WARRAMBOO PROJECT: BASELINE AQUATIC ECOSYSTEM SURVEY

WET SEASON SAMPLING 2018
FINAL REPORT

WRM Wetland Research & Management

November 2018

Warramboo Project

Baseline Aquatic Ecosystem Survey
Wet Season Sampling 2018

Prepared for:

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Frontispiece (left to right): Warramboo Creek in the upstream reference reach at WARUS1; head of a male *Branchinella* cf. *proboscida* (Anostraca) recorded from WARUS6; and, sampling macroinvertebrates at WARUS2 (photos by WRM ©).

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Disclaimer

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EXECUTIVE SUMMARY

This report summarises the results of the wet-18 aquatic survey, as part of the baseline sampling program for aquatic ecosystems of Warramboo Creek. The creek is a naturally ephemeral system adjacent to the Robe River Mining Co. Pty Ltd Mesa A / Warramboo iron ore mine, in the Pilbara region of Western Australia. The Warramboo deposit has been mined above water table since 2012, however, in order to access the orebody that lies below water table, dewatering and discharge of excess water is considered necessary. The proposed surplus water discharge location is located on Warramboo Creek, approximately 3.5 km to the south-west of current operations. The aim of the current sampling program was to document the existing ecological condition of Warramboo Creek, and procure baseline data against which any potential changes associated with dewatering discharge from the below water table development can be assessed. This report includes data collected in the initial phase of sampling in the wet-16, when two reference sites held surface water for sampling. To provide regional context for the sampling program, the current report also includes data from nearby creeks and rivers collected by WRM or the Department of Biodiversity Conservation and Attractions (DBCA) during their Pilbara Biological Survey (PBS).

The sampling design included six sites on Warramboo Creek upstream of the proposed discharge location (reference sites) and six sites downstream of the proposed discharge location (potential exposed sites). At each site, the aquatic survey included water quality sampling, habitat assessment, and sampling of microinvertebrates, hyporheos fauna, macroinvertebrates and fish. Methods used are consistent with those used by WRM for other Rio Tinto projects across the Pilbara, as well as those used by government departments (i.e. DBCA's PBS – Pinder *et al.* 2010, and the National Monitoring River Health Initiative - Department of Environment Sport and Territories *et al*. 1994).

The main findings of the baseline survey were:

- Surface water quality at Warramboo Creek indicated recent filling by rainwater and was low in alkalinity, hardness, electrical conductivity (EC), and concentrations of major ions. Waters were generally characterised by circum-neutral pH, adequate to high dissolved oxygen (DO), fresh waters, with low to moderate TSS, generally low nitrogen nutrients and dissolved metal concentrations, with low buffering capacity at some sites (i.e. low alkalinity).
- Exceedances of default ANZECC/ARMCANZ (2000) GVs included pH (at most sites), DO (at WARUS2 and WARDS6), N_NOx (at WARUS2, WARUS3 and WARUS4), total N (at WARUS2, WARUS3, WARUS4, WARUS6, and WARDS5), total P (at all upstream reference sites and potential exposed site WARDS5), dCu (all reference sites and WARDS5), and dFe (at WARUS6).
- There were a number of significant differences in water quality between the upstream reference reach, and the downstream potential exposed reach. This appears to be the natural baseline condition, and should be taken into account when interpreting future monitoring results once dewatering-discharge commences. Water quality parameters which were significantly higher in the potential exposed reach included EC, TDS, alkalinity and hardness, corresponding ionic concentrations of Ca, Mg, K and HCO_3, and dBa. In contrast, total N and concentrations of dCu were significantly higher from the reference reach.
- Despite these significant differences in water quality between reach, there were no corresponding significant differences in taxa richness of phytoplankton (or density), microinvertebrates, hyporheos fauna, or macroinvertebrates between reference and potential exposed sites. Likewise, there were no significant differences in overall

assemblage structures of the aforementioned faunal components between site type (multivariate analysis results), with the exception of macroinvertebrate assemblages.

- There appeared to be considerable temporal differences between wet-16 and wet-18 data for all components. Although due to the low sample size in the wet-16, statistical analyses could not be undertaken.
- Phytoplankton samples were collected from three reference sites and three potential exposed sites in the wet-18. From these six samples, a total of 28 phytoplankton taxa were recorded. Two species of cyanobacteria known to be 'potential, but unconfirmed toxin producers' were recorded during the current study; *Cylindrospermum licheniforme* and *Dolichospermum affine*. However, neither were recorded in sufficient densities deemed to be of concern or to warrant immediate action as detailed in the National Protocol for the Monitoring of Cyanobacteria and their Toxins in Surface Freshwater by Jones *et al.* (2002).
- In comparing Warramboo Creek phytoplankton taxa richness and density with other sites sampled by WRM, the phytoplankton assemblage from Warramboo appeared to be generally higher in richness and density than Brockman 4 (~ 140 km to the east) samples from the wet-14 and wet-15. However, overall there was no significant difference in taxa richness or density between project/year.
- Microinvertebrate taxa richness ranged from 17 (at WARUS4) to 36 (at WARUS2). Of interest within the Warramboo microinvertebrate fauna was the collection of a species listed on the IUCN Red List of Threatened Species (calanoid copepod *Eodiaptomus lumholtzi* listed as Vulnerable), and an undescribed species the cyclopoid copepod *Thermocyclops* cf. *emini*). Both species were recorded from reference and potential exposed sites (see Table 10 in section 5 for more information). In comparing wet season microinvertebrate taxa richness from nearby creeks sampled by WRM and DBCA between 2005 and 2018, richness from Warramboo Creek was found to be statistically similar to Mungarathoona Creek and the Cane River.
- A total of 44 taxa were recorded from the hyporheic zone. Stygobites were only recorded from three sites; WARUS2, WARUS3 and WARUS6. Hyporheos fauna taxa richness (combined richness of stygobites, occasional hyporheic stygophiles and possible hyporheic fauna) ranged from three taxa at WARDS1 and WARDS6, to ten taxa at WARUS4.
- 94 macroinvertebrate taxa were recorded from Warramboo Creek in the wet-18. No species listed for conservation significance were recorded. Of interest, however, was the collection of an unknown anostracan (*Branchinella* cf. *proboscida*), and a Pilbara endemic chironomid (?*Pentanuera* sp.) with a disjunct distribution (see Table 10 in section 5 for more information). Warramboo Creek had comparable macroinvertebrate richness to these other nearby creeklines (i.e. Red Hill Creek, Cane River, Mungarathoona Creek, Myanore Creek, Robe River, Jimmawarrada Creek and Yarraloola Station Claypan), with no significant difference in richness recorded between creeks.
- No fish were recorded from Warramboo Creek in the wet-18, nor previously from the two sites sampled in the wet-16. Given the highly ephemeral nature of Warramboo Creek, and the isolation from nearby systems which support fish in permanent pools, it is highly unlikely fish are present in Warramboo, unless they are artificially introduced, as has occurred elsewhere in the Pilbara. And given the current absence of permanent water, they would not survive the following dry season.

It should be noted that this report is based on two sampling events, and is unlikely to fully capture the range in temporal / seasonal variability within the survey area. There is likely to be considerable variation in water quality and aquatic fauna present depending on the timing of surveys with respect to rainfall. Further baseline surveys are planned to capture as much of this variation as possible, and ensure an adequate dataset with which to detect future impacts, if any, from the Warramboo BWT development.

1 INTRODUCTION

1.1 Background

Robe River Mining Co. Pty Ltd (Robe) currently operates the Mesa A/Warramboo iron ore mine located approximately 38 km northwest of the existing Mesa J operations, 43 km west of Pannawonica town and 245 km by rail from the Cape Lambert port facilities the Robe Valley area of the Pilbara region of Western Australia (Figure 1). Robe is proposing to undertake below water table (BWT) mining at the Warramboo deposit, in. The Warramboo deposit has been mined above water table since 2012, in accordance with environmental approvals under Ministerial Statement 756, as part of the Mesa A / Warramboo Iron Ore Project. However, as part of the proposed BWT development, dewatering and discharge of any excess water are considered likely to be necessary, introducing a new environmental factor which potentially requires a referral to the EPA under Section 38 of the *Environmental Protection Act 1986* (EP Act). The proposed surplus water discharge location is the Warramboo Creek, via a single discharge point located between the old and new North West Coastal Highway (Figure 1). Current hydrological monitoring predicts the discharge footprint will extend approximately 8 km downstream of the proposed discharge location, depending on the discharge rate before completely infiltrating/evaporating (Figure 1).

The Warramboo Creek catchment drains an area of approximately 685 km^2 and 70 km in length, flowing in a northerly direction. Climate factors such as sporadic rainfall, high temperatures and high evaporation rate in the Pilbara region, and apparent lack of groundwater contribution to surface water flow (Rio Tinto 2015a), contribute to the highly ephemeral nature of Warramboo Creek. The catchment experiences high-velocity flash flooding following infrequent but intense cyclonic and monsoonal rainfall events, which combined with impervious ground conditions and infiltration excess, have carved out a well-defined main channel.

The surplus water discharge volume and velocities modelled were considered to be significantly smaller than the volume and velocities generated by the catchment during flood events (Rio Tinto 2015a). As such, channel erosion and overtopping of the creek banks as an impact of dewatering discharge is considered unlikely to occur. However, the permanent presence of water or changed flow regimes resulting from dewatering discharge can alter the ecological composition of aquatic-dependent species, particularly invertebrates which are adapted to intermittent flows (Bunn and Arthington 2002). Temporary waters in Australia may support species richness not unlike that found in more permanent streams, and tend to have a high degree of endemism (Lake *et al*. 1985, Boulton and Suter 1986, Davis *et al*. 1993, Pontin and Shiel 1995, Williams 1998, Williams 2002, Shiel *et al.* 2002). Initial data on Warramboo groundwater quality indicate the water is brackish, compared with any surface flow which is rain-fall generated and therefore fresh. In addition, concentrations of nitrate (NO_3) in seven monitoring bores, and in all but one sample from production bores (total of 48 samples) exceeded the 95% default ANZECC toxicity guideline value (GV; 0.7 mg/L; WRM 2016). Concentrations of total nitrogen (total N) were also elevated above the default eutrophication GV (0.3 mg/L) in three production bores. Groundwaters in arid zone areas across Australia are often naturally enriched in NO_3 (Magee 2009), however, the relative contribution of anthropogenic and natural sources to nitrogen enrichment in surface and groundwater of the Warramboo area is unknown. Ultimately, discharge may have an adverse effect on the water quality, and therefore, freshwater fauna of the receiving creek. Additionally, the creation of a water source through dewatering discharge has the potential to attract cattle and feral herbivores, which contribute to increased eutrophication, erosion and sedimentation in the creek and riparian zone (Carwardine *et al.* 2014). These impacts are likely to be limited to the discharge footprint, predicted to extend up to 8 km downstream of the proposed discharge location.

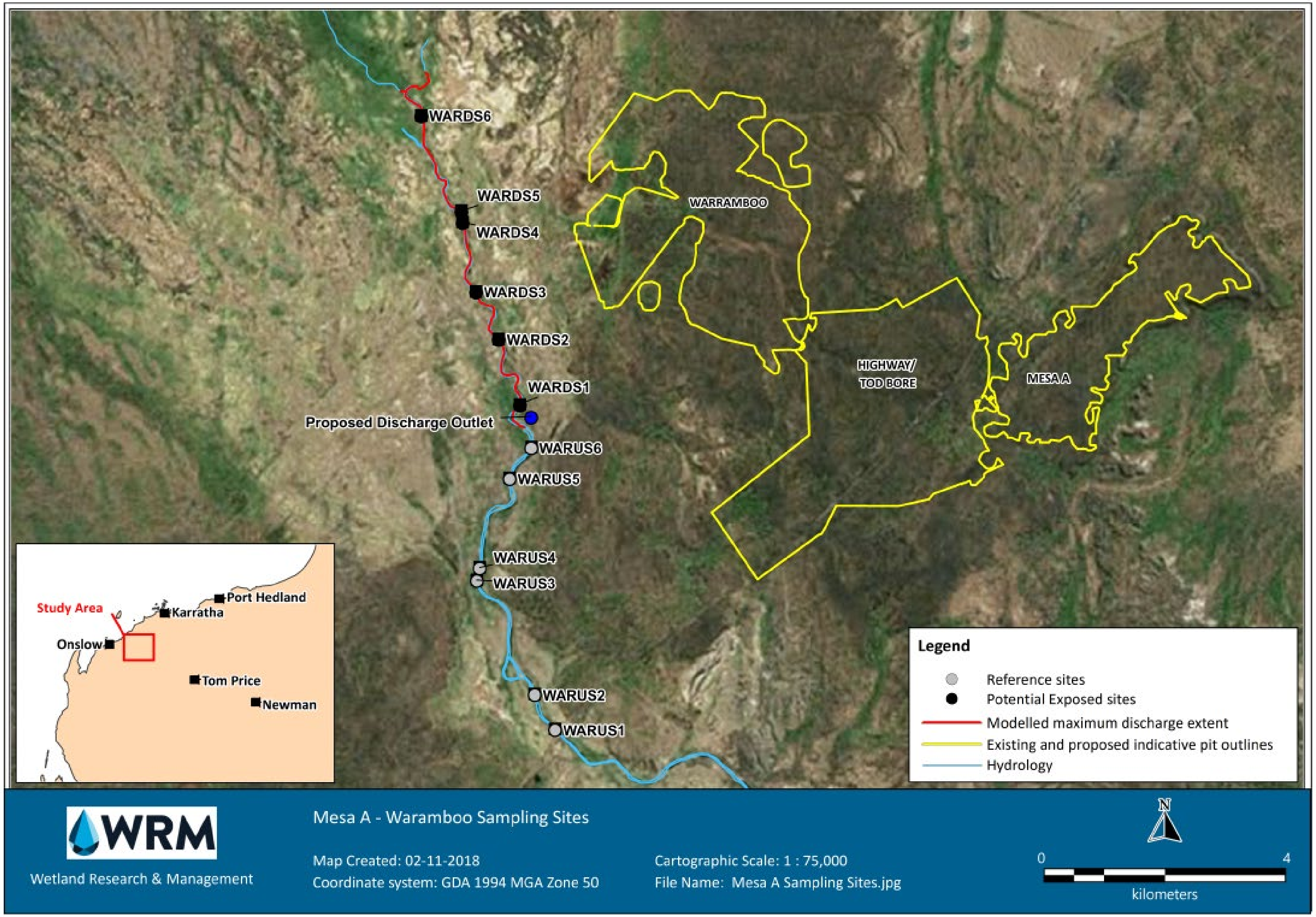


Figure 1. Baseline aquatic fauna and water quality sampling sites along Warramboo Creek, together with conceptual pit outlines for the Warramboo development, and the largest predicted discharge extent.

Wetland Research & Management (WRM) were commissioned to design and conduct a baseline sampling program for aquatic ecosystems downstream and upstream of the Warramboo BWT development. The aim of the sampling program is to document current ecological condition, including the presence of any fauna of conservation and/or regional significance, and provide a benchmark against which any future effects of the Warramboo mine may be assessed.

Wet season sampling in April 2018 constitutes the second sampling event as part of establishing a baseline dataset, with previous sampling undertaken in the wet season (April) of 2016 (see WRM 2016).

1.2 Study objectives

The aim of the current study was to sample water quality and aquatic fauna of reference and potentially exposed creekline sites previously identified in the wet-16 (WRM 2016), and develop a robust dataset with which to determine future impacts, if any, of the Warramboo development. Specifically, the scope of works included:

- Systematic sampling of aquatic fauna (microinvertebrates", hyporheic fauna, macroinvertebrates, fish), water quality (*in situ*, ions, nutrients, metals, TSS), habitat, and observations of turtle and frog species (if present) at all sites previously identified in the wet-16;
- Identification of all specimens to the lowest level possible;
- Determination of the conservation significance of all fauna, taking into account species listed as:
    - Threatened fauna under the IUCN Red List,
    - Threatened fauna under the EPBC Act (1999),
    - Scheduled fauna listed under the WA Wildlife Conservation (Rare Fauna) notice,
    - Priority fauna recognised by the Department of Biodiversity Conservation and Attractions (DBCA), and
    - Restricted or likely short-range endemic (SRE) species;
- Reporting water quality data against ANZECC/ARMCANZ (2000) [1]; guidelines;
- Preliminary analysis of spatial and temporal change in water quality and aquatic fauna by making comparison to the wet-16 data, and
- Preparation of a detailed technical report of all survey findings.

[1] The ANZECC/ARMCANZ (2000) guidelines have recently been revised and are presented in an interactive online platform. Where previously, WRM have referred to "trigger values (TVs)", these are now known as "guideline values (GVs)" (ANZECC/ARMCANZ 2018). Although actual GVs are not yet provided for fresh and marine water quality (expected to be included in late 2018), updated water quality guidance and framework are currently available online.

2 STUDY AREA

2.1 Climate

The study area is located on the western edge of the Hamersley Ranges, approximately 150 km south-west of Karratha and 53 km south-west of Pannawonica. The climate of the region is semi-arid, with relatively dry winters and hot summers.

2.1.1 Rainfall

Long-term Bureau of Meteorology (BOM) rainfall data is represented by Yarraloola Homestead (005032; 1899 to 2015) near Mesa A and Red Hill (no. 5022; 1898 to 2018) at the south-eastern catchment extent. Recent data for the Mesa A and Warramboo Mine area has been recorded by Rio Tinto at Mesa A since 2015. The Mesa A/Yarraloola data represent rainfall received across the lower Warramboo catchment area, while the Red Hill data provide a representation for the upper catchment. Total annual rainfall in the year preceding the aquatic fauna survey (2017) was 138.4 mm at Mesa A, and 366.4 mm at Red Hill.

Most rainfall occurs during the summer months (December to February; Figure 2) and is predominantly 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 (BOM 2018). Long-term average annual rainfall is 308 mm for Yarraloola/Mesa A and 365 mm for Red Hill.

Prior to sampling in April 2018, total monthly rainfall at Red Hill was above the long-term average for that station in both January and March, while at the nearby Mesa A station, rainfall was considerably lower during these months but higher in February (Figure 2). These rainfall events meant that there was sufficient surface water in Warramboo Creek at the time of the wet-18 survey to allow sampling of all sites along the 10 km stretch of creek. Both Mesa A and Red Hill also recorded substantial rainfall events in June, following the wet-18 field survey (Figure 2).

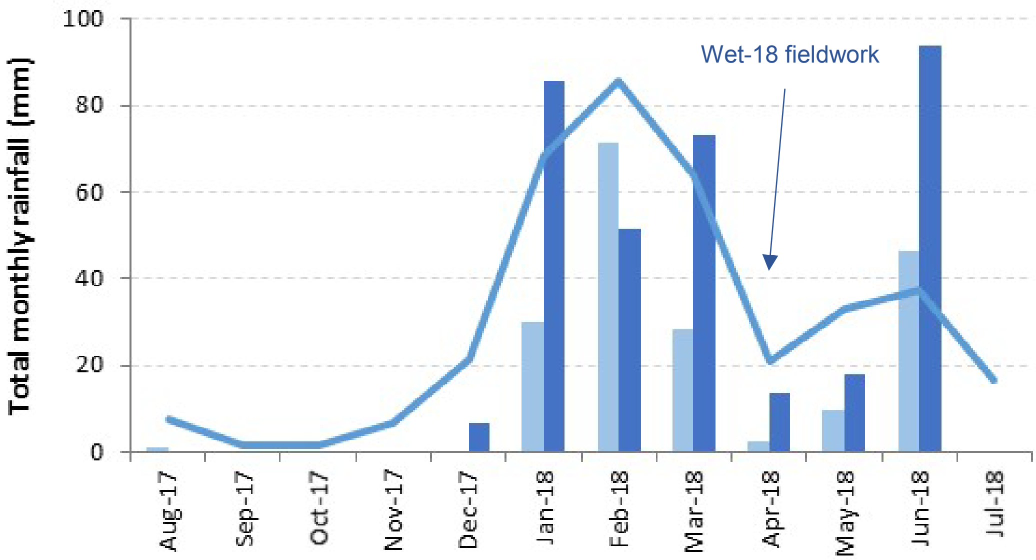


Figure 2. Total monthly rainfall (2017/18) for the two nearby gauging stations, including the long-term average monthly rainfall for BOM's Red Hill gauging station (number 5022).

2.1.2 Streamflow

Warramboo Creek is located within the Onslow Coast River Region within the Pilbara-Gascoyne Topographic Drainage Division (Rio Tinto 2015a). The catchment measures approximately 70 km in length with an equivalent uniform slope of 1.4 m/km, and drains approximately 685 km^2 in area. For the most part, the Warramboo Creek is well-defined before discharging into poorly defined scrubland in the coastal plain. Surface flow in Warramboo Creek is naturally ephemeral, typically only occurring in response to significant rainfall events and continuing for a few weeks to a couple months during wetter summers. The rainfall at Mesa A in Feb-18 and Red Hill in March-18 resulted in the presence of surface water along the creek, with a couple of short duration event-flows that filled some stream-bed lows, some of which held water to mid-March. There are no known permanent surface water pools in the immediate area of Warramboo (Rio Tinto 2013a).

The nearest streamflow gauging station to Warramboo Creek is Yarraloola (707002), located on the Robe River adjacent to the North West Coastal Highway, approximately 15 km north-east of the proposed discharge location. However, the Robe River has a catchment area of 7,100 km^2, much greater than the Warramboo Creek catchment (685 km^2), and is thus unlikely to adequately represent the hydrologic regime of the survey area. The next closest streamflow gauging station is Toolunga (707005), located on Cane River, with a catchment area of 2,330 km^2, located 43 km south of the proposed discharge location. The Cane River catchment provides a slightly better long-term representation of the Warramboo Creek catchment as it shares a common boundary at Red Hill and has similar topographic and long-term climate characteristics (Rio Tinto 2015a). Although scaled data from nearby Yarraloola and Toolunga stations can be used to infer long-term flow characteristics for Warramboo Creek, the “patchy” occurrence of rainfall across the area and significant difference in catchment size, limits the capacity to assess actual flow occurrence in the Warramboo catchment, particularly for smaller events.

Rio Tinto field observations during 2017-2018 confirmed small flow events in Warramboo Creek in February 2018, March 2018 and June 2018. Site inspections and logger data have highlighted that Warramboo Creek lacks the more prolonged recession flows and wet-season baseflows that are applicable to the Cane River and Robe River systems.

2.2 Hydrogeology

The Warramboo area of the Robe Valley is underlain by the Ashburton Formation, a very low permeability aquitard that serves as a basement for the palaeochannel aquifer (Rio Tinto 2015b). The groundwater at Warramboo is stored within an unconfined aquifer comprised of the Robe Pisolite and the Yarraloola Conglomerate (Rio Tinto 2013b, Rio Tinto 2015b). Robe Pisolite is a pisolitic alluvial sedimentary rock that fills the broad valley between ridges of the Brockman Iron Formation and outcrops along the Robe palaeochannel (EPA 1991). The Yarraloola Conglomerate is comprised of angular to rounded pebble gravel with minor beds of sand and clay and is particularly transmissive where the conglomerate underlies the pisolite (DoW 2010). The water table in the area of Warramboo is between 12 and 30 metres below ground level. Recharge to the main aquifers is predominantly via direct infiltration from rainfall and during periods of high streamflow when the Warramboo Creek breaks out over the coastal flats (Rio Tinto 2015a).

3 METHODS

3.1 Guidance and general approach

As part of the environmental impact assessment (EIA) process, key environmental factors must be identified, and these form the EPA's basis for the decision of whether a proposal's environmental impact is considered acceptable (EPA 2013). A recent review of the EPA's Guidelines and Procedures Framework has led to the two previous environmental factors relating to inland waters and aquatic ecology being combined into a single factor, Inland Waters, which is defined as:

"The occurrence, distribution, connectivity, movement, and quantity (hydrological regimes) of inland water including its chemical, physical, biological and aesthetic characteristics (quality)" (EPA 2018).

Under this factor, inland waters are considered to include groundwater systems, wetlands, estuaries, and any river, creek, stream or brook (and its floodplain), including systems that "flow permanently, for part of the year or occasionally, and parts of waterways that have been artificially modified" (EPA 2018). The objective of this factor is "to maintain the hydrological regimes and quality of groundwater and surface water so that environmental values are protected" (EPA 2018). Environmental value is defined under the EP Act as a beneficial use or an ecosystem health condition. Aquatic fauna and the ecological processes that support them are specifically listed in the revised Environmental Factor Guideline as one of the ecosystem health values that must be considered (EPA 2018).

Despite the new updated Environmental Factor relating to Inland Waters (EPA 2018), there are still (August 2018) no specific guidance statements for undertaking aquatic fauna surveys. However, field surveys by WRM employed sampling design, methods and general approaches consistent with the following:

- Australian and New Zealand Guidelines for Fresh and Marine Water Quality (ANZECC/ARMCANZ 2000);
- Environment Protection Authority (EPA) Guidance No. 20, Sampling of Short Range Endemic Invertebrate Fauna for Environmental Impact Assessment in Western Australia (EPA 2009);
- EPA Position Statement No. 3, Terrestrial Biological Surveys as an Element of Biodiversity Protection (EPA 2002);
- EPA Guidance No. 56, Terrestrial Fauna Surveys for Environmental Impact Assessment in Western Australia (EPA 2004).

Aquatic fauna sampling methods were also similar to the following:

- Streamtec/UWA surveys of benthic macroinvertebrates of the Robe River (see Dobbs and Davies 2009, Streamtec 2014);
- Parks and Wildlife surveys of benthic macroinvertebrates for the regional Pilbara Biological Survey (PBS) (see Pinder *et al.* 2010); and
- National Monitoring River Health Initiative (Department of Environment Sport and Territories *et al*. 1994).

3.2 Licences

This study was conducted under Department of Primary Industries and Regional Development (DPIRD) Fisheries Licence EXEM 2760 (Instruments of Exemption to the Fish Resources Management Act 1994 for Scientific Research Purposes), and DBCA Licence 08-000316-2 (Reg 17; Licence to Take Fauna for Scientific Purposes). As a condition of these licences, taxa lists and reports are required to be submitted to the respective authorities. Surveys were undertaken in the late wet season (4th and 8th of April) of 2018.

3.3 Sites and sampling design

The sampling design is an mBACI (multiple Controls - Before/After - Control/Impact) type design (Keough and Mapstone 1995). Location and number of sites were selected to provide data for robust statistical analysis and to meet requirements of such a design. An mBACI design is considered ideal for impact assessment, as impacts may be placed in context with natural temporo-spatial catchment changes. An mBACI type design provides both benchmark information as well as a strong basis to detect future changes. Reference sites upstream of the proposed discharge location on Warramboo Creek were selected to serve as the "control" for potentially impacted sites. Surveys conducted in May 2016 and April 2018 are part of the baseline or "before" phase against which to assess any future changes following mine development.

The sampling design included a total of 12 sites; six 'potential exposed' sites on Warramboo Creek downstream of the proposed discharge location, and six 'reference' sites located on Warramboo Creek upstream of the potential discharge, and likely outside any mining impact zones (Table 1 and Figure 1). These sites were selected as those most likely to contain suitable surface water for sampling, based on aerial imagery[2] of the creekline. The sites chosen were located within a 12 km stretch; 6 km upstream and 6 km downstream of the proposed discharge location (Figure 1). The intent was to sample six replicate sites in each 'zone' (reference and potential exposed) to characterise the fauna and current conditions along that stretch of creek, and most importantly, to allow adequate statistical power to test for spatial and temporal change in water quality and aquatic fauna currently and in the future.

Only three sites held water in the wet-16, of which one (WARUS2) was in flood at the time of survey, and as such, sediment samples were taken at all sites in order to undertake rehydration and emergence trials in the laboratory (Table 1). In the wet-18, all sites held water, and therefore all sites were sampled for the full aquatic suite, including water quality, habitat, microinvertebrates, hyporheic fauna and fish (Table 1).

