WWS (11 individuals) and BENS (one individual), and during the wet-20 from YC4 (two), WWS (18) and SS (11 individuals; Figure 4.14).

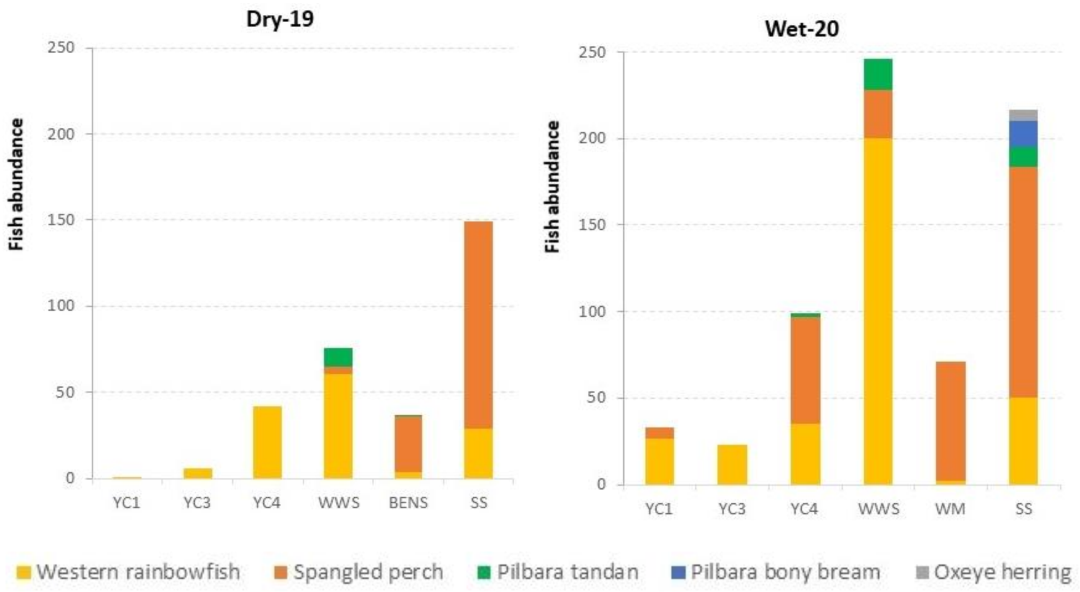


Figure 4.14: Abundance of each freshwater fish species recorded from each site.

4.10.3 Conservation significant fish species

Despite the low diversity of freshwater fishes in the Pilbara, the region does support high endemicity (56%; Morgan *et al.,* 2014). During the current study, 40% of fish species recorded were endemic to the Pilbara. Endemics included the Pilbara tandan and Pilbara bony bream. Although restricted to the region, both species are common and widespread. Neither are listed or of conservation significance.

The Fortescue grunter *Leiopotherapon aheneus* has a restricted distribution within the Pilbara and is known only from the Fortescue, Ashburton, and Robe rivers (Allen *et al.,* 2002). It is currently listed as a Priority 4 (P4) species on the DBCA Threatened and Priority Fauna Species List (DBCA, 2019b) and Endangered on the IUCN Redlist of Threatened Species (IUCN, 2019). The latter listing was recently revised and upgraded from Near Threatened (IUCN, 2019). The estimated extent of occurrence for the Fortescue grunter is 37,155 km^2, but the population is severely fragmented and a continuing decline in the number of mature individuals was noted in the listing (IUCN, 2019). Morgan *et al.,* (2009) reported that upper pools on the Fortescue River, especially Hamersley Gorge and Fern Pool in Karijini NP, are important refuges for the species. Major threats to the Fortescue grunter are considered to include livestock and the pastoral industry, mining, fire and fire suppression, and invasive species (IUCN, 2019). *L. aheneus* was not recorded in the current study and is not considered likely to occur within the Study Area. Within the Fortescue River, the most eastern extent of its distribution appears to be Fortescue Falls, although in a desktop review, FMG (2009) suggested that it may occur in the Fortescue Marsh.

4.10.4 Length-frequency analysis

The seasonal, yet unpredictable nature of rainfall and streamflow in the Pilbara is reflected in the opportunistic and periodic reproductive strategies of Pilbara freshwater fish (Beesley, 2006). Most species breed during the wet season, a time when new recruits and juveniles have the greatest chance of survival owing to the greater persistence of water/habitat, increased ecosystem productivity, and availability of food resources. Larvae have only a short window, usually in the order of a few days, with which to locate food or risk starving.

Analysis of population structure and age-classes present provides a way of characterising recruitment, the health of local fish assemblages, and therefore the environmental conditions present which can support or impede recruitment. Length-frequency analysis was undertaken for all fish species which were recorded in sufficient abundance. As few oxeye herring were recorded, and only from one reference site, this species was excluded from further analysis.

Western rainbowfish

Western rainbowfish have multiple spawning events throughout the year which take advantage of the intermittent rainfall and streamflow characteristic of the Pilbara (Beesley 2006). Maximum size is generally around 110 mm TL[12] (Morgan *et al.* 2002). Size at first maturity varies between river systems and sex, but for the purposes of this study is considered to be 50 mm SL. New recruits accounted for 30% and 35% of western rainbows recorded in the dry-19 and wet-20, respectively. The presence of relatively high abundances of new recruits and juveniles within Yandicoogina Creek suggests good levels of breeding and recruitment within the Survey Area (Figure 4.15). There was a general increase in abundance for all size classes during the wet-20, particularly at WWS.

Spangled perch

Spangled perch breed during the wet season, between late November and March (Beesley, 2006), with spawning generally coinciding with flooding events (Morgan *et al.*, 2002). Several spawning events will occur over the wet season (Beesley, 2006). Maturity is attained after the first year, at around 58 mm TL for males and 78 mm TL for females. To allow for field determination of age-class (without knowing sex), size at maturity was considered to be 70 mm SL for the purposes of this study. Maximum size is ~ 300 mm TL. During the current study, greatest proportions of spangled perch recorded in the dry-19 were sub-adults (50%), followed by adults and juveniles in wet-20 (40%; Figure 4.16). New recruits and juveniles accounted for 40% of spangled perch recorded across all sites in the wet-20. Spangled perch recorded from YC1 within the Survey Area predominantly comprised new recruits and juveniles, with some sub-adults and adults also present. At YC4, all spangled perch recorded were adults.

[12] Measurements of TL (total length) include the tail.

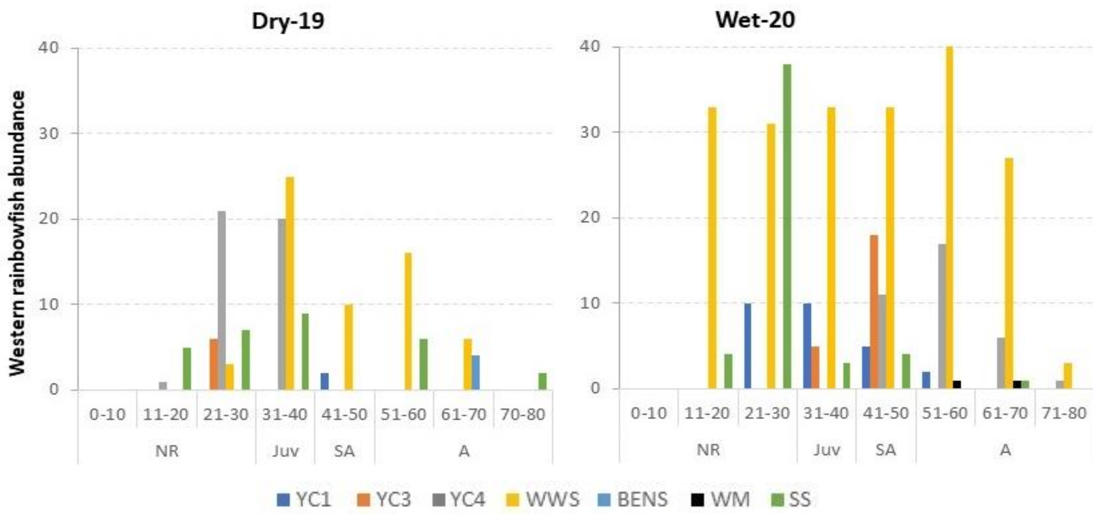


Figure 4.15. Length-frequency analysis for western rainbowfish.

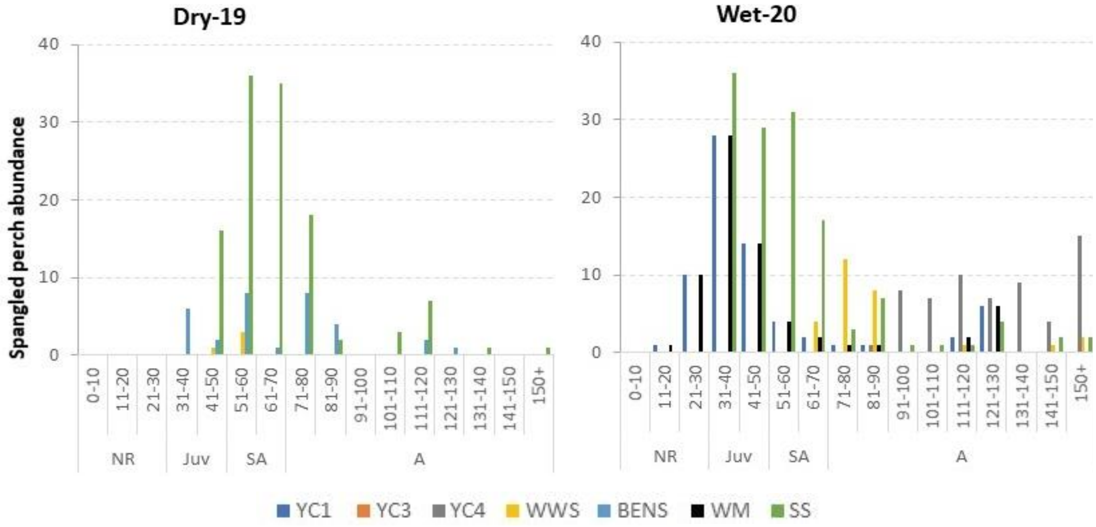


Figure 4.16: Length frequency analysis for spangled perch.

Pilbara tandan

As it is a relatively new, undescribed species, the breeding ecology of the Pilbara tandan is unknown; however, information relating to congeneric species may provide some insight. In northern populations of the closely related *Neosilurus hyrtlii,* breeding occurs early in the wet season in shallow, sandy/gravelly areas of the upper reaches of creeks (Allen *et al.*, 2002b) and fecundity ranges from 1,600 to 15,300 eggs (Orr & Milward, 1984). While other eel-tailed catfish, such as *Tandanus tandanus*, construct a unique nest into which eggs are spawned (Burndred *et al.*, 2017), the available evidence suggests that *N. hyrtlii* simply scatter fertilised eggs over the substrate (Orr & Milward, 1984). Sexual maturity in *N.hyrtlii* is attained at around 90 mm SL and they reach a maximum size of 400 mm TL (Bishop *et al.*, 2001). During a reconnaissance of the Survey Area undertaken in May 2019, Pilbara tandan were observed at a number of locations between YC2 and YC4. They are likely to occur throughout the seep area despite only being recorded from YC4, in low abundance during the wet-20. Both Pilbara tandan recorded from YC4 were adults (Figure 4.17). No new recruits were recorded from any site, and juveniles were only recorded from WWS in the wet-20 (Figure 4.17).

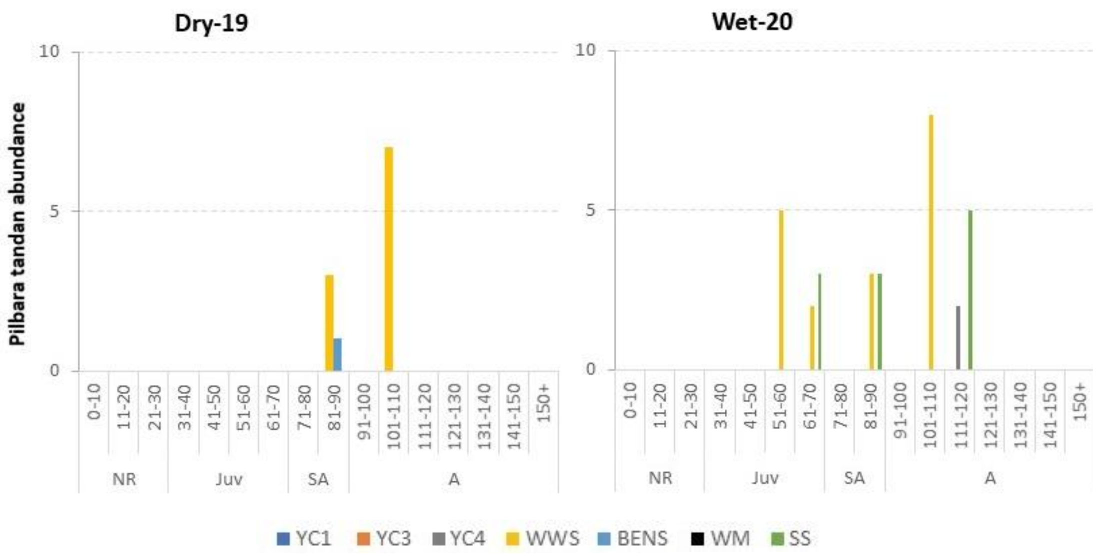


Figure 4.17: Length frequency analysis for Pilbara tandan.

<u>**Other freshwater fish species**</u>

Other freshwater fish species were recorded in the wet-20, from reference site Skull Springs; the Pilbara bony bream (*Nematalosa* sp.) and oxeye herring (*Megalops cyprinoides*). Nothing is known of breeding and life history characteristics of the Pilbara bony bream as this species is relatively new to science and remains undescribed; however, breeding in the closely related *Nematalosa erebi* may provide some indication of likely biology in the Pilbara species. *N. erebi* is highly fecund, with females producing up to 880,000 eggs, depending on fish size. Breeding is thought to be independent of flooding, with the species able to breed year-round (Puckridge & Walker, 1990); however, Morgan *et al.,* (2004) suggested that in the Pilbara, bony bream may have a protracted spawning period. In the Murray River (South Australia), *N. erebi* breeds during summer when temperatures are highest (Puckridge & Walker, 1990). Sexual maturity is attained at approximately 144 mm TL for males and 180 mm TL for females (Puckridge & Walker, 1990). A total of 15 individual Pilbara bony bream was recorded from Skull Springs, ranging in size from 50 mm to 268 mm SL.

Oxeye herring is an estuarine species which utilise freshwater systems as nursery grounds because they are relatively safe and protected habitats (Morgan & Gill, 2004). They can spend extensive periods, if not years, in the river, well beyond their juvenile stage. Maximum size tends to be around 500 mm for individuals spending their life in freshwaters. Seven oxeye herring were recorded from Skull Springs, ranging from 252 mm to 303 mm SL).

4.11 Other vertebrate fauna

Other vertebrate fauna recorded over the course of this study included the Pilbara toadlet (*Uperoleia saxatilis*) from reference site MUNJS in the dry-19, and the Pilbara olive python (*Liasis olivaceus barroni*) from reference site WWS during the reconnaissance survey in May 2019.

The Pilbara toadlet is endemic to the Pilbara region, where it occurs in rocky habitats, such as that of Munjina Spring. The species was described in 2011 when genetic analysis indicated it was distinct from its congener *Uperoleia russeli*, which is now known to be restricted to the Gascoyne region (Catullo *et al.*, 2011). Although endemic to the Pilbara, *U. saxatilis* is fairly common among rock landscapes surrounding creeks across the region and is often observed following cyclonic rains. It is not listed or considered to be of conservation significance.

The Pilbara olive python is also restricted to the Pilbara region and can be found in gorges, waterholes and on escarpments. It is currently listed as Vulnerable on both Federal (EPBC Act) and State (BC Act) conservation lists. Threats to their habitat include fire, foxes, and development of mining infrastructure. One Pilbara olive python individual was recorded from Weeli Wolli Spring only and was not observed within Yandicoogina Creek.

5 DISCUSSION

5.1 Habitat assessment

Numerous permanent and semi-permanent pool and riffle sequences occur along the length of the Survey Area. Within the pools sampled, in-stream habitat diversity was high and comprised a variety of complex, heterogenous structures with which to support aquatic fauna, including submerged and emergent macrophytes, large woody debris (LWD), root mats, detritus, and trailing vegetation. Given the consistent presence of surface water, there was little seasonal change in habitat.

5.2 Water quality

Water quality of Yandicoogina Creek was generally good and characterised by fresh, clear waters, with low dissolved oxygen saturation, neutral pH, and generally low nitrogen nutrient and dissolved metals concentrations. While all sites within Yandicoogina Creek recorded EC in excess of the default ANZECC & ARMCANZ (2000) GV, none were considered to pose a threat to aquatic life. Generally, sites with EC less than 1,500 µS/cm experience little ecological stress, but a considerable shift in aquatic fauna assemblages is known to occur above this threshold. While many Pilbara waters have wide ranging EC with large temporal and seasonal variability, sites sampled in the current study had notably low levels of seasonal variation. This likely reflects the permanent nature of the spring sites sampled, with more ephemeral waters displaying large seasonal variations due to waters receding in the drier months and evapo-concentration of ions leading to increased EC in the dry season.

Dissolved oxygen (DO) concentrations were generally in the lower range. Although oxygen needs of aquatic biota differ between species and life history stage, studies have reported DO less than 50% can lead to chronic responses in fish and macroinvertebrates, through reduced fecundity, decreased feeding activity, slowed larval and juvenile growth, suppressed emergence, impaired swimming ability, and death (Connolly *et al.*, 2004; Flint *et al.*, 2015). For six tropical, northern Australian fish species, of which one congener occurs in the Yandicoogina Creek (rainbowfish), acute toxicity was reported to be between 25% and 30% (Butler & Burrows, 2007). Therefore, the point of ecological stress for DO was considered to be 30% for the purposes of this study. As such, the aquatic biota of YC2 (in both seasons) and YC4 (in the wet-20) may be adversely affected by the low DO levels, if these levels are maintained over long periods. DO saturation recorded from these sites was likely related to the decay of algae and organic matter surrounding the abundant *Typha* beds, with bacteria consuming oxygen in the water as part of this process.

Ionic composition, along with the lack of seasonal change in ionic dominance at Yandicoogina Creek sites, suggested surface waters of Yandicoogina Creek are strongly influenced by a connection to groundwater. The dominance of calcium (Ca) and hydrogen carbonate (HCO_3) in surface waters, such as was recorded from sites within the Survey Area, often indicates connection to groundwater, while sodium (Na) dominance tends to indicate contribution by rainfall and evapo-concentration effects.

Nutrient concentrations within Yandicoogina Creek exceeded the DGVs for protection against eutrophication for nitrogen oxides (N_NOx), total nitrogen (total N) and total phosphorous (total P). The eutrophication DGV is designed to protect aquatic ecosystems from the effects of nuisance algal and macrophyte growth. Excessive plant growth can physically smother aquatic invertebrates, as well as deplete oxygen in the water, due to increased biological oxygen demand as plants decay and are decomposed by bacteria. The relationship between nitrate-enrichment and enhanced algal growth in freshwaters is well documented, often resulting in very high density/abundance but low species richness (Camargo & Alonso, 2006; Wagenhoff *et al.*, 2011). While the idea that phosphorus (as FRP or total P) is the primary limiting factor for algal growth in freshwaters has been challenged as too simplistic (Beck & Hall, 2018; Elser *et al.*, 2007; Muhid & Burford, 2012), the fact that both total N and total P are enriched in surface waters of Yandicoogina Creek, suggests there is a risk of eutrophication from additional nutrient inputs.

While dissolved metal concentrations were generally low, dB exceeded the 99% toxicity DGV at all Survey Area sites and most reference sites (except WM). The seemingly high dB concentrations recorded in the current study are not atypical for Pilbara surface waters, with many pools and springs commonly recording values within the range seen here. The ANZECC DGVs are perhaps too conservative for freshwater ecosystems of the region. In addition to dB, dFe exceeded the interim indicative working level provided in the ANZECC & ARMCANZ (2000) guidelines at only one site; YC2 in the dry-19.

5.3 Wetland Flora

A total of six macrophyte taxa were recorded from Yandicoogina Creek, including three emergent macrophyte taxa (*Cyperus vaginatus*, *Schoenoplectus subulatus* and *Typha domingensis*) and three submerged macrophytes (*Chara* spp., *Vallisneria nana* and *Ruppia* spp.). Other riparian vegetation taxa were also recorded including various *Eucalyptus*, *Melaleuca*, and *Acacia* species, as well as herbs, shrubs and grasses.

Groundwater Dependent Ecosystems (GDEs) and their associated vegetation is dependent on the presence of groundwater to meet some, or all, of their water requirements, either through surface expression or subsurface presence of groundwater (Hatton & Evans, 1998). The presence of specific phreatophytic (groundwater dependent) flora taxa indicates dependence of such vegetation on surface and/or subsurface groundwater, which in turn indicates water permanence and potential significance of the system, especially for those not associated with large river or drainage systems (Rio Tinto, 2018). All emergent macrophytic flora taxa recorded during the survey are considered phreatophytic and are known indicators of high and consistent moisture availability. In addition, the obligate phreatophyte *Melaleuca argentea* and facultative phreatophyte *Eucalyptus camaldulensis* were recorded at all Yandicoogina Creek sites, indicating that the area is a GDE.

5.4 Zooplankton

A total of 35 zooplankton taxa were recorded from Yandicoogina Creek, and included protists, rotifers, copepods and Cladocera. In general, richness recorded from Yandicoogina Creek was comparable to, if not slightly higher than, reference sites. Across all sites sampled, there was considerable seasonal variation in zooplankton richness. Reference site WWS recorded both the lowest richness in the wet season, but the highest in the dry. The disparity between seasons was largely due to the complete lack of rotifers, Cladocera and ostracods in the wet season. Being planktonic, zooplankton are highly responsive to increases in flow and flooding events, such as that which occurred in January 2020. Flooding associated with wet season cyclonic rainfall likely flushed zooplankton taxa downstream, with the population yet to fully re-establish. Within the Survey Area, YC3 also recorded a large seasonal variation in zooplankton taxa richness, but in this case, richness was higher following the wet season flooding event, indicating emergences and colonisation following rainfall.

Zooplankton richness recorded during the current study was compared to previous surveys undertaken in nearby creek systems. Results indicated that overall, there was no significant difference in zooplankton richness between creeks or between seasons. This was likely due to the high variability in zooplankton richness, within a creek system, within a season, as evidenced by the large standard error bars. Zooplankton are known to be patchily distributed, with notably high spatial and temporal variability (Klais *et al.*, 2016; Zhang *et al.*, 2019). Interestingly, variability within Yandicoogina Creek was noticeably lower than other creek systems, in both seasons. Generally, average wet season zooplankton richness recorded from the Survey Area was slightly lower than that recorded from Marillana and Weeli Wolli creeks, but average dry season richness from the Survey Area was greater. The semi-permanent and ephemeral river pools in Upper Weeli Wolli Creek recorded high average zooplankton richness in the wet season, but low in the dry. Permanent waters tend to have lower zooplankton diversity than ephemeral systems, especially of the more permanent residents which require a period of desiccation to complete their life cycle.

5.5 Hyporheos fauna

A total of 108 invertebrate taxa was recorded from hyporheic zones within Yandicoogina Creek. Of these, a total of 13% are directly dependent on groundwater for their persistence (12% stygobites and 1% permanent hyporheos stygophiles). The percentage of stygobitic taxa recorded was considerably greater than that reported previously for Pilbara hyporheic zones (i.e. only 5% stygobitic fauna recorded in Halse *et al.* 2002), further highlighting the strong groundwater connection within this reach of Yandicoogina Creek.

Across all sites sampled, those within the Survey Area recorded notably high richness of hyporheos taxa (including occasional stygophiles and possible hyporheic taxa). For example, YC5H recorded 18 hyporheos taxa, and YC7H, YC8H and YC9H all recorded 14 taxa. Only one site recorded greater hyporheos richness and this was reference site SS (20 taxa). The high hyporheos richness at these sites suggests a strong connection to groundwaters. YC2 yielded no stygobitic taxa, likely due to the dense *Typha* stands present at this site, which impeded access to the hyporheos.