Site photographs are provided in Appendix 1.

3.4 Field sampling

Sampling in the wet-18 was conducted using the same methods as previous surveys in the area, including the wet-16 Warramboo survey. Details and rationale are provided in WRM (2016). In summary, methods used were as follows:

- *In situ* measurement of dissolved oxygen (DO), water temperature (ºC) and pH using hand-held Wissenschaftlich-Technische-Werkstätten (WTW) and TPS field meters. Meters were calibrated immediately prior to field surveys.

[2] Google Earth imagery – acquisition dates: 1/3/2004, 4/7/2004, 9/1/2005, 12/21/2006, 4/10/2012, 9/3/2013, 5/7/2015, 11/3/2015.

Table 1. Information on aquatic ecosystem sampling sites on Warramboo Creek, including GPS locations, type of sample collected (full suite indicates surface water was present and a full aquatic fauna survey was able to be undertaken at that site), and whether previously sampled in the wet-16 (sediments only, indicates the site was dry and rehydration/emergence trials were undertaken on collected sediments).

Type	Site	Description	GPS co-ordinates (zone 50 K, WGS84)		Sample type	Previously sampled
			Easting	Northing		Wet-16
Reference	**WARUS1**	Warramboo upstream site 1, ~ 5.8 km upstream of the discharge point.	375798	7596721	Full aquatic survey	✓ sediments only
	WARUS2	Warramboo upstream site 2, ~ 5 km upstream of the discharge location.	375460	7597293	Full aquatic survey	×
	WARUS3	Warramboo upstream site 3, ~ 3.5 km upstream of the discharge location.	374487	7599158	Full aquatic survey	✓ sediments only
	WARUS4	Warramboo upstream site 4, ~ 2.7 km upstream of the discharge location.	374534	7599364	Full aquatic survey	✓ sediments only
	WARUS5	Warramboo upstream site 5, ~ 1 km upstream of the discharge location.	375017	7600833	Full aquatic survey	✓
	WARUS6	Warramboo upstream site 6, ~ 400 m upstream of the discharge location.	375370	7601342	Full aquatic survey	✓
Potential Exposed	**WARDS1**	Warramboo downstream site 1, ~ 200 m downstream of the discharge location.	375181	7602044	Full aquatic survey	✓ sediments only
	WARDS2	Warramboo downstream site 2, ~ 1 km downstream of the discharge location.	374818	7603116	Full aquatic survey	✓ sediments only
	WARDS3	Warramboo downstream site 3, ~ 2 km downstream of the discharge location.	374437	7603891	Full aquatic survey	✓ sediments only
	WARDS4	Warramboo downstream site 4, ~ 3 km downstream of the discharge location.	374207	7605027	Full aquatic survey	✓ sediments only
	WARDS5	Warramboo downstream site 5, ~ 4.5 km downstream of the discharge location.	374184	7605217	Full aquatic survey	✓ sediments only
	WARDS6	Warramboo downstream site 6, ~ 5.5 km downstream of the proposed discharge location, and therefore likely within the zone of dewatering discharge extent.	373511	7606775	Full aquatic survey	✓ sediments only

✓sediments only = site was dry; sediments collected for rehydration and emergence trials.

- Water depth was measured using a graduated pole.
- Collection of water samples for laboratory analyses of major ions, alkalinity, dissolved metals, nutrients, and total suspended solids (TSS). Samples for nutrients and dissolved metals were filtered in the field through 0.45 µm Millipore nitrocellulose filters. To avoid contamination, all sample bottles used for dissolved metals were acid-washed (0.1% nitric acid) prior to use, double-wrapped in polyethylene bags after collection, with samplers wearing nitrile gloves at all times, as detailed in Ahlers *et al.* (1990) and Batley (1990). All water samples were kept cool in an esky while in the field, and either refrigerated (ions & metals), or frozen (nutrients) as soon as possible for subsequent transport to the laboratory. All laboratory analyses were conducted by the Chem Centre, Bentley, WA (a NATA accredited laboratory). All water quality variables measured are summarised in Table 2.

Table 2. All water quality parameters measured, indicating units of measurement.

Parameter	Units	Parameter	Units
In situ		***Dissolved metals***	
pH	pH units	Aluminium (Al)	mg/L
Electrical conductivity	µS/cm	Arsenic (As)	mg/L
Redox potential	mV	Boron (B)	mg/L
Dissolved oxygen	% saturation	Barium (Ba)	mg/L
Dissolved oxygen	mg/L	Cadmium (Cd)	mg/L
Water temp	°C	Cobalt (Co)	mg/L
Maximum water depth	m	Chromium (Cr)	mg/L
		Copper (Cu)	mg/L
Ionic composition		Iron (Fe)	mg/L
Sodium (Na)	mg/L	Manganese (Mn)	mg/L
Potassium (K)	mg/L	Molybdenum (Mo)	mg/L
Calcium (Ca)	mg/L	Nickel (Ni)	mg/L
Magnesium (Mg)	mg/L	Lead (Pb)	mg/L
Chloride (Cl)	mg/L	Sulfur (S)	mg/L
Carbonate (CO_3)	mg/L	Selenium (Se)	mg/L
Hydrogen carbonate (HCO_3)	mg/L	Uranium (U)	mg/L
Sulfate (SO_4)	mg/L	Vanadium (V)	mg/L
Alkalinity	mg/L	Zinc (Zn)	mg/L
Hardness	mg/L		
Nutrients		***Other***	
Nitrate + nitrite (N_NO_X)	mg/L		
Ammonia (N_NH_3)	mg/L	Total suspended solids (TSS)	mg/L
Total Nitrogen (total N)	mg/L		
Total Phosphorus (total P)	mg/L		

- Water quality data were compared against the ANZECC/ARMCANZ (2000) default guideline values (GVs) [3] for physical and chemical stressors applicable to tropical northern Australia (see Appendix 2 for default ANZECC GVs). Warramboo Creek is considered a slightly to moderately disturbed ecosystem, due to impacts associated with historic pastoral use (Yarraloola Station), clearing of transport corridors (Great Northern Highway construction and realignment) and mine development. Therefore, ANZECC/ARMCANZ (2000) guideline values (GVs) for the protection of 95% of species were considered more appropriate than default GVs for 99% protection. In accordance with ANZECC/ARMCANZ (2000) however, the default 99% GVs were applied to bioaccumulating metals such as selenium. For metals and nutrients, dissolved concentrations (0.45 µm filtered samples) were compared to the default GVs. Filtered concentrations were considered a better reflection of the fraction that may be bioavailable. By contrast, comparison of the default GVs to the total metal or total nutrient concentration may overestimate the risk to the environment (ANZECC/ARMCANZ 2000).
- Qualitative visual observations of habitat characteristics were made at each site to assist in explaining any patterns in faunal assemblages in relation to possible discharge effects. WRM have standard worksheets for this task so that recordings between sites and seasons remain as comparable as possible. Habitat characteristics recorded included percent cover by inorganic sediment, submerged macrophyte, floating macrophyte, emergent macrophyte, algae, large woody debris, detritus, roots and trailing vegetation. Details of substrate composition were also recorded and included percent cover by bedrock, boulders, cobbles, pebbles, gravel, sand, silt and clay.
- Microinvertebrates (i.e. zooplankton) were collected from the water column using a 53 µm mesh plankton net to sweep over a standard 15 m distance at each site. Samples were preserved in 70% ethanol for laboratory enumeration and identification. Samples were processed by identifying the first 200-300 individuals encountered in an agitated sample decanted into a 125 mm^2 gridded plastic tray, with the tray then scanned for additional missed taxa also taken to species, and recorded as 'present'. Specimens were identified to the lowest taxon possible, i.e. species or morphotypes.
- Hyporheic fauna were collected using the Karaman-Chappuis method (Delamare Deboutteville 1960). This involved digging a 30 cm deep x 40 cm diameter hole in alluvial gravels adjacent to the water's edge. The hole was allowed to fill with water percolating through the gravel and then swept with a small 110 µm mesh hand-net (Plate 1).

Plate 1. Collection of the hyporheic sample from WARUS5, using the Karaman-Chappuis method. Photo by WRM ©

[3] The ANZECC/ARMCANZ (2000) guidelines have recently been revised and are presented in an interactive online platform. Where previously, WRM have referred to "trigger values (TVs)", these are now known as "guideline values (GVs)" (ANZECC/ARMCANZ 2018). Although actual GVs are not yet provided for fresh and marine water quality (expected to be included in late 2018), updated water quality guidance and framework are currently available online.

- Benthic macroinvertebrates were collected using a 250 µm mesh FBA d-frame dip net (Plate 2). All meso-habitats at a site were sampled, including trailing riparian vegetation, woody debris, open water column and benthic sediments, with the aim of maximising the number of species recorded. Each sample was washed through a 250 µm sieve to remove fine sediment, while leaf litter and other coarse debris were carefully washed into the sieve to remove attached animals and then discarded. Samples were preserved in 70% ethanol for laboratory enumeration and identification. Collected specimens were then identified to the lowest possible level (genus or species level) and enumerated to $\log_{10}$ abundance scale (i.e. 1 = 1 individual, 2 = 2 - 10 individuals, 3 = 11 – 100 individuals, 4 = 101-1,000 individuals, *etc.*).

Plate 2. Sampling for macroinvertebrates using a 250 µm mesh FBA d-frame dip net. Photo by WRM ©.

- Fish fauna were sampled using a variety of methods including electrofishing, seine netting, gill nets and dip nets. Electrofishing was conducted with a Smith-Root Model LR24 battery powered backpack electrofisher. 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) were used at each site and were set in deeper water for the duration of sampling at that site. Smaller light-weight fine mesh gill nets (10 m net, with a 2 m drop, 10 mm stretched mesh) were also set at each site to capture smaller fish. All gill nets were checked frequently to avoid fish deaths. Catch from the duplicate nets were combined to form one replicate sample from each sampling location. Smaller species and juveniles were sampled by beach seine (10 m net, with a 2 m drop and 6 mm mesh) deployed in shallow areas where there was little vegetation or large woody debris. Two hauls of the seine were conducted at each site to maximise the number of individuals caught. Fish were identified in the field, with standard length[4] (SL) measurements taken, and then released alive.

3.4.1 Phytoplankton

In addition to the routine, full suite of aquatic ecosystem sampling as detailed above, phytoplankton samples were also collected from Warramboo Creek in the wet-18, at the request of Rio Tinto. Three sites were sampled in each reach; WARUS3, WARUS4 and WARUS6 from the upstream reference reach, and WARDS2, WARDS3 and WARDS4 from the downstream potential exposed reach. Phytoplankton includes algae, diatoms, dinoflagellates and cyanobacteria/blue-green algae, and forms the basis of many food webs.

[4] 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).

Phytoplankton sampling involved collecting three random 1 L water samples at each site, which were amalgamated in a bucket and a 1 L sub-sample (i.e. composite sample) taken and preserved with 5 % Lugol's solution. Phytoplankton samples were allowed to settle for at least 2 weeks, and then decanted in the WRM laboratory, and the concentrated volume freighted to Ms Gosia Przybylska (AlgaeTest Consulting, Mulgrave, Victoria) for processing and identification. Phytoplankton were identified to lowest possible level (genus or species), and cell counts/ml made of all dominant species. Where specific names could not be assigned, vouchers were established. These vouchers are held by Ms Przybylska.

3.5 Data analysis

All data collected were entered into Excel 2017 spreadsheets.

3.5.1 Assessment of conservation significance of fauna

The conservation significance of all aquatic fauna recorded was assessed using established lists of conservation fauna. For invertebrates, reference was made to the IUCN Red List of Threatened Species (IUCN 2018) and DBCA Threatened and Priority Fauna Rankings (DBCA 2018). Fish species were compared against the IUCN Red List of Threatened Species (IUCN 2018), DBCA Threatened and Priority Fauna Rankings (DBCA 2018), and Australian Society for Fish Biology Conservation List (ASFB 2001). Reference was also made to other Pilbara studies, as well as databases such as The Australian Faunal Directory, The Australian National Insect Collection Database and in-house WRM database for distribution and occurrence information for all aquatic species.

3.5.2 Hyporheic fauna classifications

All taxa recorded from hyporheic samples were classified using Boulton's (2001) categories;

- stygobite – obligate groundwater species, with special adaptations to survive such conditions,
- permanent hyporheos stygophiles - epigean[5] species 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).

3.5.3 Univariate analyses

Univariate statistics were performed using SPSS software (Version 22.0 for Windows). Two-way analysis of variance (ANOVA) was applied to test for significant differences in water quality analytes, main habitat characteristics, richness of phytoplankton taxa, hyporheos fauna, and micro- and macro-invertebrates between site type (potential exposed vs reference site) and year (wet-16 or wet-18). A Levene's test was used in the first instance to test for equality of variances (Levene 1960). Data were log transformed where necessary.

3.5.4 Multivariate analyses

Multivariate analyses were performed using the PRIMER package v 7 (**P**lymouth **R**outines in **M**ultivariate **E**cological **R**esearch; Clarke *et al.* 2014) to investigate differences in water quality and aquatic fauna assemblages (hyporheos fauna, micro- and macro-invertebrates, and fish age-classes)

5 Epigean – living or occurring on or near the surface of the ground.

between site type and year. Relationships between faunal assemblages and physico-chemical characteristics were also examined. Multivariate analyses were conducted on all data collected by WRM in the wet-16 and wet-18. Analyses applied to the data included some or all of the following:

1. Describing pattern amongst the water quality and fauna assemblage data (i.e. microinvertebrates, hyporheos fauna, macroinvertebrates and fish age-classes) using cluster and ordination techniques. Similarity matrices for environmental data were based on the Euclidean Distance Measure, and environmental data were log transformed, where necessary, and standardised prior to analyses. Similarity matrices for fauna data were based on the Bray-Curtis Similarity Measure (Bray and Curtis 1957). Due to the erratic nature of the microinvertebrate and hyporheos fauna (high variability within site type within a sampling event), the data were first dispersion-weighted in PRIMER.
2. Ordination was by non-metric Multi-Dimensional Scaling (nMDS) (Clarke and Gorley 2014). Ordinations were depicted as two-dimensional plots based on the similarity matrices.
3. One-way permutational multivariate analysis of variance (PERMANOVA) was undertaken to determine if there was a significant difference between site type (potential exposed vs reference) (Anderson 2001a, b, Anderson and ter Braak 2003, Anderson *et al.* 2008).
4. The relationship between water quality and biotic data was assessed using the BVSTEP routine. This was used to calculate the minimum suite of parameters that explain the greatest percent of variation (i.e. the parameters which most strongly influence the species ordination).

The sampling design allows comparison of change in the similarity (Bray-Curtis) of all faunal assemblages over time between reference and potential exposed sites. The premise being that if the degree of similarity between exposed and reference sites differs significantly over time (compared with pre-mine similarity), this would indicate mine-related response rather than stochastic variability due to factors such as climatic change.

Wet-18 microinvertebrate and macroinvertebrate richness and overall assemblage structure was also compared with other nearby systems sampled by WRM and DBCA (during the Pilbara Biological Survey) in the wet season between the 2005 and 2018; namely Red Hill Creek, Cane River, Mungarathoona Creek, Myanore Creek, Robe River, Jimmawarrada Creek and a claypan[6] (Yarraloola Station Pool). Taxonomy was standardised across projects and years prior to analysis, to ensure updates in taxonomy over time did not influence the analysis. As such, taxa richness presented here may not necessarily be the same as reported in other project reports.

[6] NB: taxonomy was first standardised across projects and years, so taxa richness presented here may not necessarily be the same as reported above, or in other project reports.

4 RESULTS AND DISCUSSION

4.1 Water quality

Water quality data were compared against ANZECC/ARMCANZ (2000) water quality guidelines, using default guideline values (GVs) for physical and chemical stressors applicable to slightly to moderately disturbed lowland rivers from tropical northern Australia (see Appendix 2 for default GVs). All water quality data recorded during the current study are provided in Appendix 3.

4.1.1 Wet 2018 data

Surface water quality at Warramboo Creek indicated recent filling by rainwater and was low in alkalinity, hardness, electrical conductivity (EC), and concentrations of major ions (Figures 3 and 4). Waters were generally characterised by circum-neutral pH, adequate to high dissolved oxygen (DO), fresh[7] waters, with low to moderate TSS, generally low nitrogen nutrients and dissolved metal concentrations, with low buffering capacity at some sites (i.e. low alkalinity). The water quality at Warramboo Creek is likely to vary depending on the timing of sampling relative to rainfall events, with higher concentrations of ions and EC likely to be recorded as surface waters recede, due to evapoconcentration effects. This highlights the importance of obtaining an adequate baseline dataset over multiple years/events relative to rainfall and recessional flows, with which to detect impacts, if any, of the Warramboo development and discharge operation in the future.

Alkalinity ranged from 11 mg/L at WARUS2 to 58 mg/L at WARDS3 (see Appendix 3). While alkalinity at all sites was generally considered low, three sites in the upstream reference reach recorded alkalinity below the point at which waters would be considered to be poorly buffered (≤ 20 mg/L); WARUS1, WARUS2 and WARUS3 (Appendix 3). Buffering capacity of these waters would be low, and the removal of carbon dioxide during photosynthesis would result in rapidly rising pH (Sawyer and McCarty 1978, Romaire 1985, Lawson 2002).

Ionic composition was dominated by calcium (Ca^{2+}) cations and hydrogen carbonate (HCO_3^-) anions with potassium (K^+) and chloride (Cl^-) sub-dominant at all sites across the study area. This suggests groundwater ingress has less influence on the water chemistry of these sites than filling by rainwater. Concentrations of all ions was low.

While most water quality analytes recorded values within default ANZECC/ARMCANZ (2000) GVs, a number were elevated in comparison to default GVs at a number of sites, including;

- pH was elevated in comparison to the upper default GV at reference sites WARUS2 and WARUS3, and potential exposed sites WARDS4, WARDS5 and WARDS6 (Figure 3).
- The upper default GV for DO (120%) was exceeded at WARUS2 (124.4%) and WARDS6 (132.8%; Figure 3). DO at these sites was super-saturated, which occurs when net photosynthesis exceeds total oxygen consumption. Super-saturation is common in areas of high algal and macrophyte growth, and/or areas of high turbulence (e.g. riffle zones). Sites which are super-saturated during the day are likely to experience oxygen stress overnight, as respiration by plants, algae, bacteria and other aquatic fauna deplete DO. Super-saturation is also known to cause gas bubble disease in fish (Bouck 1980). Although, it is important to remember that oxygen needs of aquatic biota differ between species and between life history stages.

[7] Fresh defined as < 1500 µS/cm, Brackish = 1500 – 4500 µS/cm, Saline = 4500 – 50,000 µS/cm, Hypersaline > 50,000 µS/cm (DoE 2003). Classifications were presented as TDS (mg/L) in DoE (2003) so a conversion factor of 0.68 was used to convert to conductivity µS/cm as recommended by ANZECC/ARMCANZ (2000).

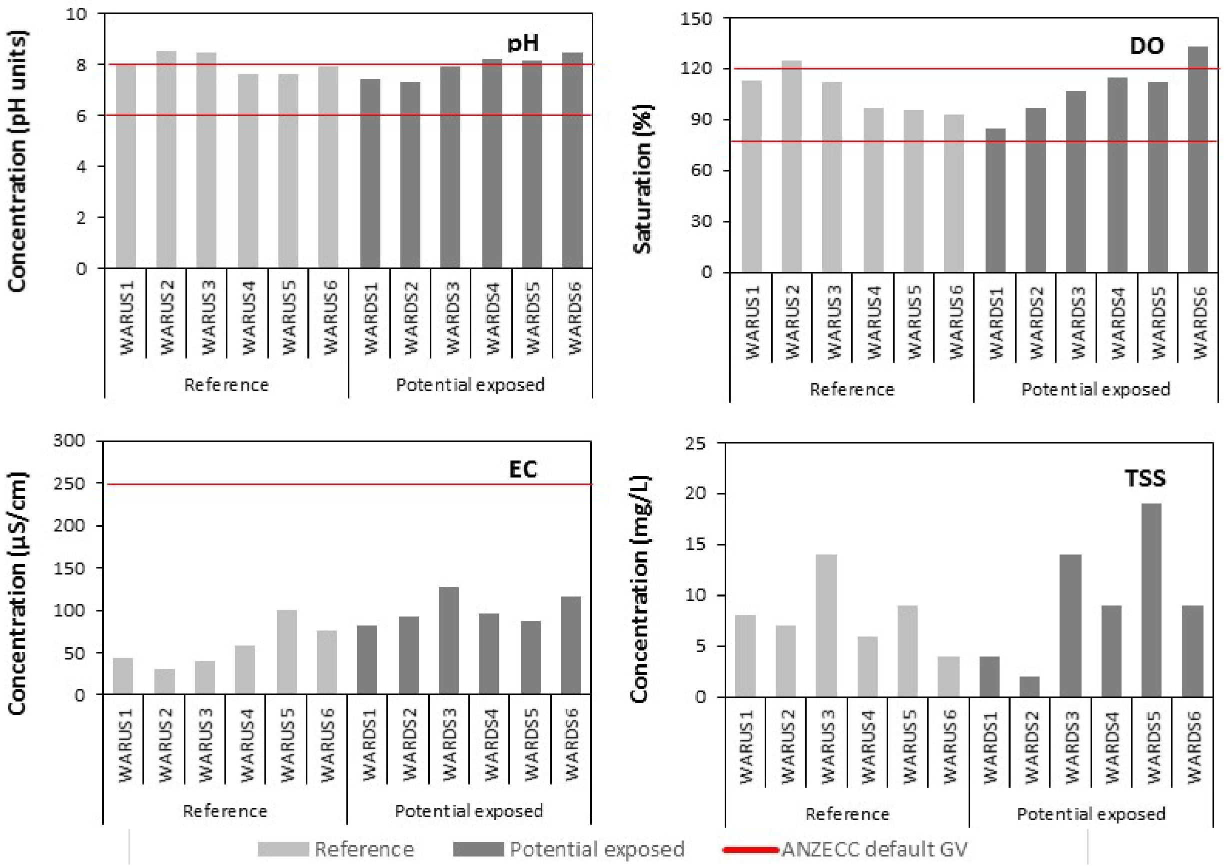


Figure 3. Comparison of pH, DO, EC and TSS amongst Warramboo Creek surface water sampling sites in the wet-18. Default ANZECC/ARMCANZ (2000) GVs are provided for comparison, where appropriate. Refer Table 1 for explanation of site codes and Figure 1 for location of sites. NB: y-axes may be different for different analytes.

- Three reference sites recorded nitrogen oxide (nitrate + nitrite; N_NOx) concentrations greater than the default eutrophication GV, including WARUS2, WARUS3 and WARUS4 (Figure 5). Particularly high N_NOx concentrations, more than ten times the ANZECC/ARMCANZ (2000) default GV were recorded from WARUS4 (0.12 mg/L; Figure 5). The natural, baseline difference in N_NOx concentrations between reference and potential exposed sites along Warramboo Creek is interesting and provides a useful background from which to detect impacts from the discharge of dewatering water from Warramboo, if any, in the future. The current source of N_NOx in the upstream reference reach is unknown, but cattle likely contribute to the high concentrations.

- Total nitrogen (total N) was recorded in concentrations greater than the default GV from reference sites WARUS2, WARUS3, WARUS4 and WARUS6, and potential exposed site WARDS5 (Figure 5).

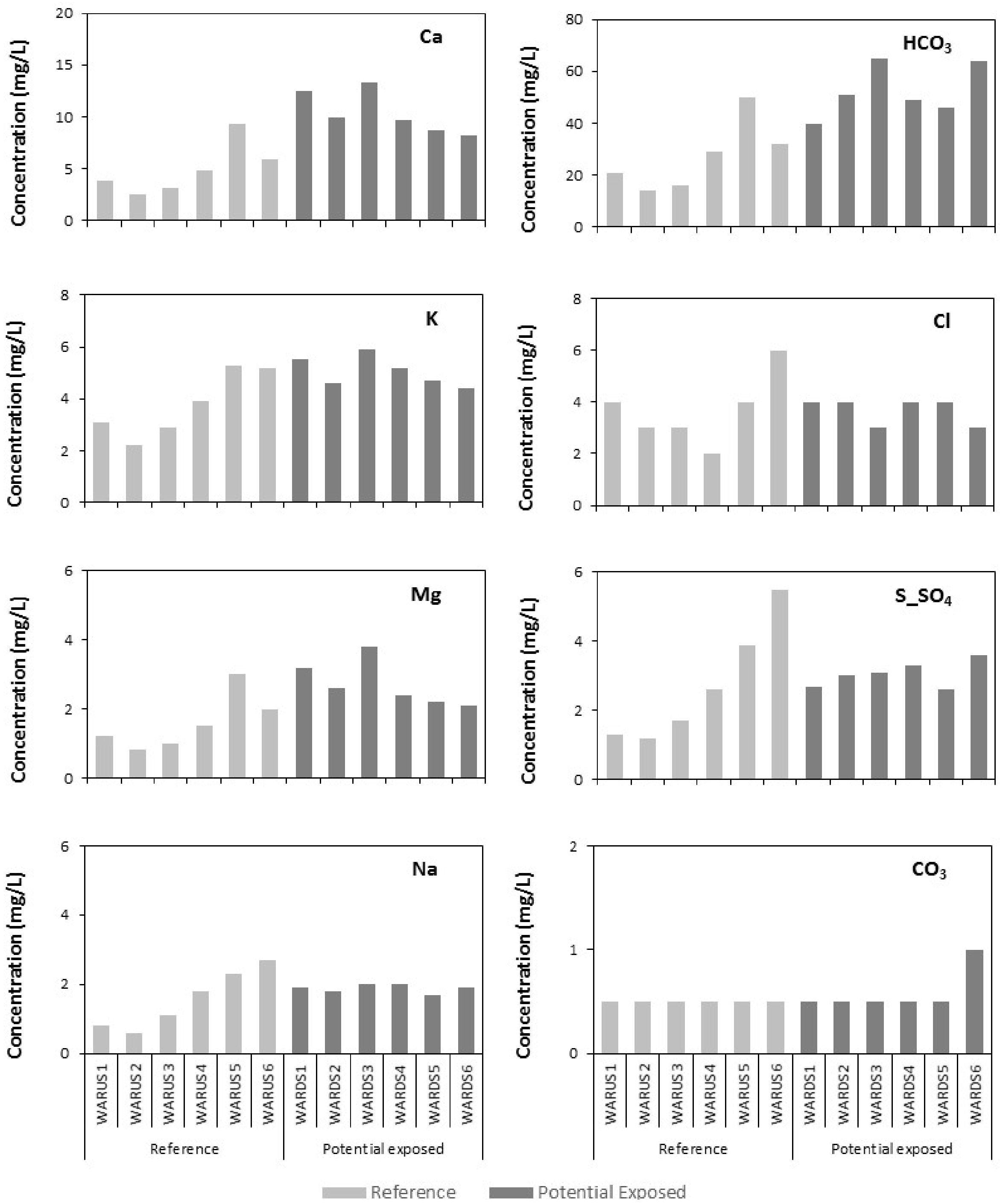


Figure 4. Comparison of alkalinity, hardness, and concentration of major ions amongst Warramboo Creek surface water sampling sites in the wet-18. NB: y-axes may be different for different analytes.

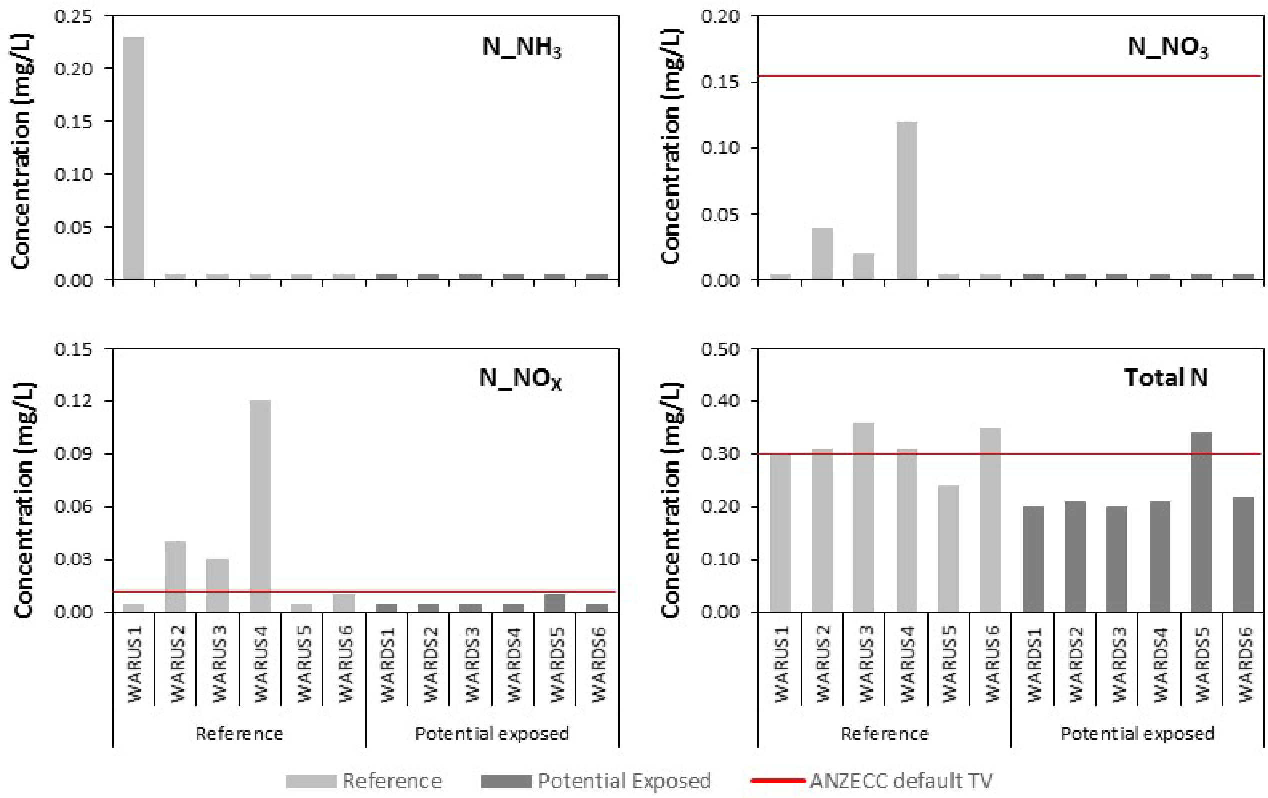


Figure 5. Comparison of nitrogen nutrient concentrations amongst Warramboo Creek surface water sampling sites in the wet-18. Default ANZECC/ARMCANZ (2000) GVs are provided for comparison. The default toxicity GV for N_NH_3 was above the limit of the y-axis (0.73 mg/L). NB: y-axes may be different for different analytes.

- Total phosphorus (total P) was elevated in comparison to the default ANZECC/ARMCANZ (2000) GV at all upstream reference sites and potential exposed site WARDS5 (Figure 6).

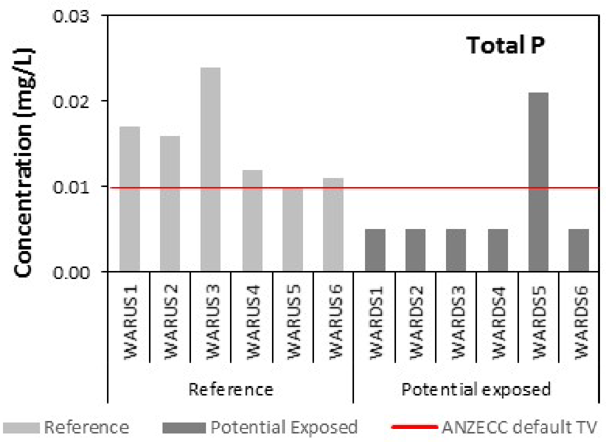


Figure 6. Comparison of total P concentrations amongst Warramboo Creek sites in the wet-18, with the default ANZECC GV indicated.

- Dissolved copper (dCu) concentrations were high across the upstream reference reach of Warramboo Creek, with all sites recording concentrations in excess of the default GV (Figure 7). Only one potential exposed site recorded elevated dCu concentrations; WARDS5 (Figure 7).

- Dissolved iron (dFe) concentrations were generally low across the study area, with only one site exceeding the default GV; WARUS6 (Figure 7). It should also be noted that ANZECC/ARMCANZ (2000) provide only a low reliability GV for dFe.

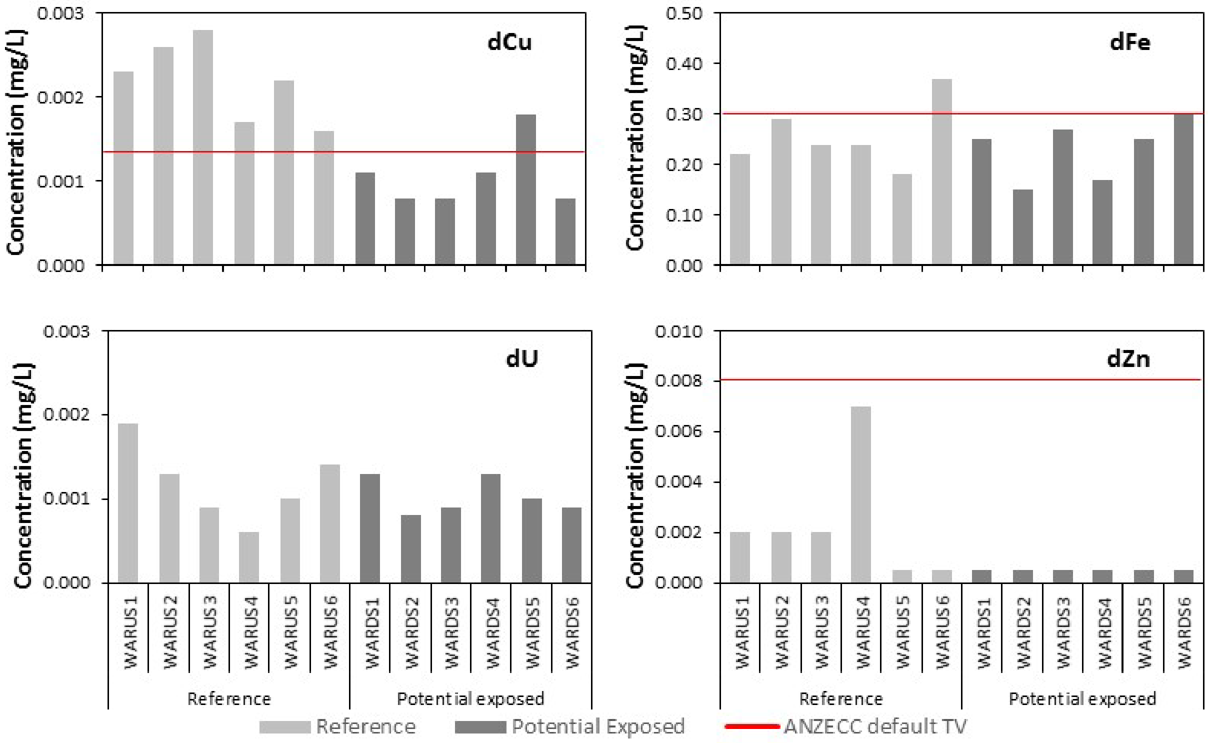


Figure 7. Comparison of some selected dissolved metal concentrations amongst Warramboo Creek surface water sampling sites in the wet-18. Default ANZECC/ARMCANZ (2000) GVs are provided for comparison, where appropriate. NB: y-axes may be different for different analytes.

4.1.2 Spatial and temporal differences in water quality

One-way ANOVA was undertaken to determine whether there was a significant difference in any water quality parameters between reference and potential exposed sites (Table 3 and Figures 8 to 10). As only two reference sites were successfully sampled in the previous survey (wet-16), no univariate temporal analysis could be undertaken at this time. However, general differences between the wet-16 and wet-18 included greater alkalinity, EC (and associated ions; Ca, Cl, HCO_3, K, Na, S_SO_4), TSS, and total N.

A number of parameters were significantly greater in concentration in the downstream potentially exposed Warramboo Creek reach, including EC, TDS, alkalinity and harness, and corresponding ionic concentrations Ca, Mg, K and HCO_3 (One-way ANOVA; df = 1, $p \leq 0.048$; Table 3 and Figure 8). Reasons for the higher EC, alkalinity and ionic concentrations from this reach are currently unknown, but are perhaps unsurprising given the transient nature of flows, and differences in recession of flows along the length of the creek.. The two most downstream sites on the reference reach, WARUS5 and WARUS6, recorded concentrations of the above analytes more similar to the potential exposed sites than the other reference sites (see Figures 3 and 4).

Concentrations of dissolved barium (dBa) were also significantly higher from potential exposed sites, in comparison to reference sites (df = 1, $p = 0.002$; Table 3 and Figure 10).

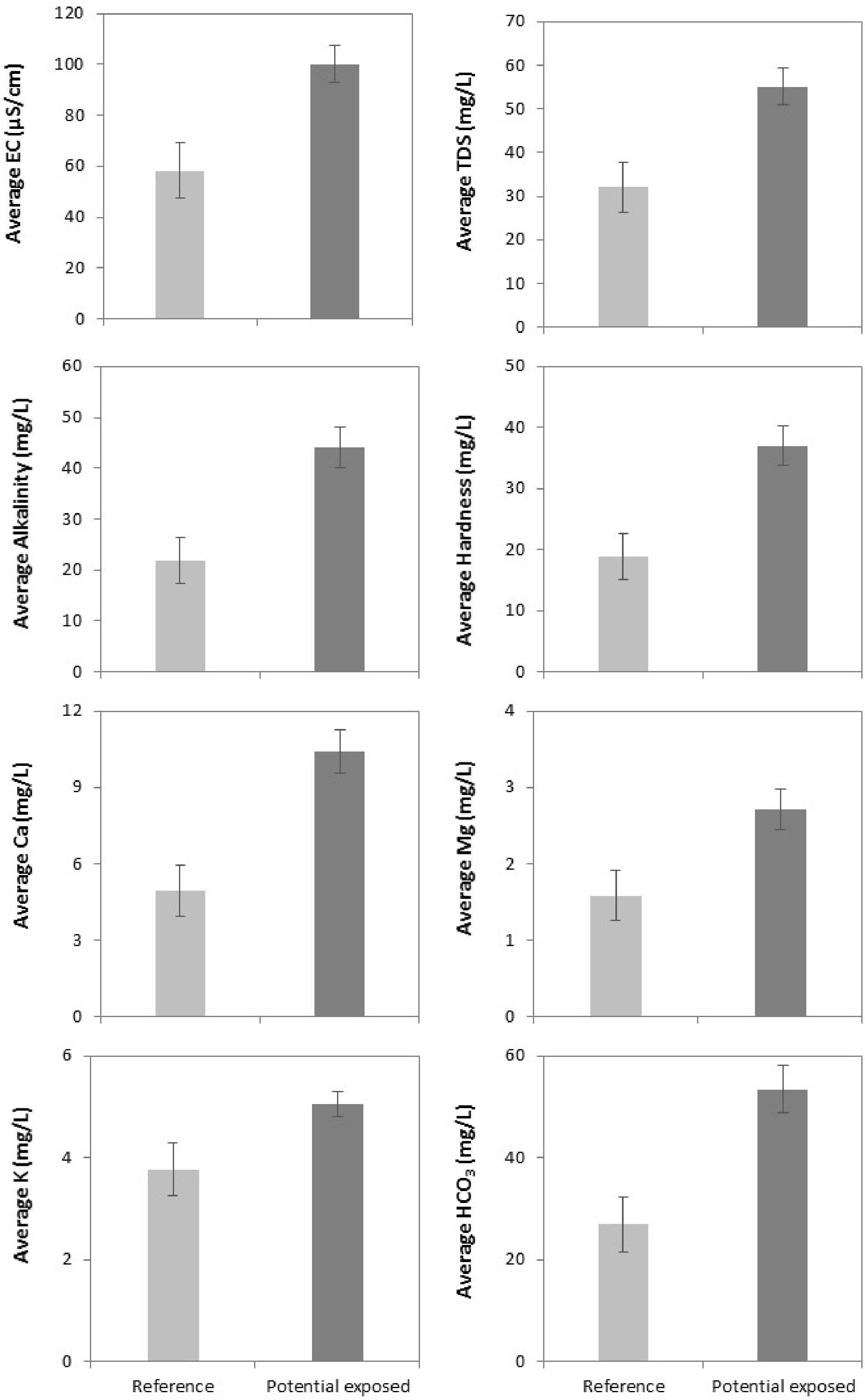


Figure 8. Average EC, TDS, alkalinity, hardness and concentrations of major ions (± se) recorded from reference and potential exposed reaches of Warramboo Creek in the wet-18. Only parameters which were significantly different between site type are shown (see Table 4).

Table 3. One-way ANOVAs testing for significant differences ($p < 0.05$) in mean concentrations of water quality parameters between site type (reference vs potential exposed). Only significant results are shown.

Variable	Source	df	F	*p*-value
EC	Between	1	10.35	**0.009**
	Total	11		
TDS	Between	1	10.42	**0.009**
	Total	11		
Alkalinity	Between	1	13.88	**0.004**
	Total	11		
Hardness	Between	1	13.47	**0.004**
	Total	11		
Ca	Between	1	17.70	**0.002**
	Total	11		
Mg	Between	1	7.06	**0.024**
	Total	11		
K	Between	1	5.06	**0.048**
	Total	11		
HCO_3	Between	1	13.46	**0.004**
	Total	11		
Total N	Between	1	8.38	**0.016**
	Total	11		
dBa	Between	1	17.56	**0.002**
	Total	11		
dCu	Between	1	20.35	**0.001**
	Total	11		

Two analytes were recorded in significantly higher concentrations from reference sites, including total N (df = 1, $p = 0.016$; Table 3 and Figure 9) and dissolved copper (dCu; df = 1, $p = 0.001$; Figure 10).

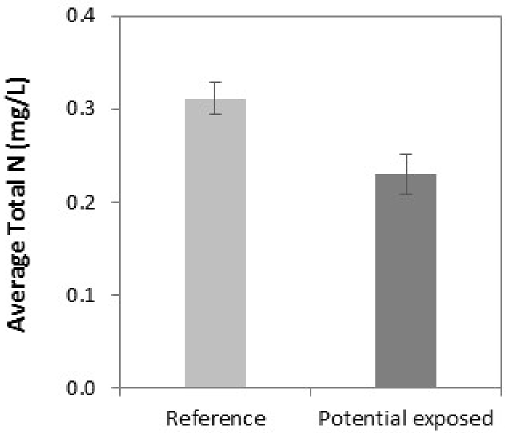


Figure 9. Average total N (± se) recorded from reference and potential exposed reaches of Warramboo Creek in the wet-18.

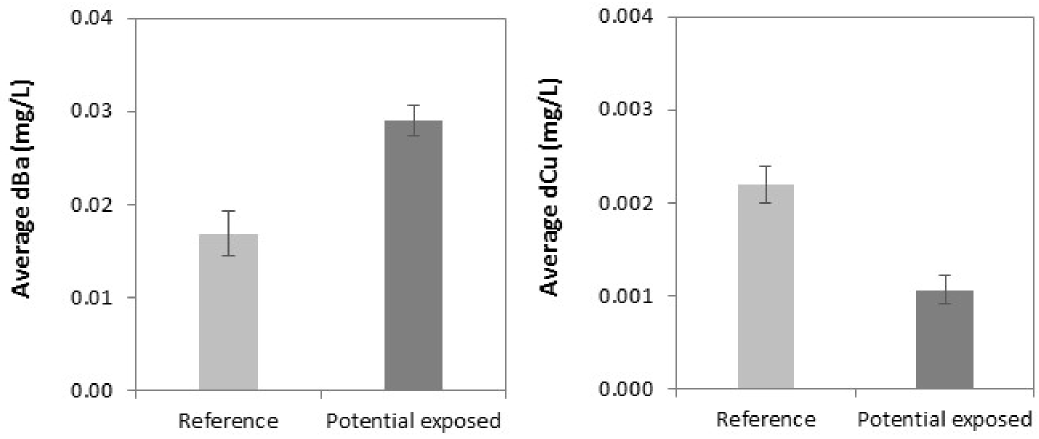


Figure 10. Average dBa and dCu (± se) recorded from reference and potential exposed reaches of Warramboo Creek in the wet-18.

Patterns in water quality

Patterns were evident in the nMDS ordination of all water quality data collected from Warramboo Creek in the wet-16 and wet-18 (Figure 11). The two reference samples from the wet-16, WARUS5 and WARUS6, separated from all others in ordination space (Figure 11). Differences between the wet-16 and wet-18 samples was influenced by the higher alkalinity, EC (and associated ions; Ca, Cl, HCO_3, K, Na, S_SO_4), TSS, and total N recorded in the wet-16. The reference samples collected in the wet-18 also separated from the wet-18 potential exposed samples, but interestingly the same two reference sites, WARUS5 and WARUS6, sat with the potential exposed sites (Figure 11). SIMPROF detected four significant cluster groups, including:

1. The two wet-16 reference sites
2. WARUS2 and WARUS3 from the wet-18
3. WARUS1 and WARUS1 from the wet-18
4. All wet-18 potential exposed samples and WARUS5 and WARUS6 from the wet-18 (Figure 11).

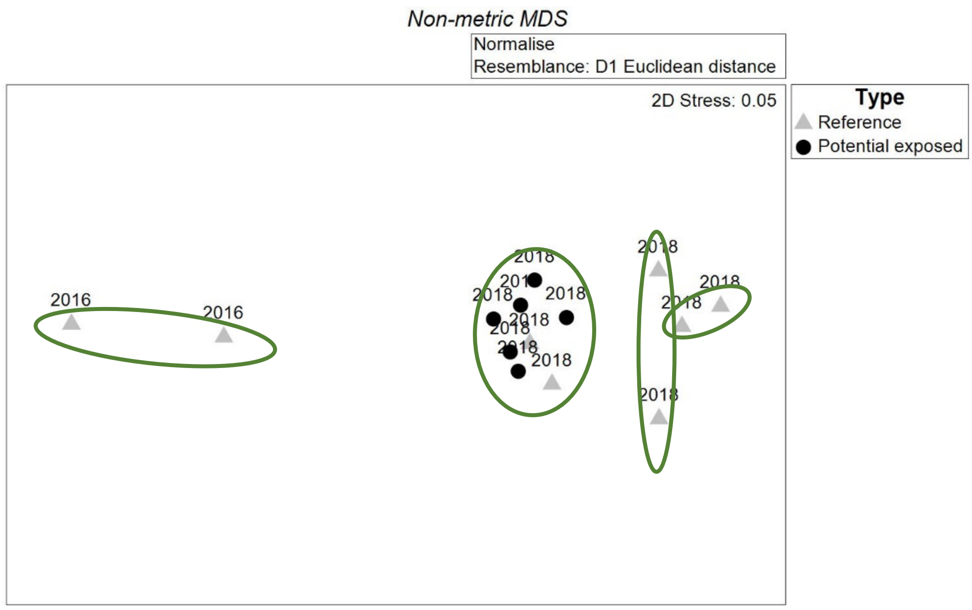


Figure 11. nMDS plots of water quality data collected from Warramboo Creek reference and potential exposed sites in the wet-16 and wet-18. Samples are identified by site type (reference v potential exposed) and labelled by year. Samples are grouped within green circles based on significant clusters as determined using SIMPROF.

The two wet-16 samples were removed from further analysis as there was insufficient replication with which to undertake temporal analysis, and no samples from potential exposed sites at that time. The ordination was repeated on 2018 data only (Figure 12).

There was a large degree of variation amongst reference samples in ordination space, but little amongst potential exposed samples, all collected in the wet-18 (Figure 12). There was some evidence of longitudinal trends in water quality within both reaches, at least amongst the first four sites within a reach. As mentioned above, the WARUS5 and WARUS6 reference sites were more similar to the potential exposed sites, than the other reference sites (Figure 12). Despite this, there was still an overall significant difference in water quality between reference and potential exposed sites (One-way PERMANOVA; df = 1, 11; pseudo-F = 3.99, p = 0.011).

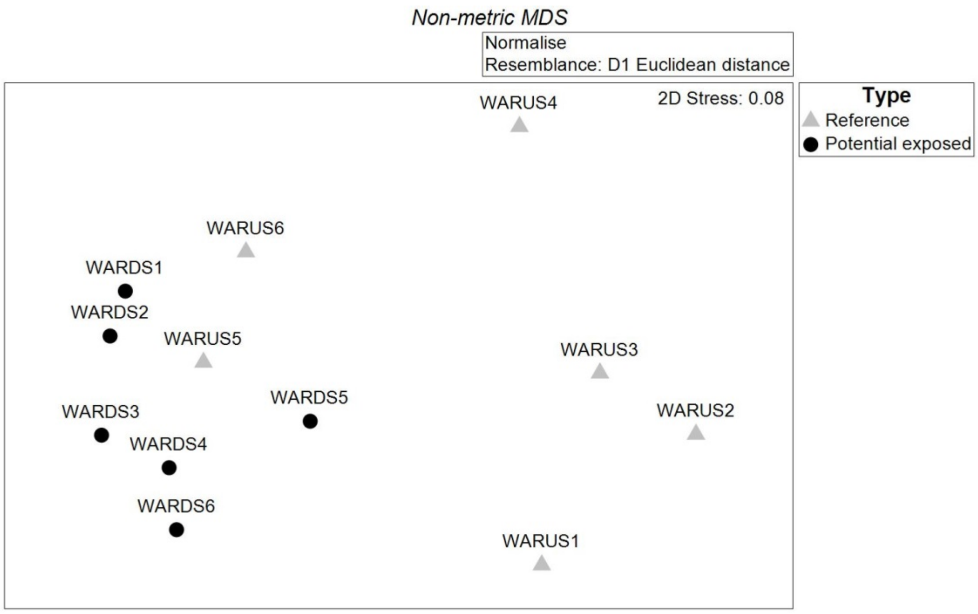


Figure 12. nMDS ordination of water quality data collected from Warramboo Creek reference and potential exposed sites in the wet-18. Samples are identified by type (reference v potential exposed) and labelled by site.

The difference between reference and potential exposed sites in ordination space, was generally related to the higher EC, alkalinity and concentrations of most ions recorded from potential exposed sites (Figure 13). As mentioned above, WARUS5 and WARUS6 recorded concentrations of these analytes more similar to potential exposed sites, and this influenced their separation from reference samples in ordination space, with these samples instead clustering with potential exposed sites. The higher total N and dCu concentrations from reference sites also influenced the nMDS ordination (Figure 13).

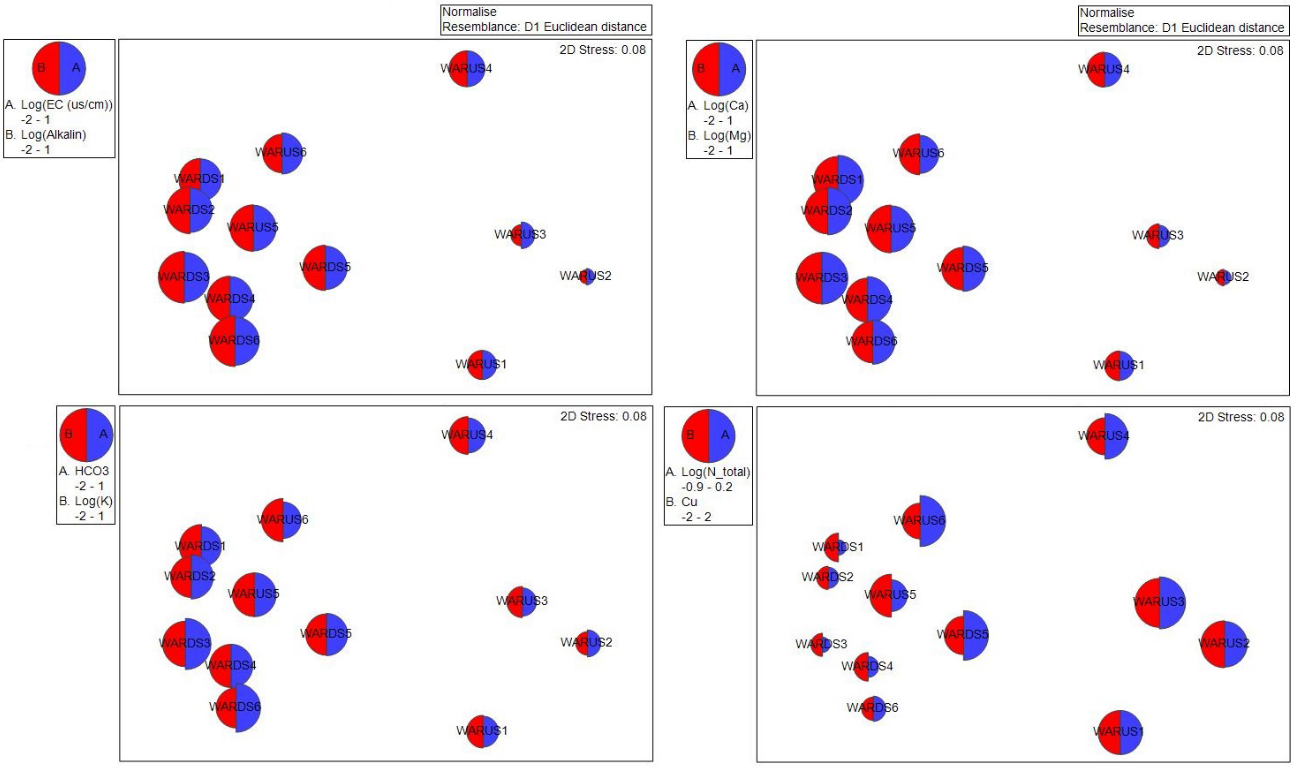


Figure 13. Bubble plots showing the influence of certain water quality analytes on the ordination.

4.2 Habitat

4.2.1 Wet 2018 data

The eight most upstream sites, including all reference sites and WARDS1 and WARDS2, were characterised by transmissive pebble/gravel and sand substrates (Figure 14). WARDS3, WARDS4 and WARDS6 were dominated by finer clay sediments. Though clay is a highly porous sediment and can hold a lot of water, the pores within the fine sediments are so small that water moves slowly through them, making clay a poorly conductive sediment (i.e. low hydraulic conductivity). WARDS5 was dominated by highly porous and highly permeable sand substrates (Figure 14).