Several Potential SRE species were recorded from the hyporheos of Yandicoogina Creek (all stygal), including:

- The ostracod *Meridiescandona facies* (YC1 and YC9H) - known from Weeli Wolli Creek and the central and eastern Fortescue (and now Yandicoogina Creek).
- The ostracod *Gomphodella yandii* (YC7H) – known only from Weeli Wolli Creek, Marillana Creek, and now Yandicoogina Creek.
- The ostracod *Gomphodella alexanderi* (YC7H and YC8H) - known only from Marillana Creek, groundwater bores at Yandi, and now Yandicoogina Creek.
- The amphipod *Chydaekata* sp. \`E\` (YC3, YC5H, YC7H, YC9H, and reference site WWS) - previously known and appears to be restricted to Marillana Creek, Upper Weeli Wolli Creek, and now Yandicoogina Creek.
- The amphipod Paramelitidae \`sp. Biologic-AMPH023\` (YC1, YC3, YC5H, and YC9H) – previously known only from Marillana Creek.
- The isopod *Pygolabis weeliwolli* (YC3, YC4, YC5H, YC7H, and YC9H) – known only from Weeli Wolli Creek, Marillana Creek, Yandicoogina Creek (now), and bores within the Yandicoogina tenement.
- The syncarid Bathynellidae sp. BES7547 (YC9H) – new, undescribed genus, currently known only from the Survey Area.

5.6 Macroinvertebrates

A total of 151 macroinvertebrate taxa was recorded within the Survey Area, comprising Nematoda, Gastropoda, Oligochaeta, Crustacea, Arachnida, Collembolla, Coleoptera, Diptera, Ephemeroptera, Hemiptera, Lepidoptera, Odonata, and Trichoptera. The greatest macroinvertebrate richness was recorded from YC4 and reference site SS in the dry-19 (72 taxa), and reference sites SS and WWS in the wet-20 (66 taxa). YC2 consistently recorded the lowest richness, likely due to difficulties associated with sampling, owing to the high abundance of *Typha* throughout the site, with little open water and limited space with which to kick-sweep effectively.

Macroinvertebrate richness was compared statistically to previous aquatic surveys undertaken in the area. Overall, there was a significant difference in macroinvertebrate richness between creek, but not between seasons. Richness recorded from Yandicoogina Creek was statistically similar to Weeli Wolli Creek and Marillana Creek, but significantly lower than Weeli Wolli Spring. It is important to note that this analysis was influenced by the slightly lower richness recorded from YC1 and YC2, which was likely a reflection of difficulties associated with sampling these sites. Individual site richness recorded from YC4 (67 taxa in the dry-19) was actually similar to site richness recorded from Weeli Wolli Spring in this (64 taxa in the dry-19) and past surveys (i.e. 67 taxa recorded from BENS in the wet-14, and 69 taxa recorded from WWS in the wet-05).

Multivariate analyses on the same dataset of current and previous surveys indicated that non-spring sites (i.e. Upper Weeli Wolli Creek and Marillana Creek) had greater within-creek variability that springs/seeps, such as Yandicoogina Creek and Weeli Wolli Springs. Assemblages from Yandicoogina Creek were found to be most similar to other springs, such as Munjina Spring, Weeli Wolli Spring and Skull Springs. This indicates a greater affinity with springs, rather than creek pools.

While most aquatic macroinvertebrates recorded from the Survey Area were common, ubiquitous species, several species were of conservation significance, including:

- the Pilbara pin damselfly *Eurysticta coolawanyah* (YC4 and a reference site BENS) - Vulnerable on the IUCN Redlist.
- the Pilbara emerald, *Hemicordulia koomina* (YC4 and a reference site BENS) - Vulnerable on the IUCN Redlist.
- the stygal Potential SRE amphipod *Chydaekata* sp. `E. (surface waters of YC3 and YC4, and reference site WWS) - known only from Yandicoogina Creek, Marillana Creek, and Upper Weeli Wolli Creek.

While no introduced macroinvertebrate taxa were recorded from the Survey Area, the introduced redclaw, *Cherax quadricarinatus* (a species of freshwater crayfish) was recorded from reference site WWS in the wet-20.

5.7 Crayfish

Juveniles and adults of both sexes were recorded from Weeli Wolli Spring in the wet-20, suggesting a healthy breeding population is present. The fact that no redclaw were recorded from this site in the dry-19 suggests the introduction is fairly recent, and that the population has established rapidly.

Redclaw are native to tropical Queensland and the Northern Territory within Australia, and south-eastern Papua New Guinea. They have since been translocated broadly, and within Western Australia has been known from the Ord River system in the Kimberley, and the Harding Dam in the Pilbara. More recently, however, several additional records have been noted for the Pilbara, including the Fortescue River in Karijini National Park, George River within Millstream-Chichester National Park, and Weelamurra Ck, a tributary of the lower Fortescue River.

The short term impacts of introduced crayfish have been widely reported in the literature and include habitat modification (Gherardi *et al.*, 2011), alteration to food webs, changes in nutrient and energy flow (Nyström *et al.*, 1999), introduction of disease, increased competition for limiting resources (Lynas *et al.*, 2006; Lynas *et al.*, 2007) and increased predation. Although there are no native crayfish species in the Pilbara, these impacts would still be considerable, especially on high conservation value aquatic ecosystems such as the Weeli Wolli Spring PEC. Some of the impacts to aquatic systems in the Pilbara have included changes to invertebrate assemblages and reduction in submerged macrophyte cover (Pinder *et al.*, 2019). Long term impacts of introduced crayfish include the possible decline of invertebrate taxa, amphibians and fish (Gherardi, 2007), and the potential to induce irreparable shifts in species diversity (Hobbs *et al.*, 1989). Given Weeli Wolli Spring has high ecological value and currently listed as a PEC, any attempts to remove redclaw would be beneficial to the ecosystem. Since the record of this invasive species at WWS in the wet-20, BHP WAIO commissioned Biologic to undertake a redclaw control program, the results of which were reported separately (Biologic, 2020b).

5.8 Fish

A total of 204 individual freshwater fish from three species were recorded from Yandicoogina Creek, including western rainbowfish, spangled perch and Pilbara tandan. The low richness recorded during the current study is not unexpected given the fish fauna of the Pilbara is known to be characterised by low species diversity due to the region's aridity (Allen *et al.*, 2002a; Masini, 1988; Morgan *et al.*, 2014). Greatest freshwater fish diversity in the region is reported from relatively clear, permanent and semi-permanent pools, as was the case in the current study, with all five species recorded at Skull Springs (Davis River) in the wet season. Wet season rains and flooding likely improved connectivity throughout the upper Davis River catchment. All species considered likely to populate the Survey Area were successfully recorded. No introduced species were recorded or are currently known from the Survey Area.

A greater abundance of fish was recorded from Yandicoogina Creek during the wet season, likely due to flooding throughout these creeklines following cyclonic rains, and increased connection with upstream systems. While YC2 recorded no fish, this likely reflected the inability to sample effectively given the shallow water depths and high abundance of *Typha* choking the channel throughout this site. Fish records from YC1 and YC3 were obtained by visual observation due to difficulties associated with sampling throughout the seep area. No fish were recorded from reference site Munjina Spring (MUNJS) either, but this was likely due to the lack of connection with other systems and/or location upstream of waterfalls which would impede dispersal.

No conservation significant fish species were recorded from the Survey Area. Although the Pilbara tandan is restricted to the region, it is relatively common and widespread. The Priority 4 (P4) listed Fortescue grunter *Leiopotherapon aheneus* is not considered likely to occur within the Survey Area.

The presence of relatively high abundances of western rainbowfish new recruits and juveniles within Yandicoogina Creek suggests good levels of breeding and recruitment. Spangled perch age-classes recorded from the Survey Area generally indicated good wet season recruitment at YC1, as well as across reference sites more broadly.

Pilbara tandan are a cryptic, benthic species and more difficult to catch using traditional fish sampling methods. As such, they were the least abundant and widespread species recorded. Despite appearing to be restricted to the large permanent pool within the Survey Area (YC4), Pilbara tandan were observed elsewhere throughout the Survey Area during the reconnaissance trip in May 2019. Both Pilbara tandan recorded from YC4 were adults.

6 CONCLUSION

6.1 Water quality and habitats

Yandicoogina Creek within the Survey Area comprises an open to closed *Eucalyptus camaldulensis* and *Melaleuca argentea* woodland over *Acacia tumida* var. *pilbarensis* shrubland, with reeds and sedges along the waterline. *Typha domingensis* was particularly dense in some parts of the Survey Area, including YC1, YC2 and YC3. Weeds were present throughout the Survey Area, though no other disturbances were apparent. Riparian vegetation was in good condition, with a number of groundwater dependant flora taxa recorded.

In-stream habitat diversity was high and comprised a variety of complex, heterogenous structures with which to support aquatic fauna, including submerged and emergent macrophytes, large woody debris (LWD), root mats, detritus, and trailing vegetation. Given the consistent presence of surface water, there was little seasonal change in habitat.

Water quality of Yandicoogina Creek was generally good and characterised by fresh, clear waters, with low dissolved oxygen saturation, neutral pH, and generally low nitrogen nutrient and dissolved metals concentrations. While water quality was generally within default ANZECC & ARMCANZ (2000) guidelines for the protection of lowland river systems of tropical north Australia, there were some exceedances. These included:

- DO – YC2 (both seasons) and YC4 (wet-20) recorded insufficient DO, below the lower default GV and below the limit of ecological stress (i.e. < 30%).
- N_NOx – YC1 and YC2 recorded nitrogen oxide concentrations in excess of the eutrophication DGV in the dry-19. Elevated N_NOx was also recorded from reference sites in the dry-19 (MUNJS) and wet-20 (WWS, MUNJS and SS).
- Total N – YC2 (dry-19) and YC3 (wet-20) recorded total N in excess of the eutrophication DGV. While total N also exceeded DGVs at reference sites (WM and MUNJS), the concentration recorded from YC2 in the dry-19 was particularly high, exceeding the eutrophication DGV by more than seven times.
- Total P – was high and exceeded the eutrophication DGV at all Yandicoogina Creek and reference sites. Concentrations from YC2 were notably high.
- dB – the 99% toxicity DGV was exceeded at all sites except WM. Elevated dB is commonly reported in surface waters of the Pilbara.
- dFe – YC2 (dry-19) exceeded the interim indicative working level provided in the ANECC/ARMCANZ (2000) guidelines.

Unlike many pools in the Pilbara, there was little seasonal variation in EC. This likely reflects the permanent nature of the spring/seep sites sampled, with more ephemeral waters displaying large seasonal variations due to waters receding in the drier months and evapo-concentration of ions. Ionic composition of surface waters at Yandicoogina Creek also indicated connection to groundwaters (dominance of calcium cations and hydrogen carbonate anions).

6.2 Wetland Flora

The vegetation found to occur in association with this reach of Yandicoogina Creek comprised GDVs, including both obligate (*Eucalyptus camaldulensis*) and facultative phreatophytes (*Melaleuca argentea*), as well as reeds and sedges (*Cyperus vaginatus*, *Schoenoplectus subulatus* and *Typha domingensis*) along the waterline, and submerged macrophytes in-stream (*Chara* spp., *Vallisneria nana* and *Ruppia* spp.).

6.3 Aquatic Fauna

Yandicoogina Creek within the Survey Area was found to support a diverse range of aquatic fauna, including 250 invertebrate taxa[13] and three freshwater fish species. Two sites (YC3 and YC4) were found to be of particularly high ecological value. These sites generally recorded high macroinvertebrate diversity, a high richness of hyporheos fauna, and high Pilbara endemic taxa richness. In addition, SRE stygal species were recorded from the hyporheic zone throughout the Survey Area, as well as surface waters of some pools. The high hyporheos richness, and presence of stygal species within surface waters, suggests a strong connection to groundwater throughout the hydrological profile. In comparisons with previous surveys from nearby creeklines, the macroinvertebrate assemblages of Yandicoogina Creek had a greater affinity with other spring assemblages, rather than creek pools.

While most of the taxa recorded from the Survey Area are generally common and ubiquitous across the Pilbara, a number are of conservation significance, and are either locally restricted or rarely collected (Table 6.1).

All freshwater fish species likely to populate the Survey Area were recorded, including the western rainbowfish *Melanotaenia australis* (Melanotaeniidae), Pilbara tandan *Neosilurus* sp. (Plotosidae), and spangled perch *Leiopotherapon unicolor* (Terapontidae). None are of conservation significance. The presence of western rainbowfish new recruits and juveniles within Yandicoogina Creek suggests good levels of breeding and recruitment within the Survey Area. Spangled perch breeding and recruitment was also evident at YC1.

[13] The total invertebrate richness includes taxa recorded in zooplankton, hyporheic and macroinvertebrate samples.

Table 6.1: Conservation significant taxa recorded from Yandicoogina Creek during the current survey.

Type	Species	Sites Recorded		
		Within Survey Area	Reference Sites	Conservation significance
Stygal ostracod	*Meridiescandona facies*	YC1 (hyporheos) YC9H (hyporheos)		SRE
	Gomphodella yandii	YC7H (hyporheos)		SRE known only from Weeli Wolli Creek, Marillana Creek and now Yandicoogina Creek
	Gomphodella alexanderi	YC7H (hyporheos) YC8H (hyporheos)		SRE known only from Marillana Creek and now Yandicoogina Creek, as well as groundwater bores at Yandi.
Syncarida	Bathynelllidae sp. BES7547	YC9H (hyporheos)		New genus. Not previously known.
Stygal amphipod	*Chydaekata* sp. \`E\`	YC3 (hyporheos & surface waters) YC4 (surface waters) YC5H (hyporheos) YC7H (hyporheos) YC9H (hyporheos)	WWS (hyporheos & surface waters)	SRE known only from Marillana Creek and Upper Weeli Wolli Creek
	Paramelitidae 'sp. Biologic-AMPH023'	YC1 (hyporheos) YC3 (hyporheos) YC5H (hyporheos) YC9H (hyporheos)	WWS (hyporheos)	SRE known only from Marillana Creek and now Yandicoogina Creek
Stygal isopod	*Pygolabis weeliwolli*	YC3 (hyporheos) YC4 (hyporheos) YC5H (hyporheos) YC7H (hyporheos) YC9H (hyporheos)		SRE, range restricted to Weeli Wolli Creek and Marillana Creek and groundwater bores within the Yandicoogina tenement
Water mite	*Wandesia* sp.	YC3 (hyporheos) YC6H (hyporheos)	SS (hyporheos)	Species identification unknown, may be uncommon, with a disjunct distribution in the Pilbara
Damselfly	*Eurysticta coolawanyah*	YC4	BENS	Vulnerable IUCN Redlist
Dragonfly	*Hemicordulia koomina*	YC4	BENS	Vulnerable IUCN Redlist

6.4 Final remarks

This study represents the first aquatic ecosystem survey undertaken in Yandicoogina Creek. Results from this survey provide an assessment of the ecological values and health of aquatic systems within the Survey Area. Yandicoogina Creek was found to support a GDE of high ecological value, characterised by mature stands of the obligate phreatophyte *Melaleuca argentea* and facultative phreatophyte *Eucalyptus camaldulensis*, with no obvious signs of canopy decline. A diversity of other mesic species was also recorded in close association with the creek, such as *Cyperus vaginatus*, *Schoenoplectus subulatus* and *Typha domingensis*. The presence of phreatophytes, and more specifically, the obligate phreatophyte *Melaleuca argentea*, suggests groundwater is persistently at or just below the surface. This is further supported by the presence of numerous permanent and semi-permanent pools and riffle sequences, which occur along the length of the Survey Area. These pools provide important habitat for aquatic fauna and a resource for terrestrial invertebrate and vertebrate species. The current study found that four of these pools support; aquatic invertebrates with restricted distributions that would be classified as potential SREs (i.e. *Chydaekata* sp. E, immature or damaged Paramelitidae sp.); a high diversity of Pilbara endemic aquatic invertebrate taxa; conservation listed species (*Eurysticta coolawanyah* and *Hemicordulia koomina*); and three species of freshwater fish. Additionally, hyporheic zones within the Survey Area supported potential SREs, including *Chydaekata* sp. E, Paramelitidae `sp. Biologic-AMPH023`, *Pygolabis weeliwolli*, *Meridiescandona facies*, *Gomphodella yandii*, *Gomphodella alexanderi*, and Bathynellidae sp. BES7547. These important ecological values are supported by the high in-stream habitat diversity and heterogeneity characteristic of the system, as well as the strong connection to groundwater in this area.

Due to the aridity of the Pilbara, rivers of the region tend to be ephemeral. Streamflow is highly seasonal and variable, and generally occurs over the summer months in response to cyclonic events and thunderstorms. As such, permanent water sources in the region are relatively scarce and restricted to springs and permanent pools. Such predictable sources of water have high conservation importance as they support richer faunas than ephemeral water-bodies and provide a refuge for many species during drought (Halse *et al.*, 2002; Kay *et al.*, 1999). This is the case in the current study, with one permanent pool in the Survey Area in particular found to support a notably high diversity of aquatic invertebrates, comparable to the Weeli Wolli Spring PEC and Skull Springs. Permanent pools are also known to provide an important source of animals for colonisation of newly flooded pools and maintenance of invertebrate species at the regional level (Halse *et al.*, 2002). Permanent springs in shaded gorges and river beds support a suite of mesic-adapted species that are otherwise rare in the region.

For riverine pools to be termed GDEs they must have demonstrated long-term connectivity to the groundwater and be maintained by groundwater discharge during drought periods. GDEs are those parts of the environment, the species composition, and natural ecological processes that are dependent on the permanent or temporary presence or influence of groundwater

(Murray *et al.*, 2003). A number of physical and ecological elements highlight the close connection to groundwaters within the Survey Area. These include:

- the presence of GDVs such as *Melaleuca argentea* and *Eucalyptus camaldulensis*;
- the relatively stable surface water levels between seasons (despite the dry-19 following on from a particularly dry period and the high flood event which occurred prior to the wet 2020 survey);
- stable electrical conductivity in surface waters between seasons, with little evidence of evapoconcentration effects associated with pool drying in the dry season;
- ionic composition dominated by calcium carbonate, similar to other spring systems of the Pilbara;
- the presence of stygofauna throughout the surface water pools; and
- the macroinvertebrate assemblages having a greater affinity with other spring assemblages, rather than creek pools (determined through multivariate analysis with the PBS data).

As such, the stretch of Yandicoogina Creek encompassing the Survey Area should likely be considered an aquatic GDE holding considerable importance in the region.

7 REFERENCES

Ahlers, W. W., Reid, M. R., Kim, J. P., & Hunter, K. A. (1990). Contamination-free sample collection and handling protocols for trace elements in natural fresh waters. *Australian Journal of Marine and Freshwater Research, 41*, 713-720. doi:https://doi.org/10.1071/MF9900713

ALA, Atlas of Living Australia. (2020). Atlas of Living Australia; Occurrence search (custom search). http://www.ala.org.au/

Allen, G. R., Midgley, S. H., & Allen, M. (2002a). *Field Guide to the Freshwater Fishes of Australia*. Melbourne, Australia: CSIRO Publishing.

Allen, G. R., Midgley, S. H., & Allen, M. (2002b). *Field guide to the freshwater fishes of Australia* (Vol. 1st). Melbourne, Australia: CSIRO Publishing.

ANZECC, Australian and New Zealand Environment and Conservation Council, & ARMCANZ, Agriculture and Resource Management Council of Australia and New Zealand. (2000). *Australian and New Zealand Guidelines for Fresh and Marine Water Quality. Australia and New Zealand Environment and Conservation Council and the Agriculture and Resource Management Council of Australia and New Zealand*. Canberra: http://www.deh.gov.au/water/quality/nwqms/index.html

ANZG. (2018). Australian and New Zealand Guidelines for Fresh and Marine Water Quality. Retrieved from www.waterquality.gov.au/anz-guidelines

Bastin, G. (2008). *Rangelands 2008 - Taking the Pulse*. Canberra: National Land and Water Resources Audit.

Batley, G. E. (1989). Collection, preparation and storage of samples for speciation analysis. In G. E. Batley (Ed.), *Trace Element Speciation: Analytical Methods and Problems* (pp. 1-24). Boca Raton: CRC Press, Inc.

Beck, W. S., & Hall, E. K. (2018). Confounding factors in algal phosphorus limitation experiments. *PLoS ONE, 13*.

Beesley, L. (2006). *Environmental stability: Its role in structuring fish communities and life history strategies in the Fortescue River, Western Australia.* (Unpublished PhD Thesis), University of Western Australia,

Bennelongia, Environmental Consultants. (2008). *Subterranean fauna sampling at Balmoral South Iron Ore Project and adjacent areas*. 2008/42.

BHP, Billiton Iron Ore. (2017). *Aquatic Fauna Assessment Methods Procedure*. Perth, W.A.:

BHP, Billiton Iron Ore. (2018). *Biological survey spatial data requirements*. Unpublished manuscript prepared by BHP Billiton.

Biologic. (2020a). *Ministers North Miscellaneous Licence Area Amendment Surveys, Sanders Seep and Yandicoogina Creek Detailed Flora and Vegetation Assessment*. East Perth:

Biologic. (2020b). *Redclaw Control Program at Weeli Wolli Spring*. Unpublished report to BHP Western Australian Iron Ore.

Biota, Environmental Sciences. (2010). *Yandicoogina subterranean fauna assessment phases I - V*. Perth:

Bishop, K. A., Allen, S. A., Plooard, D. A., & Cook, M. G. (2001). *Ecological studies of the freshwater fishes of the Alligator Rivers*

region, Northern Territory: Autecology. Darwin:

BoM, Bureau of Meteorology. (2019). Climate Data Online. Retrieved 2019 http://www.bom.gov.au./climate/data/index.shtml

BoM, Bureau of Meteorology. (2020). Groundwater dependent ecosystems atlas.

Bou, C. (1974). Les méthodes de recolte dans les eaux souterraines interstitielles. *Annales de Spéologie, 29*(4).

Boulton, A. J. (2001). Twixt two worlds: taxonomic and functional biodiversity at the surface water/groundwater interface. *Records of the Western Australian Museum Supplement, 64*, 1-13.

Boulton, A. J. (2014). Conservation of ephemeral streams and their ecosystem services: What are we missing? *Aquatic Conservation, 24*, 733-738.

Bray, J. R., & Curtis, J. T. (1957). An ordination of the upland forest communities of Southern Wisconsin. *Ecological Monographs, 27*(4), 325-349.

Bressler, D., Stribling, J., Paul, M., & HIcks, M. (2006). Stressor tolerance values for benthic macroinvertebrates in Mississippi. *Hydrobiologia, 573*, 155-172.

Brunke, M., & Gonser, T. (1997). The ecological significance of exchange processes between rivers and groundwater. *Freshwater Biology, 37*, 1-33.

Bruno, M. C., Bottazzi, E., & Rossetti, G. (2012). Downward, upstream or downstream? Assessment of meio- and macrofaunal colonization patterns in a gravel-bed stream using artificial substrates. *Annales de Limnologie - International Journal of Limnology, 48*, 371-381.

Burndred, K. R., Cockayne, B. J., & Lou, D. C. (2017). Early development of eel-tailed catfish, Tandanus tandanus (Mitchell) (Teleostei : Plotosidae), with validation of daily otolith increment formation. *Australian Journal of Zoology, 65*, 12-20.

Butler, B., & Burrows, D. W. (2007). *Dissolved oxygen guidelines for freshwater habitats of Northern Australia*. ACTFR Report No. 07/32. Townsville, QLD:

Cain, D. J., Luoma, S. N., Carter, J. L., & Fend, S. V. (1992). Aquatic insects as bioindicators of trace element contamination in cobble-bottom rivers and streams. *Canadian Journal of Fisheries and Aquatic Sciences, 49*, 2141-2154.

Camargo, J., & Alonso, A. (2006). Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assesssment. *Environment International, 32*, 831-849.

Canton, S. P., & Chadwick, J. W. (2000). Distribution of the subterranean amphipod *Stygobromus* in central Colorado streams, with notes on the interstitial community. *Western North American Naturalist, 60*, 130-138.

Carew, M. E., Pettigrove, V., Cox, R. L., & Hoffman, A. A. (2007). The response of Chironomidae to sediment pollution and other environmental characteristics in urban wetlands. *Freshwater Biology, 52*, 2444-2462.

Catullo, R. A., Doughty, P., Roberts, J. D., & Keogh, J. S. (2011). Multi-locus phylogeny and taxonomic revision of *Uperoleia* toadlets (Anura: Myobatrachidae) from the western arid zone of Australia, with a description of a new species. *Zootaxa, 2902*, 1-43.