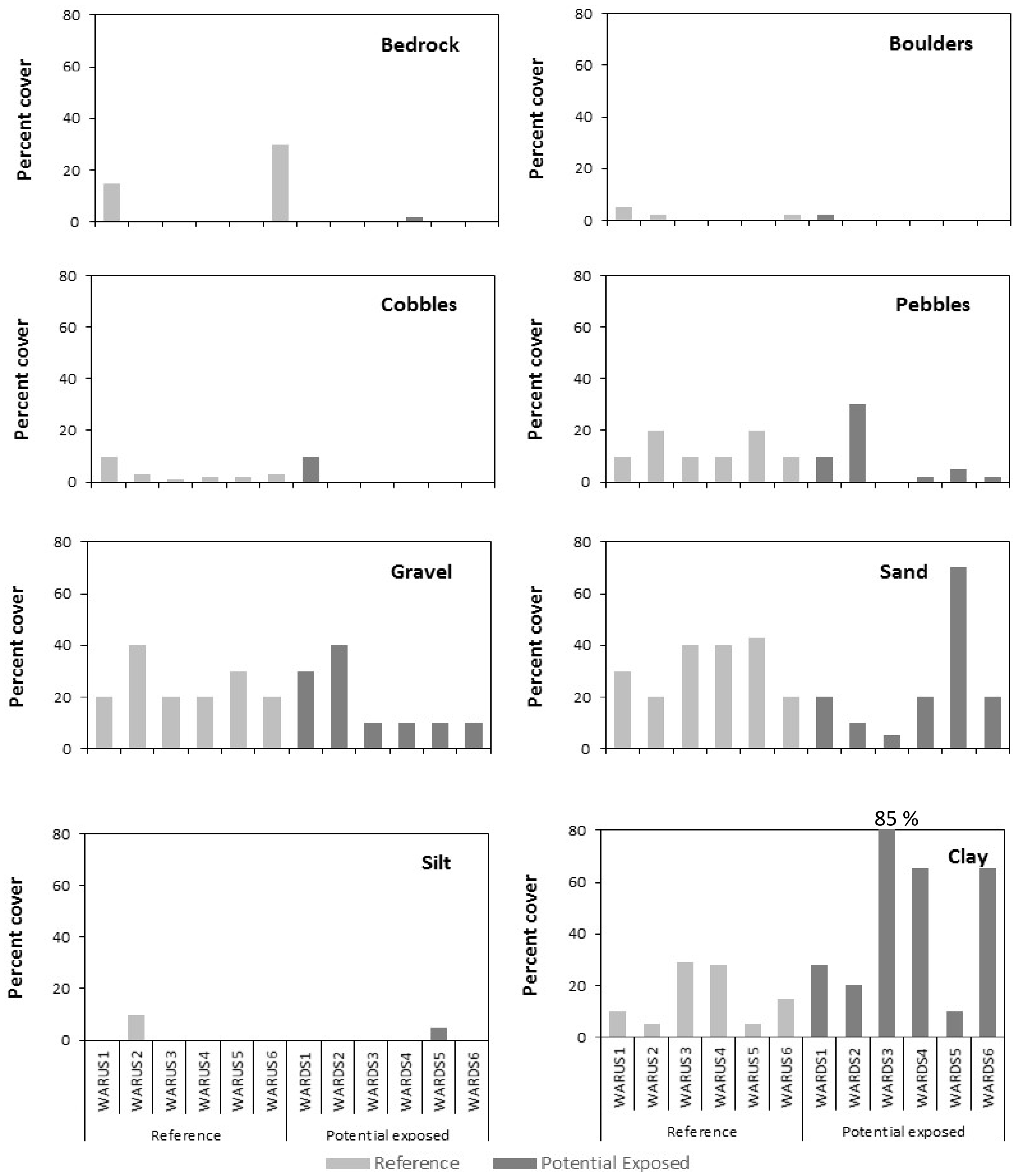


Figure 14. Plots of substrate composition, showing percentage cover by bedrock, boulders, cobbles, pebbles, gravel, sand, silt and clay at each site in the wet-18.

There was little in-stream habitat along Warramboo Creek, with only four of the eight habitat types measured being present at the time of the wet-18 surveys. There was low levels of complex and heterogeneous substrates with which to support aquatic invertebrate and fish communities, with only small amounts of large woody debris (LWD) and detritus, and no submerged or emergent macrophyte, algae or root mats present (Figure 15). All sites were dominated by cover of open mineral substrate (Figure 15). LWD and detritus were present at all sites, with highest cover by detritus at WARDS2 and by LWD at WARDS3 (Figure 15). All sites recorded low overall habitat diversity, with little variation amongst sites (Figure 16). All sites recorded either three of four different types of in-stream habitat (Figure 16).

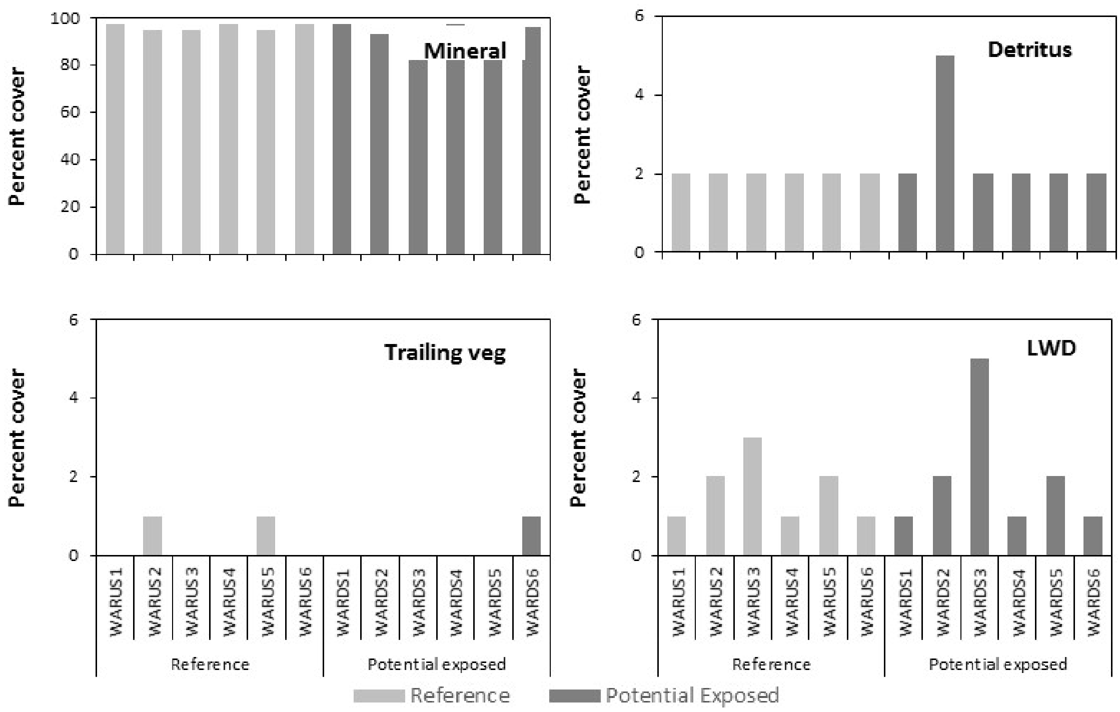


Figure 15. Plots showing percent cover by different habitat types at each site in the wet-18. NB: y-axes scales are not the same for all plots.

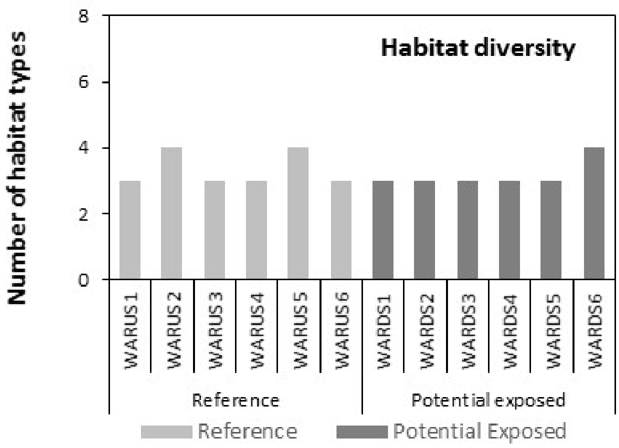


Figure 16. In-stream habitat diversity at each site sampled in the wet-18.

There was very little spatial (reference vs potential exposed sites) difference in sediment and habitat characteristics across the Warramboo Creek sites. The only sediment type which recorded a significant difference between site type was clay, with significantly greater percent cover recorded from potential exposed sites (One-way ANOVA clay arcsin proportion; df = 1, F = 5.22, p = 0.045; Figure 17). There was no significant difference in percent cover by any of the habitat variables measured between site types (One-way ANOVA; df = 1, $p \geq 0.341$).

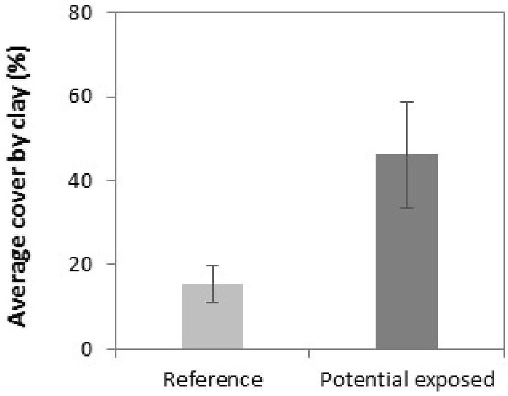


Figure 17. Average percent cover by clay (±se), recorded from reference and potential exposed sites in the wet-18.

There was also no significant difference in maximum water depth between reference and potential impact sites (One-way ANOVA; df = 1, F = 0.02, p = 0.897). Maximum water depth ranged from 0.5 m at WARUS4 and WARDS2, to 1 m at WARUS2, WARUS3, WARDS3 and WARDS5 (Figure 18).

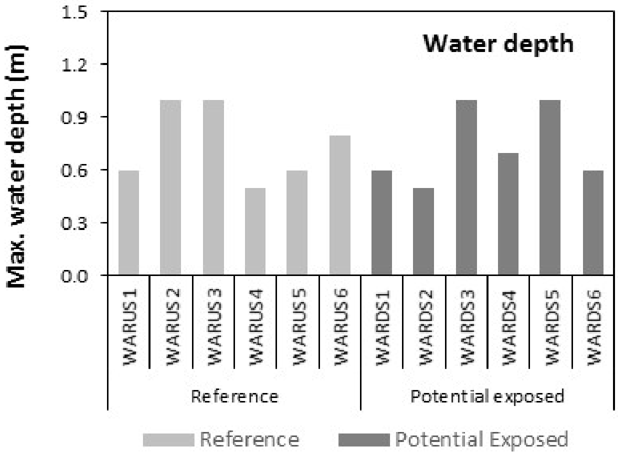


Figure 18. Maximum water depth (m) recorded from each site in the wet-18.

4.3 Phytoplankton

4.3.1 Taxonomic composition and species richness

A total of 28 phytoplankton taxa were recorded from the six samples taken within the study area; of which 24 were found at upstream reference sites, and 15 from downstream potential exposed sites (Table 4). Phytoplankton composition included Cyanophyta (blue-green algae), Charophyta (stoneworts), Chlorophyta (green algae), Pyrrophyta (dinoflagellates), Cryptophyta (cryptophytes), Chrysophyta (Golden-brown algae) and Bacillariophyceae (diatoms; Table 4 and Appendix 4). Generally, Cyanophyta and Chlorophyta dominated phytoplankton taxa at each site, with comparatively few Charophyta, Pyrrophyta or Chrysophyta. The list includes groups which could not be identified to species level due to unresolved taxonomy and/or immature specimens. Therefore, the total phytoplankton species richness is likely to be greater than 28.

Table 4. Summary of higher-order phytoplankton taxa composition in Warramboo Creek, at upstream reference sites (WARUS) and downstream potential exposed sites (WARDS) as recorded in the wet-18. Refer Appendix 4 for full species list.

Phytoplankton		Number of taxa	
Scientific name	Common name	WARUS - ref (n = 3)	WARDS - PE (n = 3)
Cyanophyta	Blue-green algae	9	6
Charophyta	Stoneworts	1	0
Chlorophyta	Green algae	5	4
Pyrrophyta	Dinoflagellates	2	1
Cryptophyta	Cryptophytes	2	2
Chrysophyta	Golden-brown algae	1	0
Bacillariophyceae	Diatoms	4	2
		24	**15**

The blue-green algae *Planktolyngbya* sp., green algae *Pyramimonas* sp. and small Chlorophyta (<5 µm), dinoflagellate *Peridinium inconspicuum*, cryptophytes *Cryptomonas* sp. < 10 µm and *Cryptomonas* sp. > 10 µm, and diatom *Pinnularia* sp. were the most commonly recorded taxa, being found at ≥ five of the six Warramboo creekline sites sampled for phytoplankton in the wet-18. The next most common taxa was the potentially toxic cyanobacteria (blue-green algae) *Cylindrospermum licheniforme*, green algae *Coccomonas orbicularis*, and diatom *Nitzschia tubicola*, all of which occurred at four sites. Half of all taxa recorded were only recorded from one site.

The cyanobacteria *Gloeotrichia raciborskii* is a non-toxic algae, but can be abundant in the right conditions, growing periphytically on aquatic plants and submerged stones and wood (Gosia Przybylska, Algaltest, pers comm.). The colonies of filaments enveloped in slime can detach from the substrate and float on the surface (Gosia Przybylska, Algaltest, pers comm.). It was recorded from WARDS4.

Phytoplankton taxa richness ranged from eight at WARDS3 to 19 at WARUS4 (Figure 19). Total cell counts (phytoplankton cells/ml of sample) were greatest at WARDS4 (14, 028 cells/ml) and lowest at WARDS3, just upstream (8, 767 cells/ml; Figure 19).

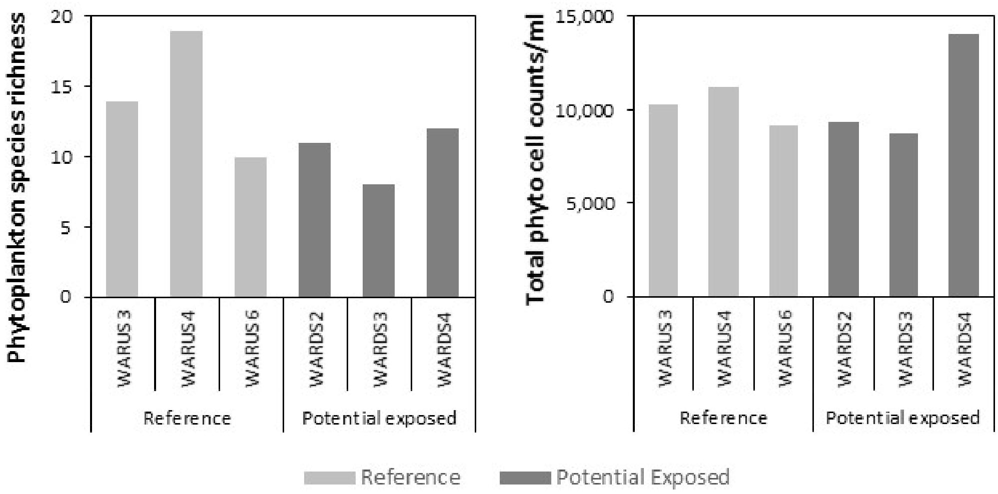


Figure 19. Phytoplankton taxa richness (left) and total phytoplankton cells/ml recorded from three reference and three potential exposed sites along Warramboo Creek in the wet-18.

4.3.2 Potentially toxic cyanobacteria

Two species of cyanobacteria known to be 'potential, but unconfirmed toxin producers' were recorded during the current study; *Cylindrospermum licheniforme* and *Dolichospermum affine*[8]. Potentially toxic cyanobacteria are genera or species known to produce substances toxic to animals and humans. Toxin is often produced during bloom in eutrophic waterbodies, but the trigger for toxin production is largely unknown. The level of toxicity is dependent on the species of cyanobacteria and the density and extent of algal blooms. Under suitable conditions, *C. lichenphorme* can produce cylindrospermopsin which is known to primarily affect the liver and kidney, and anatoxin which affects the central nervous system. The genus *Dolichospermum* appear to be able to produce three quite different types of neurotoxins, as well as the cyclic heptapeptides (Hrubec 2013). Blooms of the filamentous *D. affine* have been associated with low densities of the cladoceran *Daphnia pulex* and high densities of rotifer, with an endotoxin produced by *D. affine* being implicated in the adverse impact to Cladocera (Gilbert 1990). It was ingestion of the cyanobacteria which led to the differential response in cladocera vs rotifers (Gilbert 1990). Cyanobacteria in general, produce hepatotoxins. These are liver toxins whose short-term effects can include gastrointestinal and liver illness and occasionally death. Children are particularly vulnerable. Short exposure, as well as prolonged low-level exposure to hepatotoxins (*e.g.* ingesting water containing toxic cells or toxins) can lead to long-term chronic health effects. Hepatocellular carcinoma and tumour growth cases have been recorded in humans. Effects on animals are very similar. Obvious signs that a bloom is toxic include large numbers of dead fish, waterbirds or other animals in or around a body of water. Such signs were not present at any sites in the current study.

Although two unconfirmed but potentially toxic cyanobacteria species were recorded from Warramboo Creek in the wet-18, neither were recorded in sufficient densities deemed to be of concern or to warrant immediate action as detailed in the National Protocol for the Monitoring of Cyanobacteria and their Toxins in Surface Freshwater by Jones *et al.* (2002) (see Table 5). All densities recorded in 2018 were low, and below the detection level, being the threshold level at which regular weekly sampling is recommended (i.e. all densities < 500 cells/ml, see Table 5; Figure 20). The greatest density of *C. licheniforme* was recorded from WARDS4 (382 cells/ml) and *D. affine* from WARUS6 (10 cells/ml; see Figure 20 and Appendix 4).

[8] *Dolichospermum affine* has undergone a taxonomic name change, being previously known as *Anabaena affinis*.

Table 5. Summary of the Australian National guidelines for water managers monitoring drinking water (after Jones *et al.* 2002).

Alert Level	Density of Bloom	Action
Detection Level	>500 cells/ml of total Cyanobacteria counts	• Initiate regular weekly sampling.
Alert Level 1	>2,000 cells/ml of total Cyanobacteria counts	• Increase sampling to 2x weekly at off-take and at representative locations. • Appropriate Water Authorities must be notified.
Alert Level 2	>5,000 cells/ml of potentially toxic Cyanobacteria counts	• Sampling as per Level 1. • Appropriate Water Authorities and Health Authorities must be notified. • Toxin monitoring may be required
Alert Level 3	>50,000 cells/ml of potentially toxic Cyanobacteria counts	• Sampling as per Level 1. • Appropriate Water Authorities and Health Authorities must be notified immediately. • Toxin monitoring is required.

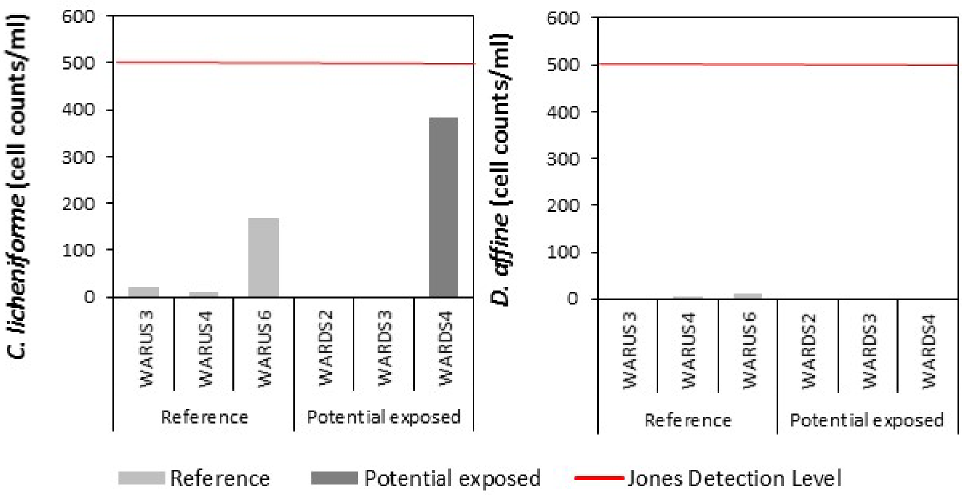


Figure 20. Cell densities (cells/ml) of the potential but unconfirmed toxin producers *Cylindrospermum licheniforme* (left) and *Dolichospermum affine* (right) recorded from Warramboo Creek, in comparison to the Detection Level trigger value associated with the national guidelines for drinking water (Jones *et al.* 2002).

4.3.3 Spatial differences in phytoplankton

There was no significant difference in taxa richness, total cell counts, or cell counts of the dominant types of phytoplankton (Cyanophyta, Charophyta, Chlorophyta, Pyrrophyta, Cryptophyta, Chrysophyta or Bacillariophyceae) between reference and potential exposed sites (One-way ANOVA; df = 1, $p \leq 0.810$). However, it must be noted that replication, and therefore statistical power, was low, with only three samples within each site type collected and available for analyses.

<u>**Patterns in phytoplankton assemblage structure**</u>

Phytoplankton assemblages were variable both within- and between- type (reference vs potential exposed sites; Figure 21). Generally, reference samples appeared to separate slightly in ordination space from potential exposed samples, however, due to the high degree of within-type variation, and low replication, there was no overall significant difference in assemblages between site type (One-way PERMANOVA; df = 1, 5, pseudo-F = 1.43, p = 0.2996; Figure 21). In addition, SIMPROF cluster analysis placed all phytoplankton samples within one large cluster group, indicating no significant difference in assemblages amongst sites. Again, these analyses have low statistical power due to the low number of replicates within each site type.

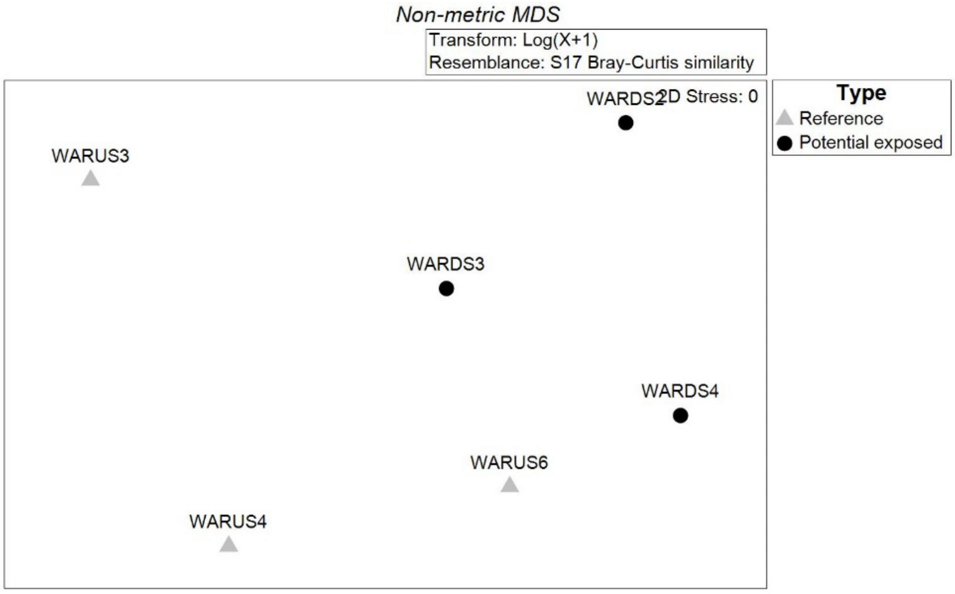


Figure 21. nMDS plot of phytoplankton assemblage data collected from Warramboo Creek reference and potential exposed sites in wet-18. Samples are identified by site type (reference v potential exposed) and labelled by year.

4.3.4 Comparisons with other WRM phytoplankton samples from the Pilbara

WRM do not routinely sample phytoplankton, however, a number of datasets do exist with which to make comparisons with phytoplankton recorded from Warramboo (Table 6). Phytoplankton samples include both wet and dry seasons and have been collected between 2012 and 2018 from Marandoo, Pilbara Regional sites (located across the Pilbara), and Brockman 4 (Table 6).

Table 6. WRM studies from the Pilbara which have included phytoplankton sampling, and with which a dataset exists for comparison with Warramboo.

Project	Sampling event	Number of sites
Marandoo	Dry-12	6
Regional	Dry-12	12
Brockman 4	Wet-14	4
	Wet-15	7
This study	Wet-18	6

Phytoplankton richness and density varied between surveys and years (Figure 22 and Figure 23). Generally, Warramboo Creek recorded high richness but low density of phytoplankton compared to other sites.

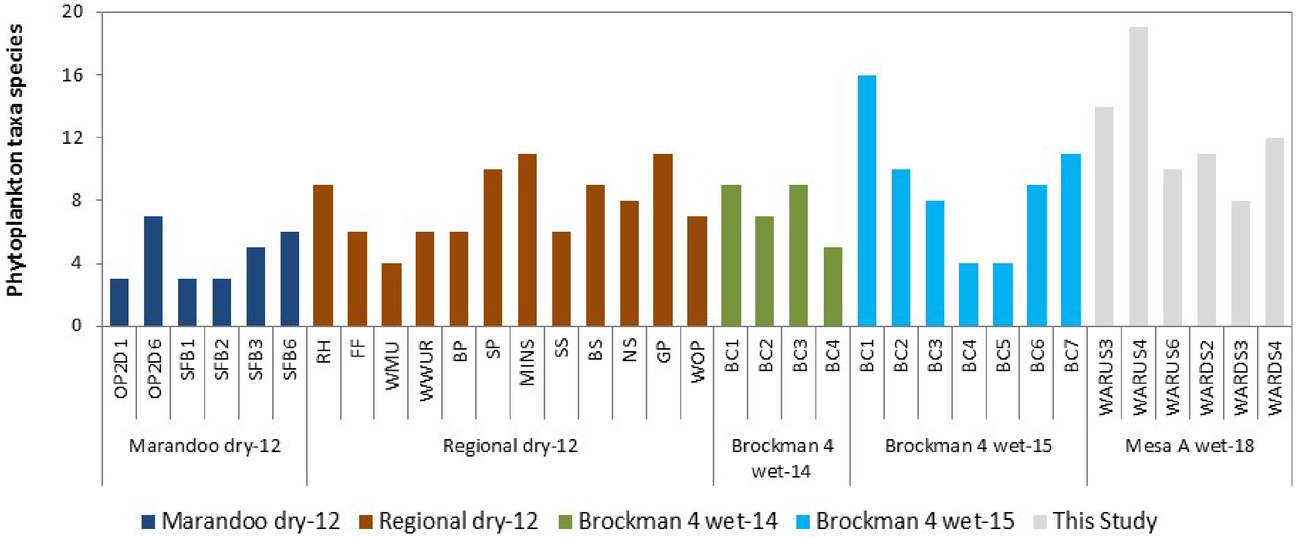


Figure 22. Comparison of phytoplankton taxa richness recorded in the current study with that recorded in previous WRM surveys between 2012 and 2018.

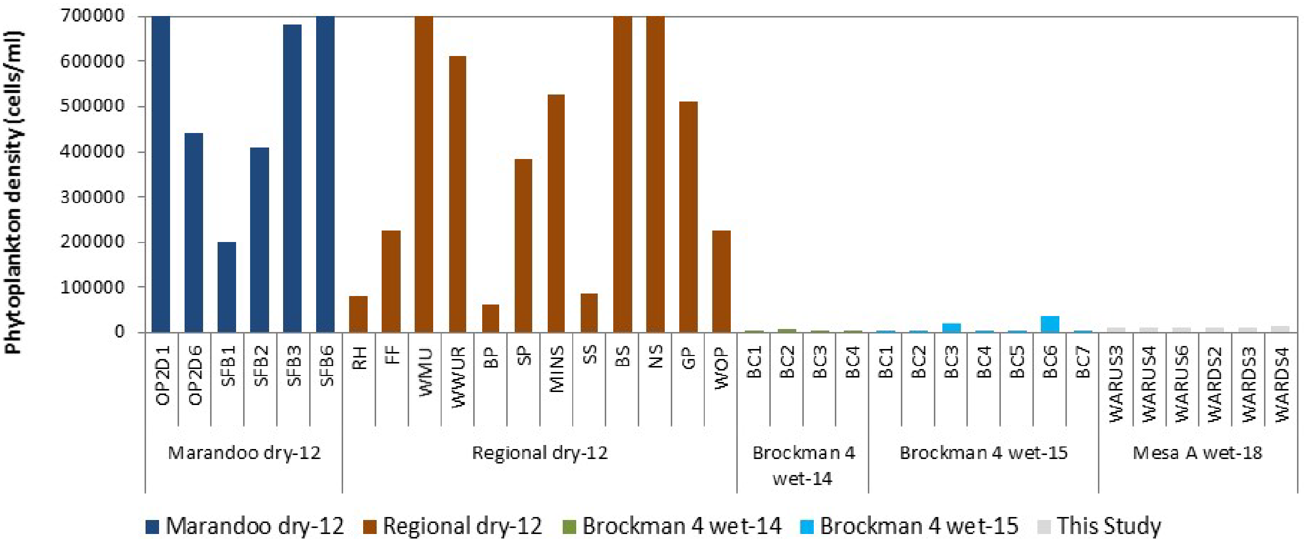


Figure 23. Comparison of phytoplankton density (cells/ml) recorded in the current study with that recorded in previous WRM surveys between 2012 and 2018. NB: Densities from OP2D1, SFB6, WMU, BS and NS were above the limit of the y-axis.