Chappuis, P. A. (1942). Eine neue Methode zur Untersuchung der Grundwasser-fauna. *Acta Scientiarum Mathematicarum et Naturalium, 6*, 1-7.

Choy, S., & Thompson, P. (1995). River Bioassessment and Monitoring using Benthic Macroinvertebrate Community Structure:

The Monitoring River Health Initiative. In H. M. Hunter, A. G. Eyles, & G. E. Rayment (Eds.), *Downstream Effects of Landuse* (pp. 53-55). Queensland: Department of Natural Resources.

Clarke, K. R., & Gorley, R. N. (2015). *PRIMER v7: User Manual/Tutorial*. Plymouth, United Kingdom: Primer.

Coe, H. J. (2001). *Distribution patterns of hyporheic fauna in a riparian floodplain terrace, Queets River, Washington.* (MSc thesis), University of Washington,

Connolly, N. M., Crossland, M. R., & Pearson, R. G. (2004). Effect of low dissolved oxygen on survival, emergence, and drift of tropical stream macroinvertebrates. *Journal of the North American Benthological Society, 23*, 251-270.

Cooling, M. P., & Boulton, A. J. (1993). Aspects of the hyporheic zone below the terminus of a south Australian arid-zone stream. *Australian Journal of Marine and Freshwater Research, 44*, 411-426.

DBCA, Department of Biodiversity, Conservation and Attractions. (2019). Threatened and Priority Fauna Database (custom search). Retrieved 2019 http://www.dpaw.wa.gov.au/plants-and-animals/threatened-species-and-communities/threatened-animals

DBCA, Department of Biodiversity, Conservation and Attractions. (2020). NatureMap: Mapping Western Australia's Biodiversity (custom search). http://naturemap.dec.wa.gov.au./default.aspx

Dole-Olivier, M. J., & Marmonier, P. (1992a). Effects of spates on the vertical distribution of the interstitial community. *Hydrobiologia, 230*, 49-61.

Dole-Olivier, M. J., & Marmonier, P. (1992b). Patch distribution of interstitial communities: prevailing factors. *Freshwater Biology, 27*, 177-191.

Doody, T. M., Barron, O. V., Dowsley, K., Emelyanova, I., Fawcett, J., Overton, I. C., . . . Warren, G. (2017). Continental mapping of groundwater dependent ecosystems: A methodological framework to integrate diverse data and expert opinion. *Journal of Hydrology: Regional Studies, 10*, 61-81.

DWER, Department of Water, Environment and Regulation,. (2019). Water Information Reporting (custom search). http://www.water.wa.gov.au/maps-and-data/monitoring/water-information-reporting

DWER, Department of Water, Environment and Regulation,. (2020). Water Information Reporting (custom search). http://www.water.wa.gov.au/maps-and-data/monitoring/water-information-reporting

Eamus, D., Fu, B., Springer, A. E., & Stevens, L. E. (2016). Groundwater dependent ecosystems: Classification, identification techniques and threats. In A. J. Jakeman, O. Barreteau, R. J. Hunt, J. D. Rinaudo, & A. Ross (Eds.), *Integrated Groundwater Management: Concepts, Approaches and Challenges* (pp. 313-346). Cham: Springer International Publishing.

Edwards, R. T. (1998). The hyporheic zone. In R. J. Naiman, R. E. Bilby, & S. Kantor (Eds.), *River Ecology and Management: Lessons from the Pacific Coastal Ecoregion*. New York, United States of America: Springer-Verlag.

Elser, J. J., Bracken, M. E. S., & Cleland, E. E. (2007). Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine, and terrestrial ecosystems. *Ecology Letters, 10*, 1135-1142.

Environment Australia. (2001). *A Directory of Important Wetlands in Australia* (Third Edition ed.). Canberra Australian Capital Territory: Environment Australia.

EPA, Environmental Protection Authority. (2018). *Environmental Factor Guideline: Inland Waters*. http://www.epa.wa.gov.au/sites/default/files/Policies_and_Guidance/Guideline-Inland-Waters

Flint, N., Crossland, M. R., & Pearson, R. G. (2015). Sublethal effects of fluctuating hypoxia on juvenile tropical Australian freshwater fish. *Marine and Freshwater Research, 66*, 293-304.

Gherardi, F. (2007). Understanding the impact of invasive crayfish. In F. Gherardi (Ed.), *Biological Invaders in Inland Waters: Profiles, Distribution and Threats*: Springer.

Gherardi, F., Aquiloni, L., Dieguez-Uribeondo, J., & Tricarico, E. (2011). Managing invasive crayfish: is there hope? *Aquatic Sciences, 73*, 185-200.

Gibson, L. A., Williams, K. J., Pinder, A. M., Harwood, T. D., McKenzie, N. L., Ferrier, S., . . . Manion, G. (2015). Compositional patterns in terrestrial fauna and wetland flora and fauna across the Pilbara biogeographic region of Western Australia and the representativeness of its conservation reserve system. *Records of the Western Australian Museum, 78*, 515–545. doi:http://doi.og/10.18195/issn.0313-122x.78(2).2015.515-545

Guzik, M. T., Abrams, K. M., Cooper, S. J. B., Humphreys, W. F., Cho, J.-L., & Austin, A. D. (2008). Phylogeography of the ancient Parabathynellidae (Crustacea: Bathynellacea) from the Yilgarn region of Western Australia. *Invertebrate Systematics, 22*, 205-216.

Halse, S. A., Scanlon, M. D., & Cocking, J. S. (2002). *Do springs provide a window to the ground water fauna of the Australian arid zone?* Paper presented at the Balancing the ground water budget: Proceedings of an International Ground water Conference, Darwin.

Halse, S. A., Scanlon, M. D., Cocking, J. S., Barron, H. J., Richardson, J. B., & Eberhard, S. (2014). Pilbara stygofauna: deep groundwater of an arid landscape contains globally significant radiation of biodiversity. *Records of the Western Australian Museum, Supplement, 78*, 443-483.

Harvey, M. S. (2002). Short range endemism in the Australian fauna: some examples from non-marine environments. *Invertebrate Systematics, 16*, 555-570.

Hatton, T., & Evans, R. (1998). *Dependence of ecosystems on groundwater and its significance to Australia*. Occasional Paper No. 12/98, Canberra.

Hobbs, H. H., Jass, J. P., & Hunter, J. V. (1989). A review of global crayfish introductions with particular emphasis on two North American species (Decapoda, Cambaridae). *Crustaceana, 56*, 299-316.

Hodkinson, I. D., & Jackson, J. K. (2005). Terrestrial and aquatic invertebrates as bioindicators for environmental monitoring, with particular reference to mountain ecosystems. *Environmental Management, 35*(5), 649-666.

Hose, G. C., Jones, P., & Lim, R. P. (2005). Hyporheic macroinvertebrates in riffle and pool areas of temporary streams in south-eastern Australia. *Hydrobiologia, 532*, 81-90.

Jacobi, G. Z., & Cary, S. J. (1996). Winter stoneflies (Plecoptera) in seasonal habitats in New Mexico, USA. *Journal of the North American Benthological Society, 15*(4), 690-699.

Karaman, S. (1935). Die Fauna der unterirdischen Gewässer Jugoslaviens. *Internationale Vereinigung für Theoretische und Angewandte Limnologie: Verhandlungen, 7*, 46-73.

Karanovic, I. (2007). *Candonidae (Ostracoda) from the Pilbara region in Western Australia* (Vol. 7): Brill.

Karanovic, I., & Humphreys, W. F. (2014). Phylogeny and diversity of Timiriaseviinae ostracods (Podocopida, Cytheroidea) with description of one new species from arid Western Australia. *Systematics and Biodiversity, 12*, 93-110.

Kay, W. R., Smith, M. J., Pinder, A. M., McRae, J. M., Davis, J. A., & Halse, S. A. (1999). Patterns of distribution of macroinvertebrate families in rivers of north-western Australia. *Freshwater Biology, 41*, 299-316.

Kendrick, P. (2001). Pilbara 3 (PIL3 - Hamersley subregion). In J. May & N. McKenzie (Eds.), *A Biodiversity Audit of Western Australia's 53 Biogeographical Subregions in 2002* (pp. 568-580). Kensington, Western Australia: Department of Conservation and Land Management.

Klais, R., Lehtiniemi, M., Rubene, G., Semenova, A., Margonski, P., Ikauniece, A., . . . Ojaveer, H. (2016). Spatial and temporal variability of zooplankton in a temperate semi-enclosed sea: implications for monitoring design and long-term studies. *Journal of Plankton Research, 38*(3), 652-661.

Leigh, C., Stubbington, R., Sheldon, F., & Boulton, A. J. (2013). Hyporheic invertebrates as bioindicators of ecological health in temporary rivers: A meta-analysis. *Ecological Indicators, 32*, 62-73.

Lynas, J., Storey, A. W., Armstrong , K., Prince, J., & Knott, B. (2006). Invasion by the exotic crayfish, *Cherax destructor* Clark (Parastacidae), into habitats of local crayfish near Perth, Western Australia. *Freshwater Crayfish, 15*, 176-188.

Lynas, J., Storey, A. W., & Knott, B. (2007). Aggressive interactions between three species of freshwater crayfish of the genus Cherax (Decapoda: Parastacidae): potential for competitive exclusion. *Marine and Freshwater Behaviour and Physiology, 40*, 105-116.

Madrid, Y., & Zayas, Z. P. (2007). Water sampling: Traditional methods and new approches in water sampling strategy. *Trends in Analytical Chemistry, 26*(4), 293-299.

Masini, R. J. (1988). *Inland waters of the Pilbara, Western Australia (Part 1)*. Perth, WA:

Moldovan, O. T., Meleg, I. N., Levei, E., & Terente, M. (2013). A simple method for assessing biotic indicators and predicting biodiversity in the hyporheic zone of a river polluted with metals. *Ecological Indicators, 24*, 412-420.

Morgan, D., Allen, M., Bedford, P., & Horstman, M. (2002). *Inland fish fauna of the Fitzroy River Western Australia (including the Bunuba, Gooniyandi, Ngarinyin, Nyikina, and Walmajarri names)*. Unpublished report to the Natural Heritage Trust.

Morgan, D. L., Allen, M. G., Beatty, S. J., Ebner, B. C., & Keleher, J. J. (2014). *A Field Guide to the Freshwater Fishes of Western Australia's Pilbara province*. Maddington, Western Australia: Printsmart Online.

Morgan, D. L., & Gill, H. S. (2004). Fish fauna in inland waters of the Pilbara (Indian Ocean) Drainage Division of Western Australia – evidence for three subprovinces. *Zootaxa, 636*, 1-43.

Muhid, P., & Burford, M. A. (2012). Assessing nutrient limitation in a subtropical reservoir. *Inland Waters, 2*, 185-192.

Murray, B. B. R., Zeppel, M. J. B., Hose, G. C., & Eamus, D. (2003). Groundwater-dependent ecosystems in Australia: It's more than just water for rivers. *Ecological Management & Restoration, 4*(2), 110-113. doi:10.1046/j.1442-8903.2003.00144.x

NIWA. (2013). *Updating nitrate toxicity effects on freshwater aquatic species*. HAM2013-009. Hamilton, New Zealand:

Nyström, P., Brönmark, C., & Granëli, W. (1999). Influence of an exotic and a native crayfish species on a littoral benthic community. *Oikos, 85*, 545-553.

Orr, T. M., & Milward, N. E. (1984). Reproduction and development of Neosilurus ater and

Neosilurus hyrtlii in a tropical Queensland stream. *Marine and Freshwater Research, 35*, 187-195.

Pacioglu, O., & Moldovan, O. T. (2016). Response of invertebrates from the hyporheic zone of chalk rivers to eutrophication and land use. *Environmental Science and Pollution Research, 23*, 4729–4740.

Palmer, M. A., Bely, A. E., & Berg, K. E. (1992). Response of invertebrates to lotic disturbance: a test of the hyporheic refuge hypothesis. *Oecologia, 89*, 182-194.

Pinder, A., Harman, A., Bird, C., Quinlan, K. L., Angel, F., Cowan, M., . . . Thillainath, E. (2019). Spread of non-native redclaw crayfish Cherax quadricarinatus (von Martens, 1868) into natural waters of the Pilbara region of Western Australia, with observations on potential adverse ecological effects. *Bioinvasions Records, 8*, 882-897.

Pinder, A. M., Halse, S. A., Shiel, R. J., & McRae, J. M. (2010). An arid zone awash with diversity: patterns in the distribution of aquatic invertebrates in the Pilbara region of Western Australia. *Records of the Western Australian Museum*, 205-246.

Puckridge, J. T., & Walker, K. F. (1990). Reproductive biology and larval development of a gizzard shad *Nematalosa* erebi (Dorosomatinae: Teleostei) in the River Murray, South Australia. *Australian Journal of Marine and Freshwater Research, 41*, 695-712.

Ramchandra, T. V., Rishiram, R., & Karthik, B. (2006). *Zooplankton as bioindicators: hydrobiological investigations in selected Bangalore lakes*. Bangalore:

Rio Tinto, Rio Tinto Iron Ore. (2018). *Assessment of Groundwater Dependent Vegetation distribution on the Robe River - Targeted Riparian Vegetation Survey - Stage 1. High confidence mapping of the distribution of Obligate and Facultative Phreatophytic Vegetation between Mesa A and East Deepdale deposits on the Robe River*. Unpublished report by Rio Tinto Iron Ore.

Strayer, D., & Bannon-O'Donnell, E. (1988). Aquatic Microannelids (Oligochaeta & Aphanoneura) of Underground Waters of Southeastern New York. *American Midland Naturalist, 119*, 327-335.

Streamtec. (2004). *Aquatic Ecosystem Surveys of the Upper Fortescue River Catchment, Final Report.*

Thackway, R., & Cresswell, I. D. (1995). *An Interim Biogeographic Regionalisation for Australia: A Framework for Setting Priorities in the National Reserves System Cooperative Program*. Canberra: Australian Nature Conservation Agency.

Unmack, P. J. (2013). Biogeography. In P. Humphries & K. Walker (Eds.), *Ecology of Australian Freshwater Fishes* (pp. 25-48). Collingwood: CSIRO Publishing.

van Etten, E. J. B. (2009). Inter-annual rainfall variability of arid Australia: Greater than elsewhere? *Australian Geographer, 40*(1), 109-120.

Wagenhoff, A., Townsend, C. R., Phillips, N., & Matthaei, C. D. (2011). Subsidy-stress and multiple-stressor effects along gradients of deposited fine sediment and dissolved nutrients in a regional set of streams and rivers *Freshwater Biology, 56*, 1916-1936.

WAM, Western Australian Museum. (2019a). Arachnid and Myriapod Collection Database (custom search). Retrieved 2019 http://www.museum.wa.gov.au

WAM, Western Australian Museum. (2019b). Crustacea Collection Database (custom search). Retrieved 2019 http://www.museum.wa.gov.au

WAM, Western Australian Museum. (2019c). Mollusc Collection Database (custom search). Retrieved 2019 http://www.museum.wa.gov.au

Warne, M. S., Batley, G. E., van Dam, R. A., Chapman, J. C., Fox, D. R., Hickey, C. W., & Stauber, J. L. (2018). *Revised Method for Deriving Australian and New Zealand Water Quality Guidelines Values for Toxicants*. Canberra:

Western Australian Herbarium. (1998–). FloraBase—the Western Australian Flora. Retrieved from https://florabase.dpaw.wa.gov.au/

WRM, Wetland Research & Management. (2013a). *Jinidi: Baseline Aquatic Surveys at Weeli Wolli Creek. Wet 2011, Dry 2011 and Wet 2012 sampling. Final Report*.

WRM, Wetland Research & Management. (2013b). *Lake Robinson Aquatic Invertebrate Fauna and Water Quality Surveys. Wet seasons of 2011 and 2012. Final Report*.

WRM, Wetland Research & Management. (2015). *Yandi Aquatic Fauna Survey. Wet & Dry Season Sampling 2014*.

WRM, Wetland Research & Management. (2018). *Yandi: Marillana Creek Aquatic Fauna Survey. Wet & Dry Sampling*.

Zhang, K., Jiang, F., Chen, H., Dibar, D. T., Wu, Q., & Zhou, Z. (2019). Temporal and spatial variations in zooplankton communities in relation to environmental factors in four floodplain lakes located in the middle reach of the Yangtze River, China. *Environmental Pollution, 251*, 277-284. doi:https://doi.org/10.1016/j.envpol.2019.04.139

APPENDICES

Appendix A: Conservation Status Codes

International Union for Conservation of Nature

Category	Definition
Extinct (EX)	A taxon is Extinct when there is no reasonable doubt that the last individual has died. A taxon is presumed Extinct when exhaustive surveys in known and/or expected habitat, at appropriate times (diurnal, seasonal, annual), throughout its historic range have failed to record an individual. Surveys should be over a time frame appropriate to the taxon's life cycle and life form.
Extinct in the Wild (EW)	A taxon is Extinct in the Wild when it is known only to survive in cultivation, in captivity or as a naturalized population (or populations) well outside the past range. A taxon is presumed Extinct in the Wild when exhaustive surveys in known and/or expected habitat, at appropriate times (diurnal, seasonal, annual), throughout its historic range have failed to record an individual. Surveys should be over a time frame appropriate to the taxon's life cycle and life form.
Critically Endangered (CR)	A taxon is Critically Endangered when the best available evidence indicates that it meets any of the criteria A to E for Critically Endangered (see Section V), and it is therefore considered to be facing an extremely high risk of extinction in the wild.
Endangered (EN)	A taxon is Endangered when the best available evidence indicates that it meets any of the criteria A to E for Endangered (see Section V), and it is therefore considered to be facing a very high risk of extinction in the wild.
Vulnerable (VU)	A taxon is Vulnerable when the best available evidence indicates that it meets any of the criteria A to E for Vulnerable (see Section V), and it is therefore considered to be facing a high risk of extinction in the wild.
Near Threatened (NT)	A taxon is Near Threatened when it has been evaluated against the criteria but does not qualify for Critically Endangered, Endangered or Vulnerable now, but is close to qualifying for or is likely to qualify for a threatened category in the near future
Data Deficient (DD)	A taxon is Data Deficient when there is inadequate information to make a direct, or indirect, assessment of its risk of extinction based on its distribution and/or population status. A taxon in this category may be well studied, and its biology well known, but appropriate data on abundance and/or distribution are lacking. Data Deficient is therefore not a category of threat. Listing of taxa in this category indicates that more information is required and acknowledges the possibility that future research will show that threatened classification is appropriate. It is important to make positive use of whatever data are available. In many cases, great care should be exercised in choosing between DD and a threatened status. If the range of a taxon is suspected to be relatively circumscribed, and a considerable period of time has elapsed since the last record of the taxon, threatened status may well be justified.

Environment Protection and Biodiversity Conservation Act 1999

Category	Definition
Extinct (EX)	Taxa not definitely located in the wild during the past 50 years.
Extinct in the Wild (EW)	Taxa known to survive only in captivity.
Critically Endangered (CE)	Taxa facing an extremely high risk of extinction in the wild in the immediate future.
Endangered (EN)	Taxa facing a very high risk of extinction in the wild in the near future.
Vulnerable (VU)	Taxa facing a high risk of extinction in the wild in the medium-term future.
Migratory (MG)	Consists of species listed under the following International Conventions: Japan-Australia Migratory Bird Agreement (JAMBA) China-Australia Migratory Bird Agreement (CAMBA) Convention on the Conservation of Migratory Species of Wild animals (Bonn Convention)

Biodiversity Conservation Act 2016

Category	Definition
CR	Rare or likely to become extinct, as *critically endangered* fauna.
EN	Rare or likely to become extinct, as *endangered* fauna.
VU	Rare or likely to become extinct, as *vulnerable* fauna.
EX	Being fauna that is presumed to be extinct.
MI	Birds that are subject to international agreements relating to the protection of migratory birds.
CD	Special conservation need being species dependent on ongoing conservation intervention. (Conservation Dependant)
OS	In need of special protection, otherwise than for the reasons pertaining to Schedule 1 through to Schedule 6 Fauna. (Other specially protected species

Department of Biodiversity, Conservation and Attractions Priority codes

Category	Definition
Priority 1 (P1)	Taxa with few, poorly known populations on threatened lands.
Priority 2 (P2)	Taxa with few, poorly known populations on conservation lands; or taxa with several, poorly known populations not on conservation lands.
Priority 3 (P3)	Taxa with several, poorly known populations, some on conservation lands.
Priority 4 (P4)	Taxa in need of monitoring. Taxa which are considered to have been adequately surveyed, or for which sufficient knowledge is available, and which are considered not currently threatened or in need of special protection but could be if present circumstances change.

Appendix B: Default ANZECC & ARMCANZ (2000) water quality guidelines

Default trigger values for some physical and chemical stressors for tropical Australia for slightly disturbed ecosystems (TP = total phosphorus; FRP = filterable reactive phosphorus; TN = total nitrogen; NOx = total nitrates/nitrites; NH4+ = ammonium). Data derived from trigger values supplied by Australian states and territories, for the Northern Territory and regions north of Carnarvon in the west and Rockhampton in the east (ANZECC & ARMCANZ, 2000).

Aquatic Ecosystem	Analyte						
	TP mg/L	FRP mg/L	TN mg/L	NOx mg/L	NH_4^+ mg/L	DO % saturation[f]	pH
Upland River[e]	0.01	0.005	0.15	0.03	0.006	90-120	6.0-7.5
Lowland River[e]	0.01	0.004	0.2-0.3[h]	0.01[b]	0.01	85-120	6.0-8.0
Lakes	0.01	0.005	0.35[c]	0.01[b]	0.01	90-120	6.0-8.0
Wetlands[3]	0.01-0.05[g]	0.05-0.025[g]	0.35-1.2[g]	0.01	0.01	90[b]-120[b]	6.0-8.0

b = Northern Territory values are 0.005mg/L for NO_x, and < 80 (lower limit) and >110% saturation (upper limit) for DO;

c = this value represents turbid lakes only. Clear lakes have much lower values;

e = no data available for tropical WA estuaries or rivers. A precautionary approach should be adopted when applying default trigger values to these systems;

f = dissolved oxygen values were derived from daytime measurements. Dissolved oxygen concentrations may vary diurnally and with depth. Monitoring programs should assess this potential variability;

g = higher values are indicative of tropical WA river pools;

h = lower values from rivers draining rainforest catchments.

Default trigger values for salinity and turbidity for the protection of aquatic ecosystems, applicable to tropical systems in Australia (ANZECC & ARMCANZ, 2000).

Salinity	(µs/cm)	Comments
Aquatic Ecosystem		
Upland & lowland rivers	20-250	Conductivity in upland streams will vary depending on catchment geology. The first flush may result in temporarily high values
Lakes, reservoirs & wetlands	90-900	Higher conductivities will occur during summer when water levels are
Turbidity	**(NTU)**	
Aquatic Ecosystem		
Upland & lowland rivers	2-15	Can depend on degree of catchment modification and seasonal
Lakes, reservoirs & wetlands	2-200	Most deep lakes have low turbidity. However, shallow lakes have higher turbidity naturally due to wind-induced re-suspension of sediments. Wetlands vary greatly in turbidity depending on the general condition of the catchment, recent flow events and the water

Guideline values for toxicants at alternative levels of protection (in mg/L). Values in grey shading are applicable to typical *slightly-moderately disturbed systems* (ANZECC & ARMCANZ, 2000).