As there was a significant seasonal difference in phytoplankton taxa richness (One-way ANOVA; df = 1, p = 0.010) and density (df = 1, p = 0.020; Figure 24), only wet season data were included in further comparisons. Phytoplankton taxa richness was significantly greater in the wet season, but density was significantly higher in the dry (Figure 24).

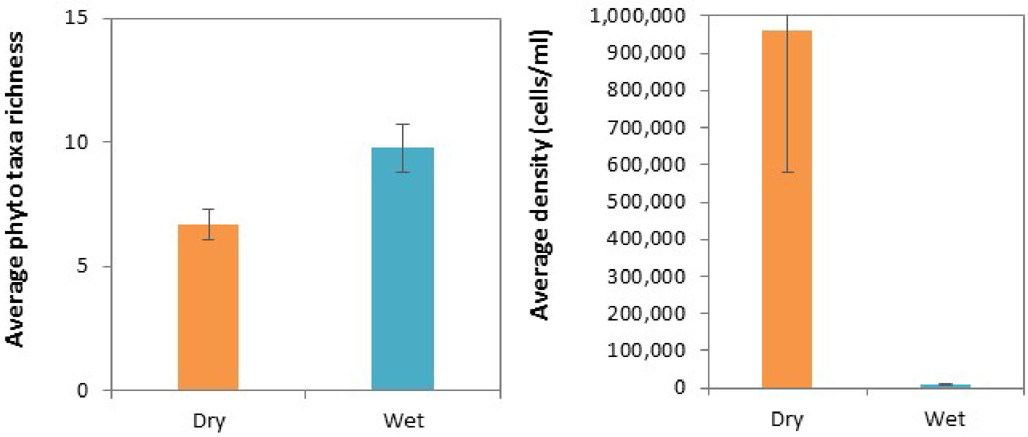


Figure 24. Average seasonal phytoplankton taxa richness (left) and density (right; ±se) recorded from all WRM Pilbara surveys listed in Table 6, including the current study.

Using all wet season phytoplankton data listed in Table 6 in the analysis, there was no significant difference in taxa richness (One-way ANOVA; df = 2, F = 2.44, p = 0.124), or density (log density; df = 2, F = 2.80, p = 0.095) between project/year (i.e. Brockman 4 wet-14, Brockman 4 wet-15 or Warramboo wet-18). Despite the lack of significant difference, the phytoplankton assemblage from Warramboo appeared to be generally high in richness and density in comparison to other WRM wet season phytoplankton surveys (Figure 25). The high variation within years likely contributed to the lack of significant difference overall (Figure 25). Average taxa richness from Warramboo in the wet-18 was more than 1.5 times that recorded from Brockman 4 in the wet-14, and average density was almost five times greater (Figure 25).

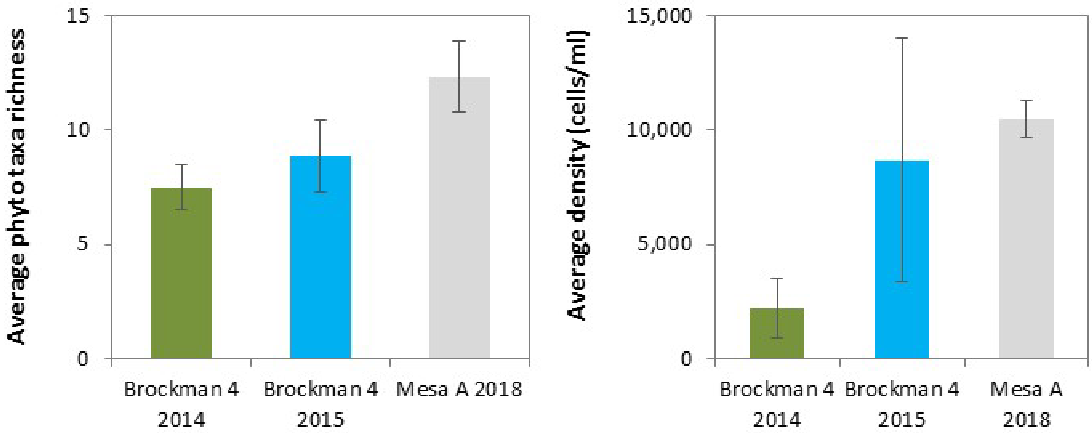


Figure 25. Average phytoplankton taxa richness (left) and density (right; ±se) recorded during the wet season from Brockman 4 in 2014, Brockman 4 in 2015, and Warramboo in 2018.

Patterns in phytoplankton assemblage structure

The seasonal difference was also evident in the overall phytoplankton assemblage structures of all samples collected between 2012 and 2018 (see Table 6 for a list of data included in the analysis; One-way PERMANOVA; df = 1, 34; pseudo F = 5.17, p = 0.0000; Figure 26). Therefore, further analyses were conducted on wet season samples only, in order to compare with the recent Warramboo wet-18 samples (see Figure 27).

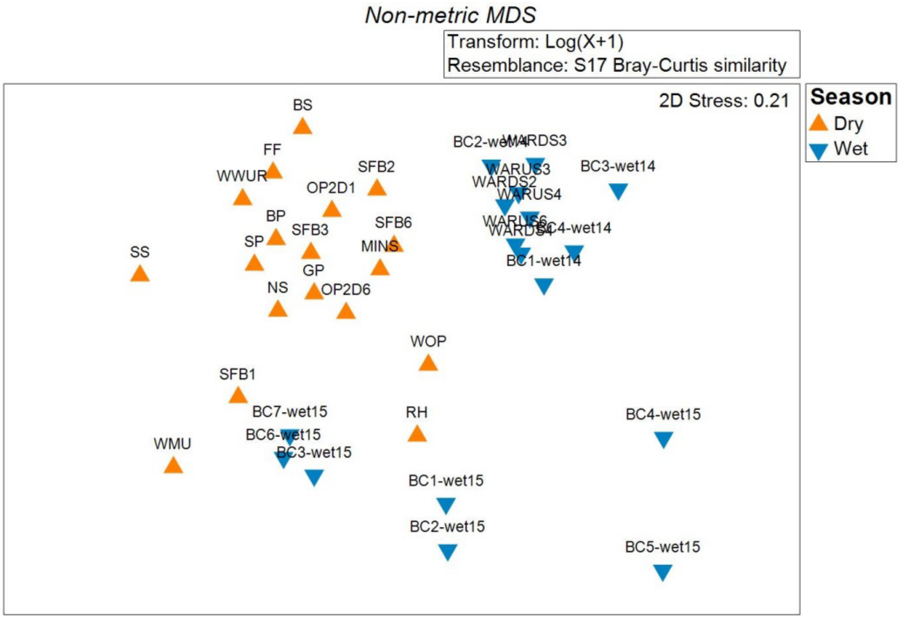


Figure 26. nMDS plot of phytoplankton assemblage data collected all WRM projects listed in Table 6 between 2012 and 2018. Samples are identified by season and labelled by site.

There was very little variation amongst phytoplankton assemblages from Warramboo sites, with all samples collected in the wet-18 grouping together in a tight cluster in ordination space (Figure 27). In contrast, variability amongst wet-15 Brockman 4 samples was high (Figure 27). Warramboo assemblages were most similar to the 2014 assemblages from Brockman 4 (Figure 27), although even these groups only had 37.35 % average similarity between them (SIMPER results). Overall, there was a significant difference in phytoplankton assemblages between project (PERMANOVA; df = 2, 16, pseudo-F = 5.92, p = 0.000). Pairwise post-hoc analysis indicated all projects/surveys were significantly different from each other ($p \leq 0.005$).

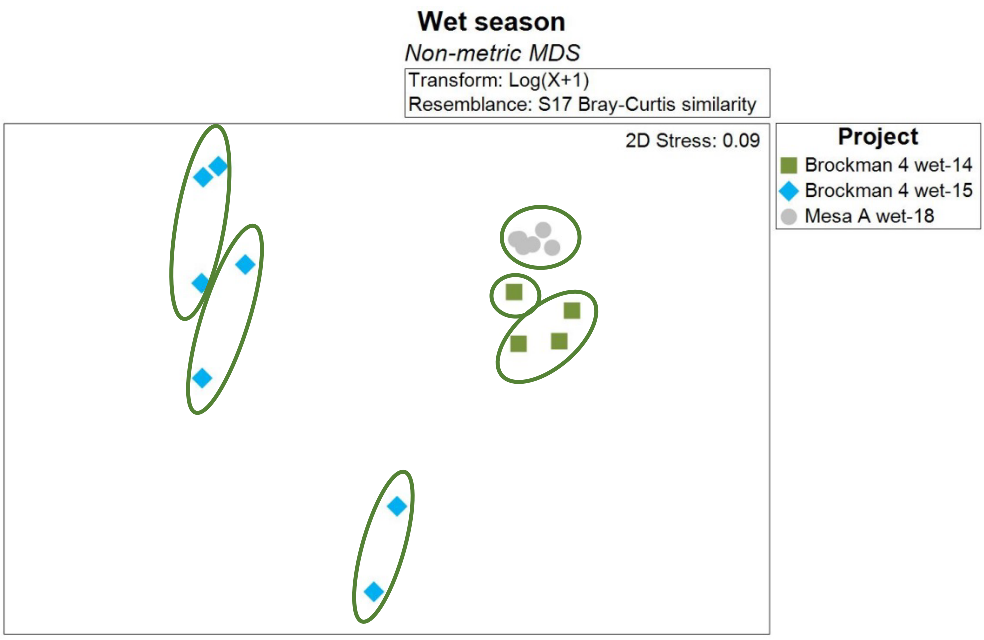


Figure 27. nMDS plot of wet season phytoplankton assemblage data collected from Brockman 4 in 2014 and 2015, and Warramboo in 2018. Samples are identified by project and grouped within green circles based on significant clusters as determined using SIMPROF.

4.4 Microinvertebrates

4.4.1 Taxonomic composition and species richness

A total of 59 microinvertebrate taxa were recorded from Warramboo Creek in the wet-18, with 51 being recorded from the upstream reference reach, and 43 from the downstream potential exposed reach (Table 7, Appendix 5). In this context, "taxa" includes groups which could not be identified to species level, due to unresolved taxonomy and/or immaturity of specimens. Therefore, the total microinvertebrate taxa richness at each site is likely greater than reported here. The microinvertebrate fauna comprised Protista, Rotifera, Cladocera (water fleas), Copepoda, and Ostracoda (seed shrimp; Table 7). The microinvertebrate fauna were typical of tropical systems reported elsewhere (e.g. Koste and Shiel 1983, Tait *et al.* 1984, Smirnov and De Meester 1996, Segers *et al*. 2004). For example, Brachionidae (five species recorded) within the Rotifera were overshadowed by Lecanidae (ten species recorded), as is generally the case in tropical systems.

Table 7. Summary of higher-order microinvertebrate taxa composition in Warramboo Creek, upstream (WARUS) and downstream (WARDS) of the proposed Warramboo discharge location. Refer Appendix 5 for full species list.

Microinvertebrates		Number of taxa	
Scientific name	Common name	WARUS (*n* = 6)	WARDS (*n* = 6)
Protista		7	6
Rotifera		29	22
Cladocera	Water fleas	4	5
Copepoda		8	7
Ostracoda	Seed shrimp	3	3
	Total taxa richness	**51**	**43**

There were 18 singleton taxa recorded (*i.e.* those recorded from only one site), and 12 taxa were considered common, *i.e.* they were present at over 80% of sites. These included the protist *Netzelia corona*, the rotifers *Keratella procurva*, *Conochilus* sp. [sm], *Polyarthra* sp. and *Trichocerca similis*, Cladocera *Diaphanosoma excisum*, copepods *Eodiaptomus lumholtzi*, and cyclopoid and calanoid copepodites, and cyclopoid and calanoid nauplii, and the ostracod *Cypretta* sp.

Microinvertebrate taxa richness ranged from 17 (at WARUS4) to 36 (at WARUS2; Figure 28). There was no significant difference in average microinvertebrate taxa richness between site types (One-way ANOVA; df = 1, F = 0.01, *p* = 0.921; Figure 29).

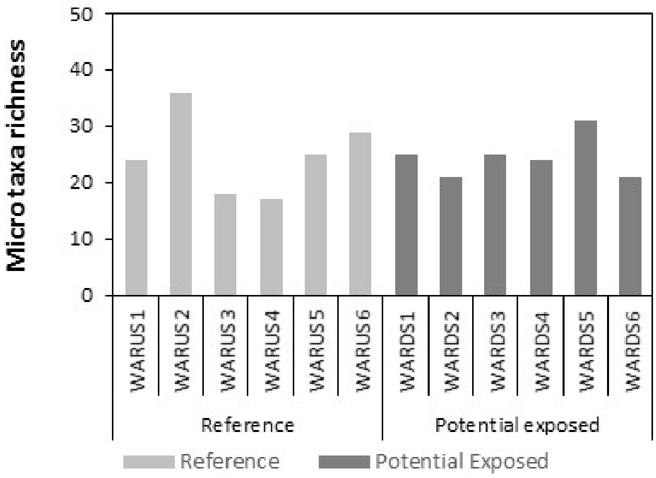


Figure 28. Microinvertebrate taxa richness recorded from Warramboo Creek sampling sites in the wet-18.

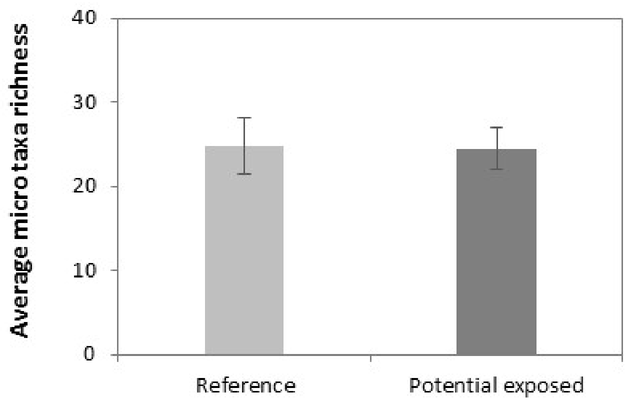


Figure 29. Average microinvertebrate taxa richness recorded from reference and potential exposed sites in the wet-18.

4.4.2 Conservation significance of microinvertebrates

The majority of microinvertebrate taxa recorded during the current study were common ubiquitous species, with distributions extending throughout Australasia or the world (cosmopolitan species). However, of interest within the Warramboo microinvertebrate fauna was the collection of a species listed on the IUCN Red List of Threatened Species, and an undescribed species.

The calanoid copepod *Eodiaptomus lumholtzi*, currently listed on the IUCN Red List of Threatened Species as Vulnerable (IUCN 2018) was recorded from all Warramboo Creek sites in the wet-18. This species was assessed as Vulnerable due to being known only from a small number of localities including Lake Woods in Northern Territory, Collinson's Lagoon at Ayr and Saltern Lagoon in the Valley of Lagoons, west of Ingham, Queensland (Reid 1996). However, IUCN (2018) indicates that this assessment requires updating because *E. lumholtzi* has since been recorded from a number of other localities across the Australasian region. *E. lumholtzi* has been recorded previously from sites along Fortescue River, Coondiner Creek, Kalgan Creek, Weeli Wolli Creek, Koodaideri Springs, Caves Creek, Duck Creek and Cane River (WRM unpub. data). *E. lumholtzi* has also been recorded from Papua New Guinea, and is considered to have a pan-tropical distribution (Vlaardingerbroek 1989, WRM unpub. data).

A potentially new species of cyclopoid copepod was also recorded during the current study. The specimen was conservatively named *Thermocyclops* cf. *emini* for the purposes of this study as it was morphologically most similar to *Thermocyclops emini*. However, this is a Madagascan species not currently known from the Australian continent. There are a number of previous records of *Thermocyclops* cf. *emini* from the Pilbara; one from Myanmore Creek Pool, on Red Hill Creek (Robe River system) which was recorded during the PBS (Pinder *et al*. 2010), one record by Bennelongia from the Fortescue River near Opthalmia Dam, Newman (Jane McRae, Bennelongia, pers. comm.); collections from the HD4 area by the authors (sites KCDS4 in the wet-15 and BP in the wet-16), and a number of records along the middle to upper Fortescue Valley (Pinder *et al.* 2017). During the current study, *T*. cf. *emini* was recorded in low numbers from all sites except WARUS3, WARUS4, and WARUS5.

4.4.3 Spatial and temporal differences in microinvertebrate fauna

Taxa richness

Although it was not possible to statistically compare between years due to the lack of replication in the wet-16, considerably greater taxa richness was recorded from Warramboo Creek in the wet-18 compared with the wet-16; range of 17-36, compared to a maximum of 15 in the wet-16 (from WARUD6; Figure 30).

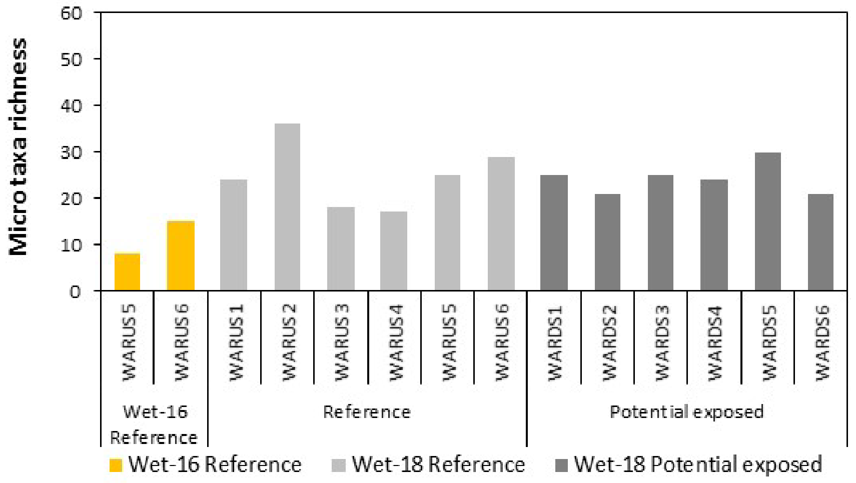
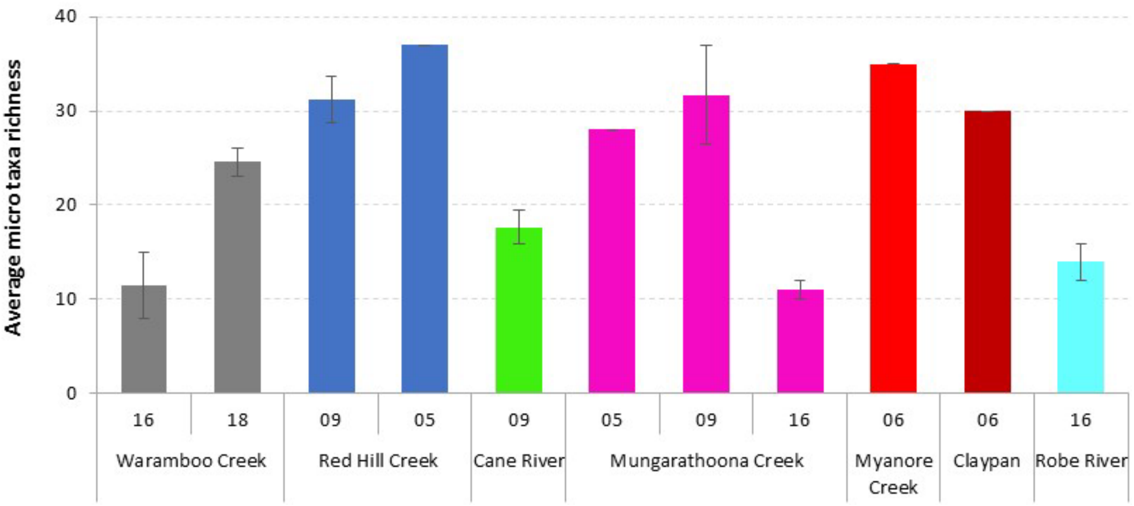


Figure 30. Microinvertebrate taxa richness (using the amalgamated taxonomy combined dataset) recorded from each site in the wet-16 and wet-18.

In comparing microinvertebrate taxa richness from nearby creeks sampled by WRM and DBCA between 2005 and 2018 (wet season only), there was a large variation in richness between creeks and between sampling years (Figure 31). However, microinvertebrate richness recorded from Warramboo Creek appeared to be comparable to most other creeks sampled, including Mungarathoona Creek, Myanore Creek and the Yarraloola Station claypan (Figure 31). Highest average richness was recorded from Red Hill Creek in the wet-05 (Figure 31). One-way ANOVA found that whilst there was a significant difference in microinvertebrate richness between creeks (df = 4[9], F = 6.85, p = 0.000), richness from Warramboo Creek was statistically similar to Mungarathoona Creek and the Cane River.



[9] The Myanore Creek and Yarraloola claypan samples were removed from this analysis as there was only one wet season replicate for each of these creeks.

Figure 31. Average microinvertebrate taxa richness (amalgamated taxonomy) (±se) recorded in the wet season from Warramboo Creek, and other nearby creeklines between 2005 and 2018. X-axis labels indicate year of sampling and system sampled.

Patterns in microinvertebrate assemblage structure

Patterns were evident in the nMDS ordination of all microinvertebrate data collected from Warramboo Creek in the wet-16 and wet-18 (Figure 32). Most samples from the wet-18 clustered together in ordination space, all falling within one significant SIMPROF cluster group, with the exception of WARUS4 and WARUS1 (Figure 32). The two reference samples from the wet-16, sat apart from all other samples, as well as each other (Figure 32). SIMPROF detected five significant cluster groups, including:

1. WARUS5 from the wet-16
2. WARUS6 from the wet-16
3. WARUS1 from the wet-18
4. WARUS4 from the wet-16
5. All other reference, and all potential exposed samples from the wet-18 (Figure 32).

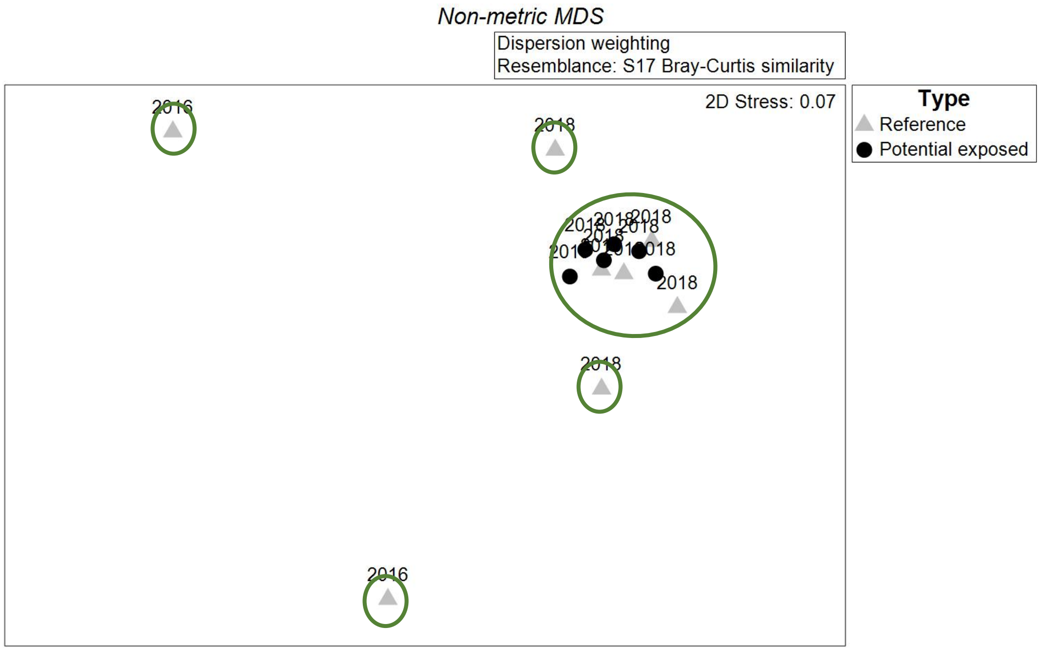


Figure 32. nMDS plots of microinvertebrate assemblage data (log_{10} abundance, dispersion weighted by type) collected from Warramboo Creek reference and potential exposed sites in the wet-16 and wet-18. Samples are identified by site type (reference v potential exposed) and labelled by year. Samples are grouped within green circles based on significant clusters as determined using SIMPROF.

The two wet-16 samples were removed from further analysis as there was insufficient replication with which to undertake temporal analysis, and no samples from potential exposed sites at that time. The ordination was repeated on 2018 data only (Figure 33).

Once the 2016 samples were removed from the analysis, the large degree of variation amongst wet-18 microinvertebrate samples became evident (Figure 33). There were no separations by site type apparent in the ordination, with all samples falling within a large SIMPROF cluster group, except the reference site WARUS1. Overall, there was no significant difference in microinvertebrate assemblages between reference and potential exposed sites in the wet-18 (One-way PERMANOVA; df = 1, 11; pseudo-F = 1.08, p = 0.3826).

There was a high correlation between water quality variables and microinvertebrate assemblages (BVSTEP; Rho = 0.75, p = 0.024). Two water quality variables were found to have the greatest influence on the microinvertebrate assemblages; N_NH3 and dZn.

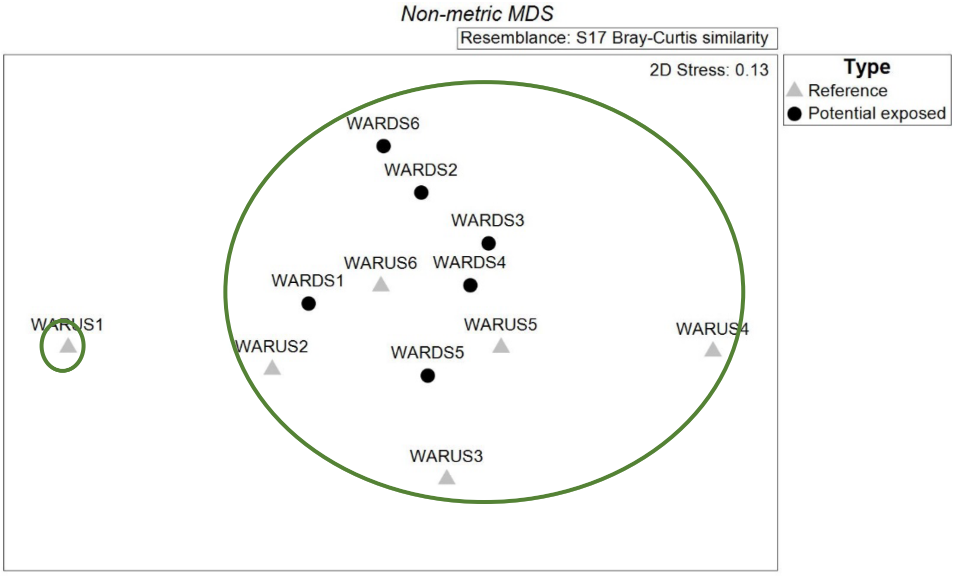


Figure 33. nMDS ordination of microinvertebrate assemblage data (log_{10} abundance) collected from Warramboo Creek reference and potential exposed sites in the wet-18. Samples are identified by type (reference v potential exposed) and labelled by site.