Chemical			Guideline values for freshwater mg/L Level of protection (% species)			
			99%	95%	90%	80%
Metals and metalloids						
Aluminium	pH > 6.5		0.027	0.055	0.08	0.15
Aluminium	pH < 6.5		ID	ID	ID	ID
Arsenic (As III)			0.001	0.024	0.094^C	0.36^C
Arsenic (AsV)			0.0008	0.013	0.042	0.14^C
Boron			0.09	0.37^C	0.68^C	1.3^C
Cadmium		H	0.00006	0.0002	0.0004	0.0008^C
Chromium (Cr III)		H	ID	ID	ID	ID
Chromium (Cr IV)			0.00001	0.001^C	0.006^A	0.04^A
Cobalt			ID	ID	ID	ID
Copper		H	0.001	0.0014	0.0018^C	0.0025^C
Iron		G	ID	ID	ID	ID
Lead		H	0.001	0.0034	0.0056	0.0094^C
Manganese			1.2	1.9^C	2.5^C	3.6^C
Mercury (inorganic)		B	0.00006	0.0006	0.0019^C	0.0054^A
Mercury (methyl)			ID	ID	ID	ID
Molybdenum			ID	ID	ID	ID
Nickel		H	0.008	0.011	0.013	0.017^C
Selenium (Total)		B	0.005	0.011	0.018	0.034
Selenium (SeIV)		B	ID	ID	ID	ID
Uranium			ID	ID	ID	ID
Vanadium			ID	ID	ID	ID
Zinc		H	0.0024	0.008^C	0.015^C	0.031^C
Non-metallic inorganics						
Ammonia		D	0.32	0.9^C	1.43^A	2.3^A
Chlorine		E	0.0004	0.003	0.006^A	0.013^A
Nitrate		J	1.0	2.4	3.4^C	17^A

Notes:

Most guideline values listed here for metals and metalloids are *High Reliability* figures, derived from field or chronic NOEC data (see 3.4.2.3). The exceptions are *Moderate Reliability* for freshwater aluminium (ph>6.5) and manganese.

Most non-metallic inorganics are *Moderate Reliability* figures, derived from acute LC50 data (see section 3.4.2.3). The exception is *High Reliability* for freshwater ammonia

A = Figure may not protect key test species from acute toxicity (and chronic) (Section 8.3.4.4)

B = Chemicals for which possible bioaccumulation and secondary poisoning effects should be considered (see Sections 8.3.3.4 and 8.3.5.7

C = Figure may not protect key test species from chronic toxicity (this refers to experimental chronic figures or geometric mean for species) - check Section 8.3.7 for spread of data and its significance.

D = Ammonia as TOTAL ammonia as [NH_3_N] at pH 8. For changes in trigger value with pH refer to Section 8.3.7.2

E = Chlorine as Total Chlorine, as [Cl]; see Section 8.3.7.2

F = Figures protect against toxicity and do not relate to eutrophication issues. Refer to Section 3.3 if eutrophication is a concern.

G = There were insufficient data to derive a reliable guideline value for iron. The current Canadian guideline level is 0.3 mg/L which could be used as an interim working level. However, further data are required to establish a figure appropriate for Australian and New Zealand waters.

H = Chemicals for which algorithms have been provided in table 3.4.3 to account for the effects of hardness. The values have been calculated using a hardness of 30 mg/L $CaCO_3$. These should be adjusted to the site-specific hardness (see Section 3.4.3).
J = Figures relate to toxicity (not eutrophication). The ANZECC & ARMCANZ (2000) DGVs for nitrate have been found to be erroneous (ANZG, 2018). In the absence of updated values, ANZG (2018) suggest reference is made to current New Zealand nitrate toxicity guidelines, specifically the 'Grading' GVs published in the '*Updating Nitrate Toxicity Effects on Freshwater Aquatic Species*' report (NIWA, 2013). These New Zealand Grading DGVs for N_NO_3 are provided above.

Appendix C: Habitat results

Percentage cover by each of the in-stream substrate types.

Dry-19

Type	Site	Bedrock	Boulders	Cobbles	Pebbles	Gravel	Sand	Silt	Clay
Yandicoogina Creek	YC1	40	3	10	11	10	5	15	6
	YC2	1	1	1	7	37	20	25	8
	YC3	1	0	4	30	45	11	9	0
	YC4	3	0	5	11	10	12	28	31
Reference Sites	WWS	6	4	9	26	40	10	5	0
	BENS	4	3	9	20	44	10	10	0
	MUNJS	96	0	0	0	0	0	4	0
	SS	2	3	7	20	38	20	10	0

Wet-20

Type	Site	Bedrock	Boulders	Cobbles	Pebbles	Gravel	Sand	Silt	Clay
Yandicoogina Creek	YC1	40	3	10	11	10	5	15	6
	YC2	1	1	1	7	37	20	25	8
	YC3	1	0	4	30	40	13	12	0
	YC4	3	1	2	8	19	10	28	29
Reference Sites	WWS	9	4	15	21	36	10	5	0
	WM	3	1	0	20	26	38	12	0
	MUNJS	89	1	0	0	0	0	10	0
	SS	3	3	2	18	41	22	11	0

Percentage cover by each of the in-stream habitat types. NB: Sub. Mac = submerged macrophyte, Emerg. Mac. = emergent macrophyte and Trailing Veg. = trailing vegetation.

Dry-19

Type	Site	Inorganic sed.	Sub. Mac	Emerg. Mac	Algae	LWD	Detritus	Roots	Trailing Veg.	Habitat diversity
Yandicoogina Creek	YC1	16	15	40	5	5	10	3	6	**8**
	YC2	10	0	70	0	5	10	2	3	**6**
	YC3	14	0	50	20	3	10	2	1	**7**
	YC4	49	18	16	0	4	11	1	1	**7**
Reference Sites	WWS	54	0	6	10	11	15	2	2	**7**
	BENS	58	0	4	0	12	18	6	2	**6**
	MUNJS	42	15	12	7	8	11	3	2	**8**
	SS	12	50	7	9	6	11	3	2	**8**

Wet-20

Type	Site	Inorganic sed.	Sub. Mac	Emerg. Mac	Algae	LWD	Detritus	Roots	Trailing Veg.	Habitat diversity
Yandicoogina Creek	YC1	10	10	40	11	8	14	5	2	**8**
	YC2	10	0	70	0	5	10	2	3	**6**
	YC3	6	15	45	18	3	10	2	1	**8**
	YC4	40	19	11	5	9	13	2	1	**8**
Reference Sites	WWS	66	0	2	8	10	9	2	3	**7**
	WM	61	0	0	9	17	13	0	0	**4**
	MUNJS	39	15	12	10	8	11	3	2	**8**
	SS	5	50	5	20	7	10	2	1	**8**

Appendix D: Water quality results

Highlighted cells refer to values which are in excess of: ▪ > the 99% ANZECC D GV, and ▪ > the 95% DGV.

Dry-19

	Units	ANZECC default GV 99% GV	95%	YC1	YC2	YC3	YC4	WWS	BENS	MUNJS	SS
Temp	°C			23.6	24.5	26.4	23.3	27.5	20.6	25.6	26.41
pH	pH units		6-8	7.22	6.8	7.23	7.45	7.25	7.84	8.08	7.74
Redox	mV			-46.8	-43.7	-43	-41.1	-56.2	-62	-29.7	-90.5
EC	µS/cm		250	598	571	664	621	1030	890	922	729
DO	%		85-120	53.8	27.0	55.6	70.3	83.6	52.0	108.8	58.1
Turbidity	NTU		15	3.6	3.8	0.3	2.4	0.5	<0.1	2.0	2.2
TSS	mg/L			8	23	36	11	1	<1	5	5
Alkalinity	mg/L			266	251	295	267	256	395	426	328
Hardness	mg/L			228	202	238	209	284	382	443	259
Na	mg/L			35.2	31.6	42.9	39.5	84.8	42.5	28.2	53.6
Ca	mg/L			47.1	42.3	48	41.9	37.5	67.3	68.4	47.7
Mg	mg/L			26.8	23.4	28.8	25.4	46.3	52.1	66.2	33.9
K	mg/L			9.8	9.5	11	10	12.2	9.6	7.6	4.9
HCO3	mg/L			266	251	295	267	246	395	426	328
Cl	mg/L			38	38	44	45	201	71	54	51
S_SO4	mg/L			9.17	8.37	8.83	9.13	4.27	17.9	11.1	6.63
CO3	mg/L			<1	<1	<1	<1	11	<1	<1	<1
dAl	mg/L	0.027	0.055	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
dAs	mg/L	0.001	0.024	<0.0002	<0.0002	<0.0002	<0.0002	0.0002	0.0004	0.0006	0.0002
dB	mg/L	0.09	0.37	0.144	0.126	0.172	0.157	0.252	0.376	0.157	0.144
dBa	mg/L			0.0166	0.0165	0.028	0.0185	0.0618	0.0103	0.0698	0.472
dCd	mg/L	0.00006	0.0002	<0.00005	<0.00005	<0.00005	<0.00005	<0.00005	<0.00005	<0.00005	<0.00005
dCo	mg/L			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0003	0.0004
dCr	mg/L	0.00001	0.001	0.0004	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002
dCu	mg/L	0.001	0.0014	<0.00005	0.00009	<0.00005	<0.00005	0.00011	0.00008	0.00048	0.00013
dFe	mg/L			0.054	0.02	0.032	0.038	0.025	<0.002	0.004	0.046
dMn	mg/L	1.2	1.9	0.0406	0.0022	0.0039	0.0014	0.0019	<0.0005	0.0741	0.553
dMo	mg/L			0.0002	0.0002	0.0002	0.0002	<0.0001	0.0003	0.0005	0.0005
dNi	mg/L	0.008	0.011	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	0.0008	<0.0005
dPb	mg/L	0.001	0.0034	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
dS	mg/L			9.9	8.8	9.5	8.9	4.9	18.1	11.6	7.2
dSe	mg/L	0.005	0.011	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002
dU	mg/L			0.00011	0.0001	0.00019	0.00017	<0.00005	0.00065	0.00046	0.00048
dV	mg/L			<0.0001	0.0007	0.0011	0.0014	0.0002	0.0029	0.0012	0.0007
dZn	mg/L	0.0024	0.008	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
N_NH_3	mg/L	0.32	0.90	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.02	<0.01
N_NO_3	mg/L	1.00	2.40	0.02	0.04	<0.01	0.01	<0.01	<0.01	0.02	0.01
N_NOx	mg/L		0.01	0.02	0.04	<0.01	0.01	<0.01	<0.01	0.02	0.01
TN	mg/L		0.30	0.17	2.20	0.06	0.27	0.25	0.02	0.71	0.12
TP	mg/L		0.01	0.04	0.09	0.03	0.06	0.04	0.03	0.09	0.04

Highlighted cells refer to values which are in excess of: ■ > the 99% ANZECC D GV, ■ > the 95% DGV, and ■ > low reliability ANZECC DGV.

Wet-20

		ANZECC default GV		Yandicoogina Creek				Reference Sites			
	Units	99% GV	95%	YC1	YC2	YC3	YC4	WWS	WM	MUNJS	SS
Temp	°C			25.8	25.7	27.8	24.5	29.1	25.3	23.3	27.2
pH	pH units		6-8	7.36	7.39	7.45	7.68	7.94	8.22	7.96	8.09
Redox	mV			45.8	-63.7	105.9	53.7	138	92	92.6	37.9
EC	µS/cm		250	620	554	639	641	883	525	833	581
DO	%		85-120	38.2	25.5	39.5	23.3	53.2	92.4	73.1	76.5
Turbidity	NTU		15	7.6	10.9	2.0	1.5	0.4	3.2	1.1	0.4
TSS	mg/L			3	4	5	4	2	5	<1	<1
Alkalinity	mg/L			241	218	261	254	324	81	162	294
Hardness	mg/L			263	240	274	262	413	193	294	248
Na	mg/L			36.2	32.7	39.6	43.4	42.3	36.9	63.5	37.5
Ca	mg/L			54.4	49.7	55.3	52.7	72.8	41.6	44.2	49.1
Mg	mg/L			30.9	28.2	32.9	31.7	56.2	21.6	44.6	30.5
K	mg/L			11	10.8	12.2	12	10	6	13	5.5
HCO3	mg/L			241	218	261	254	324	81	162	292
Cl	mg/L			39	37	40	45	71	58	150	43
S_SO_4	mg/L			11.1	8.3	10.3	9.67	19.8	31.5	22.8	6.47
CO_3	mg/L			<1	<1	<1	<1	<1	<1	<1	2
dAl	mg/L	0.027	0.055	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
dAs	mg/L	0.001	0.024	0.0003	0.0002	<0.0002	<0.0002	0.0005	0.0004	0.0004	0.0002
dB	mg/L	0.09	0.37	0.18	0.15	0.18	0.17	0.34	0.06	0.25	0.13
dBa	mg/L			0.020	0.020	0.029	0.022	0.012	0.031	0.062	0.243
dCd	mg/L	0.00006	0.0002	<0.00005	<0.00005	<0.00005	<0.00005	<0.00005	<0.00005	<0.00005	<0.00005
dCo	mg/L			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
dCr	mg/L	0.00001	0.001	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002
dCu	mg/L	0.001	0.0014	<0.00005	0.00005	0.00008	<0.00005	0.00009	0.00164	0.00009	0.00010
dFe	mg/L			0.030	0.468	0.014	0.038	<0.002	0.009	0.080	0.021
dMn	mg/L	1.2	1.9	0.0393	0.0344	0.0083	0.0049	<0.0005	0.0016	0.0041	0.111
dMo	mg/L			0.0002	0.0002	0.0004	0.0001	0.0003	0.0001	0.0001	0.0003
dNi	mg/L	0.008	0.011	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	0.0006	<0.0005	<0.0005
dPb	mg/L	0.001	0.0034	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
dS	mg/L			9.6	7.3	9.9	7.6	17.0	28.9	20.6	5.6
dSe	mg/L	0.005	0.011	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002	<0.0002
dU	mg/L			0.00011	0.00006	0.00017	0.00015	0.00069	0.00006	<0.00005	0.00044
dV	mg/L			<0.0001	0.0002	0.0007	0.0006	0.0032	0.0019	0.0002	0.0016
dZn	mg/L	0.0024	0.008	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
N_NH_3	mg/L	0.26	0.73	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
N_NO_3	mg/L	1.00	2.40	0.01	<0.01	0.01	0.01	0.04	<0.01	0.02	0.08
N_NOx	mg/L		0.01	0.01	<0.01	0.01	0.01	0.04	<0.01	0.02	0.08
TN	mg/L		0.30	0.21	0.11	0.40	0.12	0.06	0.34	0.22	0.12
TP	mg/L		0.01	0.04	0.05	0.06	0.03	0.03	0.03	0.04	0.02

Appendix E: Zooplankton taxonomic list

Values are total abundances.

Dry-19

Class/Order	Family	Lowest taxon	Yandicoogina Creek YC1	YC3	YC4	Reference sites WWS	BENS	MUNJS	SS
PROTISTA									
		Testate Amoeba	5	0	0	0	0	0	0
Ciliophora		Ciliate indet.	10	3	3	8	0	2	2
		cf. Hypotrichia	0	0	0	3	0	0	0
	Habrotrochidae	Habrotrochidae spp.	0	0	1	16	1	3	0
ROTIFERA									
		Unidentified Rotifera	10	0	0	21	1	0	0
Bdelloidea		Bdelloidea spp. indet.	30	0	7	26	0	2	1
Monogononta									
	Brachionidae	*Brachionus angularis*	0	1	2	0	0	0	0
		Brachionus cf. *quadrata*	0	0	0	1	0	0	0
		Keratella tropica	0	0	0	2	0	0	0
		Plationus patulus	0	0	1	0	0	0	0
	Colurellidae	*Lepadella sp.*	10	0	2	0	0	1	0
	Dicranophoridae	*Dicranophorous* sp.	15	0	3	0	0	0	0
		cf. Erignatha sp.	0	0	1	0	0	0	0
	Notommatidae	*Cephalodella* sp.	0	0	1	0	0	0	0
		Notommata sp.	0	0	1	0	0	0	0
	Lecanidae	*Lecane bulla*	20	0	0	0	0	0	0
		Lecane sp.	5	0	0	0	0	5	0
		Lecane hamata	0	0	0	3	0	0	0
	Proalidae	*Proales* sp.	5	0	0	11	0	0	0
	Trichotriidae	*Trichocerca* sp.	10	0	0	3	1	4	1
		Trichocerca cf. *similis*	45	0	49	0	0	12	1
ARTHROPODA									
CRUSTACEA									
MAXILLIPODA			0	0	0	0	0	0	0
Calanoida	**Centropagidae**	*Boeckella* sp.	0	0	0	0	0	1	0
Cyclopoida		Cyclopoid copepodite	0	0	0	0	1	0	8
		Cyclopoid nauplii	105	0	158	79	4	37	16
	Cyclopidae	*Mesocyclops* sp.	2350	2	0	1	0	0	0
		Mesocyclops notius	0	0	0	3	0	13	0
		Tropocyclops confinis confinis	6925	7	210	14	2	134	2
Harpacticoid		Harpacticioida spp.	0	0	0	1	0	0	0
DIPLOSTRACA									
CLADOCERA	**Daphnidae**	*Ceriodaphnia* sp.	325	0	25	0	0	0	0
	Bosminidae	*Bosmina meriodionalis*	0	1	0	0	0	0	0
	Chydoridae	*Chydorus* sp.	0	0	0	1	0	0	0
OSTRACODA									
PODOCOPIDA		Ostracoda spp. juv.	0	0	0	1	0	0	3
	Ilyocyprididae	*Ilyocypris australiensis*	0	0	0	1	0	0	0
	Notodromadidae	*Newnhamia* sp.	0	0	0	0	0	2180	0
		Taxa richness	**15**	**5**	**14**	**18**	**6**	**12**	**8**

Wet-20

Class/Order	Family	Lowest level of identification	Yandicoogina Creek YC1	YC3	YC4	Reference sites WWS	WM	MUNJS	SS
PROTISTA									
		Testate Amoeba	0	3	0	0	14	0	4
Ciliophora		Ciliate indet.	6	2	0	1	16	1	5
		Coleops sp.	0	0	41	0	0	0	0
	Habrotrochidae	Habrotrochidae spp.	1	0	0	0	0	0	0
ROTIFERA									
		Unidentified Rotifera	0	1	2	0	2	1	1
Bdelloidea		Bdelloidea spp. indet.	4	12	3	0	0	0	2
Monogononta									
	Brachionidae	*Brachionus angularis*	0	0	0	0	5	0	0
		Brachionus falcatus	0	0	0	0	36	0	0
		Brachionus quadridentatus	0	0	18	0	0	0	0
		Keratella cf. *serrulata*	0	0	0	0	32	0	0
		Keratella valga	0	0	0	0	2	0	0
		Keratella sp.	0	0	0	0	0	0	3
		Plationus patulus	0	0	0	0	24	0	0
	Colurellidae	*Lepadella sp.*	0	2	7	0	0	0	0
	Euchlanidae	*Euchlanis* cf. *dilata*	3	0	0	0	0	0	0
	Lecanidae	*Lecane cf. curvicornis*	0	1	0	0	0	0	0
		Lecane luna	4	0	0	0	0	0	0
		Lecane lunaris	1	2	0	0	0	0	0
		Lecane cf. hastata	4	8	2	0	0	0	2
		Lecane cf. pertica	1	0	0	0	0	0	0
		Lecane cf. styrax	3	1	0	0	0	0	8
		Lecane thalera	4	4	1	0	0	0	0
		Lecane sp.	5	4	0	0	0	0	0
	Synchaetidae	*Polyarthra* sp.	0	0	0	0	148	0	7
	Trichotriidae	*Trichocerca* sp.	0	0	0	0	0	0	1
ARTHROPODA									
CRUSTACEA									
MAXILLIPODA									
Calanoida	**Centropagidae**	*Eodiaptomus lumholtzi*	0	0	0	0	0	104	0
Cyclopoida		Cyclopoid copepodite	1	0	0	2	0	61	46
		Cyclopoid nauplii	8	7	564	0	159	117	34
	Cyclopidae	*Mesocyclops* sp.	0	0	0	0	20	22	0
		Mesocyclops notius	0	0	300	0	0	0	0
		Tropocyclops confinis confinis	1	160	14,100	0	11,800	236	15
DIPLOSTRACA									
CLADOCERA	**Daphnidae**	*Simocephalus* sp. (juv.)	0	0	0	0	0	1	0
	Chydoridae	*Alona sp.*	0	0	0	0	0	1	0
		Chydorus sp.	0	10	0	0	0	0	0
	Ilyocryptidae	*Ilyocryptus* sp.	0	0	0	0	10	0	0
	Sididae	*Diaphanosoma* sp.	0	0	0	0	0	3	0
OSTRACODA									
PODOCOPIDA		Ostracoda spp. juv.	0	0	0	0	0	1	0
	Ilyocyprididae	*Ilyocypris australiensis*	0	0	0	0	0	1	0
	Notodromadidae	*Newnhamia* sp.	0	0	0	0	0	148	1
		Taxa richness	**14**	**14**	**10**	**2**	**13**	**13**	**13**

Appendix F: Hyporheos fauna taxonomic list

Values are log abundances (i.e. 1=1 individual, 2 = 2-10, 3 = 11-100, 4 = 101-1000).

***Indicates stygobitic and permanent hyporheos stygophile species**

<u>Dry-19</u>

			Yandicoogina Creek			Reference sites			
Phylum/Class/Order	**Family**	**Lowest taxon**	**YC2**	**YC3**	**YC4**	**WWS**	**BENS**	**MUNJ**	**SS**
CNIDARIA									
HYDROZOA									
Antoathecata	**Hydridae**	*Hydra* sp.	0	0	0	0	3	0	0
PLATYHELMINTHES		Turbellaria spp.	0	0	0	2	2	0	2
NEMATODA		Nematoda spp.	0	0	0	0	0	0	2
ANNELIDA									
OLIGOCHAETA									
Tubificida		Oligochaeta spp. (imm./dam.)	0	3	0	0	0	0	0
	Naididae	*Nais variabilis*	0	2	0	0	0	0	0
		Pristina leidyi	0	3	0	0	0	2	0
		Pristina longiseta	0	0	0	1	2	0	1
		Pristina jenkinae	1	0	0	0	0	0	0
		Pristina sp.	0	0	4	0	3	0	2
		Naidinae spp.	2	0	2	2	2	0	2
	Phreodrilidae	Phreodrilidae spp.	0	0	6	1	0	0	1
ARTHROPODA									
CRUSTACEA									
BRANCHIOPODA									
Ostracoda	**Candonidae**	*Candonopsis tenuis**	0	0	0	0	1	0	0
		Humphreyscandona sp. `BOS1402`*	0	0	0	0	0	0	2
		*Notacandona boultoni**	0	0	0	1	0	0	0
	Cyprididae	*Cypridopsis* sp. `BOS1401`*	0	2	0	0	1	2	1
		Cyprinopsinae sp. (imm.)	0	0	1	0	0	0	0
	Darwinulidae	*Vestalenula marmonieri**	0	2	0	0	0	1	2
		*Vestalenula matildae**	0	0	0	0	0	0	2
		Vestalenula sp. (imm.)*	0	0	0	0	1	0	0
	Limnocytheridae	*Limnocythere dorsosicula**	0	0	2	0	0	0	0
MAXILLOPODA									
Copepoda									
Cyclopoida		Cyclopoida copepodite	0	0	0	0	0	0	1
	Cyclopidae	*Diacyclops cockingi**	0	0	0	2	0	0	0
		Diacyclops humphreysi s.l.*	0	2	0	0	0	0	0
		Ectocyclops phaleratus	0	0	1	3	0	0	0
		Mesocyclops darwini	0	0	0	0	0	0	1
		Microcyclops varicans	0	0	0	0	0	0	1
		Paracyclops chiltoni	0	0	0	0	0	2	0
		Paracyclops intermedius	0	0	2	0	0	0	0
		Tropocyclops confinis confinis	0	0	0	0	0	0	2
Harpacticoida	**Canthocamptidae**	Canthocamptidae sp. B02*	0	0	0	0	0	0	2
	Parastenocarididae	*Kinnecaris* sp.*	0	0	0	0	0	0	2
MALACOSTRACA									
Bathynellacea	**Parabathynellidae**	*Atopobathynella* sp.*	0	0	0	0	0	0	2
Amphipoda	**Paramelitidae**	*Chydaekata* sp. `E`*	0	3	0	2	0	0	0
		Chydaekata sp.*	0	0	0	0	0	0	1
		Paramelitidae 'sp. Biologic-AMPH023'	0	1	0	0	0	0	0
Isopoda	**Tainisopidae**	*Pygolabis weeliwolli**	0	1	0	0	0	0	0
CHELICERATA									
ARACHNIDA									
		Acari sp. (imm/damm)	0	0	0	0	0	1	2
Trombidiformes	**Halacaridae**	Halacaridae sp.	0	0	0	0	0	0	1
	Hydrophantidae	*Wandesia* sp.*	0	1	0	0	0	0	1
COLLEMBOLLA									
Entomobryomorpha		Entomobryoidea spp.	1	1	2	0	0	0	0
Symphypleona		Symphypleona sp.	0	1	0	0	0	0	0
HEXAPODA									
INSECTA									
Coleoptera	**Carabidae**	Carabidae sp. (A)	0	0	0	0	2	0	0
		Carabidae sp. (L)	0	0	2	0	0	0	0
	Elmidae	*Austrolimnius* sp. (L)	0	0	0	0	0	0	2
	Hydraenidae	*Hydraena* sp.	0	0	0	0	3	0	0
		Hydraenidae sp. (L)	0	0	0	0	3	0	0
	Hydrophilidae	*Chaetarthria* sp. (L)	0	1	1	2	0	0	0
		Helochares sp. (L)	0	0	0	0	1	0	0
		Paracymus spenceri	0	0	0	0	2	0	0
		Paracymus sp. (L)	0	0	0	0	0	0	1
		Hydrophilidae sp. (L) (hypo)	0	2	0	0	2	1	0
	Noteridae	*Neohydrocoptus subfaciatus*	0	0	0	0	0	1	0

Wet-20

Phylum/Class/Order	Family	Lowest taxon	Yandicoogina Creek YC1	YC2	YC3	YC4	YC5H	YC6H	YC7H	YC8H	YC9H	Reference sites WWS	WM	MUNJ	SS
CNIDARIA															
HYDROZOA															
Antoathecata	Hydridae	*Hydra* sp.	0	0	0	0	0	0	0	0	0	2	0	0	0
ANNELIDA															
OLIGOCHAETA															
Tubificida	Phreodrilidae	Phreodrilidae sp.	0	0	0	0	1	0	0	0	0	0	0	0	0
	Naididae	*Allonais pectinata*	0	1	0	0	0	0	0	0	0	0	0	0	3
		Allonais ranauna	0	0	0	0	0	0	0	2	0	0	0	0	3
		Bratislavia unidentata	1	0	0	1	0	0	0	0	0	0	0	0	0
		Pristina aequiseta	0	0	0	0	2	3	0	0	2	0	0	0	3
		Pristina leidyi	0	0	3	1	3	0	2	2	0	0	0	0	0
		Pristina longiseta	0	0	2	1	2	0	0	0	0	1	3	0	0
		Pristina jenkinae	0	2	0	0	0	1	2	2	1	0	0	0	0
		Pristina sima	0	0	0	0	0	0	2	1	0	0	0	0	0
		Pristina spp.	2	2	3	2	0	0	1	0	2	1	3	1	0
		Naidinae spp. (imm./dam.)	2	3	2	2	2	2	2	3	2	2	3	2	3
GASTROPODA															
	Planorbidae	*Ferrissia petterdi*	0	0	0	0	3	0	0	0	0	0	0	0	0
		Gyraulus sp.	0	0	0	0	2	0	0	0	1	0	0	0	0
ARTHROPODA															
CRUSTACEA															
BRANCHIOPODA															
Ostracoda	Candonidae	*Meridiescandona facies**	1	0	0	0	0	0	0	0	2	0	0	0	0
	Cyprididae	*Ilyodromus* sp.*	0	0	0	0	0	0	0	0	0	0	0	0	1
		Cypretta seurati	0	0	0	0	2	2	2	2	0	0	0	0	0
		Cypridopsis sp.	0	0	0	0	0	2	0	1	0	0	0	0	0
		Riocypris fitzroyi	0	0	0	0	2	0	0	0	0	0	0	0	0
		Stenocypris major	0	0	0	0	0	1	0	1	0	0	0	0	0
	Limnocytheridae	*Gomphodella alexanderi**	0	0	0	0	0	0	1	1	0	0	0	0	0
		*Gomphodella yandii**	0	0	0	0	0	0	2	0	0	0	0	0	0
		*Limnocythere dorsosicula**	0	0	0	0	0	0	0	2	0	0	0	0	0
	Darwinulidae	*Penthesilenula brasiliensis*	0	0	0	0	2	0	2	0	0	0	0	0	0
MAXILLOPODA															
Copepoda															
Cyclopoida	Cyclopidae	*Ectocyclops phaleratus*	0	1	1	0	0	0	0	0	0	0	0	0	2
		Microcyclops varicans	0	2	2	0	2	1	0	3	3	2	0	0	2
		Mesocyclops darwini	0	0	0	0	1	1	0	0	0	0	0	0	0
		Mesocyclops notius	0	0	0	0	0	0	0	1	0	0	0	0	0
		Paracyclops intermedius	0	0	0	0	0	2	0	0	0	0	0	0	0
		Thermocyclops sp.	0	0	0	0	0	0	3	0	0	0	0	0	0
Harpacticoida		Harpacticoida sp.*	0	0	0	0	0	0	2	0	0	0	0	0	0
MALACOSTRACA															
Bathynellacea		Bathynellidae sp. BES7547*	0	0	0	0	0	0	0	0	3	0	0	0	0
Amphipoda	Paramelitidae	*Chydaekata* sp. E*	0	0	3	0	2	0	2	0	2	3	0	0	0
		Paramelitidae 'sp. Biologic-AMPH023'*	2	0	3	0	1	0	0	0	2	0	0	0	0
		Paramelitidae 'sp. Biologic-AMPH024'*	0	0	0	0	0	0	0	0	0	2	0	0	0
		Paramelitidae spp. (imm./dam.)*	2	0	3	1	2	0	0	0	3	3	0	0	0
Isopoda	Tainisopidae	*Pygolabis weeliwolli**	0	0	1	2	2	0	2	0	1	0	0	0	0
CHELICERATA															
ARACHNIDA		Acari sp. (imm/damm)	0	0	0	0	0	0	0	0	2	0	0	0	0
Trombidiformes		Trombidioidea sp.	0	0	0	0	0	0	2	0	0	0	0	0	0
	Halacaridae	Halacaridae sp.	0	0	0	0	0	1	2	0	0	0	0	0	0
	Unionicolidae	*Recifella* sp.	0	0	1	0	0	0	0	2	0	0	0	0	0
		Neumania sp.	0	0	0	0	0	2	0	0	0	0	0	0	0
	Arrenuridae	*Arrenurus* sp.	0	0	0	0	0	0	0	1	0	0	0	0	0
	Hydryphantidae	*Wandesia* sp*	0	0	0	0	0	1	0	0	0	0	0	0	0
	Limnesiidae	*Limnesia* sp.	0	0	0	0	0	0	0	1	0	0	0	0	0
	Oxidae	*Oxus orientalis*	0	0	0	0	0	0	0	1	0	0	0	0	0
	Limnocharidae	*Limnochares* sp.	0	0	1	0	0	0	0	0	0	0	0	0	0
Mesostigmata		Mesostigmata spp.	0	1	0	0	0	1	0	0	0	1	0	0	2
Sarcoptiformes		Oribatida sp.	0	0	0	0	3	0	3	3	2	0	0	0	0
COLLEMBOLLA															
Entomobryomorpha		Entomobryoidea spp.	1	2	2	1	2	0	1	2	1	1	0	0	0
Poduroidea		Poduroidea spp.	2	0	0	0	1	2	0	0	0	0	0	0	0
		Symphypleona sp.	0	0	0	0	0	0	0	1	0	0	0	0	0
HEXAPODA															
INSECTA															
Coleoptera	Carabidae	Carabidae sp. (L)	0	0	0	0	0	0	2	0	0	0	2	0	0
	Dytiscidae	Dytiscidae sp. (L)	0	0	1	0	0	1	0	2	0	0	0	0	0
		Neobidessodes denticulatus	0	0	0	0	0	0	0	1	0	0	0	0	0
		Limbodessus compactus	0	0	0	0	0	0	0	2	0	0	0	0	0
		Limbodessus occidentalis	0	0	0	0	1	0	0	0	0	0	0	0	0
	Elmidae	*Austrolimnius* sp. (L)	0	0	0	0	2	2	0	0	0	0	0	0	0
	Hydraenidae	*Hydraena* sp.	0	0	2	0	2	0	3	2	0	0	0	0	0
		Hydraenidae sp. (L)	0	0	0	0	0	0	0	0	0	0	2	0	0
	Hydrophilidae	*Chaetarthria nigerrima*	0	0	0	0	0	0	0	0	0	0	0	0	1
		Chaetarthria nigerrima (L)	0	0	1	0	0	0	0	0	0	2	0	0	3
		Enochrus sp. (L)	0	0	0	0	0	0	0	0	0	0	0	0	1
		Helochares sp. (L)	0	0	0	0	0	0	0	0	0	0	0	0	2
		nr. *Anacaena* sp.	0	0	0	0	0	1	2	0	1	0	0	0	0
		Hydrophilidae sp. (L)	0	1	0	0	0	0	0	0	0	2	0	0	1
		Laccobius billi	0	0	0	0	0	0	0	0	0	0	0	0	1
		Laccobius sp. (L)	0	0	0	0	0	0	0	0	0	0	0	0	2
		Paracymus spenceri	0	0	1	0	1	0	0	1	0	0	0	0	0
		Paracymus sp. (L)	0	0	0	0	0	0	0	1	0	0	0	0	0
	Limnichidae	Limnichidae spp.	0	0	0	0	0	0	0	0	0	0	1	0	3
	Scirtidae	Scirtidae sp. (L)	0	2	3	0	3	2	3	2	3	2	3	2	0
	Staphylinidae	Staphylinidae sp.	0	0	0	0	1	0	1	0	0	0	0	0	0

			Yandicoogina Creek									Reference sites			
Phylum/Class/Order	**Family**	**Lowest taxon**	**YC1**	**YC2**	**YC3**	**YC4**	**YC5H**	**YC6H**	**YC7H**	**YC8H**	**YC9H**	**WWS**	**WM**	**MUNJ**	**SS**
Diptera	**Ceratopogonidae**	Ceratopogonidae sp. (P)	0	1	0	0	1	0	0	0	0	1	2	0	2
		Ceratopogoninae spp.	3	3	2	3	3	3	3	3	2	3	3	2	3
		Dasyhelea spp.	2	3	2	2	2	0	0	0	3	0	0	0	3
		Forcipomyiinae spp.	0	0	0	2	0	0	0	0	0	0	0	0	0
	Chironomidae														
	Chironominae	Chironomini sp.	2	0	0	0	2	0	2	0	0	0	0	0	0
		Chironomus aff. *alternans*	0	0	0	0	0	2	0	0	0	0	0	0	0
		Cladotanytarsus sp.	0	0	0	0	1	2	0	0	2	0	0	0	0
		Cryptochironomus grideidorsum	0	0	0	0	1	0	0	0	0	0	0	0	0
		Paratendipes sp. \`K1\`	0	2	0	0	1	0	0	0	0	0	0	0	1
		Polypedilum sp. S01 (PSW)	0	0	0	0	0	0	0	0	0	0	0	2	0
		Tanytarsus sp. D (SAP)	0	0	2	0	0	0	0	0	0	0	0	0	2
		Tanytarsus sp.	0	0	0	0	3	3	0	1	2	0	0	0	0
		Dicrotendipes sp.	0	0	0	0	2	0	0	0	0	0	0	0	0
		Paratanytarsus sp.	0	0	0	0	2	0	0	0	0	0	0	0	0
		Kiefferulus sp.	0	0	0	0	0	1	0	0	0	0	0	0	0
	Tanypodinae	*Ablabesmyia notabilis*	0	0	0	0	0	0	2	0	0	0	0	0	0
		?*Australopelopia* sp.	0	0	0	0	0	0	0	0	0	0	0	0	2
		Larsia ?albiceps	0	2	2	0	3	0	3	3	0	3	2	0	0
		Paramerina sp. 1	0	0	2	0	3	0	2	0	0	0	3	0	0
		Paramerina sp. 2	0	0	0	0	1	2	0	0	0	0	0	0	0
		Tanypodinae sp. WWT14	0	0	2	0	0	0	0	0	0	0	0	0	0
		Procladius spp.	0	0	0	0	2	0	2	2	0	0	1	0	0
	Dolichopodidae	Dolichopodidae spp.	1	0	0	0	0	0	0	0	0	2	1	0	0
	Ephydridae	Ephydridae spp.	0	1	0	0	0	0	0	0	0	0	0	1	0
	Psychodidae	Psychodidae sp.	0	0	0	0	0	0	0	0	0	0	1	0	0
	Sciaridae	Sciaridae sp.	0	1	0	0	0	0	0	0	0	0	0	0	0
	Tipulidae	Tipulidae spp.	0	1	0	0	0	1	0	0	0	0	3	0	6
	Muscidae	Muscidae sp.	0	0	0	0	0	0	0	0	1	0	0	0	0
Trichoptera	**Ecnomidae**	*Ecnomus pilbarensis*	0	0	0	0	0	0	1	0	0	0	0	0	0
Ephemeroptera	**Caenidae**	Caenidae sp.	0	0	0	0	3	0	3	1	0	1	0	0	0
		Tasmanocoenis sp. P/arcuata	0	0	0	0	1	0	0	0	0	0	0	0	0
	Baetidae	Baetidae sp.	0	0	0	0	1	0	0	0	0	0	0	0	0
Hemiptera	**Gelastocoridae**	*Nerthra* sp.	0	0	0	0	0	0	0	2	0	1	0	0	0
	Gerridae	*Limnogonus fossarum gilguy*	0	0	0	0	0	0	0	2	0	0	0	0	0
	Mesoveliidae	Mesoveliidae sp.	0	0	0	0	0	0	0	2	0	0	0	0	0
Lepidoptera	**Crambidae**	Acentropinae sp.	0	1	0	0	1	0	0	0	0	0	0	0	0
Anisoptera Odonata		Anisoptera spp. (imm./dam.)	0	0	2	0	2	0	0	0	0	2	0	0	0
		Taxa richness	**12**	**19**	**25**	**11**	**44**	**25**	**30**	**34**	**22**	**20**	**15**	**6**	**23**

Appendix G: Biologic Molecular Report



Ministers North: Yandicoogina Creek Amphipod DNA Analysis

Biologic Environmental Survey

Report to BHP Western Australia Iron Ore

November 2020

DOCUMENT STATUS				
Version No.	Authors	Review / Approved for Issue	Approved for Issue to	
			Name	Date
1	J. Huey S. Floeckner	N. Gunawardene J. Delaney	S. Wild (BHP)	31/07/2020
2	J. Huey	J. Delaney	S. Wild (BHP)	03/11/2020
Final	J. Delaney	N. Gunawardene	S. Wild (BHP)	27/11/2020

We have prepared this report for the sole purposes of BHP ("Client") for the specific purpose only for which it is supplied. This report is strictly limited to the Purpose and the facts and matters stated in it do not apply directly or indirectly and will not be used for any other application, purpose, use or matter.

In preparing this report we have made certain assumptions. We have assumed that all information and documents provided to us by the Client or as a result of a specific request or enquiry were complete, accurate and up-to-date. Where we have obtained information from a government register or database, we have assumed that the information is accurate. Where an assumption has been made, we have not made any independent investigations with respect to the matters the subject of that assumption. We are not aware of any reason why any of the assumptions are incorrect.

This report is presented without the assumption of a duty of care to any other person (other than the Client) ("Third Party"). The report may not contain sufficient information for the purposes of a Third Party or for other uses. Without the prior written consent of Biologic:

a) This report may not be relied on by a Third Party; and

b) Biologic will not be liable to a Third Party for any loss, damage, liability or claim arising out of or incidental to a Third Party publishing, using or relying on the facts, content, opinions or subject matter contained in this report.

If a Third Party uses or relies on the facts, content, opinions or subject matter contained in this report with or without the consent of Biologic, Biologic disclaims all risk and the Third Party assumes all risk and releases and indemnifies and agrees to keep indemnified Biologic from any loss, damage, claim or liability arising directly or indirectly from the use of or reliance on this report.

In this note, a reference to loss and damage includes past and prospective economic loss, loss of profits, damage to property, injury to any person (including death) costs and expenses incurred in taking measures to prevent, mitigate or rectify any harm, loss of opportunity, legal costs, compensation, interest and any other direct, indirect, consequential or financial or other loss.

GLOSSARY

Bootstrap Value between 0 and 100 that indicates the robustness of the node in a phylogenetic tree

COI Cytochrome Oxidase subunit 1, a mitochondrial gene commonly used in phylogenetic studies and used as a DNA barcode to identify species

GenBank Annotated open access sequence database of all publicly available nucleotide sequences and their protein translations

OTU Operational taxonomic unit – species-equivalent taxonomic unit based on COI or 12S cluster similarity

1 INTRODUCTION

Biologic Environmental Survey (Biologic) conducted a two-season baseline aquatic ecosystem (Level 2) survey of Yandicoogina Creek located in the Ministers North area (the Study Area). Three stygal Amphipoda morphospecies were identified from this survey and represent potential short-range endemic fauna. These were *Chydaekata* sp. `E`, Paramelitidae Genus 2 sp. B02, and Paramelitidae spp. Twenty amphipod specimens representing these morphospecies collected from this survey were selected for molecular systematics analysis (DNA barcoding). Molecular analysis of the current specimens will provide information on the distribution of these species along Yandicoogina Creek, and whether they are genetically similar to known species from nearby creeks, namely, Weeli Wolli Creek and Marillana Creek.

1.1 Aims and objectives

The aims and objectives of the molecular systematics analysis were to:

- Undertake DNA sequencing of 20 amphipod specimens to obtain barcoding sequences of the mitochondrial gene Cytochrome Oxidase I (COI; Hebert *et al*., 2003a);
- Investigate the inter- and intra-specific relationships between sequences (*i.e.* how many different species/Operational Taxonomic Units [OTUs] are likely to occur within each genus or relevant higher taxon, based on published species-thresholds and results from the DNA analysis); and,
- Investigate the relationships between sequences from the Study Area and relevant previous sequences from the wider Pilbara region, using available DNA databases (*i.e.* undertake comparison of the current analysis with accessible DNA databases to assess whether any of the species/ OTUs from the Study Area have been collected previously and/or are known to be more widely distributed beyond the Study Area).

This document reports the methods and results of the molecular systematics analysis. All sequence data will be uploaded to GenBank (https://www.ncbi.nlm.nih.gov/genbank/) as per Biologic Molecular Systematics standard procedure.

2 METHODS

2.1 Sub-sample preparation

A total of 20 specimens collected from the Study Area by Biologic aquatic ecologists were selected for molecular systematics analysis. The specimens were selected to include representatives of each of the OTUs determined from morphological identification, as well as their geographic spread across the Study Area to assist with understanding species distributions. Adequate redundancy in specimen selection was incorporated to account for any potential sequence generation failure. Specimens in good condition were chosen to increase their DNA extraction potential. Specimens were preserved in 100% ethanol and kept as cool as possible in the field and during transport, before being stored at -20°C until required for genetic analysis.

Where whole specimens were available, tissue preparation was undertaken by removing a leg or another body part less important for taxonomic identification, briefly drying off the ethanol, and placing the tissue in ATL buffer. In some instances, for very small and/or juvenile specimens, the entire animal was utilised. Again, these were briefly dried and placed in ATL buffer. Greatest care was taken to decontaminate all tools and equipment between samples, using bleach and repeated rinsing in deionised water. Table 2.1 provides details of the taxonomic orders chosen for molecular analysis. Further taxonomic clarification for each specimen included in the analysis can be found in Appendix A.

Table 2.1: Taxonomic groups from the Study Area included in the analysis, with a summary of PCR and sequencing success.

Class/Subclass	Order	Number of samples	PCR success	Sequence success	% sequence success
Malacostraca	Amphipoda	20	19	19	95%
TOTAL		**20**	**19**	**19**	**95%**

2.2 DNA extraction, amplification and sequencing

DNA extraction and sequencing methods followed Cullen and Harvey (2017, 2018), as follows:

Subsampled tissue/specimen was placed directly into ATL buffer for extraction using the *QIAGEN DN-easy* Blood and Tissue extraction kit, and DNA extraction followed the manufacturer's protocols. DNA extractions were amplified by Polymerase Chain Reaction (PCR) using Folmer PCR primers (LCO1490, HCO2198; Folmer *et al.*, 1994) to assess the variability of COI.

The resulting PCR product was cleaned and sequenced by the Australian Genomic Research Facility (AGRF) Perth node. Molecular laboratory workflows were managed using GENEIOUS Prime (Kearse *et al.*, 2012) with the Biocode plugin (http://www.mooreabiocode.org). Raw sequence data were edited and assembled in GENEIOUS, and final consensus sequences were then available for downstream analysis.

2.3 Specimen selection for comparative analysis

Comparison was made with sequences in GenBank (a publicly available DNA sequence database), as well as Biologic's unpublished DNA sequence libraries (767 amphipod sequences). This was undertaken using two separate methods.

- BLAST (Basic Local Alignment Search Tool): a method for rapidly searching a DNA sequence library to identify similar sequences. Sequences were searched using the "blastn" function, which returns similar matches.
- Taxonomic Curation: BLAST occasionally fails to identify sequences that could be considered useful for comparison, such as species that might be genetically distant, but are required to be included in the analysis for comparison. Taxonomically relevant specimens were identified using the available taxonomic classifications and identifications in those databases.

The final phylogeny and distance matrix in this report were reduced to those sequences that are publicly available and can be provided to the Client, with any matches to sequences that cannot be provided discussed in the relevant sections.

2.4 Analysis and interpretation of sequence alignments/divergence

For each taxonomic group, the selected sequences were aligned using the MAFFT (Multiple Alignment using Fast Fourier Transform) algorithm (Katoh *et al.*, 2002). Trees were constructed on resulting alignments using the RaxML (Stamatakis, 2014) plugin in GENEIOUS Prime, using 1,000 bootstrap replicates and the GTR+G substitution model.

To delimit taxonomic units using molecular data, we applied a genetic distance-based threshold method, combined with our morphological identifications. Fauna-specific genetic distance thresholds for delimiting species and OTUs were used wherever possible, based on published literature and available previous reports. Where these thresholds were not available, the assessment used average divergence thresholds for related groups or higher taxa developed by broad-level studies (e.g. Hebert *et al.*, 2003b).

In general, ≤ 8% COI divergence is seen as appropriate to determine OTUs (Hebert *et al.,* 2003b), however, higher or lower divergences are sometimes justified depending on the organism studied. Unless otherwise stated, we considered sequences that exhibited COI divergences ≤ 8% to belong to the same OTU.

2.5 Constraints and limitations

The analysis was constrained by the breadth of data available to undertake comparisons and the accessibility of pre-existing regional sequences. Generally, the success rate of genetic sequencing can be affected by specimen collection, preservation, storage methods and contamination. Best practises were followed during specimen collection, preservation, and storage, prior to specimens arriving at Biologic's laboratories. All care was taken to ensure that the risks of laboratory contamination, data handling issues, and specimen management issues were minimised within Biologic's laboratories throughout the subsampling, processing and genetic analysis. As such, a high success rate was achieved (95%) in the current study.

The databases used for regional comparisons included GenBank and unpublished data in Biologic's database. While these sequence databases, in combination, comprise a large portion of the subterranean fauna genetic work undertaken in the Pilbara region, it is acknowledged that there may be many other relevant sequences from third party project areas nearby or elsewhere in the region that were not available for comparison at the time of the study. GenBank is dynamic database, and the addition of new sequences and altered taxonomic classifications were not included in this report if they occurred after the 9th July 2020.

DNA barcoding using the mitochondrial gene COI, while useful for explaining genetic differences between closely related or moderately related species, is limited in its ability to resolve deeper phylogenetic relationships among taxa at higher taxonomic levels (*e.g.* genus, family, order). In the current study, phylogenetic relationships among species/OTUs at >25% COI divergence are treated with caution. If further resolution of deeper phylogeny is important for project goals, this could be investigated using a multiple gene approach.

3 RESULTS AND DISCUSSION

Of the 20 specimens processed by Biologic, sequences were successfully derived for 19 (95% of specimens), with one failing to produce a PCR product. Of these 19 sequences, all were of high quality and of the target taxa. Specimens from the Study Area were designated to three OTUs (Table 3.1). The results of each taxonomic group's analysis are described in the subsequent sections.

Table 3.1: Summary of species and OTUs recovered from 19 samples successfully sequenced in this study, organised by taxon.