When comparing overall microinvertebrate assemblages of Warramboo Creek with those from other nearby creeklines, Warramboo Creek samples grouped closely together in ordination space, with the exception of the WARUS6 wet-16 sample (Figure 34). Warramboo microinvertebrate samples sat closest to samples from the Cane River, Myanore Creek, and Yarraloola Station Claypan, suggesting assemblages were more similar than those from the Robe River, which sat further apart from Warramboo in ordination space (Figure 34). Overall, there was a significant difference in microinvertebrate assemblages between creek (One-way PERMANOVA; df = 6, 47; pseudo-F = 4.23, p = 0.000). However, the pairwise post-hoc test indicated that Warramboo microinvertebrate assemblages were statistically similar to Myanore Creek (t = 1.79, p = 0.066), and the Yarraloola Station claypan site (t = 1.84, p = 0.067).

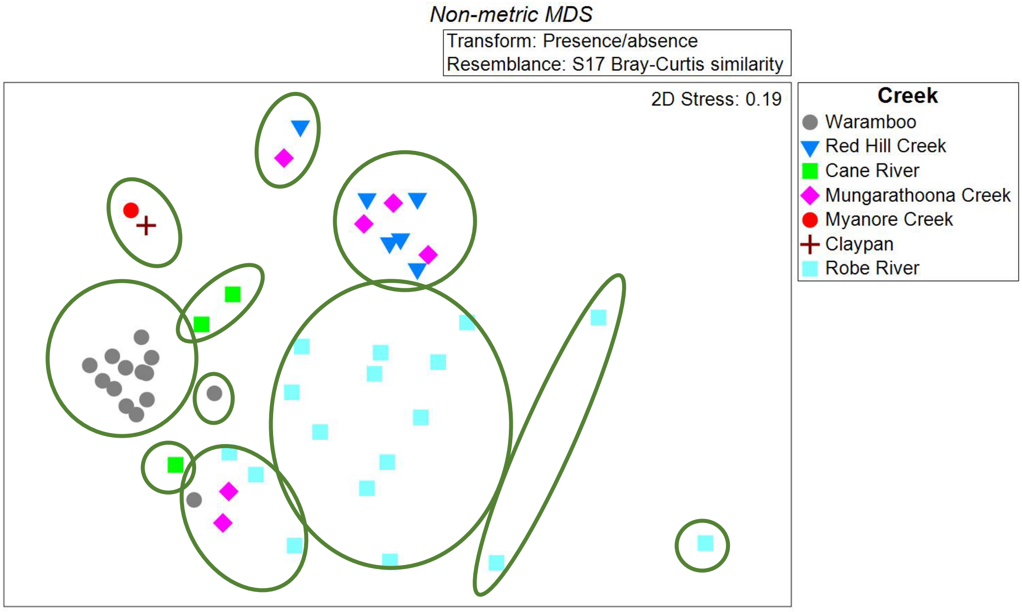


Figure 34. nMDS ordination of microinvertebrate assemblage data (presence/absence) collected from Warramboo Creek and other nearby creeklines in the wet season between 2005 and 2018. Samples are identified by creek, labelled by site, and grouped within green circles based on significant SIMPROF cluster groups.

4.5 Hyporheic fauna

4.5.1 Taxonomic composition and species richness

A total of 44 taxa were recorded from the hyporheic zones of sites sampled in the current study, with 34 being recorded from the upstream reference reach, and 27 from the downstream potential exposed reach (Appendix 6). The majority of these taxa were classified as stygoxene (57%), *i.e.* species that appear in groundwater habitats by accident or seeking refuge during drought, and not specially adapted to subterranean inhabitation. Of the remaining taxa, 7% were classified as stygobitic, *i.e.*, obligate groundwater inhabitants with specialised morphological adaptations to survive in such environments (stygofauna), 14% were considered occasional hyporheic stygophiles (species that use the hyporheic zone seasonally or during early life history stages), and 23% were possible hyporheic taxa. Although classifications followed those of Boulton (2001), this type of analysis should be treated with some caution, as results are likely affected by available information on life history, taxonomic resolution, and interpretation of classification categories. Stygobites were only recorded from three sites; WARUS2, WARUS3 and WARUS6. The greatest number of stygobitic species was recorded from WARUS2 (two taxa).

Hyporheos fauna taxa richness (combined richness of stygobites, occasional hyporheic stygophiles and possible hyporheic fauna) ranged from three taxa at WARDS1 and WARDS6, to ten taxa at WARUS4 (Figure 35). The high taxa richness encountered in the hyporheos of WARUS4 suggests at least some connectivity between ground- and surface waters.

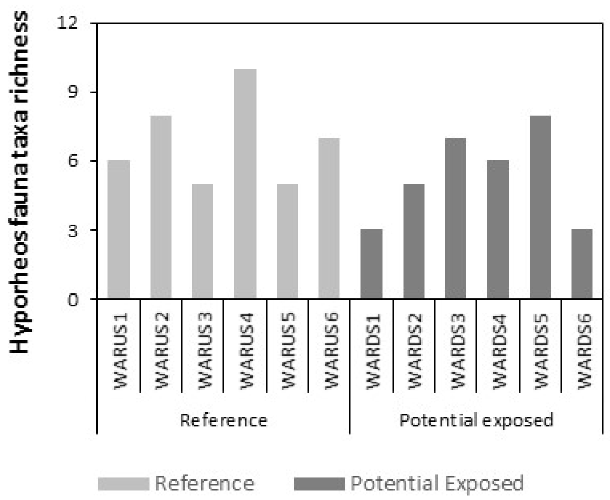


Figure 35. Hyporheos fauna taxa richness (stygobites, occasional hyporheos stygophiles and possible hyporheic taxa) recorded from Warramboo Creek in the wet-18.

There was no significant difference in average hyporheos fauna taxa richness between site types (One-way ANOVA; df = 1, F = 1.68, p = 0.224; Figure 36).

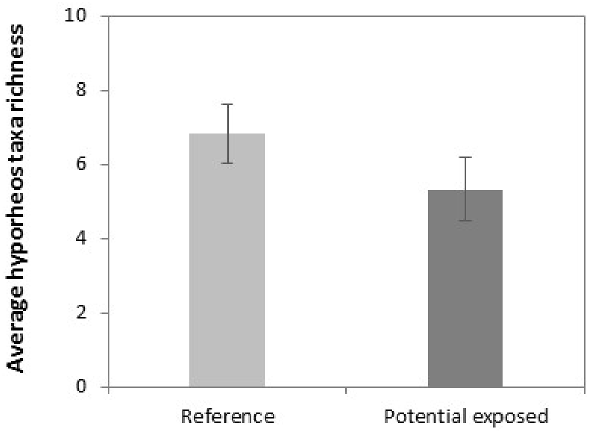


Figure 36. Average hyporheos fauna taxa richness recorded from reference and potential exposed sites in the wet-18.

4.5.2 Conservation significance of hyporheos fauna

Species classified as hyporheos fauna included;

Stygobites

- Ostracods *Candonopsis tenuis* and *Vestalenula marmonieri*
- Copepod *Parastenocaris jane*

Occasional hyporheos stygophiles

- Oligochaete *Pristina longiseta*
- Ostracod *Cypretta* sp.
- Copepod *Microcyclops varicans*
- Collembolla (spring-tails) Entomobryoidea spp. and Symphypleona spp.
- Beetles *Hydraena* sp.

Potential hyporheos fauna

- Turbellaria spp.
- Nematoda spp.
- Oligochaetes Phreodrilidae spp. and immature or damaged Oligochaeta spp. (imm/dam)
- Indeterminate chydorid Cladocera
- Indeterminate juvenile ostracods
- Ostracod cf. *Ilyodromus* sp. (dam.)
- Cyclopoid copepodites
- Acarina spp.
- Immature or damaged Baetidae sp. ephemeroptera

None of these aforementioned taxa have restricted distributions nor are any listed for conservation significance.

4.5.3 Spatial and temporal differences in hyporheos fauna

It was not possible to statistically compare between seasons or years, given Warramboo Creek is ephemeral and does not hold water in the dry season, and only two reference sites had surface water in the wet-16. However, generally similar hyporheos taxa richness was recorded in the wet season of 2016 as was recorded in 2018 (Figure 37).

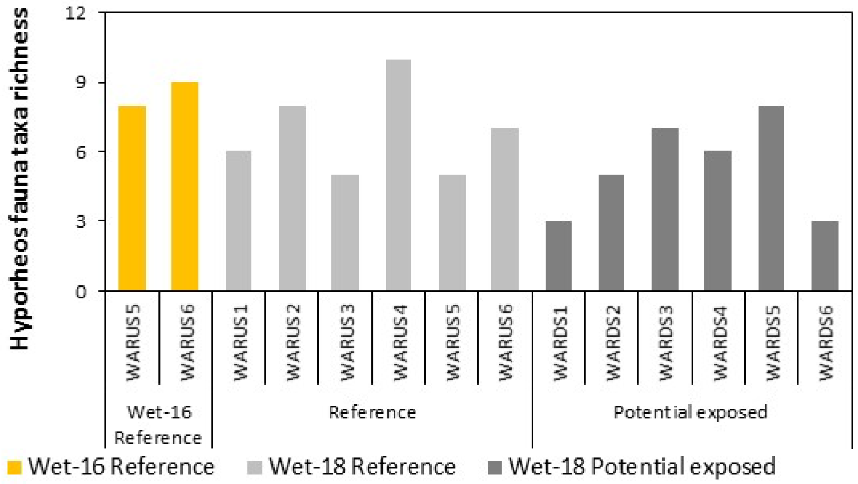


Figure 37. Macroinvertebrate taxa richness recorded from each site in the wet-16 and wet-18.

Patterns in hyporheos fauna assemblage structure

Hyporheos fauna samples were highly variable, with no apparent separation by site type or year in ordination space (Figure 38). All hyporheos fauna samples fell within one SIMPROF cluster group. The two wet-16 samples fell close to other reference site samples from the wet-18. Overall, there was no significant difference in hyporheos fauna assemblages between reference and potential exposed sites (One-way PERMANOVA; df = 1; pseudo-F = 2.33, *p* = 0.072).

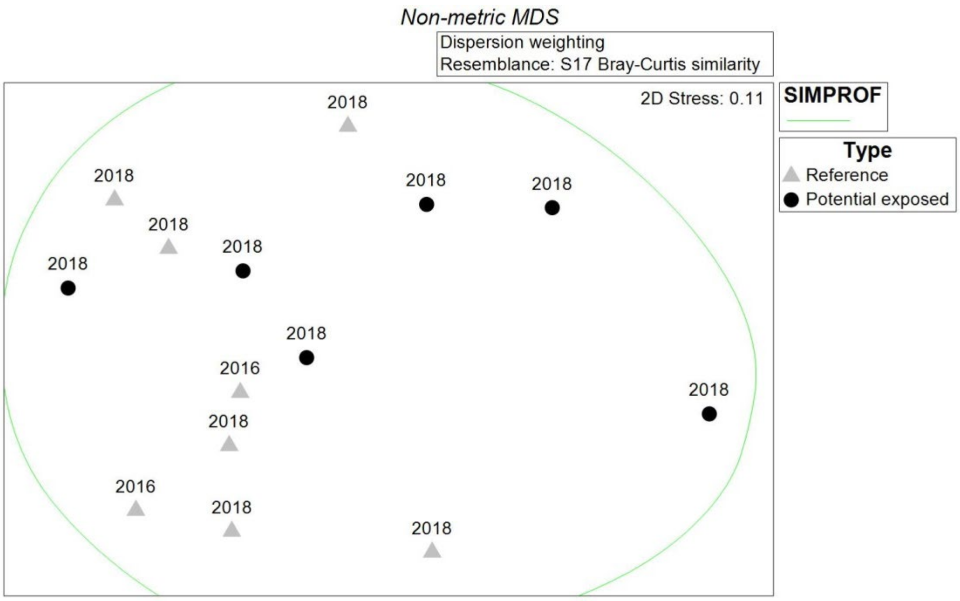


Figure 38. nMDS plots of hyporheos fauna assemblage data ($\log_{10}$ abundance, dispersion weighted) collected from Warramboo Creek reference and potential exposed sites in the wet-16 and wet-18. Samples are identified by site type (reference v potential exposed) and labelled by year. Samples are grouped within green circles based on significant clusters as determined using SIMPROF.

4.6 Macroinvertebrates

4.6.1 Taxonomic composition and species richness

A total of 94 macroinvertebrate taxa were recorded from Warramboo Creek in the wet-18, with 78 being recorded from the upstream reference reach, and 71 from the downstream potential exposed reach (Table 8, Appendix 7). In this context, "taxa" includes groups which could not be identified to species level, due to unresolved taxonomy and/or immaturity of specimens. Therefore, the total macroinvertebrate taxa richness at each site is likely greater than reported here.

The macroinvertebrate fauna comprised Gastropoda (freshwater snails), Oligochaeta (aquatic segmented worms), Acarina (water mites), Collembolla (springtails), Odonata (dragonflies and damselflies), Trichoptera (caddisflies), Ephemeroptera (mayflies), Hemiptera (true bugs), Coleoptera (aquatic beetles), and Diptera (two-winged or "true" fly larvae (Table 8). Insecta were the dominant group, with 92% of all taxa recorded belonging to this class (Table 8). Typically, insects constitute around 80% of all aquatic fauna in freshwater systems of the Pilbara (Pinder *et al*. 2010). Of the insects, the best represented taxa were Coleoptera (28 taxa, 32% of Insecta) and Diptera (28 taxa, 32%), followed by the Hemiptera (20 taxa, 23%). Diptera are typically the most diverse order of insects in freshwater systems (Hutchinson 1993), and this was also the case at Warramboo Creek. Coleopterans were also highly diverse, due largely to the high richness of dysticids (diving beetles). Mollusca (freshwater snails and bivalves) comprised only 2% of the total macroinvertebrate fauna collected. Only one species of macro-crustacea was recorded; the Anostraca (fairy shrimp) *Branchinella* cf. *proboscisda*. While the conchostracan *Caenestheriella packardi* was recorded previously from the creek (WARUS5 and WARUS6; WRM 2016), it was not collected in the wet-18.

Table 8. Summary of higher-order microinvertebrate taxa composition in Warramboo Creek, upstream (WARUS) and downstream (WARDS) of the proposed Warramboo discharge location. Refer Appendix 7 for full species list.

Macroinvertebrates Scientific name	Common name	Number of taxa WARUS (n = 6)	 WARDS (n = 6)
Gastropoda	Freshwater snails	2	1
Oligochaeta	Aquatic worms	1+	1+
Crustacea	Fairy shrimp	1	1
Acarina	Water mites	2+	1+
Collembolla	Spring tails	0	1
Odonata	Dragonflies & damselflies	3	5
Trichoptera	Caddiflies	1	1
Ephemeroptera	Mayflies	5	5
Hemiptera	True bugs	14	14
Coleoptera	Aquatic beetles	25	22
Diptera	Two-winged flies	24	19
	Total taxa richness	**78+**	**71+**

+ indicates a taxa could only be identified to genus/family/order level (not species level), and as such more than one species is likely to be present within this taxonomic group.

There were 26 singleton taxa recorded (*i.e.* those recorded from only one site), and 14 taxa were considered common, *i.e.* they were present at over 80% of sites. These included water mites Acarina sp., the mayflies *Tasmanocoenis* sp. M and immature or damaged Caenidae, immature or damaged Corixoidea spp. water boatmen, backswimmers *Anisops nasutus*, *Anisops* spp. and immature or damaged Notonectidae spp., the dytiscid *Laccophilus sharpi*, biting midge larvae Ceratopogoninae spp., non-biting midges *Polypedilum* spp., *Tanytarsus* spp., *Paramerina* sp. (WWT1), *Larsia albiceps* and *Procladius* sp. (WWT5).

Macroinvertebrate taxa richness varied greatly between sites (Figure 39). Lowest macroinvertebrate richness was recorded from WARUS4 (21 taxa) and greatest from WARDS2 (41 taxa; Figure 39). The low richness recorded from WARUS4 may be due to the fact that this site contained a small, highly receded volume of water at the time of sampling, with low habitat diversity and high nutrient concentrations.

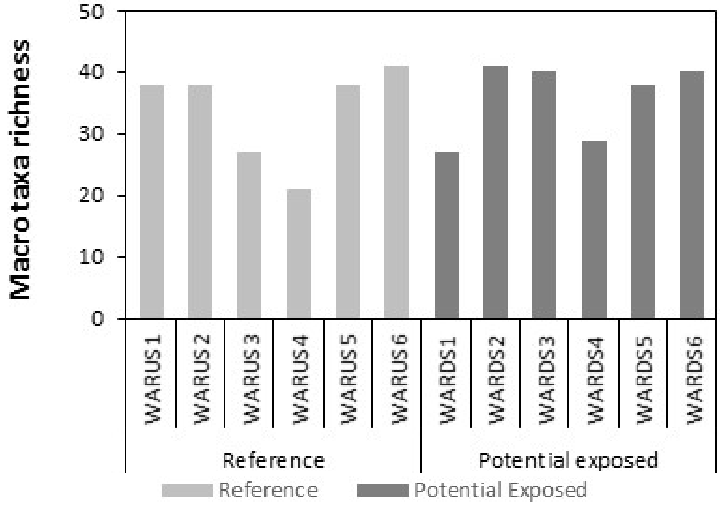


Figure 39. Macroinvertebrate taxa richness recorded from Warramboo Creek sampling sites in the wet-18.

There was no significant difference in average macroinvertebrate taxa richness between site types (One-way ANOVA; df = 1, F = 0.35, p = 0.566; Figure 40).

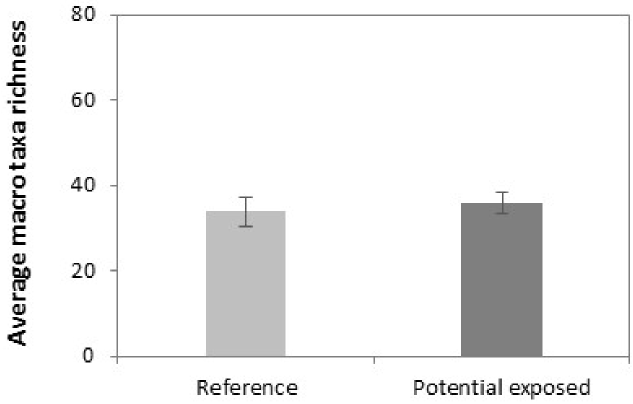


Figure 40. Average macroinvertebrate taxa richness recorded from reference and potential exposed sites in the wet-18.

4.6.2 Conservation significance of macroinvertebrates

The majority of macroinvertebrate taxa recorded were common, ubiquitous species, with distributions extending across Northern Australia, Australasia, and the world (cosmopolitan species). No species listed for conservation significance were recorded. Of interest, however, was the collection of an unknown anostracan, and a Pilbara endemic chironomid with a disjunct distribution.

A species of Anostraca (fairy shrimp) was recorded from three reference sites along Warramboo Creek in the wet-18 (WARUS3, WARUS4 and WARUS6); *Branchinella* cf. *proboscida* (see Appendix 7).

These specimens were collected in the microinvertebrate sweep sample, but are reported here as they are macro-crustacea, and hence macroinvertebrates. Specimens have been sent to the Australian Anostracan expert Brian Timms for confirmation, but from photographs, he suggested they may belong to the species *Branchinella proboscida*, or alternatively may be an undescribed species, new to science. As such, the species is referred to as *Branchinella* cf. *proboscida* here, until confirmation of its identification is obtained. *Branchinella proboscida* has a wide distribution across the Australian continent, being known from all states and territories except Victoria and Tasmania (Timms 2012). It was recorded from one site only during the PBS, Minderoo Claypan, located approximately 90 km south-west of Warramboo Creek, and has not been previously recorded by the authors from the Pilbara. *B. proboscida* is known to prefer turbid claypans (Timms 2008).

Plate 3. Head of a male *Branchinella* cf. *proboscida* fairy shrimp recorded from WARUS6 in the wet-18 (photo by Russ Shiel/University of Adelaide).

The hyporheic chironomid ?*Pentanuera* sp. was recorded from surface waters of WARUS4 in the wet-18. This chironomid is considered likely to be a hyporheic species due to its small size (they are at least half the size of other Tanyponids) and characteristic reduced eye (Dr Don Edward, The University of Western Australia, pers. comm.). ?*Pentanuera* sp. is currently undescribed and appears to have a highly disjunct distribution in the Pilbara, being known from Weeli Wolli Creek, Marillana Creek, upper Fortescue River, Fortescue River South, Caves Creek (Palm Spring), as well as within the Robe River and DeGrey River catchments (WRM unpub. data). It was not recorded from its more typical habitat in hyporheic samples during the current study, but rather was found in surface waters.

Additional macroinvertebrate taxa restricted to the Pilbara recorded previously in the wet-16 included the haliplid *Haliplus halsei* and the hydrophilid *Laccobius billi* (WRM 2016).

4.6.3 Spatial and temporal differences in macroinvertebrate fauna

__Taxa richness__

It was not possible to statistically compare between seasons or years, given Warramboo Creek is ephemeral and does not hold water in the dry season, and only two reference sites had surface water in the wet-16. However, generally higher macroinvertebrate taxa richness was recorded in the wet season of 2016, with 53 taxa recorded from WARUS5 and 57 from WARUS6 (Figure 41).

Macroinvertebrate assemblages of Warramboo Creek were compared to other nearby systems sampled by WRM and DBCA (during the Pilbara Biological Survey) in the wet season between the 2005 and 2018; namely Red Hill Creek, Cane River, Mungarathoona Creek, Myanore Creek, Robe

River, Jimmawarrada Creek and a claypan[10] (Yarraloola Station Pool). Warramboo Creek had comparable macroinvertebrate richness to these other nearby creeklines (Figure 42), with no significant difference in richness recorded between creeks (One-way ANOVA; df = 4[11], F = 1.00, p = 0.418).

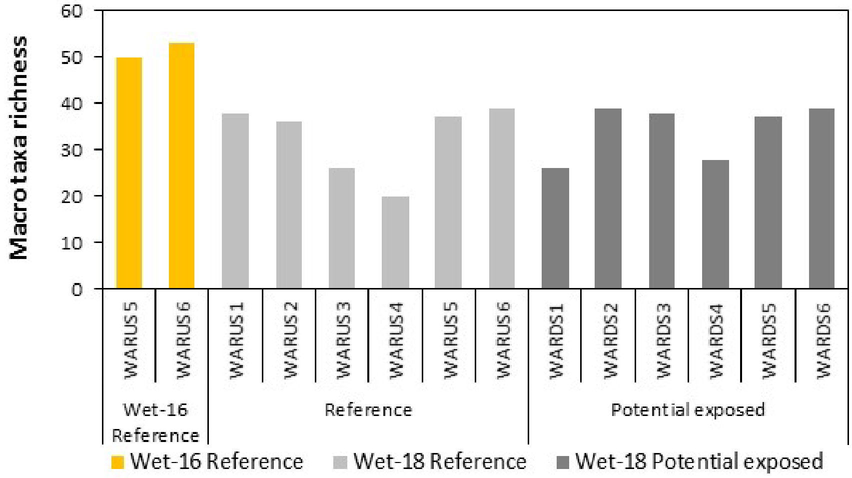


Figure 41. Macroinvertebrate taxa richness (using the amalgamated taxonomy combined dataset) recorded from each site in the wet-16 and wet-18.

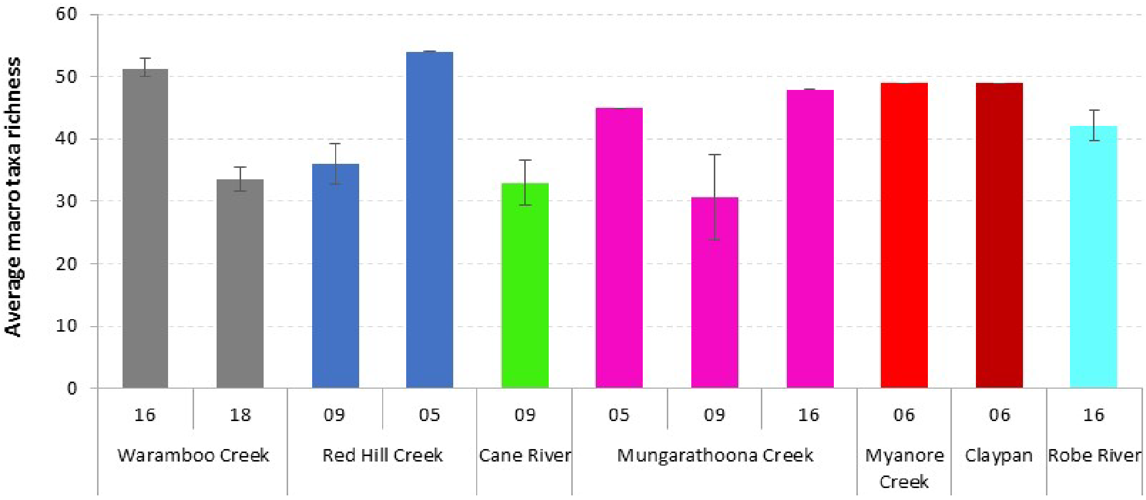


Figure 42. Average macroinvertebrate taxa richness (amalgamated taxonomy) (±se) recorded in the wet season from Warramboo Creek, and other nearby creeklines between 2005 and 2018.

Patterns in macroinvertebrate assemblage structure

Patterns were evident in the nMDS ordination of all macroinvertebrate data collected from Warramboo Creek in the wet-16 and wet-18 (Figure 43). The two reference samples from the wet-16, WARUS5 and WARUS6, separated from all others in ordination space (Figure 43). There was little variation amongst the potential exposed wet-18 sites, with all samples clustering close together in ordination space (Figure 43). The macroinvertebrate assemblages of the reference sites were

[10] NB: taxonomy was first standardised across projects and years, so taxa richness presented here may not necessarily be the same as reported above, or in other project reports.
[11] The Myanore Creek and Yarraloola claypan samples were removed from this analysis as there was only one wet season replicate for each of these creeks.

more variable, with the wet-18 samples splitting between three significant SIMPROF cluster groups (Figure 43). SIMPROF detected four significant cluster groups, including:

1. The two wet-16 reference sites
2. WARUS2 from the wet-18
3. All WARDS potential exposed sites and WARUS 1, WARUS5 and WARUS6 from the wet-18
4. WARUS3 and WARUS4 from the wet-18 (Figure 43).

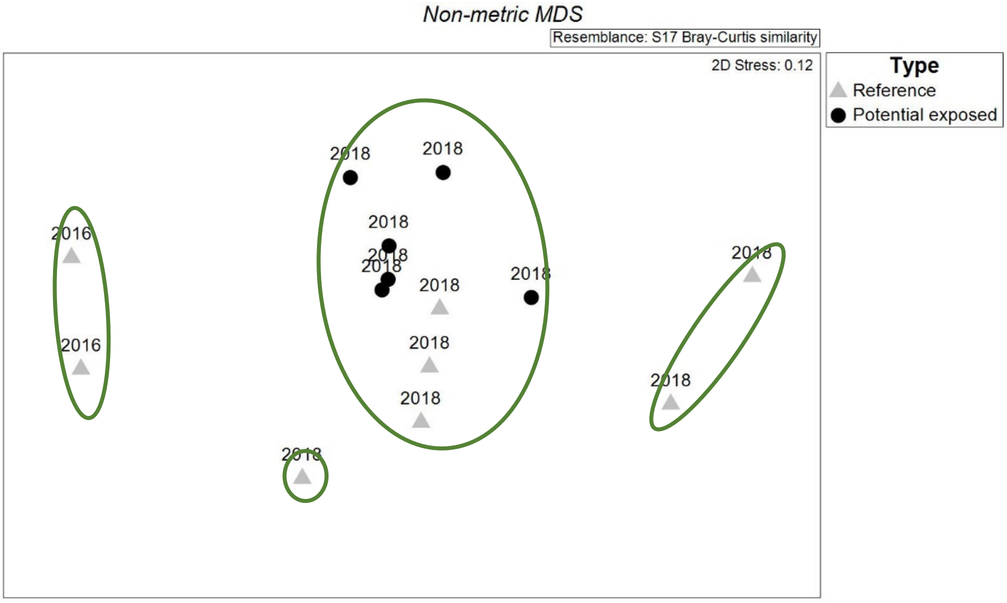


Figure 43. nMDS plots of macroinvertebrate assemblage data (log_{10} abundance) collected from Warramboo Creek reference and potential exposed sites in the wet-16 and wet-18. Samples are identified by site type (reference v potential exposed) and labelled by year. Samples are grouped within green circles based on significant clusters as determined using SIMPROF.