Species/OTU	Number of samples	PCR success	Sequence success	% sequence success
Amphipoda				
Chydaekata `sp. E TLF-2008`	14	13	13	93
Paramelitidae `sp. Biologic-AMPH023`	5	5	5	100
Paramelitidae `sp. Biologic-AMPH024`	1	1	1	100
Total	**20**	**19**	**19**	**95**

3.1 Amphipoda

The 19 specimens that produced high quality sequences at COI revealed three distinct OTUs (Table 3.1, Fig 3.1.1). Thirteen specimens matched a previously published OTU, *Chydaekata* `sp. E TLF-2008` (Finston *et al.*, 2007). In this study, *Chydaekata* sp. E was recorded across the Study Area (Yandicoogina Creek), as well as from the nearby reference site Weeli Wolli Spring. It is previously also known from Marillana Creek and Weeli Wolli Creek (downstream of the spring) (Finston *et al.,* 2007). *Chydaekata* `sp. E TLF-2008` specimens from this survey were closely related, with an intraspecific genetic distance of 3.19% (Table 3.1.1). The most closely related OTU to *C.* `sp. E TLF-2008` was *Chydaekata* `sp. D TLF-2008`, with an interspecific distance of ~14.5% on average. *Chydaekata* `sp. E TLF-2008` included specimens morphologically identified as Paramelitidae `Genus 2 sp. B03` suggesting that further morphological resolution of these morphospecies is required.

Paramelitidae `sp. Biologic-AMPH023` was represented by five specimens with an intraspecific distance of <1%. Only one Paramelitidae `sp. Biologic-AMPH024` OTU was recorded in the current study. These two OTU's were closely related, being ~10% divergent from one another, on average. (Table 3.1.1). These two OTUs were restricted to separate hydrological systems; Paramelitidae `sp. Biologic-AMPH023` was found in Yandicoogina Creek and Paramelitidae `sp. Biologic-AMPH024` was found in Weeli Wolli Spring.

Incidentally, both of these OTUs were morphologically identified as Paramelitidae `Genus 2 sp. B02`, and this morphospecies has been recorded in Marillana Creek and throughout Weeli Wolli Creek (Jess

Delaney, unpub. data). An analysis of sequences from Biologic's Sequence Library confirms that Marillana Creek specimens of Paramelitidae `Genus 2 sp. B02` belong to the genetically delineated OTU, Paramelitidae `sp. Biologic-AMPH023`. This is consistent with their geographic proximity and hydrological connectivity. Similarly, analysis of unpublished sequences confirms that Weeli Wolli Creek specimens of Paramelitidae `Genus 2 sp. B02` belong to OTU Paramelitidae `sp. Biologic-AMPH024`. While these two OTUs were considered a single unit based on morphological identification, there is sufficient genetic divergence (9.88-10.33%) to consider them as separate OTUs.

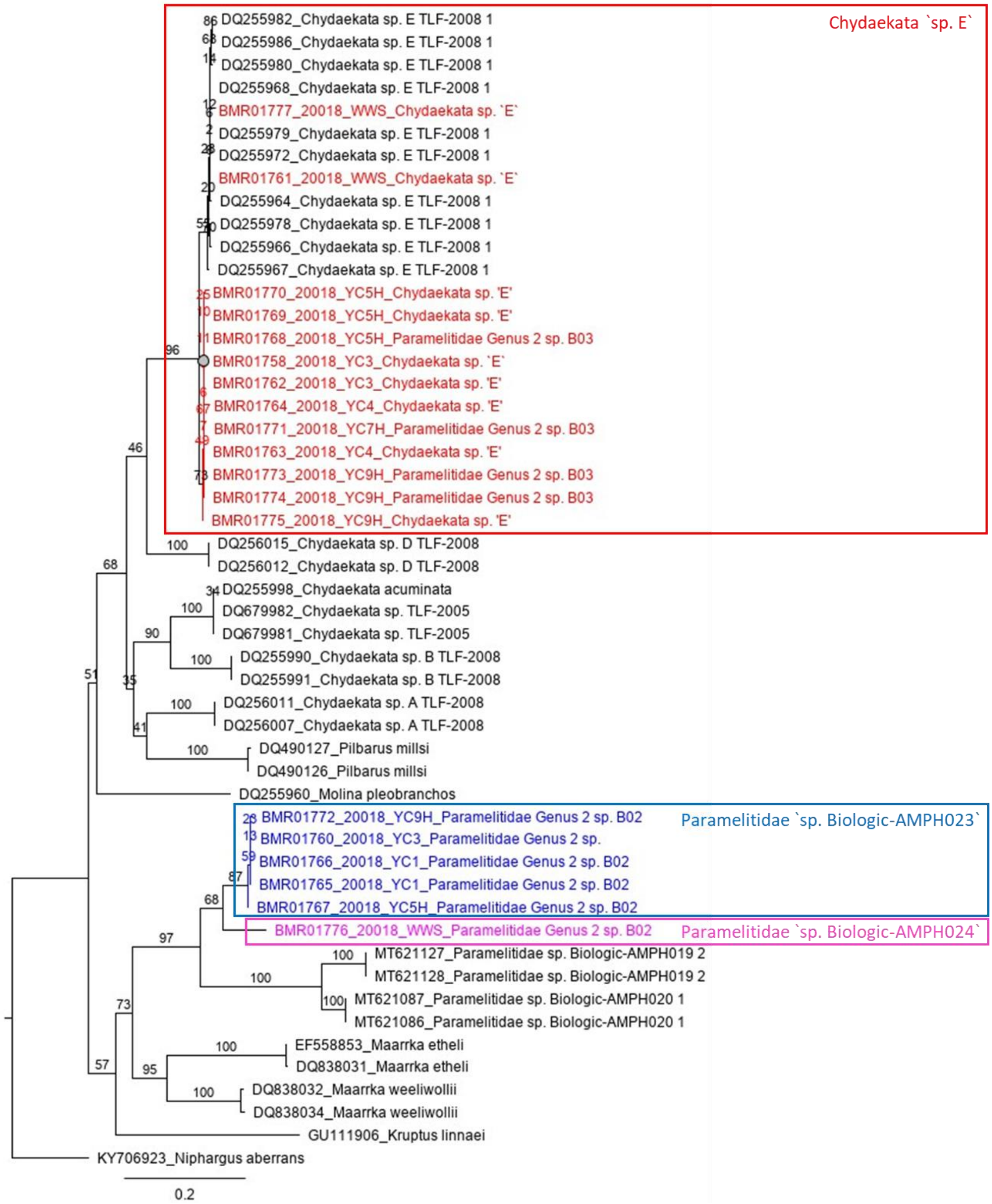


Figure 3.1.1: Maximum Likelihood phylogeny, with bootstrap values.

Table 3.1.1: Pairwise distances for the dataset included in Fig 3.1.1. Colours of OTUs match those in Fig 3.1.1.

COI pairwise divergence (%)	BMR01758	BMR01762	BMR01768	BMR01769	BMR01770	BMR01763	BMR01773	BMR01774	BMR01775	BMR01764	BMR01771	BMR01761	DQ255972	BMR01777	DQ255979	DQ255968	DQ255964	DQ255967	DQ255982	DQ255986	DQ255980	DQ255978	DQ255966	DQ256015	DQ256012	DQ256007	DQ256011	DQ255998	DQ679981	DQ679982	DQ255990	DQ255991	DQ490126	DQ490127	DQ255960	DQ838034	DQ838032	DQ838031	EF558853	BMR01760	BMR01765	BMR01766	BMR01767	BMR01772	BMR01776	MT621127	MT621128	MT621086	MT621087	KY706923	GU111906
BMR01758_20018_YC3_Chydaekata sp. `E`		0.00	0.00	0.00	0.00	0.15	0.15	0.15	0.15	0.30	0.30	2.28	2.28	2.43	2.13	2.43	2.28	2.28	2.89	2.74	2.89	2.74	2.58	14.13	14.13	17.48	17.48	16.13	16.13	16.13	15.50	15.50	17.32	17.32	19.16	22.01	22.64	24.32	24.01	22.34	22.34	22.19	22.19	22.04	23.10	23.96	23.96	23.96	23.96	19.76	21.31
BMR01762_20018_YC3_Chydaekata sp. 'E'	0.00		0.00	0.00	0.00	0.15	0.15	0.15	0.15	0.30	0.30	2.28	2.28	2.43	2.13	2.43	2.28	2.28	2.89	2.74	2.89	2.74	2.58	14.13	14.13	17.48	17.48	16.13	16.13	16.13	15.50	15.50	17.32	17.32	19.16	22.01	22.64	24.32	24.01	22.34	22.34	22.19	22.19	22.04	23.10	23.96	23.96	23.96	23.96	19.76	21.31
BMR01768_20018_YC5H_Paramelitidae Genus 2 sp. B03	0.00	0.00		0.00	0.00	0.15	0.15	0.15	0.15	0.30	0.30	2.28	2.28	2.43	2.13	2.43	2.28	2.28	2.89	2.74	2.89	2.74	2.58	14.13	14.13	17.48	17.48	16.13	16.13	16.13	15.50	15.50	17.32	17.32	19.16	22.01	22.64	24.32	24.01	22.34	22.34	22.19	22.19	22.04	23.10	23.96	23.96	23.96	23.96	19.76	21.31
BMR01769_20018_YC5H_Chydaekata sp. 'E'	0.00	0.00	0.00		0.00	0.15	0.15	0.15	0.15	0.30	0.30	2.28	2.28	2.43	2.13	2.43	2.28	2.28	2.89	2.74	2.89	2.74	2.58	14.13	14.13	17.48	17.48	16.13	16.13	16.13	15.50	15.50	17.32	17.32	19.16	22.01	22.64	24.32	24.01	22.34	22.34	22.19	22.19	22.04	23.10	23.96	23.96	23.96	23.96	19.76	21.31
BMR01770_20018_YC5H_Chydaekata sp. 'E'	0.00	0.00	0.00	0.00		0.15	0.15	0.15	0.15	0.30	0.30	2.28	2.28	2.43	2.13	2.43	2.28	2.28	2.89	2.74	2.89	2.74	2.58	14.13	14.13	17.48	17.48	16.13	16.13	16.13	15.50	15.50	17.32	17.32	19.16	22.01	22.64	24.32	24.01	22.34	22.34	22.19	22.19	22.04	23.10	23.96	23.96	23.96	23.96	19.76	21.31
BMR01763_20018_YC4_Chydaekata sp. 'E'	0.15	0.15	0.15	0.15	0.15		0.30	0.30	0.30	0.46	0.46	2.13	2.13	2.28	1.98	2.28	2.13	2.13	2.74	2.58	2.74	2.58	2.43	14.29	14.29	17.63	17.63	16.29	16.29	16.29	15.35	15.35	17.32	17.32	19.01	22.01	22.80	24.47	24.16	22.49	22.49	22.34	22.34	22.19	23.25	24.12	24.12	24.12	24.12	19.91	21.31
BMR01773_20018_YC9H_Paramelitidae Genus 2 sp. B03	0.15	0.15	0.15	0.15	0.15	0.30		0.30	0.30	0.46	0.46	2.43	2.43	2.58	2.28	2.58	2.43	2.43	3.04	2.89	3.04	2.89	2.74	14.13	14.13	17.63	17.63	16.13	16.13	16.13	15.50	15.50	17.32	17.32	19.16	22.01	22.49	24.16	24.16	22.34	22.34	22.19	22.19	22.04	23.10	23.96	23.96	23.96	23.96	19.76	21.31
BMR01774_20018_YC9H_Paramelitidae Genus 2 sp. B03	0.15	0.15	0.15	0.15	0.15	0.30	0.30		0.30	0.46	0.46	2.43	2.43	2.58	2.28	2.58	2.43	2.43	3.04	2.89	3.04	2.89	2.74	13.98	13.98	17.63	17.63	15.98	15.98	15.98	15.35	15.35	17.49	17.49	19.32	22.17	22.80	24.47	24.16	22.49	22.49	22.34	22.34	22.19	23.25	24.12	24.12	24.12	24.12	19.61	21.50
BMR01775_20018_YC9H_Chydaekata sp. 'E'	0.15	0.15	0.15	0.15	0.15	0.30	0.30	0.30		0.46	0.46	2.13	2.13	2.28	1.98	2.28	2.13	2.13	2.74	2.58	2.74	2.58	2.43	14.13	14.13	17.33	17.33	16.29	16.29	16.29	15.35	15.35	17.32	17.32	19.01	21.85	22.49	24.32	24.01	22.19	22.19	22.04	22.04	21.88	22.95	24.12	24.12	23.81	23.81	19.76	21.11
BMR01764_20018_YC4_Chydaekata sp. 'E'	0.30	0.30	0.30	0.30	0.30	0.46	0.46	0.46	0.46		0.30	2.58	2.58	2.74	2.43	2.74	2.58	2.58	3.19	3.04	3.19	3.04	2.89	14.13	14.13	17.48	17.48	16.13	16.13	16.13	15.50	15.50	17.15	17.15	19.16	22.17	22.80	24.16	23.86	22.49	22.49	22.34	22.34	22.19	22.80	24.12	24.12	24.12	24.12	19.91	21.31
BMR01771_20018_YC7H_Paramelitidae Genus 2 sp. B03	0.30	0.30	0.30	0.30	0.30	0.46	0.46	0.46	0.46	0.30		2.58	2.58	2.74	2.43	2.74	2.58	2.58	3.19	3.04	3.19	3.04	2.89	14.44	14.44	17.78	17.78	16.44	16.44	16.44	15.65	15.65	17.49	17.49	19.47	22.33	22.95	24.01	23.71	22.64	22.64	22.49	22.49	22.19	23.10	23.96	23.96	23.96	23.96	20.06	21.50
BMR01761_20018_WWS_Chydaekata sp. `E`	2.28	2.28	2.28	2.28	2.28	2.13	2.43	2.43	2.13	2.58	2.58		0.00	0.15	0.15	0.15	0.30	0.30	0.61	0.46	0.61	0.61	0.46	14.59	14.59	17.63	17.63	16.59	16.59	16.59	15.50	15.50	18.17	18.17	19.16	22.65	23.25	24.16	23.86	22.04	22.04	21.88	21.88	21.73	22.95	25.35	25.35	24.58	24.58	20.67	21.69
DQ255972_Chydaekata sp. E TLF-2008 1	2.28	2.28	2.28	2.28	2.28	2.13	2.43	2.43	2.13	2.58	2.58	0.00		0.15	0.15	0.15	0.30	0.30	0.61	0.46	0.61	0.61	0.46	14.59	14.59	17.63	17.63	16.59	16.59	16.59	15.50	15.50	18.17	18.17	19.16	22.65	23.25	24.16	23.86	22.04	22.04	21.88	21.88	21.73	22.95	25.35	25.35	24.58	24.58	20.67	21.69
BMR01777_20018_WWS_Chydaekata sp. `E`	2.43	2.43	2.43	2.43	2.43	2.28	2.58	2.58	2.28	2.74	2.74	0.15	0.15		0.30	0.30	0.46	0.46	0.76	0.61	0.76	0.76	0.61	14.44	14.44	17.63	17.63	16.44	16.44	16.44	15.35	15.35	18.00	18.00	19.16	22.65	23.25	24.01	23.71	21.88	21.88	21.73	21.73	21.58	22.80	25.35	25.35	24.58	24.58	20.67	21.50
DQ255979_Chydaekata sp. E TLF-2008 1	2.13	2.13	2.13	2.13	2.13	1.98	2.28	2.28	1.98	2.43	2.43	0.15	0.15	0.30		0.30	0.46	0.46	0.76	0.61	0.76	0.76	0.61	14.44	14.44	17.48	17.48	16.44	16.44	16.44	15.35	15.35	18.00	18.00	19.01	22.49	23.10	24.32	24.01	21.88	21.88	21.73	21.73	21.58	22.80	25.19	25.19	24.58	24.58	20.52	21.50
DQ255968_Chydaekata sp. E TLF-2008 1	2.43	2.43	2.43	2.43	2.43	2.28	2.58	2.58	2.28	2.74	2.74	0.15	0.15	0.30	0.30		0.46	0.46	0.76	0.61	0.76	0.76	0.61	14.44	14.44	17.78	17.78	16.74	16.74	16.74	15.65	15.65	18.34	18.34	19.32	22.81	23.40	24.01	23.71	21.88	21.88	21.73	21.73	21.58	22.80	25.50	25.50	24.73	24.73	20.52	21.88
DQ255964_Chydaekata sp. E TLF-2008 1	2.28	2.28	2.28	2.28	2.28	2.13	2.43	2.43	2.13	2.58	2.58	0.30	0.30	0.46	0.46	0.46		0.61	0.91	0.76	0.91	0.61	0.76	14.29	14.29	17.48	17.48	16.29	16.29	16.29	15.35	15.35	18.17	18.17	19.16	22.65	23.25	24.16	23.86	21.73	21.73	21.58	21.58	21.43	22.80	25.35	25.35	24.58	24.58	20.37	21.69
DQ255967_Chydaekata sp. E TLF-2008 1	2.28	2.28	2.28	2.28	2.28	2.13	2.43	2.43	2.13	2.58	2.58	0.30	0.30	0.46	0.46	0.46	0.61		0.91	0.76	0.91	0.76	0.61	14.44	14.44	17.48	17.48	16.44	16.44	16.44	15.65	15.65	18.34	18.34	18.86	22.65	23.25	24.16	23.86	21.88	21.88	21.73	21.73	21.58	22.80	25.19	25.19	24.42	24.42	20.52	21.88
DQ255982_Chydaekata sp. E TLF-2008 1	2.89	2.89	2.89	2.89	2.89	2.74	3.04	3.04	2.74	3.19	3.19	0.61	0.61	0.76	0.76	0.76	0.91	0.91		0.15	0.61	1.22	1.06	14.59	14.59	17.33	17.33	16.59	16.59	16.59	15.81	15.81	17.83	17.83	18.55	22.49	23.10	24.16	23.86	22.04	22.04	21.88	21.88	21.73	22.95	25.04	25.04	24.27	24.27	20.82	21.50
DQ255986_Chydaekata sp. E TLF-2008 1	2.74	2.74	2.74	2.74	2.74	2.58	2.89	2.89	2.58	3.04	3.04	0.46	0.46	0.61	0.61	0.61	0.76	0.76	0.15		0.46	1.06	0.91	14.44	14.44	17.17	17.17	16.44	16.44	16.44	15.65	15.65	17.66	17.66	18.70	22.33	22.95	24.01	23.71	21.88	21.88	21.73	21.73	21.58	22.80	24.89	24.89	24.12	24.12	20.67	21.50
DQ255980_Chydaekata sp. E TLF-2008 1	2.89	2.89	2.89	2.89	2.89	2.74	3.04	3.04	2.74	3.19	3.19	0.61	0.61	0.76	0.76	0.76	0.91	0.91	0.61	0.46		1.22	1.06	14.74	14.74	17.48	17.48	16.29	16.29	16.29	15.81	15.81	18.00	18.00	19.16	22.65	23.25	24.32	24.01	22.19	22.19	22.04	22.04	21.88	23.25	24.89	24.89	24.27	24.27	20.97	21.88
DQ255978_Chydaekata sp. E TLF-2008 1	2.74	2.74	2.74	2.74	2.74	2.58	2.89	2.89	2.58	3.04	3.04	0.61	0.61	0.76	0.76	0.76	0.61	0.76	1.22	1.06	1.22		0.46	14.44	14.44	17.78	17.78	16.44	16.44	16.44	15.50	15.50	18.00	18.00	19.62	22.49	23.10	24.16	23.86	21.88	21.88	21.73	21.73	21.58	22.80	25.35	25.35	24.73	24.73	20.52	21.50
DQ255966_Chydaekata sp. E TLF-2008 1	2.58	2.58	2.58	2.58	2.58	2.43	2.74	2.74	2.43	2.89	2.89	0.46	0.46	0.61	0.61	0.61	0.76	0.61	1.06	0.91	1.06	0.46		14.59	14.59	17.78	17.78	16.59	16.59	16.59	15.50	15.50	18.34	18.34	19.47	22.65	23.25	24.47	24.16	22.04	22.04	21.88	21.88	21.73	22.95	25.19	25.19	24.42	24.42	20.67	21.88
DQ256015_Chydaekata sp. D TLF-2008	14.13	14.13	14.13	14.13	14.13	14.29	14.13	13.98	14.13	14.13	14.44	14.59	14.59	14.44	14.44	14.44	14.29	14.44	14.59	14.44	14.74	14.44	14.59		0.00	14.44	14.44	15.68	15.68	15.68	15.96	15.96	19.02	19.02	20.70	22.01	22.95	23.56	23.40	19.30	19.15	19.00	19.15	18.85	21.88	23.50	23.50	22.43	22.43	21.28	24.18
DQ256012_Chydaekata sp. D TLF-2008	14.13	14.13	14.13	14.13	14.13	14.29	14.13	13.98	14.13	14.13	14.44	14.59	14.59	14.44	14.44	14.44	14.29	14.44	14.59	14.44	14.74	14.44	14.59	0.00		14.44	14.44	15.68	15.68	15.68	15.96	15.96	19.02	19.02	20.70	22.01	22.95	23.56	23.40	19.30	19.15	19.00	19.15	18.85	21.88	23.50	23.50	22.43	22.43	21.28	24.18
DQ256007_Chydaekata sp. A TLF-2008	17.48	17.48	17.48	17.48	17.48	17.63	17.63	17.63	17.33	17.48	17.78	17.63	17.63	17.63	17.48	17.78	17.48	17.48	17.33	17.17	17.48	17.78	17.78	14.44	14.44		0.00	15.53	15.53	15.53	17.93	17.93	17.15	17.15	20.39	21.37	22.04	24.62	24.32	20.97	20.82	20.97	21.13	20.82	22.80	24.73	24.73	22.89	22.89	20.06	21.88
DQ256011_Chydaekata sp. A TLF-2008	17.48	17.48	17.48	17.48	17.48	17.63	17.63	17.63	17.33	17.48	17.78	17.63	17.63	17.63	17.48	17.78	17.48	17.48	17.33	17.17	17.48	17.78	17.78	14.44	14.44	0.00		15.53	15.53	15.53	17.93	17.93	17.15	17.15	20.39	21.37	22.04	24.62	24.32	20.97	20.82	20.97	21.13	20.82	22.80	24.73	24.73	22.89	22.89	20.06	21.88
DQ255998_Chydaekata acuminata	16.13	16.13	16.13	16.13	16.13	16.29	16.13	15.98	16.29	16.13	16.44	16.59	16.59	16.44	16.44	16.74	16.29	16.44	16.59	16.44	16.29	16.44	16.59	15.68	15.68	15.53	15.53		0.00	0.00	13.09	13.09	16.98	16.98	21.16	18.82	19.33	22.83	22.68	21.46	21.61	21.46	21.46	21.61	22.37	25.81	25.81	25.04	25.04	21.46	24.57
DQ679981_Chydaekata sp. TLF-2005	16.13	16.13	16.13	16.13	16.13	16.29	16.13	15.98	16.29	16.13	16.44	16.59	16.59	16.44	16.44	16.74	16.29	16.44	16.59	16.44	16.29	16.44	16.59	15.68	15.68	15.53	15.53	0.00		0.00	13.09	13.09	16.98	16.98	21.16	18.82	19.33	22.83	22.68	21.46	21.61	21.46	21.46	21.61	22.37	25.81	25.81	25.04	25.04	21.46	24.57
DQ679982_Chydaekata sp. TLF-2005	16.13	16.13	16.13	16.13	16.13	16.29	16.13	15.98	16.29	16.13	16.44	16.59	16.59	16.44	16.44	16.74	16.29	16.44	16.59	16.44	16.29	16.44	16.59	15.68	15.68	15.53	15.53	0.00	0.00		13.09	13.09	16.98	16.98	21.16	18.82	19.33	22.83	22.68	21.46	21.61	21.46	21.46	21.61	22.37	25.81	25.81	25.04	25.04	21.46	24.57
DQ255990_Chydaekata sp. B TLF-2008	15.50	15.50	15.50	15.50	15.50	15.35	15.50	15.35	15.35	15.50	15.65	15.50	15.50	15.35	15.35	15.65	15.35	15.65	15.81	15.65	15.81	15.50	15.50	15.96	15.96	17.93	17.93	13.09	13.09	13.09		0.00	17.83	17.83	20.85	21.37	21.88	22.49	22.34	24.16	24.01	24.16	24.16	23.71	25.08	25.81	25.81	24.89	24.89	22.64	22.65
DQ255991_Chydaekata sp. B TLF-2008	15.50	15.50	15.50	15.50	15.50	15.35	15.50	15.35	15.35	15.50	15.65	15.50	15.50	15.35	15.35	15.65	15.35	15.65	15.81	15.65	15.81	15.50	15.50	15.96	15.96	17.93	17.93	13.09	13.09	13.09	0.00		17.83	17.83	20.85	21.37	21.88	22.49	22.34	24.16	24.01	24.16	24.16	23.71	25.08	25.81	25.81	24.89	24.89	22.64	22.65
DQ490126_Pilbarus millsi	17.32	17.32	17.32	17.32	17.32	17.32	17.32	17.49	17.32	17.15	17.49	18.17	18.17	18.00	18.00	18.34	18.17	18.34	17.83	17.66	18.00	18.00	18.34	19.02	19.02	17.15	17.15	16.98	16.98	16.98	17.83	17.83		0.34	21.39	22.75	22.75	23.09	22.92	23.26	22.92	23.09	23.09	23.26	22.92	26.32	26.32	25.81	25.81	24.11	25.14
DQ490127_Pilbarus millsi	17.32	17.32	17.32	17.32	17.32	17.32	17.32	17.49	17.32	17.15	17.49	18.17	18.17	18.00	18.00	18.34	18.17	18.34	17.83	17.66	18.00	18.00	18.34	19.02	19.02	17.15	17.15	16.98	16.98	16.98	17.83	17.83	0.34		21.39	22.75	22.75	23.26	23.09	23.26	22.92	23.09	23.09	23.26	22.92	26.32	26.32	25.98	25.98	24.11	25.14
DQ255960_Molina pleobranchos	19.16	19.16	19.16	19.16	19.16	19.01	19.16	19.32	19.01	19.16	19.47	19.16	19.16	19.16	19.01	19.32	19.16	18.86	18.55	18.70	19.16	19.62	19.47	20.70	20.70	20.39	20.39	21.16	21.16	21.16	20.85	20.85	21.39	21.39		22.81	23.00	22.85	22.70	22.39	21.93	22.08	22.08	21.93	23.16	23.62	23.62	25.00	25.00	22.54	23.99
DQ838034_Maarrka weeliwollii	22.01	22.01	22.01	22.01	22.01	22.01	22.01	22.17	21.85	22.17	22.33	22.65	22.65	22.65	22.49	22.81	22.65	22.65	22.49	22.33	22.65	22.49	22.65	22.01	22.01	21.37	21.37	18.82	18.82	18.82	21.37	21.37	22.75	22.75	22.81		1.28	19.78	19.62	21.37	21.21	21.05	20.89	20.89	20.10	24.24	24.24	23.13	23.13	23.45	23.80
DQ838032_Maarrka weeliwollii	22.64	22.64	22.64	22.64	22.64	22.80	22.49	22.80	22.49	22.80	22.95	23.25	23.25	23.25	23.10	23.40	23.25	23.25	23.10	22.95	23.25	23.10	23.25	22.95	22.95	22.04	22.04	19.33	19.33	19.33	21.88	21.88	22.75	22.75	23.00	1.28		20.21	20.21	22.19	22.04	21.88	21.73	21.73	21.13	24.58	24.58	22.89	22.89	23.71	23.99
DQ838031_Maarrka etheli	24.32	24.32	24.32	24.32	24.32	24.47	24.16	24.47	24.32	24.16	24.01	24.16	24.16	24.01	24.32	24.01	24.16	24.16	24.16	24.01	24.32	24.16	24.47	23.56	23.56	24.62	24.62	22.83	22.83	22.83	22.49	22.49	23.09	23.26	22.85	19.78	20.21		0.30	23.86	23.40	23.56	23.40	23.25	23.10	27.96	27.96	26.73	26.73	26.75	24.95
EF558853_Maarrka etheli	24.01	24.01	24.01	24.01	24.01	24.16	24.16	24.16	24.01	23.86	23.71	23.86	23.86	23.71	24.01	23.71	23.86	23.86	23.86	23.71	24.01	23.86	24.16	23.40	23.40	24.32	24.32	22.68	22.68	22.68	22.34	22.34	22.92	23.09	22.70	19.62	20.21	0.30		23.71	23.25	23.40	23.25	23.10	22.95	27.80	27.80	26.58	26.58	26.60	24.76
BMR01760_20018_YC3_Paramelitidae Genus 2 sp.	22.34	22.34	22.34	22.34	22.34	22.49	22.34	22.49	22.19	22.49	22.64	22.04	22.04	21.88	21.88	21.88	21.73	21.88	22.04	21.88	22.19	21.88	22.04	19.30	19.30	20.97	20.97	21.46	21.46	21.46	24.16	24.16	23.26	23.26	22.39	21.37	22.19	23.86	23.71		0.46	0.30	0.61	0.91	10.33	18.59	18.59	18.43	18.43	20.82	22.84
BMR01765_20018_YC1_Paramelitidae Genus 2 sp. B02	22.34	22.34	22.34	22.34	22.34	22.49	22.34	22.49	22.19	22.49	22.64	22.04	22.04	21.88	21.88	21.88	21.73	21.88	22.04	21.88	22.19	21.88	22.04	19.15	19.15	20.82	20.82	21.61	21.61	21.61	24.01	24.01	22.92	22.92	21.93	21.21	22.04	23.40	23.25	0.46		0.15	0.46	0.76	9.88	18.28	18.28	18.13	18.13	20.37	22.84
BMR01766_20018_YC1_Paramelitidae Genus 2 sp. B02	22.19	22.19	22.19	22.19	22.19	22.34	22.19	22.34	22.04	22.34	22.49	21.88	21.88	21.73	21.73	21.73	21.58	21.73	21.88	21.73	22.04	21.73	21.88	19.00	19.00	20.97	20.97	21.46	21.46	21.46	24.16	24.16	23.09	23.09	22.08	21.05	21.88	23.56	23.40	0.30	0.15		0.30	0.61	10.03	18.43	18.43	18.28	18.28	20.52	22.65
BMR01767_20018_YC5H_Paramelitidae Genus 2 sp. B02	22.19	22.19	22.19	22.19	22.19	22.34	22.19	22.34	22.04	22.34	22.49	21.88	21.88	21.73	21.73	21.73	21.58	21.73	21.88	21.73	22.04	21.73	21.88	19.15	19.15	21.13	21.13	21.46	21.46	21.46	24.16	24.16	23.09	23.09	22.08	20.89	21.73	23.40	23.25	0.61	0.46	0.30		0.91	9.88	18.43	18.43	18.13	18.13	20.37	22.65
BMR01772_20018_YC9H_Paramelitidae Genus 2 sp. B02	22.04	22.04	22.04	22.04	22.04	22.19	22.04	22.19	21.88	22.19	22.19	21.73	21.73	21.58	21.58	21.58	21.43	21.58	21.73	21.58	21.88	21.58	21.73	18.85	18.85	20.82	20.82	21.61	21.61	21.61	23.71	23.71	23.26	23.26	21.93	20.89	21.73	23.25	23.10	0.91	0.76	0.61	0.91		10.33	18.28	18.28	18.13	18.13	20.67	23.23
BMR01776_20018_WWS_Paramelitidae Genus 2 sp. B02	23.10	23.10	23.10	23.10	23.10	23.25	23.10	23.25	22.95	22.80	23.10	22.95	22.95	22.80	22.80	22.80	22.80	22.80	22.95	22.80	23.25	22.80	22.95	21.88	21.88	22.80	22.80	22.37	22.37	22.37	25.08	25.08	22.92	22.92	23.16	20.10	21.13	23.10	22.95	10.33	9.88	10.03	9.88	10.33		21.35	21.35	18.59	18.59	23.10	24.95
MT621127_Paramelitidae sp. Biologic-AMPH019 2	23.96	23.96	23.96	23.96	23.96	24.12	23.96	24.12	24.12	24.12	23.96	25.35	25.35	25.35	25.19	25.50	25.35	25.19	25.04	24.89	24.89	25.35	25.19	23.50	23.50	24.73	24.73	25.81	25.81	25.81	25.81	25.81	26.32	26.32	23.62	24.24	24.58	27.96	27.80	18.59	18.28	18.43	18.43	18.28	21.35		0.00	8.60	8.60	25.04	25.91
MT621128_Paramelitidae sp. Biologic-AMPH019 2	23.96	23.96	23.96	23.96	23.96	24.12	23.96	24.12	24.12	24.12	23.96	25.35	25.35	25.35	25.19	25.50	25.35	25.19	25.04	24.89	24.89	25.35	25.19	23.50	23.50	24.73	24.73	25.81	25.81	25.81	25.81	25.81	26.32	26.32	23.62	24.24	24.58	27.96	27.80	18.59	18.28	18.43	18.43	18.28	21.35	0.00		8.60	8.60	25.04	25.91
MT621086_Paramelitidae sp. Biologic-AMPH020 1	23.96	23.96	23.96	23.96	23.96	24.12	23.96	24.12	23.81	24.12	23.96	24.58	24.58	24.58	24.58	24.73	24.58	24.42	24.27	24.12	24.27	24.73	24.42	22.43	22.43	22.89	22.89	25.04	25.04	25.04	24.89	24.89	25.81	25.98	25.00	23.13	22.89	26.73	26.58	18.43	18.13	18.28	18.13	18.13	18.59	8.60	8.60		0.00	23.66	24.76
MT621087_Paramelitidae sp. Biologic-AMPH020 1	23.96	23.96	23.96	23.96	23.96	24.12	23.96	24.12	23.81	24.12	23.96	24.58	24.58	24.58	24.58	24.73	24.58	24.42	24.27	24.12	24.27	24.73	24.42	22.43	22.43	22.89	22.89	25.04	25.04	25.04	24.89	24.89	25.81	25.98	25.00	23.13	22.89	26.73	26.58	18.43	18.13	18.28	18.13	18.13	18.59	8.60	8.60	0.00		23.66	24.76
KY706923_Niphargus aberrans	19.76	19.76	19.76	19.76	19.76	19.91	19.76	19.61	19.76	19.91	20.06	20.67	20.67	20.67	20.52	20.52	20.37	20.52	20.82	20.67	20.97	20.52	20.67	21.28	21.28	20.06	20.06	21.46	21.46	21.46	22.64	22.64	24.11	24.11	22.54	23.45	23.71	26.75	26.60	20.82	20.37	20.52	20.37	20.67	23.10	25.04	25.04	23.66	23.66		24.18
GU111906_Kruptus linnaei	21.31	21.31	21.31	21.31	21.31	21.31	21.31	21.50	21.11	21.31	21.50	21.69	21.69	21.50	21.50	21.88	21.69	21.88	21.50	21.50	21.88	21.50	21.88	24.18	24.18	21.88	21.88	24.57	24.57	24.57	22.65	22.65	25.14	25.14	23.99	23.80	23.99	24.95	24.76	22.84	22.84	22.65	22.65	23.23	24.95	25.91	25.91	24.76	24.76	24.18	