The two wet-16 samples were removed from further analysis as there was insufficient replication with which to undertake temporal analysis, and no samples from potential exposed sites at that time. The ordination was repeated on 2018 data only (Figure 44).

As with the water quality data, there was a large degree of variation amongst macroinvertebrate reference samples in ordination space, but little amongst potential exposed samples (Figure 44). As mentioned above, the WARUS5 and WARUS6 reference sites were more similar to the potential exposed sites, than the other reference sites (Figure 44). Generally, macroinvertebrate assemblages were similar across the length of the creek, with most sites falling within the same significant SIMPROF cluster group (Figure 44). Only WARUS2 (which sat apart from all other samples in ordination space), and WARUS3 and WARUS4 were within different SIMPROF cluster groups (Figure 44). Despite this, overall, there was a significant difference in macroinvertebrate assemblages between site type (reference vs potential exposed) in the wet-18 (One-way PERMANOVA; df = 1, 11; pseudo-F = 1.88, p = 0.019).

There was a high correlation between water quality variables and macroinvertebrate assemblages (BVSTEP; Rho = 0.73, p = 0.001), with concentrations of N_NO_3, total N, Na and dZn found to have the greatest influence on assemblages.

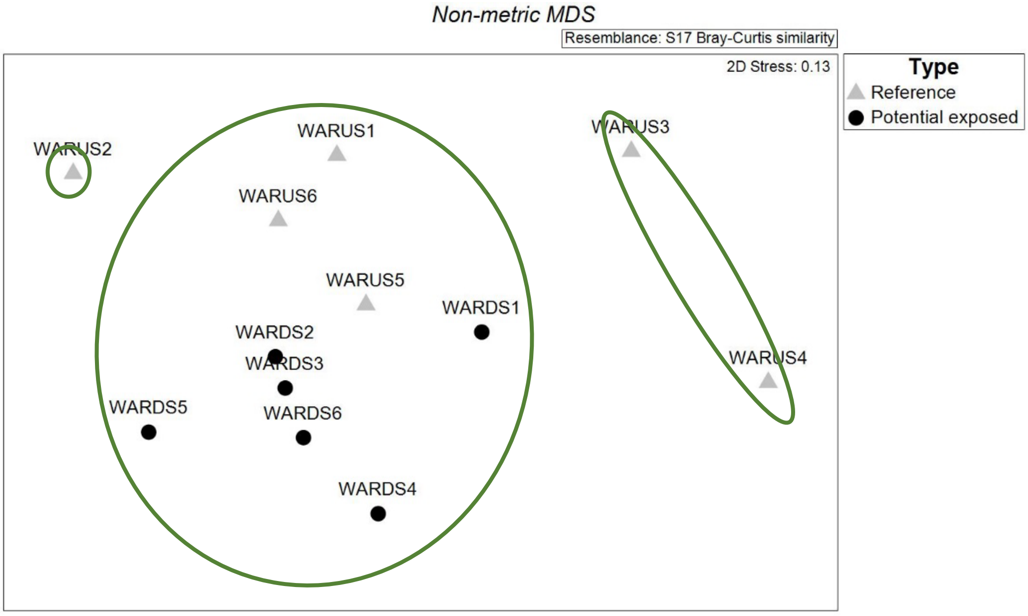


Figure 44. nMDS ordination of macroinvertebrate assemblage data (log_{10} abundance) collected from Warramboo Creek reference and potential exposed sites in the wet-18. Samples are identified by type (reference v potential exposed) and labelled by site.

Macroinvertebrate assemblages of Warramboo Creek were also compared to other nearby systems sampled by WRM and DBCA (during the Pilbara Biological Survey) in the wet season between the 2005 and 2018; namely Red Hill Creek, Cane River, Mungarathoona Creek, Myanore Creek, Robe River, Jimmawarrada Creek and a claypan (Yarraloola Station Pool; Figure 45). Macroinvertebrate assemblages tended to group by creekline, with the Warramboo samples sitting closest to other ephemeral creeklines, including Myanore Creek and Red Hill Creek (Figure 45). Whilst overall there was a significant difference in macroinvertebrate assemblages between creek (One-way PERMANOVA; df = 6, 45; pseudo-F = 5.71, p = 0.000), Warramboo Creek assemblages were statistically similar to Myanore Creek (t = 1.42, p = 0.067) and the Yarraloola Station claypan site (t = 1.65, p = 0.065).

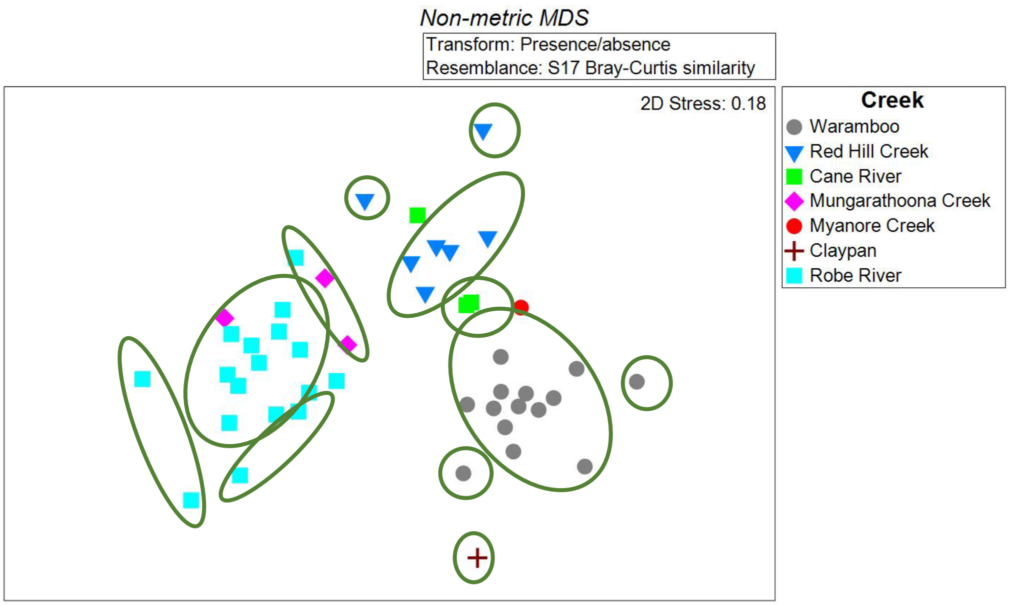


Figure 45. nMDS ordination of macroinvertebrate assemblage data (presence/absence) collected from Warramboo Creek and other nearby creeklines in the wet season between 2003 and 2018. Samples are identified by creek and labelled by site.

4.7 Fish

No fish were recorded from Warramboo Creek in the wet-18. Although recent rainfall had led to surface water being present, there was no connection with any other major system, with which fish could migrate into Warramboo Creek. No fish were recorded from the two reference sites successfully sampled in the wet-16 either.

The Fortescue Grunter, and other species of fish, are unlikely to currently reside within Warramboo Creek, as no permanent pools are known to exist in the Warramboo catchment. It is also highly unlikely fish could migrate to Warramboo Creek from permanent pools in the nearby Robe River catchment during flooding, as Warramboo Creek and the Robe River do not appear to share any drainage lines on the coastal plains. Given the highly ephemeral nature of Warramboo Creek, and the isolation from nearby systems which sustain permanent pools, it is highly unlikely fish are present in Warramboo, unless they are artificially introduced, as has occurred elsewhere in the Pilbara (WRM unpub. data).

4.8 Other vertebrate fauna

Frogs are difficult to survey in the Pilbara region, as captures are typically dependent on rainfall that is spatially and temporally variable (Doughty *et al*. 2011). Many frog species of the Pilbara aestivate over dry periods to avoid desiccation, emerging following rains to opportunistically breed and spawn (Tyler and Doughty 2009). Although not specifically surveyed in the current study, desert tree frogs (*Litoria rubella*; Plate 4) were observed at most Warramboo Creek sites over the course of the survey, with the exception of WARUS2 and WARDS5 (Table 9). Tadpoles were also present at WARDS3, which may have been this, or another species. *L. rubella* is a common species in the Pilbara and is known to occur in a wide range of habitats across wider northern Australia (Tyler and Knight 2011). They can commonly be found sheltering under stones or bark around creeks and waterholes, and are able to breed at any time of year if water is present.

Plate 4. A desert tree frog, *Litoria rubella*, at WARDS5 in the wet-18 (photo by WRM ©).

Table 9. Observations of the desert tree frog, *Litoria rubella*, from Warramboo sites in the wet-18.

Site type	Site	Present	Low abundance	High abundance	Tadpoles present
Reference	WARUS1		*		
	WARUS2				
	WARUS3		*		
	WARUS4			*	
	WARUS5			*	
	WARUS6			*	
Potential exposed	WARDS1			*	
	WARDS2			*	
	WARDS3			*	✓
	WARDS4	*			
	WARDS5				
	WARDS6	*			

Biota (2006a, b) recorded an additional two species during baseline surveys for Mesa A; the Hydalidae (tree frog) *Cyclorana maini*, and the Myobatrachidae (southern frog) *Uperoleia russelli*. None of these aforementioned species are of conservation significance (IUCN Redlist, or DBCA Threatened and Priority Fauna).

5 SUMMARY AND CONCLUSIONS

This report summarises the results of wet season baseline sampling of aquatic ecosystems of Warramboo Creek, upstream and downstream of the proposed discharge location, in April 2018. Comparisons are also made with the wet-16 dataset, when two reference sites held surface water for sampling. It should be noted that given this report is based on two sampling events, it is unlikely to fully capture the range in temporal / seasonal variability within the survey area. There is likely to be considerable variation in water quality and aquatic fauna present depending on the timing of surveys with respect to rainfall. Further baseline surveys are planned to capture as much of this variation as possible, and ensure an adequate dataset with which to detect future impacts, if any, from the Warramboo BWT development.

Surface water quality at Warramboo Creek indicated recent filling by rainwater and was low in alkalinity, hardness, electrical conductivity (EC), and concentrations of major ions. Waters were generally characterised by circum-neutral pH, adequate to high dissolved oxygen (DO), fresh waters, with low to moderate TSS, generally low nitrogen nutrients and dissolved metal concentrations, with low buffering capacity at some sites (i.e. low alkalinity). Exceedances of default ANZECC/ARMCANZ (2000) GVs included pH (at most sites), DO (at WARUS2 and WARDS6), N_NOx (at WARUS2, WARUS3 and WARUS4), total N (at WARUS2, WARUS3, WARUS4, WARUS6, and WARDS5), total P (at all upstream reference sites and potential exposed site WARDS5), dCu (all reference sites and WARDS5), and dFe (at WARUS6).

Interestingly, there were a number of significant differences in water quality between the upstream reference reach, and the downstream potential exposed reach. This appears to be the natural baseline condition, and should be taken into account when interpreting future monitoring results once dewatering-discharge commences. Water quality parameters which were significantly higher in the potential exposed reach in the wet-18 included EC, TDS, alkalinity and hardness, corresponding ionic concentrations of Ca, Mg, K and HCO_3, and dBa. The two most downstream sites on the reference reach, WARUS5 and WARUS6, recorded concentrations of the above analytes more similar to the potential exposed sites than the other reference sites. Total N and concentrations of dCu were significantly higher from the reference reach in the wet-18.

Despite these significant differences in water quality between reach, there were no corresponding significant differences in taxa richness of phytoplankton (or density), microinvertebrates, hyporheos fauna, or macroinvertebrates between reference and potential exposed sites. Likewise, there were no significant differences in overall assemblage structures of the aforementioned faunal components between site type (multivariate analysis results), with the exception of macroinvertebrate assemblages.

While temporal statistical analyses could not be undertaken due to the low replication in the wet-16, nMDS ordination indicated water quality, microinvertebrate, hyporheos fauna, and macroinvertebrate assemblages were all considerably different in the wet-16 to the wet-18.

Phytoplankton samples were collected from three reference sites and three potential exposed sites in the wet-18. From these six samples, a total of 28 phytoplankton taxa were recorded. Generally, Cyanophyta (blue-green algae) and Chlorophyta (green algae) dominated phytoplankton taxa at each site. Phytoplankton taxa richness ranged from eight at WARDS3 to 19 at WARUS4.

Two species of cyanobacteria known to be 'potential, but unconfirmed toxin producers' were recorded during the current study; *Cylindrospermum licheniforme* and *Dolichospermum affine*[12]. However, neither were recorded in sufficient densities deemed to be of concern or to warrant immediate action as detailed in the National Protocol for the Monitoring of Cyanobacteria and their Toxins in Surface Freshwater by Jones *et al.* (2002). The greatest density of *C. licheniforme* was recorded from WARDS4 (382 cells/ml) and *D. affine* from WARUS6 (10 cells/ml).

In comparing Warramboo Creek phytoplankton taxa richness and density with other sites sampled by WRM, the phytoplankton assemblage from Warramboo appeared to be generally higher in richness and density than Brockman 4 (~ 140 km to the east) samples from the wet-14 and wet-15. However, overall there was no significant difference in taxa richness or density between project/year. Multivariate nMDS ordination indicated that Warramboo phytoplankton assemblages were most similar to the 2014 assemblages from Brockman 4, although average similarity between these groups was only 37.35 %.

Microinvertebrate taxa richness ranged from 17 (at WARUS4) to 36 (at WARUS2). The majority of microinvertebrate taxa recorded during the current study were common ubiquitous species, with distributions extending throughout Australasia or the world (cosmopolitan species). However, of interest within the Warramboo microinvertebrate fauna was the collection of a species listed on the IUCN Red List of Threatened Species (calanoid copepod *Eodiaptomus lumholtzi* listed as Vulnerable), and an undescribed species the cyclopoid copepod *Thermocyclops* cf. *emini*). Both species were recorded from reference and potential exposed sites.

While it wasn't possible to compare statistically, microinvertebrate taxa richness appeared to be generally higher in 2018 compared with 2016. In comparing wet season microinvertebrate taxa richness from nearby creeks sampled by WRM and DBCA between 2005 and 2018, richness from Warramboo Creek was found to be statistically similar to Mungarathoona Creek and the Cane River.

A total of 44 taxa were recorded from the hyporheic zone with 34 being recorded from the upstream reference reach, and 27 from the downstream potential exposed reach. Stygobites were only recorded from three sites; WARUS2, WARUS3 and WARUS6. Hyporheos fauna taxa richness (combined richness of stygobites, occasional hyporheic stygophiles and possible hyporheic fauna) ranged from three taxa at WARDS1 and WARDS6, to ten taxa at WARUS4.

94 macroinvertebrate taxa were recorded from Warramboo Creek in the wet-18, with 78 taxa being recorded from reference sites and 71 from potential exposed sites. Lowest macroinvertebrate richness was recorded from WARUS4 (21 taxa) and greatest from WARDS2 (41 taxa). The low richness recorded from WARUS4 may be due to the fact that this site contained a small, highly volume of water at the time of sampling, with low habitat diversity and high nutrient concentrations. No species listed for conservation significance were recorded. Of interest, however, was the collection of an unknown anostracan (*Branchinella* cf. *proboscida*), and a Pilbara endemic chironomid (?*Pentanuera* sp.) with a disjunct distribution.

Generally higher macroinvertebrate taxa richness was recorded in the wet season of 2016, with 53 taxa recorded from WARUS5 and 57 from WARUS6. Warramboo Creek had comparable macroinvertebrate richness to these other nearby creeklines (i.e. Red Hill Creek, Cane River, Mungarathoona Creek, Myanore Creek, Robe River, Jimmawarrada Creek and Yarraloola Station Claypan), with no significant difference in richness recorded between creeks. Overall assemblages were also statistically similar to Myanore Creek and the Yarraloola Station claypan site.

[12] *Dolichospermum affine* has undergone a taxonomic name change, being previously known as *Anabaena affinis*.

As with the water quality data, there was a large degree of variation amongst wet-18 macroinvertebrate reference samples in ordination space, but little variation amongst potential exposed samples, and the WARUS5 and WARUS6 reference sites were more similar to the potential exposed sites, than the other reference sites.

No fish were recorded from Warramboo Creek in the wet-18, nor previously from the two sites sampled in the wet-16. Given the highly ephemeral nature of Warramboo Creek, and the isolation from nearby systems which support fish in permanent pools, it is highly unlikely fish are present in Warramboo, unless they are artificially introduced, as has occurred elsewhere in the Pilbara. And given the current absence of permanent water, they would not survive the following dry season.

Seven species of conservation significance/scientific value were recorded in the Proposal area, three of which were present at sites potentially exposed to dewatering discharge. These are summarised in Table 10 below:

Table 10.. Summary of aquatic species of conservation and/or scientific value (recorded from the Project area in baseline surveys to-date (wet-16 and wet-18 sampling).

Species	Type	Conservation / Scientific value	Site recorded	Occurrence within 50 km of survey area	Occurrence elsewhere
Microinvertebrates					
Lecane nitida	Rotifer	Not previously known from Western Australia	WARUS6	No records	Cosmopolitan distribution, previously recorded from Laos, India and Brazil.
Epiphanes spinosa	Rotifer	Not previously known from Western Australia	WARDS3	No records	Cosmopolitan distribution, previously recorded from Laos, India and Brazil.
Eodiaptomus lumholtzi	Copepod (micro-crustacean)	**IUCN, Vulnerable** (needs updating)	All sites	No records	Fortescue River, Coondiner Creek, Kalgan Creek, Weeli Wolli Creek, Koodaideri Springs, Caves Creek, Duck Creek, Cane River. Known also from Papua New Guinea.
Thermocyclops cf. *emini*	Copepod	Undescribed species	WARUS1, WARUS2, WARUS6, and all WARDS sites	Myanore Creek, Red Hill Creek	Fortescue River near Opthalmia Dam, Kalgan Creek, Un-named Creek (Bella Pool), and a number of records along the middle to upper Fortescue Valley
Macroinvertebrates					
Branchinella cf. *proboscida*	Fairy shrimp	May be an undescribed species, new to science[13]	WARUS3, WARUS4 and WARUS6	Not known	Not known.
Haliplus halsei	Aquatic haliplid beetle	Pilbara endemic; relatively new to science	WARUS5, WARUS6	House Pool (Cane River), Chalyarn Pool and Myannore Creek Pool	Glen Ross Ck, Coondiner Pool, the Fortescue Marsh, Moreton Pool, Paradise Pool, Munreemya Billabong, Wackilina Ck Pool, West Peawah Creek Pool, Harding River Pool, and an un-named creek in Millstream.
Laccobius billi	Aquatic hydrophilid beetle	Pilbara endemic; rarely collected	WARUS5	Mungarathoona Creek and Red Hill Creek	Cangan Pool on Yule River, Weeli Wolli Creek, Coondiner Creek, Mindy Mindy Creek and the Ashburton River.

[13] Waiting on confirmation by the Australian Anostraca expert Dr Brian Timms.

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APPENDICES

Appendix 1. Site photographs – wet season 2018

WARRAMBOO CREEK
Reference sites

WARUS1

WARUS2

WARUS3

WARUS4

WARUS5

WARUS6

Potential exposed sites

WARDS1

WARDS2

WARDS3

WARDS4

WARDS5

WARDS6

Appendix 2. ANZECC/ARMCANZ (2000) trigger values for the protection of aquatic systems in tropical northern Australia

Table A2-1. 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).

	TP ($\mu g\ L^{-1}$)	*FRP ($\mu g\ L^{-1}$)*	*TN ($\mu g\ L^{-1}$)*	*NOx ($\mu g\ L^{-1}$)*	*NH_4^+ ($\mu g\ L^{-1}$)*	*DO % saturation[f]*	*pH*
Aquatic Ecosystem							
Upland River[e]	10	5	150	30	6	90-120	6.0-7.5
Lowland River[e]	10	4	200-300[h]	10[b]	10	85-120	6.0-8.0
Lakes & Reservoirs	10	5	350[c]	10[b]	10	90-120	6.0-8.0
Wetlands[3]	10-50[g]	5-25[g]	350-1200[g]	10	10	90[b]-120[b]	6.0-8.0

b = Northern Territory values are 5µgL-1 for NOx, 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.

Table A2-2. Default trigger values for salinity and turbidity for the protection of aquatic ecosystems, applicable to tropical systems in Australia (ANZECC/ARMCANZ 2000).

Aquatic Ecosystem		*Comments*
Salinity	***(µs/cm)***	
Aquatic Ecosystem		
Upland & lowland rivers	20-250	Conductivity in upland streams will vary depending on catchment geology. The fist flush may result in temporarily high values
Lakes, reservoirs & wetlands	90-900	Higher conductivities will occur during summer when water levels are reduced due to evaporation
Turbidity	***(NTU)***	
Aquatic Ecosystem		
Upland & lowland rivers	2-15	Can depend on degree of catchment modification and seasonal rainfall runoff
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 level in the wetland.

Table A2-3. Trigger values for toxicants at alternative levels of protection (µg/L).

Compound	Trigger values for freshwater Level of protection (% species) 99%	95%	90%	80%
METALS & METALLOIDS				
Aluminium pH > 6.5	27	55	80	150
Aluminium pH < 6.5	ID	ID	ID	ID
Arsenic (As III)	1	24	94	360
Arsenic (As IV)	0.9	13	42	140
Boron	90	370	680	1300
Cadmium	0.06	0.2	0.4	0.8
Cobalt	ID	ID	ID	ID
Chromium (Cr III)	ID	ID	ID	ID
Chromium (Cr VI)	0.01	1	6	40
Copper	1	1.4	1.8	2.5
Iron	ID	ID	ID	ID
Manganese	1200	1900	2500	3600
Molybdenum	ID	ID	ID	ID
Nickel	8	11	13	17
Lead	1	3.4	5.6	9.4
Selenium (Se total)	5	11	18	34
Selenium (Se IV)	ID	ID	ID	ID
Uranium	ID	ID	ID	ID
Vanadium	ID	ID	ID	ID
Zinc	2.4	8	15	31
NON-METALLIC INORGANICS				
Ammonia	320	900	1430	2300
Chlorine	0.4	3	6	13
Nitrate	17	700	3400	17000

Appendix 3. Water quality data recorded in the wet-18.

ANZECC/ARMCANZ (2000) default GVs for protection of 95% of freshwater species are also provided for comparison; ☐ ≥ default GV, ☐ ≥ 2x default GV, ☐ ≥ 10x default GV; note, for DO, values highlighted may be either greater than or less than the lower default GV.

					Reference						Potential exposed					
Parameter	Method Code	LOR	Units	ANZECC GV	WARUS1	WARUS2	WARUS3	WARUS4	WARUS5	WARUS6	WARDS1	WARDS2	WARDS3	WARDS4	WARDS5	WARDS6
pH (field)	WTW	0.1	[H+]	6.0 - 8.0	8.00	8.55	8.49	7.61	7.62	7.93	7.45	7.34	7.91	8.23	8.17	8.48
DO (field)	TPS	0.01	% sat.	85 - 120	112.9	124.4	112.0	96.8	96.1	93.3	84.4	96.4	106.9	115.0	111.9	132.8
DO (field)	TPS	0.01	mg/L	NP	8.15	8.91	8.38	7.46	7.59	7.33	6.73	7.36	7.61	8.1	8.41	9.38
EC	iEC1WZSE	2	uS/cm	250	44	30	40	59	100	77	81	92	128	97	87	116
Temp (field)	TPS	-5	°C	NP	31.8	32.8	30.7	27.5	29.1	26.8	27	28.4	34.1	35.3	29.3	34.1
TDS_calc	iSOL1WDCA	5	mg/L	NP	24	17	22	32	55	42	44	51	71	53	48	64
TSS	iSOL1WPGR	1	mg/L	NP	8	7	14	6	9	4	4	2	14	9	19	9
Alkalinity	iALK1WATI	1	mg/L	NP	17	11	13	23	41	26	33	42	58	40	38	54
Hardness	iHTOT2WACA	1	mg/L	NP	15	10	12	18	35	23	44	36	49	34	30	29
Ca	iMET1WCICP	0.1	mg/L	NP	3.9	2.6	3.1	4.9	9.3	5.9	12.5	10	13.3	9.7	8.7	8.3
K	iMET1WCICP	0.1	mg/L	NP	3.1	2.2	2.9	3.9	5.3	5.2	5.5	4.6	5.9	5.2	4.7	4.4
Mg	iMET1WCICP	0.1	mg/L	NP	1.2	0.8	1	1.5	3	2	3.2	2.6	3.8	2.4	2.2	2.1
Na	iMET1WCICP	0.1	mg/L	NP	0.8	0.6	1.1	1.8	2.3	2.7	1.9	1.8	2	2	1.7	1.9
HCO_3	iALK1WATI	1	mg/L	NP	21	14	16	29	50	32	40	51	71	49	46	64
Cl	iCO1WCDA	1	mg/L	NP	4	3	3	2	4	6	4	4	3	4	4	3
S_SO_4	iMET1WCICP	0.1	mg/L	NP	1.3	1.2	1.7	2.6	3.9	5.5	2.7	3	3.1	3.3	2.6	3.6
CO_3	iALK1WATI	1	mg/L	NP	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	1
N_NH_3	iAMMN1WFIA	0.01	mg/L	0.73[N]	0.23	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
N_NO_2	iNTRN1WFIA	0.01	mg/L	NP	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
N_NO_3	iNTAN1WCALC	0.01	mg/L	0.16	<0.01	0.04	0.02	0.12	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
N_NO_x	iNTAN1WFIA	0.01	mg/L	0.01	<0.01	0.04	0.03	0.12	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	0.01	<0.01
N_total	iNP1WTFIA	0.01	mg/L	0.3	0.30	0.31	0.36	0.31	0.24	0.35	0.20	0.21	0.20	0.21	0.34	0.22
P_total	iPP1WTFIA	0.005	mg/L	0.01	0.017	0.016	0.024	0.012	0.010	0.011	<0.010	<0.010	<0.010	<0.010	0.021	<0.010
Al	iMET1WCICP	0.005	mg/L	0.055	0.29	0.33	0.28	0.32	0.24	0.56	0.40	0.18	0.33	0.25	0.35	0.41
As	iMET1WCMS	0.001	mg/L	0.013[A]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
B	iMET1WCICP	0.02	mg/L	0.37	<0.02	<0.02	<0.02	<0.02	<0.02	0.05	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
Ba	iMET1WCICP	0.002	mg/L	NP	0.013	0.012	0.012	0.02	0.027	0.017	0.032	0.029	0.035	0.027	0.027	0.024
Cd	iMET1WCMS	1E-04	mg/L	0.0002	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Co	iMET1WCMS	1E-04	mg/L	ID	0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0001	0.0001	<0.0001	0.0001	0.0002	<0.0001	<0.0001
Cr	iMET1WCMS	5E-04	mg/L	0.001	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Cu	iMET1WCMS	1E-04	mg/L	0.0014	0.0023	0.0026	0.0028	0.0017	0.0022	0.0016	0.0011	0.0008	0.0008	0.0011	0.0018	0.0008
Fe	iMET1WCICP	0.005	mg/L	0.3*	0.22	0.29	0.24	0.24	0.18	0.37	0.25	0.15	0.27	0.17	0.25	0.30
Mn	iMET1WCICP	0.001	mg/L	1.9	0.002	0.002	0.003	0.007	0.005	0.003	0.011	0.004	0.007	0.003	0.002	0.003
Mo	iMET1WCMS	0.001	mg/L	ID	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Ni	iMET1WCMS	0.001	mg/L	0.011	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Pb	iMET1WCMS	1E-04	mg/L	0.034	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
S	iMET1WCICP	0.1	mg/L	NP	0.5	0.4	0.6	0.9	1.3	1.8	0.9	1.0	1.0	1.1	0.9	1.2
Se	iMET1WCMS	0.001	mg/L	0.011	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Si	iMET1WCICP	0.05	mg/L	NP	3.5	2.4	3.0	5.1	4.0	3.8	3.6	3.3	4.8	3.9	3.7	4.9
U	iMET1WCMS	1E-04	mg/L	ID	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
V	iMET1WCMS	1E-04	mg/L	ID	0.0019	0.0013	0.0009	0.0006	0.001	0.0014	0.0013	0.0008	0.0009	0.0013	0.001	0.0009
Zn	iMET1WCMS	0.001	mg/L	0.008[H]	0.002	0.002	0.002	0.007	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

*ANZECC/ARMCANZ (2000) provide only a low reliability GV for iron (Fe);
A = value is for AsV as no default GV is provided for total arsenic;
H = default GV should be modified for water hardness using algorithms provided in Table 3.4.3 of ANZECC/ARMCANZ (2000);
N = value is for N_NH_3 (converted from NH_3 by dividing by a factor of 1.24 mg/L) as ANZECC/ARMCANZ (2000) provide a GV for NH_3
NN = value is for N_NO_3 (converted from NO_3 by dividing by a factor of 4.40 mg/L) as ANZECC/ARMCANZ (2000) provide a GV for NO_3

Appendix 4. Phytoplankton taxa list recorded in the wet-18. Values are cells/ml.