4 SUMMARY

Using well-established DNA extraction and sequencing methods, this molecular systematics analysis designated three distinct species/ OTUs to 19 high quality sequences from the Study Area. All OTUs, the areas in which they were found, and the specimen numbers per OTU are shown in Appendix A. The following are the key findings at the species/ OTU level:

- *Chydaekata* `sp. E TLF-2008`: found in Yandicoogina Creek, Weeli Wolli Spring in this study and matching sequences from Marillana Creek and Weeli Wolli Creek.
- Paramelitidae `sp. Biologic-AMPH023`: found in Yandicoogina Creek and Marillana Creek.
- Paramelitidae `sp. Biologic-AMPH024`: found in Weeli Wolli Spring in this study, and previously also known from Weeli Wolli Creek downstream of the spring.

This study highlights the importance of using genetic analysis alongside traditional morphological identification methods, particularly for groups which are notoriously difficult to distinguish morphologically, such as stygal amphipods. It seems likely that juvenile *Chydaekata* sp. E from Yandicoogina Creek were mis-identified as Paramelitidae Genus 2 sp. B03 in the current study. In addition, specimens which were morphologically identified as Paramelitidae Genus 2 sp. B02 corresponded to two different OTUs based on genetic sequences.

5 REFERENCES

Cullen, K., & Harvey, M. (2017). *Molecular Systematics of Subfauna from Mesa B, C, and Warramboo, Western Australia.* Unpublished report prepared for Rio Tinto Iron Ore.

Cullen, K., & Harvey, M. (2018). *Molecular Systematics of Subfauna from Greater Paraburdoo – Final Report*. Unpublished report prepared for Rio Tinto Iron Ore.

Finston, T. L., Johnson, M. S., Humphreys, W. F., Eberhard, S. M., & Halse, S. A. (2007). Cryptic speciation in two widespread subterranean amphipod genera reflects historical drainage patterns in an ancient landscape. *Molecular Ecology, 16*(2), 355-365.

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology, 3*(5), 294-299.

Hebert, P. D., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003a). Biological identifications through DNA barcodes. *Proceedings of the Royal Society B, 270*(1512), 313-321.

Hebert, P. D., Ratnasingham, S., & deWaard, J. R. (2003b). Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society B, 270 Suppl 1*, S96-99.

Katoh, K., Misawa, K., Kuma, K., & Miyata, T. (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research, 30*(14), 3059-3066.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., . . . Drummond, A. (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics, 28*(12), 1647-1649.

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics, 30*(9), 1312-1313.

Appendix A: All Operational Taxonomic Units (OTUs) found in the Study Area.

BMR	Unique ID code	Site	Latitude	Longitude	Lowest ID Legacy	OTU Name (molecular ID)	Reaction State
Amphipoda							
BMR01758	7083	YC3	-22.8245425	119.1636157	Chydaekata sp. \`E\`	Chydaekata \`sp. E TLF-2008\`	PASS
BMR01759	7336	YC3	-22.8245425	119.1636157	Chydaekata sp. \`E\`	-	FAIL; PCR
BMR01760	7083	YC3	-22.8245425	119.1636157	Paramelitidae Genus 2 sp.	Paramelitidae \`sp. Biologic-AMPH023\`	PASS
BMR01761	6156	WWS	-22.9178499	119.199844	Chydaekata sp. \`E\`	Chydaekata \`sp. E TLF-2008\`	PASS
BMR01762	8701	YC3	-22.8245425	119.1636157	Chydaekata sp. 'E'	Chydaekata \`sp. E TLF-2008\`	PASS
BMR01763	8702	YC4	-22.8258712	119.1628575	Chydaekata sp. 'E'	Chydaekata \`sp. E TLF-2008\`	PASS
BMR01764	8703	YC4	-22.8258712	119.1628575	Chydaekata sp. 'E'	Chydaekata \`sp. E TLF-2008\`	PASS
BMR01765	8704	YC1	-22.8281657	119.149978	Paramelitidae Genus 2 sp. B02	Paramelitidae \`sp. Biologic-AMPH023\`	PASS
BMR01766	8705	YC1	-22.8281657	119.149978	Paramelitidae Genus 2 sp. B02	Paramelitidae \`sp. Biologic-AMPH023\`	PASS
BMR01767	8706	YC5H	-22.8245072	119.1637513	Paramelitidae Genus 2 sp. B02	Paramelitidae \`sp. Biologic-AMPH023\`	PASS
BMR01768	8707	YC5H	-22.8245072	119.1637513	Paramelitidae Genus 2 sp. B03	Chydaekata \`sp. E TLF-2008\`	PASS
BMR01769	8708	YC5H	-22.8245072	119.1637513	Chydaekata sp. 'E'	Chydaekata \`sp. E TLF-2008\`	PASS
BMR01770	8709	YC5H	-22.8245072	119.1637513	Chydaekata sp. 'E'	Chydaekata \`sp. E TLF-2008\`	PASS
BMR01771	8710	YC7H	-22.8253506	119.1601651	Paramelitidae Genus 2 sp. B03	Chydaekata \`sp. E TLF-2008\`	PASS
BMR01772	8711	YC9H	-22.8256262	119.1581974	Paramelitidae Genus 2 sp. B02	Paramelitidae \`sp. Biologic-AMPH023\`	PASS
BMR01773	8712	YC9H	-22.8256262	119.1581974	Paramelitidae Genus 2 sp. B03	Chydaekata \`sp. E TLF-2008\`	PASS
BMR01774	8713	YC9H	-22.8256262	119.1581974	Paramelitidae Genus 2 sp. B03	Chydaekata \`sp. E TLF-2008\`	PASS
BMR01775	8714	YC9H	-22.8256262	119.1581974	Chydaekata sp. 'E'	Chydaekata \`sp. E TLF-2008\`	PASS
BMR01776	8715	WWS	-22.9178499	119.199844	Paramelitidae Genus 2 sp. B02	Paramelitidae \`sp. Biologic-AMPH024\`	PASS
BMR01777	8716	WWS	-22.9178499	119.199844	Chydaekata sp. \`E\`	Chydaekata \`sp. E TLF-2008\`	PASS

Appendix H: Macroinvertebrate taxonomic list

Values are log abundances (i.e. 1=1 individual, 2 = 2-10, 3 = 11-100, 4 = 101-1000, and so on).

Dry-19

Phylum/Class/Order	Family	Lowest taxon	Yandicoogina Creek				Reference sites			
			YC1	YC2	YC3	YC4	WWS	BENS	MUNJS	SS
CNIDARIA										
HYDROZOA	Hydridae	*Hydra* sp.	0	0	0	0	0	0	2	0
NEMATODA		Nematoda sp.	3	0	0	3	1	0	0	0
MOLLUSCA			0	0	0	0	0	0	0	0
GASTROPODA	Lymnaeidae	*Bullastra vinosa*	2	0	0	2	2	0	3	3
	Planorbidae	*Ferrissia petterdi*	2	2	0	3	0	1	0	0
		Gyraulus sp.	3	2	1	3	2	4	2	3
ANNELIDA										
OLIGOCHAETA										
Tubificida	Naididae	Naidinae spp. (imm./dam.)	2	1	0	2	3	0	0	3
		Nais communis	0	0	2	0	3	0	0	2
		Pristina spp. (imm./dam.)	2	0	3	2	3	0	0	2
ARTHROPODA										
CRUSTACEA										
MALACOSTRACA										
Amphipoda	Paramelitidae	*Chydaekata* sp. 'E'	0	0	2	2	3	0	0	0
		Paramelitidae sp. (imm/dam)	0	0	0	0	2	0	0	0
ARACHNIDA										
		Acari sp. (imm)	0	0	0	0	0	2	0	0
Trombidiformes		Prostigmata sp.	1	0	0	2	0	0	0	0
	Aturidae	*Albia rectifrons*	0	0	0	0	0	1	0	0
		Albia sp.	0	0	0	0	2	0	0	0
		Austraturus sp. P2 (PSW)	0	0	0	2	0	1	0	0
	Hydrodromidae	*Hydrodroma* sp.	0	0	0	1	2	0	0	0
	Hydryphantidae	*Diplodontus* sp.	0	0	0	0	0	1	0	0
		Hydryphantes sp.	0	0	0	0	0	0	0	2
	Hygrobatidae	*Australiobates* sp. P4 (PSW)	0	0	0	0	1	0	0	0
		Coaustraliobates minor	0	0	0	2	0	2	0	2
	Limnesiidae	*Limnesia* sp.	0	2	0	0	0	0	0	0
		Limnesia sp. 4 (PSW)	3	0	2	2	2	3	2	3
	Limnocharidae	*Limnochares australica*	0	0	0	0	1	0	0	0
	Momoniidae	*Momoniella australica*	0	0	0	1	0	0	0	0
	Oxidae	*Oxus orientalis*	0	1	0	0	0	0	0	2
	Unionicolidae	*Neumania* sp.	2	1	0	2	0	0	0	2
		Recifella sp.	0	0	0	0	0	0	0	2
		Unionicola sp.	0	0	0	2	0	2	0	0
Sarcoptiformes		Oribatida spp.	0	2	0	0	1	2	0	0
COLLEMBOLLA										
Entomobryomorpha		Entomobryoidea sp.	1	0	0	0	0	0	0	0
HEXAPODA										
INSECTA										
Coleoptera	Dytiscidae	*Austrodytes plateni*	0	0	0	1	0	1	0	1
		Cybister sp. (L)	0	0	0	0	1	0	2	0
		Hydaticus consanguineus	0	0	1	0	0	0	1	0
		Hydaticus daemeli	0	0	0	1	0	0	0	0
		Hydroglyphus grammopterus	0	0	0	0	0	1	0	1
		Hydroglyphus orthogrammus	0	0	0	2	0	3	2	3
		Hydrovatus opacus	0	2	0	1	0	0	0	0
		Hydrovatus sp. (L)	1	0	2	2	0	3	0	1
		Hyphydrus elegans	0	0	0	0	0	1	0	0
		Hyphydrus lyratus	0	0	0	1	0	0	0	2
		Limbodessus compactus	0	0	1	0	0	0	0	0
		Necterosoma regulare	0	0	0	0	0	2	0	0
		Neobidessodes denticulatus	2	0	0	3	0	0	0	0
		Platynectes decempunctatus	0	2	0	2	0	0	0	0
		Rhantaticus congestus	0	0	0	0	0	1	1	0
		Rhantus suturalis	0	0	1	0	0	0	0	0
		Tiporus tambreyi	0	0	0	0	0	2	0	1
	Elmidae	*Austrolimnius* sp. (L)	0	0	0	0	0	0	0	3
	Gyrinidae	*Dineutus australis*	0	0	0	0	0	2	2	2
		Macrogyrus darlingtoni	0	0	0	0	2	0	0	0
		Macrogyrus sp.	0	0	0	0	2	0	0	0
	Hydraenidae	*Hydraena* sp.	0	1	2	3	1	3	0	1
		Limnebius sp.	0	0	0	0	0	0	0	2
	Hydrophilidae	*Agraphydrus coomani*	0	0	0	0	0	0	0	1
		Anacaena horni	2	0	2	2	0	1	1	0
		nr. *Anacaena* sp.	0	0	0	1	0	0	0	0
		Berosus dallasae	0	0	0	0	0	0	0	3

			Yandicoogina Creek				Reference sites			
Phylum/Class/Order	**Family**	**Lowest taxon**	**YC1**	**YC2**	**YC3**	**YC4**	**WWS**	**BENS**	**MUNJS**	**SS**
		Berosus sp. (L)	0	0	0	0	0	0	0	1
		Chaetarthria nigerrima	0	0	0	0	0	0	0	1
		Enochrus deserticola	1	0	0	0	0	0	0	0
		Helochares sp. (L)	0	1	0	0	0	1	0	1
		Hydrochus burdekinensis	0	0	0	0	0	0	0	1
		Hydrochus eurypleuron	0	0	0	2	0	0	0	2
		Hydrochus interioris	0	0	0	0	0	0	0	1
		Hydrochus macroaquilonius	0	0	0	0	0	0	0	2
		Hydrochus obscuroaeneus	2	0	0	0	0	2	0	2
		Hydrochus sp. P1 (PSW)	0	0	0	0	0	0	0	2
		Paracymus spenceri	0	0	1	2	0	2	2	2
		Regimbartia attenuata	0	0	2	0	0	0	0	0
		Sternolophus australis	1	0	2	1	0	0	0	0
		Sternolophus immarginatus	2	0	2	0	0	0	0	0
		Sternolophus marginicollis	0	2	0	2	0	0	2	1
		Sternolophus sp. (L)	0	0	0	1	0	0	0	0
	Limnichidae	Limnichidae spp.	0	2	0	1	0	0	0	0
	Noteridae	*Neohydrocoptus subfasciatus*	0	1	0	0	0	0	0	0
	Scirtidae	Scirtidae sp. (L)	0	3	0	2	2	2	0	2
	Staphylinidae	Staphylinidae sp.	0	0	1	0	0	0	0	0
Diptera	**Cecidomyiidae**	Cecidomyiidae sp.	0	0	0	0	1	0	0	0
	Ceratopogonidae	Ceratopogonidae sp. (P)	1	1	2	0	2	0	2	2
		Ceratopogoninae spp.	2	2	3	2	3	2	2	2
		Dasyhelea spp.	2	1	3	2	3	0	3	2
		Forcipomyiinae spp.	0	2	1	0	0	0	0	1
	Chironomidae	Chironomidae spp. (P)	2	2	0	2	3	2	3	2
	Chironominae	Chironomini sp. K2 (unknown genus)	0	0	0	1	0	0	0	0
		Chironomus aff. *alternans*	2	2	3	0	0	3	0	1
		Cryptochironomus griseidorsum	0	0	0	0	2	1	0	0
		Dicrotendipes `CA1` Pilbara type 3	0	0	0	0	1	0	2	0
		Dicrotendipes jobetus	0	0	0	0	0	3	0	0
		Dicrotendipes sp. P4 (PSW)	0	0	0	0	3	0	0	0
		Kiefferulus intertinctus	0	3	0	2	0	0	0	0
		Polypedilum (*Pentapedilum*) *leei*	0	0	0	2	0	0	0	0
		Polypedilum (*Polypedilum*) *nubifer*	0	0	0	0	0	3	0	0
		Polypedilum nr *vespertinus* (M2) (SAP)	3	0	0	2	0	0	0	0
		Polypedilum sp. K1 (PSW)	0	2	2	0	0	0	0	0
		Polypedilum sp. S01 (PSW)	0	0	0	2	0	0	0	0
		Skusella subvittata	0	0	2	0	0	0	0	0
		Stenochironomus (*Stenochironomus*) *watsoni*	0	0	0	2	1	0	1	0
		Cladotanytarsus aff. K4 (PSW)	0	0	0	0	3	0	0	2
		Tanytarsus sp. `K12`	0	0	0	2	0	0	0	2
		Tanytarsus sp. D (SAP)	2	0	0	0	0	0	3	0
		Tanytarsus sp. H (SAP)	0	3	3	0	2	0	0	0
		Tanytarsus sp. P01 (PSW)	0	0	0	0	2	0	0	0
		Tanytarsus sp. P07 (PSW)	0	0	0	0	0	0	3	0
		Tanytarsus sp. P09 (PSW)	3	0	0	0	0	0	0	3
	Orthocladiinae	*Corynoneura* sp.	0	0	0	0	2	0	0	0
		nr. *Gymnometriocnemus* sp.	0	0	0	0	0	0	3	0
		nr. *Parametriocnemus* sp.	0	0	0	0	2	0	0	0
		Thienemanniella sp.	0	0	0	0	1	1	0	1
		Nanocladius sp.	0	0	0	0	1	0	0	0
		Parakiefferiella sp.	0	0	0	0	0	0	3	0
	Tanypodinae	*Ablabesmyia hilli*	0	0	0	2	0	0	2	0
		Ablabesmyia notabilis	0	0	0	0	0	0	2	0
		Fittkauimyia ?disparipes	1	2	1	2	0	0	0	3
		Larsia ?albiceps	0	0	2	2	3	0	3	3
		Paramerina sp. 1	2	2	2	2	3	3	2	3
		Procladius spp.	0	0	2	3	0	4	2	3
		Rheocricotopus sp.	0	0	0	0	3	0	0	0
	Culicidae	*Anopheles* sp.	2	0	2	0	2	0	0	1
		Culex sp.	1	0	0	0	1	0	2	0
		Culicidae sp. (P)	3	0	0	2	1	0	0	0
	Dolichopodidae	Dolichopodidae spp.	0	0	0	0	2	0	0	0
	Ephydridae	Ephydridae sp.	0	0	0	0	0	1	0	0
	Psychodidae	Psychodidae sp.	0	1	0	0	0	0	0	0
	Sciomyzidae	Sciomyzidae sp.	0	0	0	0	0	1	0	0
	Stratiomyidae	Stratiomyidae spp.	0	2	2	2	0	2	3	2
	Syrphidae	Syrphidae sp.	0	1	0	0	0	0	0	0
	Tabanidae	Tabanidae spp.	0	0	1	2	2	2	1	2
	Tipulidae	Tipulidae sp.	0	3	1	0	0	0	0	0
Ephemeroptera	**Baetidae**	Baetidae sp. (imm/dam)	2	0	3	0	3	3	3	3
		Cloeon sp. Redstripe	3	0	2	2	2	0	3	2
		Cloeon fluviatile	0	0	2	2	0	0	0	0
		Cloeon sp. (imm/dam)	0	0	0	3	0	1	0	0
		Offadens G1 sp.WA2	0	0	0	0	2	0	0	0
		Offadens sp. (imm/dam)	0	0	0	0	2	0	0	0
	Caenidae	Caenidae spp. (imm/dam)	0	0	2	0	2	2	2	1
		Tasmanocoenis arcuata	0	0	2	3	3	2	3	2
		Tasmanocoenis spp. (imm/dam)	0	0	0	0	2	0	0	3
		Tasmanocoenis sp. M	0	0	0	0	0	0	1	1

			Yandicoogina Creek				Reference sites			
Phylum/Class/Order	Family	Lowest taxon	YC1	YC2	YC3	YC4	WWS	BENS	MUNJS	SS
	Leptophlebiidae	*Atalophlebia* sp. AV17	0	0	0	0	0	0	3	0
		Leptophlebiidae spp. (imm/dam)	0	0	0	0	0	0	2	0
		Thraulus sp. AV1	0	0	0	0	0	0	1	0
Hemiptera	Belostomatidae	Belostomatidae spp. (imm/dam)	0	0	2	0	2	0	2	0
		Diplonychus eques	2	1	3	2	2	0	0	3
	Gerridae	Gerridae sp. (imm/dam)	0	0	0	1	0	0	0	0
		Limnogonus fossarum gilguy	1	0	0	0	0	0	0	0
		Limnogonus luctuosus	1	0	2	2	0	0	0	0
		Limnogonus sp. (imm/dam)	0	0	0	0	0	2	0	0
	Hydrometridae	*Hydrometra papuana*	0	0	1	0	0	0	0	0
	Micronectidae	Micronectidae spp. (imm/dam)	0	0	0	0	0	2	0	0
	Nepidae	*Laccotrephes tristis*	0	0	0	1	0	0	0	0
	Notonectidae	*Anisops elstoni*	0	0	1	0	0	0	0	0
		Anisops hackeri	0	0	0	0	0	0	1	0
		Anisops sp. (imm/dam)	1	0	1	0	0	0	3	0
		Enithares sp. (imm/dam)	0	0	0	0	0	0	2	0
		Notonectidae spp. (imm/dam)	0	0	0	1	0	1	0	1
	Pleidae	*Paraplea brunni*	0	0	0	2	0	0	0	0
		Paraplea sp. (imm/dam)	1	0	0	0	0	1	1	3
	Veliidae	*Nesidovelia peramoena*	0	0	0	0	0	2	0	0
		Microvelia sp. (F)	0	0	0	0	0	2	0	0
		Veliidae sp. (imm/dam)	0	0	0	0	2	3	2	0
Lepidoptera	Crambidae	Acentropinae sp. (imm/dam)	0	0	0	0	0	0	1	0
		Margarosticha sp. 3	0	0	0	0	3	0	0	3
		Nymphulinae sp. 43	0	0	0	0	0	0	0	3
Odonata										
Anisoptera		Anisoptera spp. (imm/dam)	0	0	2	0	2	2	3	2
	Aeshnidae	*Hemianax papuensis*	0	0	0	0	0	0	2	0
	Corduliidae	*Hemicordulia koomina*	0	0	0	0	0	1	0	0
	Gomphidae	*Austrogomphus gordoni*	0	0	0	0	2	2	0	0
	Libellulidae	*Crocothemis nigrifrons*	0	0	2	0	0	0	0	0
		Diplacodes haematodes	0	0	0	2	2	0	2	0
		Orthetrum caledonicum	0	0	0	0	1	0	0	0
		Rhodothemis lieftincki	0	0	1	0	0	0	0	0
		Tramea sp.	0	0	0	0	0	0	2	0
		Zyxomma elgneri	0	0	0	2	0	2	0	0
Zygoptera		Zygoptera spp. (imm/dam)	2	2	1	3	2	3	3	2
	Coenagrionidae	*Argiocnemis rubescens*	2	2	0	2	2	1	3	2
		Ischnura aurora	0	0	0	2	1	0	3	2
		Pseudagrion aurefrons	0	0	0	2	0	1	2	0
		Pseudagrion microcephalum	0	0	0	0	1	0	0	0
	Isostictidae	*Eurysticta coolawanyah*	0	0	0	2	0	3	0	0
Trichoptera		Trichoptera sp. (imm/dam)	0	0	0	0	0	0	0	2
	Ecnomidae	Ecnomidae spp.	0	0	0	0	0	2	0	0
		Ecnomina F group cf. sp. AV16 (PSW)	0	0	0	0	0	0	3	0
		Ecnomus pilbarensis	0	0	0	0	0	0	2	0
		Ecnomus sp.	0	0	0	2	0	0	0	0
	Hydropsychidae	*Cheumatopsyche wellsae*	0	0	0	0	3	0	0	3
	Hydroptilidae	*Hellyethira* sp.	0	0	0	1	0	2	0	3
		Orthotrichia sp.	0	0	0	0	0	2	0	0
	Leptoceridae	Leptoceridae sp. (imm/dam)	0	0	0	0	0	0	1	2
		Oecetis spp. (imm)	0	0	0	0	2	0	0	0
		Oecetis sp. Pilbara 1 (PSW)	0	0	0	0	0	2	2	0
		Oecetis sp. Pilbara 2 (PSW)	0	0	0	2	0	0	0	0
		Oecetis sp. Pilbara 4 (PSW)	0	0	1	0	0	0	0	0
		Oecetis sp. Pilbara 5 (PSW)	0	0	2	0	0	2	0	0
		Triaenodes sp.	0	0	0	1	2	1	1	0
		Triplectides ciuskus seductus	0	0	0	0	4	3	2	2
		Triplectides sp. (imm/dam)	0	0	0	0	2	0	0	0
	Philopotamidae	*Chimarra* sp. AV17	0	0	0	0	1	0	0	0
		Taxa richness	39	33	49	72	68	64	59	72

Wet-20

Phylum/Class/Order	Family	Lowest taxon	Yandicoogina Creek YC1	YC2	YC3	YC4	Reference sites WWS	WM	MUNJS	SS
CNIDARIA										
HYDROZOA	**Hydridae**	*Hydra* sp.	0	0	0	0	2	0	1	0
NEMATODA		Nematoda sp.	0	0	0	0	1	0	0	0
PLATYHELMINTHES		Turbellaria sp.	0	0	0	0	0	0	1	0
MOLLUSCA										
GASTROPODA	**Lymnaeidae**	*Bullastra vinosa*	2	0	0	3	0	0	1	2
	Planorbidae	*Bayardella* sp.	0	0	0	0	0	1	0	0
		Ferrissia petterdi	0	2	1	2	2	0	1	0
		Gyraulus sp.	2	0	1	0	0	0	2	0
ANNELIDA										
OLIGOCHAETA										
		Oligochaeta spp. (imm./dam.)	0	0	0	0	1	0	0	2
Tubificida	**Naididae**	*Allonais ranauana*	0	0	2	0	0	0	0	0
		Bratislavia unidentata	1	0	0	0	0	0	0	0
		Naidinae spp. (imm./dam.)	0	0	2	2	2	0	1	2
		Nais communis	0	0	0	0	0	1	1	1
		Pristina leidyi	0	2	0	0	0	0	0	1
		Pristina longiseta	0	0	2	0	2	1	0	2
		Pristina spp. (imm./dam.)	0	1	2	0	0	2	0	2
	Phreodrilidae	Phreodrilidae spp.	0	0	0	0	1	0	0	2
ARTHROPODA										
CRUSTACEA										
MALACOSTRACA										
Amphipoda	**Paramelitidae**	*Chydaekata* sp. E	0	0	0	0	3	0	0	0
		Maarrka weeliwolli	0	0	0	0	1	0	0	0
		Paramelitidae spp. (imm./dam.)	0	0	1	0	3	0	0	0
Decapoda	**Parastacidae**	*Cherax quadricarinatus*	0	0	0	0	2	0	0	0
ARACHNIDA										
		Acarina sp. (imm./dam.)	2	0	0	0	0	0	0	0
TROMBIDIFORMES	**Antisitsiellidae**	*Rutacerus* sp.	0	0	0	0	0	0	1	0
	Arrenuridae	*Arrenurus* sp.	0	0	0	0	0	0	1	0
		Arrenurus sp. F	0	0	0	0	0	0	1	0
	Aturidae	*Albia rectifrons*	0	0	0	0	2	0	0	0
	Eylaidae	*Eylais* sp.	0	0	0	0	0	1	0	0
	Hydrodromidae	*Hydrodroma* sp.	0	0	0	0	2	0	0	0
	Hydryphantidae	*Diplodontus* sp.	0	0	0	1	1	0	1	0
		Pseudohydrophantes sp.	0	0	0	0	0	0	0	1
	Hygrobatidae	*Australiobates* sp. P4 (PSW)	0	0	0	0	2	0	0	0
		Coaustraliobates minor	0	0	0	0	0	1	0	0
	Limnesiidae	*Limnesia* sp. 4	2	0	2	3	2	2	3	2
		Limnesia sp.	0	0	2	0	0	2	2	2
	Limnocharidae	*Limnochares australica*	0	0	0	0	1	0	0	0
	Mideopsidae	*Gretacarus* sp.	0	0	0	1	0	0	0	0
	Oxidae	*Oxus orientalis*	1	0	0	0	0	0	0	0
	Piersigiidae	*Stygolimnochares* nr *australica*	0	0	0	0	1	0	0	0
	Unionicolidae	*Neumania* sp.	1	0	0	0	0	0	1	0
COLLEMBOLLA										
Entomobryomorpha		Entomobryoidea spp.	2	1	0	1	0	0	0	0
HEXAPODA										
INSECTA										
Coleoptera	**Carabidae**	Carabidae sp.	1	1	0	0	0	0	0	0
	Dytiscidae	*Allodessus bistrigatus*	0	0	0	0	0	2	2	0
		Austrodytes plateni	0	0	1	0	0	0	0	0
		Copelatus irregularis	1	0	0	0	0	0	0	0
		Cybister sp. (L)	0	0	1	0	0	0	2	0
		Eretes australis	0	0	0	0	0	0	2	0
		Hydaticus consanguineus	2	0	0	0	0	0	0	0
		Hydaticus daemeli	0	0	0	0	0	0	2	0
		Hydroglyphus grammopterus	0	0	0	0	0	1	0	0
		Hydroglyphus orthogrammus	0	0	2	2	0	2	3	2
		Hydrovatus opacus	0	0	0	0	0	0	1	0
		Hydrovatus sp. (L)	0	0	2	0	0	0	0	0
		Hyphydrus elegans	0	0	0	0	0	2	0	0
		Hyphydrus lyratus	0	0	0	0	0	3	2	0
		Laccophilus sharpi	0	0	0	1	1	0	0	0
		Limbodessus compactus	0	0	1	0	0	1	0	1
		Necterosoma regulare	0	0	0	0	0	2	0	0
		Neobidessodes denticulatus	1	0	1	3	0	0	0	0
		Platynectes decempunctatus var. *decempunctatus*	0	0	1	0	0	0	1	0
		Rhantaticus congestus	0	0	0	1	0	0	1	0

			Yandicoogina Creek				Reference sites			
Phylum/Class/Order	**Family**	**Lowest taxon**	**YC1**	**YC2**	**YC3**	**YC4**	**WWS**	**WM**	**MUNJS**	**SS**
	Elmidae	*Austrolimnius* sp. (L)	0	0	0	0	0	0	0	3
	Gyrinidae	*Dineutus australis*	0	0	1	0	2	2	2	0
		Macrogyrus paradoxus	0	0	0	0	3	0	0	0
	Hydraenidae	*Hydraena* sp.	2	3	2	2	1	2	2	0
	Hydrophilidae	*Anacaena horni*	1	2	0	2	2	0	0	0
		nr. *Anacaena* sp.	1	0	0	0	0	0	0	0
		Berosus dallasae	0	0	0	0	0	0	0	2
		Coelostoma fabricii	0	2	0	1	0	0	0	0
		Enochrus deserticola	0	3	2	0	1	0	1	1
		Helochares tatei	1	0	0	0	0	0	0	2
		Hydrochus eurypleuron	0	0	0	0	0	0	0	1
		Hydrochus interioris	0	0	0	0	0	0	0	2
		Hydrochus macroaquilanius	0	0	0	0	0	0	0	1
		Hydrochus obscuroaeneus	2	1	1	0	0	1	0	2
		Hydrochus sp. Group 3	0	0	0	0	0	0	0	1
		Hydrochus sp. P1	0	0	0	0	0	0	0	1
		Paracymus spenceri	2	0	2	0	1	0	2	2
		Paracymus sp. (L)	0	0	0	0	0	0	1	0
		Regimbartia attenuata	0	0	0	0	1	0	0	0
		Sternolophus australis	2	0	3	0	0	1	2	0
		Sternolophus immarginatus	0	0	0	0	0	2	0	0
		Sternolophus marginicollis	3	2	3	3	0	0	0	0
	Limnichidae	Limnichidae sp.	0	0	0	1	0	0	0	0
	Scirtidae	Scirtidae sp. (L)	0	2	1	2	2	0	0	2
Diptera	**Ceratopogonidae**	Ceratopogonidae spp. (P)	0	0	0	1	0	0	0	2
		Ceratopogoninae spp.	2	3	3	2	2	2	2	2
		Dasyhelea spp.	1	2	4	0	2	2	0	2
	Chironomidae	Chironomidae spp. (P)	0	0	2	2	2	2	2	2
	Chironominae	*Chironomus* aff. *alternans*	3	2	4	2	0	0	0	2
		Cyptochironomus griseidorsum	0	0	0	0	0	0	0	2
		Dicrotendipes jobetus	1	0	0	0	0	0	0	0
		Dicrotendipes sp. 4 (PSW)	0	0	0	0	3	0	0	3
		Dicrotendipes sp. 'CA1'	0	0	0	0	0	0	0	2
		Kiefferulus intertinctus	0	0	0	2	0	1	0	0
		Polypedilum nubifer	0	0	0	1	3	0	0	0
		Polypedilum sp.	0	0	0	0	0	2	0	0
		Polypedilum sp. S01 (PSW)	0	1	0	0	0	0	0	0
		Stenochironomous watsoni	0	0	0	0	3	2	0	2
		Rheotanytarsus sp.	0	0	0	0	0	2	0	0
		Rheotanytarsus trivittatus	0	0	0	0	2	0	0	0
		Tanytarsini spp.	2	2	3	2	4	2	3	2
		Tanytarsus sp. `K12`	0	0	0	0	0	2	0	0
		Tanytarsus fuscithorax/*semibarbitarsus*	0	0	2	0	0	0	0	2
		Tanytarsus sp. H (SAP)	0	0	0	2	0	0	0	0
		Tanytarsus sp. P01 (PSW)	0	2	0	0	2	2	0	0
		Tanytarsus sp. P07 (PSW)	0	0	2	0	0	0	0	0
		Tanytarsus sp. P09 (PSW)	0	0	0	0	0	0	1	1
	Orthocladiinae	*Corynoneura* sp.	0	0	0	0	2	0	0	0
		Cricotopus sp. 2	0	0	0	0	0	0	1	0
		Thienemanniella sp.	0	0	0	0	2	0	0	0
	Tanypodinae	*Larsia ?albiceps*	1	3	3	2	3	4	4	4
		Paramerina sp. 1	1	3	2	2	2	3	0	3
		Procladius spp.	1	0	2	2	0	4	3	4
		Rheocricotopus sp.	0	0	0	0	0	0	0	2
		Thienemannimyia sp.	0	0	0	0	0	0	0	2
	Culicidae	*Aedes* sp.	0	0	0	0	0	0	1	0
		Anopheles sp.	0	0	0	0	1	0	2	0
		Culex sp.	2	0	2	0	0	0	2	2
	Dolichopodidae	Dolichopodidae spp.	0	0	0	0	2	0	0	2
	Simuliidae	Simuliidae spp.	0	0	0	0	0	0	2	0
	Stratiomyidae	Stratiomyidae spp.	0	1	1	1	0	0	2	1
	Syrphidae	Syrphidae sp.	1	1	0	0	0	0	0	0
	Tabanidae	Tabanidae spp.	0	2	0	1	0	0	2	1
	Tipulidae	Tipulidae spp.	2	0	0	0	0	0	0	0
Ephemeroptera	**Baetidae**	Baetidae spp. (imm./dam.)	2	0	2	2	4	4	4	3
		Cloeon fluviatile	0	0	0	0	0	0	0	1
		Cloeon sp. Red Stripe	0	0	2	0	2	0	3	0
		Offadens G1 sp. WA2	0	0	0	0	2	0	0	2
	Caenidae	Caenidae spp. (imm./dam.)	0	0	1	2	2	4	3	3
		Tasmanocoenis sp.	0	0	0	1	0	0	0	0
		Tasmanocoenis sp. M	0	0	0	0	0	3	0	0
		Tasmanocoenis sp. P/arcuata	0	0	0	0	1	3	0	3
	Leptophlebiidae	*Atalophlebia* sp. AV17	0	0	0	0	0	0	1	0
Hemiptera	**Belostomatidae**	*Diplonychus eques*	2	0	2	2	3	0	2	2
		Diplonychus sp.	0	1	0	2	0	0	0	0
	Gerridae	*Limnogonus fossarum gilguy*	0	0	1	1	0	0	0	0
		Limnogonus luctuosus	2	0	0	0	0	1	0	0
		Limnogonus sp.	0	0	0	2	1	0	1	0
	Mesoveliidae	*Mesovelia hungerfordi*	0	0	0	1	1	0	1	0
		Mesovelia sp.	0	0	1	2	0	0	0	0
	Micronectidae	*Austronecta bartzarum*	0	0	0	0	0	0	0	1
		Austronecta micra	0	0	0	1	0	0	0	0
		Micronecta annae	0	0	0	0	0	1	0	0

			Yandicoogina Creek				Reference sites			
Phylum/Class/Order	**Family**	**Lowest taxon**	**YC1**	**YC2**	**YC3**	**YC4**	**WWS**	**WM**	**MUNJS**	**SS**
	Nepidae	*Ranatra diminuta*	1	0	0	0	0	0	0	0
		Ranatra occidentalis	0	0	0	0	2	1	0	0
	Notonectidae	*Anisops hackeri*	1	0	0	0	0	2	2	0
		Anisops sp.	1	0	0	0	0	1	2	0
		Enithares woodwardi	0	0	0	0	0	0	2	0
	Pleidae	*Paraplea brunni*	0	0	0	0	0	0	0	1
		Paraplea sp. (imm.)	0	0	0	0	0	0	1	2
	Veliidae	*Nesidovelia peramoena*	2	0	0	0	0	0	0	0
		Veliidae spp.	0	1	1	0	2	0	2	0
Lepidoptera	**Crambidae**	Acentropinae spp. (imm.)	0	0	0	1	2	0	0	3
		Eoophyla repetitalis	0	0	0	0	0	0	1	0
		Margarosticha sp. 3	0	0	0	0	2	0	1	3
		Parapoynx sp.	0	0	0	0	0	0	2	0
Odonata										
Anisoptera		Anisoptera spp. (imm./dam.)	2	3	2	2	4	0	4	2
	Aeshnidae	*Hemianax papuensis*	0	0	0	0	0	0	2	0
	Corduliidae	*Hemicordulia koomina*	0	0	0	2	0	0	0	0
	Gomphidae	*Austrogomphus gordoni*	0	0	0	0	1	1	0	1
	Libellulidae	*Diplacodes haematodes*	0	0	0	0	2	2	2	0
		Nannophlebia injibandi	0	0	0	0	0	0	0	1
		Orthetrum caledonicum	0	1	0	1	1	0	0	0
		Tramea sp.	0	0	0	0	0	0	2	0
		Zygoptera spp. (imm./dam.)	2	0	2	3	4	0	3	2
	Coenagrionidae	*Argiocnemis rubescens*	0	0	1	0	0	0	0	0
		Ischnura aurora	0	0	0	2	0	0	2	0
		Pseudagrion aureofrons	0	0	0	2	2	0	0	0
		Pseudagrion microcephalum	0	0	0	1	0	0	0	0
Trichoptera	**Ecnomidae**	Ecnomidae sp.	0	0	0	0	1	0	0	0
		Ecnomus pilbarensis	0	0	0	2	0	4	0	0
	Hydropsychidae	*Cheumatopsyche wellsae*	0	0	0	0	2	0	0	3
		Cheumatopsyche spp. (imm.)	0	0	0	0	0	0	0	4
	Hydroptilidae	*Hellyethira* sp.	0	0	0	0	3	0	2	0
		Orthotrichia sp.	0	0	0	0	4	0	0	0
	Leptoceridae	*Leptocerus* sp. AV2	0	0	0	0	0	0	0	1
		Triaenodes sp.	0	0	0	0	2	0	0	0
		Triplectides ciuskus seductus	0	0	0	0	2	0	0	2
	Philopotamidae	*Chimarra* sp. AV17	0	0	0	0	0	0	0	3
	Polycentropodidae	*Paranyctiophylax* sp. AV5	0	0	0	1	0	0	0	0
		Taxa richness	**40**	**27**	**46**	**50**	**66**	**45**	**64**	**66**