Taxa	Reference			Potential exposed		
	WARUS3	WARUS4	WARUS6	WARDS2	WARDS3	WARDS4
Cyanophyta						
Aphanocapsa sp.	0	98	0	0	0	0
Chroococcus minimus	0	5	0	0	0	0
Cylindrospermum licheniforme ‼	21	10	169	0	0	382
Dolichospermum affine ‼	0	3	10	0	0	0
Gloeotrichia raciborskii	0	0	0	0	0	54
Lyngbya sp.	0	98	0	0	0	0
Microcystis sp.	49	0	0	74	0	0
Oscillatoria sp.	0	0	0	0	0	49
Phormidium sp.	0	12	0	0	0	0
Planktolyngbya sp.	15	78	96	0	81	98
Plectonema wollei	0	15	0	0	0	0
Pseudoanabaena sp.	0	0	0	74	0	0
Subtotal	**85**	**319**	**275**	**148**	**81**	**583**
Charophyta						
Staurastrum sp.	18	0	0	0	0	0
Subtotal	**18**	**0**	**0**	**0**	**0**	**0**
Chlorophyta						
Chlorella sp	0	0	0	135	0	167
Coccomonas orbicularis	0	87	103	143	0	167
Dictyosphaerium sp.	381	249	0	0	0	0
Monoraphidium sp.	63	0	0	0	0	0
Pyramimonas sp.	127	151	127	119	286	222
Small *Chlorophyta* <5µm	8231	8580	6566	7708	7026	10991
Subtotal	**8802**	**9067**	**6796**	**8105**	**7312**	**11547**
Pyrrophyta						
Peridinium inconspicuum	81	215	80	181	269	102
Peridinium sp.	9	0	0	0	0	0
Subtotal	**90**	**215**	**80**	**181**	**269**	**102**
Cryptophyta						
Cryptomonas sp. <10µm	1079	1269	1380	904	968	1681
Cryptomonas sp. >10µm	103	206	650	14	95	103
Subtotal	**1182**	**1475**	**2030**	**918**	**1063**	**1784**
Chrysophyta						
Dinobryon sp.	71	0	0	0	0	0
Subtotal	**71**	**0**	**0**	**0**	**0**	**0**
Bacillariophyceae						
Encyonema minutum	0	11	0	0	0	0
Nitzschia tubicola	78	80	0	22	19	0
Nitzschia sp.	0	15	0	0	0	0
Pinnularia sp.	0	10	11	10	23	12
Subtotal	**78**	**116**	**11**	**32**	**42**	**12**
Total dominant algae	10326	11192	9192	9384	8767	14028
Total algae	10530	11561	9426	9536	8850	14106
Taxa richness	**14**	**19**	**10**	**11**	**8**	**12**

Appendix 5. Microinvertebrate taxa list recorded in the wet-18. Values are log10 abundance categories, where 1= 1 individual, 2 = 2-10 individuals, 3 = 11-100, 4 = 101-1000, and so on

			Reference						Potential exposed					
Phylum/Class/Order	Family	Lowest taxon	WARUS1	WARUS2	WARUS3	WARUS4	WARUS5	WARUS6	WARDS1	WARDS2	WARDS3	WARDS4	WARDS5	WARDS6
PROTISTA														
Rhizopoda	Arcellidae	*Arcella bathystoma*	0	0	0	0	3	0	0	0	0	0	0	0
		Arcella discoides	0	0	0	0	0	3	0	0	0	0	0	0
		Arcella hemisphaerica	1	0	0	0	0	0	0	0	0	0	0	0
		Arcella [sm., disc]	0	1	0	0	0	0	0	0	0	0	0	0
		Arcella [med., domed]	0	0	0	0	0	0	1	0	0	0	0	0
	Difflugidae	*Difflugia australis*	0	0	0	0	0	0	4	0	0	4	0	0
		Difflugia cf. *limnetica*	0	5	0	0	4	0	4	0	0	3	5	3
	Lesquereusiidae	*Netzelia corona*	0	1	1	1	4	1	1	1	0	1	1	1
		Netzelia gramen	0	1	1	3	4	0	1	0	3	4	5	0
		Netzelia tuberculata	0	0	0	0	0	0	0	0	0	0	0	1
ROTIFERA														
Bdelloidea		indet. bdelloid [med.]	0	0	0	0	1	0	0	3	0	0	0	0
Monogononta	Brachionidae	*Anuraeopsis fissa*	5	1	4	0	4	0	0	0	3	0	0	0
		Brachionus falcatus	1	4	4	0	0	0	1	0	0	0	0	0
		Keratella procurva	5	5	4	3	5	5	5	4	4	4	5	5
		Keratella tropica	0	0	5	0	4	4	0	0	3	3	5	0
		Plationus patulus	0	1	0	0	0	0	0	0	0	0	0	0
	Conochilidae	*Conochilus* [sm]	0	5	5	4	3	3	5	4	3	3	5	0
		Conochilus natans	0	5	0	0	0	3	1	4	3	3	1	3
	Dicranophoridae	*Dicranophorus epicharis*	0	0	0	0	0	0	0	0	0	0	4	0
	Euchlanidae	*Euchlanis dilatata*	1	1	0	0	0	0	0	0	0	0	0	0
		Euchlanis incisa	1	1	0	0	0	1	0	0	0	0	0	0
	Gastropodidae	*Ascomorpha ecaudis*	0	0	0	0	0	0	0	0	0	0	4	0
		Ascomorpha ?indica	0	4	0	0	4	4	0	0	3	4	4	4
	Hexarthridae	*Hexarthra intermedia*	5	5	0	0	0	0	0	0	0	3	0	0
	Lecanidae	*Lecane bulla*	4	1	0	0	0	3	0	0	3	0	1	0
		Lecane leontina	0	1	0	0	0	0	0	0	0	0	0	0
		Lecane nitida	1	4	0	0	0	1	1	0	3	0	1	0
		Lecane papuana	0	0	0	0	0	0	0	0	3	0	0	1
		Lecane quadridentata	0	0	0	0	0	3	0	0	0	0	1	0
		Lecane signifera	0	1	0	0	0	0	0	0	3	0	1	0
		Lecane unguitata	0	0	0	3	3	0	0	0	0	1	4	0
		Lecane ungulata	0	0	0	0	0	0	0	0	0	1	0	0
		Lecane (*M.*) sp.	0	1	0	0	0	0	0	0	0	0	0	0
		Lecane (s. str..) sp.	0	1	0	0	0	0	0	0	0	0	0	0
	Notommatidae	*Cephalodella forficula*	0	0	4	0	0	0	0	0	0	0	0	0

			Reference						Potential exposed					
Phylum/Class/Order	**Family**	**Lowest taxon**	**WARUS1**	**WARUS2**	**WARUS3**	**WARUS4**	**WARUS5**	**WARUS6**	**WARDS1**	**WARDS2**	**WARDS3**	**WARDS4**	**WARDS5**	**WARDS6**
		Cephalodella gibba	0	1	0	0	0	0	0	0	0	0	0	0
		Notommata cf. *cerberus*	0	0	0	0	0	1	0	0	0	0	0	0
		Notommata copeus	1	0	0	0	0	0	0	0	0	0	0	0
	Synchaetidae	*Polyarthra* sp.	1	5	5	0	5	5	5	5	5	5	5	5
		Synchaeta sp.	5	0	0	0	0	0	0	0	0	0	0	0
	Testudinellidae	*Testudinella amphora*	0	0	0	0	4	0	0	3	3	0	0	0
	Trichocercidae	*Trichocerca similis*	4	5	5	0	5	5	5	4	4	4	5	5
		Trichocerca sp.	4	4	0	0	4	4	1	0	0	0	5	1
ARTHROPODA														
CRUSTACEA														
DIPLOSTRACA														
CLADOCERA	**Chydoridae**	*Anthalona harti occidentalis*	0	0	0	0	0	0	0	0	1	0	1	0
	Daphnidae	*Ceriodaphnia cornuta*	0	1	0	3	0	3	1	3	0	0	0	1
		Simocephalus heilongjiangensis	0	0	0	3	1	1	1	1	1	0	1	1
	Moinidae	*Moina micrura*	1	4	4	1	0	1	1	0	0	0	1	0
	Sididae	*Diaphanosoma excisum*	1	4	0	1	1	3	1	1	3	1	5	1
MAXILLIPODA														
Cyclopoida	**Cyclopidae**	*Mesocyclops thermocyclopoides*	0	0	0	3	1	0	0	0	0	0	0	0
		Thermocyclops decipiens	1	4	0	0	0	1	4	1	0	1	0	1
		Thermocyclops cf. *emini*	1	1	0	0	0	1	1	3	3	1	1	3
		Cyclopoid copepodites	1	5	4	3	1	4	0	3	3	1	5	1
		Cyclopoid nauplii	5	5	5	3	4	5	5	5	4	4	5	0
Calanoida	**Diaptomidae**	*Eodiaptomus lumholtzi*	1	1	5	5	4	3	1	3	4	3	5	4
		Calanoid copepodites	1	1	4	4	1	3	4	1	3	3	5	1
		Calanoid nauplii	5	5	5	4	5	5	5	5	4	4	5	5
PODOCOPIDA														
OSTRACODA	**Cyprididae**	*Bennelongia* sp.	0	1	0	1	1	1	0	1	1	1	1	1
		Cypretta sp.	1	1	1	3	1	1	1	1	1	4	1	1
	Notodromadidae	*Newnhamia fenestrata*	0	0	1	0	0	1	1	1	0	0	1	0
		Taxa richness	**24**	**36**	**18**	**17**	**25**	**29**	**25**	**21**	**25**	**24**	**31**	**21**

Appendix 6. Taxa collected from the hyporheic zone in the wet-18. Values are log_{10} abundance categories, where 1= 1 individual, 2 = 2-10 individuals, 3 = 11-100, 4 = 101-1000, and so on. * indicates stygal species.

			Reference						Potential exposed					
Phylum/Class/Order	Family	Lowest Taxon	WARUS1	WARUS2	WARUS3	WARUS4	WARUS5	WARUS6	WARDS1	WARDS2	WARDS3	WARDS4	WARDS5	WARDS6
TURBELLARIA		Turbellaria sp.	0	0	0	0	1	0	0	0	0	0	0	0
NEMATODA		Nematoda spp.	0	2	0	0	0	0	0	0	0	0	1	0
ANNELIDA														
OLIGOCHATEA		Oligochaeta spp. (imm/dam)	3	3	0	0	2	0	0	0	2	2	0	0
Turbificida	Naididae	*Pristina longiseta*	3	4	3	4	4	4	3	3	0	3	3	3
	Phreodrilidae	Phreodrilidae spp.	0	0	0	0	0	3	0	0	0	0	0	0
POLYCHAETA	Aeolosomatidae	Aeolosomatidae spp.	0	3	0	0	0	0	0	0	0	0	0	0
ARTHROPODA														
CRUSTACEA														
BRANCHIOPODA														
Cladocera	Chydoridae	indet. alonine chydorid	0	0	0	0	0	0	0	0	1	0	0	0
	Daphnidae	*Simocephalus* sp.	0	0	0	0	0	0	1	0	0	0	0	0
OSTRACODA		indet. juv. ostracod	0	0	0	0	0	0	0	0	2	0	0	0
	Candonidae	*Candonopsis tenuis* *	0	1	0	0	0	0	0	0	0	0	0	0
	Cyprididae	*Cypretta* sp.	0	0	0	2	0	0	0	0	0	0	1	0
		cf. *Ilyodromus* sp. (dam.)	0	0	0	2	0	0	0	0	1	0	0	0
	Darwinulidae	*Vestalenula marmonieri* *	0	0	0	0	0	3	0	0	0	0	0	0
MAXILLOPODA														
COPEPODA														
Cyclopoida		cyclopoid copepodites	3	3	2	3	0	3	2	0	1	3	3	0
	Cyclopidae	*Microcyclops varicans*	1	1	2	2	0	2	2	2	0	2	2	0
Harpacticoida	Parastenocarididae	*Parastenocaris jane* *	0	2	1	0	0	0	0	0	0	0	0	0
CHELICERATA														
ARACHNIDA		Acarina spp.	0	0	2	2	1	2	0	2	0	0	1	0
Sarcoptiformes		Oribatida sp.	0	0	0	0	0	0	1	0	0	0	0	0
HEXAPODA														
COLLEMBOLA														
Entomobryomorpha		Entomobryoidea spp.	1	0	0	3	0	0	0	4	3	2	2	4
Symphypleona		Symphypleona spp.	1	2	0	4	2	1	0	2	4	2	1	3

			Reference						Potential exposed					
Phylum/Class/Order	**Family**	**Lowest Taxon**	**WARUS1**	**WARUS2**	**WARUS3**	**WARUS4**	**WARUS5**	**WARUS6**	**WARDS1**	**WARDS2**	**WARDS3**	**WARDS4**	**WARDS5**	**WARDS6**
INSECTA														
Odonata		Anisoptera sp. (imm/dam)	0	1	0	0	0	0	0	0	0	0	0	0
	Libellulidae	*Orthetrum caledonicum*	0	0	0	0	0	0	0	0	1	0	0	0
Ephemeroptera	**Baetidae**	Baetidae sp. (imm/dam)	0	0	0	1	0	0	0	0	0	0	0	0
	Caenidae	Caenidae spp. (imm/dam)	0	0	0	0	0	0	0	0	1	0	2	0
Hemiptera	**Micronectidae**	*Micronecta* sp. (imm/dam)	0	1	0	0	0	0	0	0	0	0	0	0
Coleoptera	**Dytiscidae**	*Hydroglyphus grammopterus*	0	0	0	0	0	0	0	0	1	0	0	0
	Hydraenidae	*Hydraena* sp.	0	0	0	1	0	0	0	0	0	0	0	0
	Hydrophilidae	*Paracymus pygmaeus*	0	0	0	0	0	0	1	0	0	0	0	0
Diptera	**Ceratopogonidae**	Ceratopogonidae spp. (P)	0	2	0	0	3	0	0	0	0	1	0	0
		Ceratopogoninae spp.	3	4	3	2	4	3	2	2	0	2	2	0
		Dasyheleinae spp.	0	0	0	0	0	0	4	3	3	4	4	2
	Chironomidae	Chironomidae spp. (P)	0	1	0	0	0	2	0	0	0	0	0	0
	Chironominae	Chironominae spp.	2	0	0	2	0	0	0	0	0	0	0	0
		Chironomus sp.	0	1	0	0	0	0	0	0	0	0	0	0
		Cryptochironomus spp.	0	2	0	0	0	0	1	0	0	0	0	0
		Dicrotendipes spp.	3	0	1	0	2	0	0	0	0	1	0	1
		Polypedilum spp.	3	2	0	0	0	0	2	2	2	0	2	3
	Tanytarsini	*Cladotanytarsus* spp.	1	1	0	0	0	0	0	0	0	0	0	0
		Rheotanytarsus sp.	0	0	0	0	0	0	0	0	0	0	1	0
	Tanypodinae	*Paramerina* spp. (WWT1)	0	2	0	0	0	0	0	0	0	0	0	0
		Larsia albiceps	2	3	0	2	3	2	0	2	0	2	2	0
		Procladius spp. (WWT5)	2	2	0	0	0	0	2	2	0	1	2	0
	Muscidae	Muscidae spp.	0	0	0	1	1	0	0	0	0	0	0	0
	Tipulidae	Tipulidae spp.	1	0	0	0	2	1	0	0	0	0	0	0
		Taxa Richness	**14**	**21**	**7**	**14**	**11**	**11**	**11**	**10**	**12**	**12**	**15**	**6**

Appendix 7. Macroinvertebrate taxa list. Values are log_{10} abundance categories, where 1= 1 individual, 2 = 2-10 individuals, 3 = 11-100, 4 = 101-1000, and so on.

			Reference						Potential exposed					
Phylum/Class/Order	**Family**	**Lowest taxon**	**WARUS1**	**WARUS2**	**WARUS3**	**WARUS4**	**WARUS5**	**WARUS6**	**WARDS1**	**WARDS2**	**WARDS3**	**WARDS4**	**WARDS5**	**WARDS6**
MOLLUSCA														
GASTROPODA														
Hygrophila	**Planorbidae**	*Bayardella* spp.	1	2	2	0	2	0	0	2	0	0	2	0
		Leichhardtia sisurnius	0	0	0	0	0	2	0	0	0	0	0	0
ANNELIDA														
OLIGOCHAETA		Oligochaeta spp.	2	0	0	0	1	2	2	0	2	0	1	0
ARTHROPDA														
CRUSTACEA														
BRANCHIOPODA	**Thamnocephalidae**	*Branchinella* cf. *proboscida*	0	0	2	3	0	2	0	0	0	0	0	0
CHELICERATA														
ARACHNIDA		Acarina sp.	1	2	1	2	2	2	2	2	2	2	2	2
Sarcoptiformes		Oribatida sp.	0	2	1	2	0	0	0	0	0	0	0	0
HEXAPODA														
ENTOGNATHA														
Symphypleona		Symphypleona spp.	0	0	0	0	0	0	0	0	0	0	0	2
INSECTA														
Odonata														
Anisoptera	**Corduliidae**	*Hemicordulia tau*	0	0	0	0	0	0	0	2	0	0	0	2
	Libellulidae	*Diplacodes haematodes*	2	2	0	0	0	1	0	3	2	2	2	3
		Orthetrum caledonicum	0	0	0	0	0	0	0	0	1	1	0	2
		Pantala flavescens	0	0	1	0	0	1	1	2	1	1	0	2
Zygoptera		Zygoptera spp. (imm./dam.)	0	0	0	0	0	2	1	2	0	0	0	0
Tricoptera	**Ecnomidae**	*Ecnomus* spp.	1	0	0	0	2	2	2	0	0	1	2	2
Ephemeroptera	**Baetidae**	Baetidae spp. (imm./dam.)	1	0	0	0	1	2	1	0	0	0	0	0
		Cloeon sp. Red Stripe	0	0	0	1	0	1	0	0	0	0	1	2
	Caenidae	Caenidae spp. (imm./dam.)	3	1	0	2	2	3	2	3	3	2	2	3
		Tasmanocoenis sp. M	2	2	1	0	2	2	2	3	3	2	1	3
		Tasmanocoenis sp. *P/arcuata*	0	0	0	0	2	3	2	2	2	1	1	2
Hemiptera	**Gerridae**	Gerridae spp. (imm./dam.)	0	0	0	0	0	1	0	0	0	0	0	0
		Limnogonus fossarum gilguy	0	0	0	0	0	2	0	0	0	0	1	0
	Veliidae	*Nesidovelia peramoena*	0	0	0	0	0	0	0	0	0	0	1	1

			Reference						Potential exposed					
Phylum/Class/Order	Family	Lowest taxon	WARUS1	WARUS2	WARUS3	WARUS4	WARUS5	WARUS6	WARDS1	WARDS2	WARDS3	WARDS4	WARDS5	WARDS6
		Nesidovelia sp.	0	0	0	0	0	0	0	0	0	0	1	0
		Veliidae spp. (imm./dam)	0	1	0	0	0	1	0	1	0	0	1	0
	Corixoidea	Corixoidea spp. (imm./dam)	2	1	0	2	2	2	2	1	1	2	0	2
	Hebridae	Hebridae sp. (imm./dam)	0	0	0	0	0	0	0	0	1	0	0	0
		Hebrus axillaris	0	1	0	0	0	0	0	0	0	0	0	0
		Hebrus nourlangiei	0	0	0	0	0	0	0	0	0	0	1	0
	Micronectidae	*Austronecta bartzarum*	0	0	0	0	2	0	0	0	0	0	0	0
		Micronecta adelaidae	0	1	0	0	0	1	0	0	0	0	0	0
		Micronecta annae	0	2	0	0	0	0	0	1	1	0	1	0
		Micronecta gracilis	0	0	0	1	0	0	0	0	0	0	0	0
		Micronecta spp. (imm./dam.)	0	2	0	0	0	0	0	1	2	0	0	2
	Notonectidae	*Anisops gratus*	0	0	0	0	0	0	0	0	0	0	1	0
		Anisops hackeri	0	0	0	0	0	0	0	0	0	2	2	0
		Anisops nasutus	2	0	2	2	1	2	2	2	2	2	2	2
		Anisops spp.	2	3	2	2	2	2	2	3	2	3	2	3
		Notonectidae spp. (imm./dam)	2	3	3	3	3	3	2	3	3	3	2	3
	Pleidae	*Paraplea* sp.	0	0	0	0	1	0	0	0	0	0	0	0
Coleoptera	**Dytiscidae**	*Allodessus bistrigatus*	0	2	0	0	0	0	0	0	1	0	0	1
		Bidessini spp. (L)	0	2	0	0	0	0	0	0	0	0	0	0
		Copelatus nigrolineatus	0	2	0	0	0	0	0	0	0	0	0	0
		Cybister tripunctatus	0	0	1	0	0	0	1	0	0	0	2	2
		Dytiscidae spp. *(L)*	0	0	1	0	0	0	0	0	0	0	0	0
		Eretes australis	1	0	1	0	2	0	0	3	1	2	3	2
		Hydroglyphus grammopterus	2	3	0	1	2	0	1	2	3	2	0	2
		Hydroglyphus orthogrammus	0	0	0	0	0	0	0	0	2	0	2	0
		Hyphydrus lyratus	1	0	2	2	1	0	1	2	0	0	0	0
		Hyphydrus spp. (L)	2	2	2	2	2	2	0	2	2	0	0	1
		Laccophilus clarki	0	0	0	0	0	1	0	2	1	0	0	0
		Laccophilus sharpi	2	3	2	0	2	2	2	3	2	1	3	2
		Laccophilus spp. (L)	2	0	1	0	0	1	0	0	1	0	0	0
		Rhantaticus congestus	0	0	1	0	0	2	1	0	0	0	0	0
		Tiporus lachlani	0	0	1	0	1	0	2	2	0	0	0	0
		Tiporus spp. (L)	0	0	0	0	1	0	0	0	0	0	1	0
	Gyrinidae	*Dineutus australis*	0	2	0	0	0	2	0	0	2	2	1	2
		Dineutus spp. (L)	1	0	1	0	1	0	0	0	0	0	0	0
	Hydrochidae	*Hydrochus* spp.	2	3	1	0	2	1	0	2	2	0	2	0
	Hydrophilidae	*Berosus dallasae*	0	0	0	0	0	0	0	0	0	0	0	1
		Berosus pulchellus	2	3	0	0	0	0	0	1	0	1	2	2
		Enochrus deserticola	2	3	0	0	1	1	0	2	2	0	1	0
		Enochrus elongatulus	0	1	0	0	0	0	0	0	0	0	0	0

Phylum/Class/Order	Family		Lowest taxon	Reference WARUS1	WARUS2	WARUS3	WARUS4	WARUS5	WARUS6	Potential exposed WARDS1	WARDS2	WARDS3	WARDS4	WARDS5	WARDS6
			Laccobius matthewsi	0	0	0	0	0	0	0	0	0	0	0	1
			Paracymus pygmaeus	0	2	0	0	0	0	0	0	0	0	0	0
			Paracymus spenceri	0	2	0	0	0	0	0	1	2	0	0	1
			Regimbartia attenuata	0	2	0	0	0	0	0	1	0	0	1	0
	Hydraenidae		*Hydraena* spp.	0	3	0	0	1	1	0	2	3	0	2	2
Diptera	**Tipulidae**		Tipulidae spp.	1	1	0	0	0	0	0	0	0	0	0	0
	Culicidae		*Anopheles* sp.	1	0	0	0	0	0	0	0	0	0	0	0
			Culex spp.	3	4	0	0	1	0	0	0	0	0	0	0
			Culicidae spp. (P)	2	0	0	0	0	0	0	0	0	0	0	0
	Ceratopogonidae		Ceratopogoninae spp.	3	3	1	1	3	3	2	2	3	4	2	2
			Dasyheleinae spp.	0	0	1	0	2	0	2	1	3	2	0	2
	Chironomidae		Chironomidae spp. (P)	2	0	0	0	0	2	0	1	0	0	0	0
		Chironominae	Chironominae spp. (imm./dam)	2	0	0	0	0	0	0	0	0	0	0	1
			Chironomus spp.	3	4	2	2	0	0	1	0	0	0	0	1
			Cryptochironomus spp.	0	0	0	0	1	0	0	0	1	2	0	0
			Polypedilum spp.	2	3	2	2	2	3	1	1	2	2	1	2
			Harnischia sp.	0	0	0	0	0	0	0	1	0	0	0	0
			Harrisius/Stenochironomus spp.	0	0	0	0	2	0	0	3	1	2	2	2
			Kiefferulus sp.	0	0	0	0	0	0	0	1	0	0	0	0
			Stenochironomus spp.	2	0	0	0	0	3	0	0	0	0	0	0
			Dicrotendipes spp.	0	3	0	0	2	2	0	0	0	2	0	0
			Parachironomus spp.	1	0	0	0	1	3	0	0	0	0	0	0
			Cladotanytarsus spp.	1	0	0	0	0	0	0	0	0	1	0	0
			Tanytarsus spp.	1	0	0	2	3	3	1	2	2	1	2	3
			Paratanytarsus sp.	0	0	0	1	0	0	0	0	0	0	0	0
			Rheotanytarsus spp.	0	0	0	0	0	0	0	0	2	0	0	0
		Tanypodinae	*Paramerina* spp. (WWT1)	3	3	1	0	2	3	3	3	3	0	2	3
			Larsia albiceps	3	4	2	0	2	3	3	3	3	3	2	3
			Procladius spp. (WWT5)	2	3	2	2	2	3	4	3	4	4	2	4
			Ablabesmyia hilli	0	0	0	2	0	0	0	3	3	3	0	3
			?Pentanuera sp. (WWT9)	0	0	0	1	0	0	0	0	0	0	0	0
			Coelopymia pruinosa	0	0	0	0	1	3	0	0	0	0	0	0
	Muscidae		Muscidae sp.	0	0	0	0	0	0	0	0	1	0	0	0
			Taxa richness	**38**	**38**	**27**	**21**	**38**	**41**	**27**	**41**	**40**	**29**	**38**	**40**