Tutunup Mineral Sands Project

Aquatic Biology Desktop Review and Aquatic Fauna Field Survey 2020

April 2021

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Prepared for:

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Final Report
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Frontispiece (left to right): Inundated paperbark woodland at Tutunup Wetland site TUT1-3 in August 2020 (photo A. Hickling, WRM).

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Disclaimer

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EXECUTIVE SUMMARY

This report presents the findings of a recent desktop review and baseline aquatic biology field survey for a seasonal wetland, "Tutunup Wetland", adjacent to Iluka Resources Limited (Iluka) Tutunup Deposit in the South-West SWA IBRA region of Western Australia. Tutunup Wetland is currently classified as part Conservation Category Wetland (CCW) and part Resource Enhancement Wetland (REW) under Department of Biodiversity, Conservation and Attractions (DBCA) management categories. The wetland also correlates to a Busselton Ironstone Threatened Ecological Community (TEC).

The desktop review indicated there has only been one previous aquatic fauna survey for the wetland, that by WRM in August 2008. Aquatic fauna of seasonal wetlands in general has been historically understudied, especially microinvertebrate communities in coloured, acidic wetlands such as Tutunup Wetland. Other than surveys by WRM (WRM 2006a-c, 2008a-b) of more disturbed rural wetlands, there appear are no published datasets for seasonal wetlands in the South-West SWA bioregion. Large scale studies of wetlands in the SWA and Warren (WAR) bioregions have previously been undertaken by state agencies. However species datasets are only available for one of these studies; the *Wetlands of the Swan Coastal Plain* study undertaken by Davies *et al*. (1993) for wetlands in the Greater Perth Metropolitan region.

The 2008 survey was limited by the extent of surface water at the time the survey was requested. As data from the 2008 survey were considered insufficient for adequate characterisation of aquatic ecosystem values, Iluka requested WRM re-survey the wetland in August 2020, to provide additional baseline data to that recorded in 2008.

Field methods used for the 2020 survey were consistent with methods used for the 2008 survey, and align with those routinely used by government departments, *i.e.* DBCA, Department of Water Environmental Regulation (DWER) and the National Monitoring River Health Initiative (NMRHI). In accordance with the National Water Quality Management Framework (ANZG 2018), water quality (physical and chemical stressors and toxicants) and several aquatic fauna receptors, representative of different trophic groups (*i.e.* microinvertebrates, macroinvertebrates, and fish) were surveyed.

Surface waters in the study area were found to be sodium-chloride dominated, highly coloured, generally acidic (pH 5.66 - 6.66), and with low alkalinity and conductivity (229 - 605 µS/cm). Conductivity in the only farm dam sampled for water quality, was considerably higher (1,930 µS/cm) than that of the surrounding wetland. Background levels of dissolved aluminium exceeded the ANZG (2018) DGV for 80% species protection at a number of sites, noting that ANZG (2018) provide only a low reliability DGV for aluminium at pH < 6.5.

The 2020 field survey yielded an additional 17 microinvertebrate and 37 macroinvertebrate taxa to those recorded in 2008, with combined totals of 27 microinvertebrate and 59 macroinvertebrate taxa across the two surveys. Consistent with the 2008 survey, no fish were recorded. None of the listed conservation significant species identified in the desktop review were recorded in 2020.

The minute freshwater snail *Glacidorbis occidentalis* (DBCA Priority 3), collected in 2008, is likely still present in the study area but not recorded in 2020 due to the species characteristically low abundance. As the species occurs widely throughout the SWA and WAR bioregions, disturbance to the local population would not result in direct loss of regional biodiversity.

The listed black-stripe minnow *Galaxiella nigrostriata* (EPBC Endangered, DBCA Endangered), identified in the desktop review as potentially occurring, is considered to have negligible-low chance of occurring based on the combined results of the 2008 and 2020 field surveys. Though the study area is within the known range for this species, suitable habitat is limited by the very short persistence time and shallow depth of surface waters, coupled with the (likely) limited spatial extent of cool soils that are sufficiently deep for this minnow to aestivate in over the summer.

The 2020 field survey collected two potential short range endemic (SRE) macroinvertebrate species, in addition to the epigean diving beetle, *Paroster* sp. nov. (family Dytiscidae), recorded in 2008. Potential SRE species recorded in 2020 were another species of *Paroster*, *P. ellenbrookensis*, and the stygobitic amphipod *Wesniphargus nichollsi* (family Neoniphargidae). None of these species are listed as conservation significant under the Commonwealth EPBC Act or state BC Act, however few aquatic invertebrates are formally listed as conservation significant due to data deficiency. *Paroster* sp. nov. is new to science and has not been recorded elsewhere. *P. ellenbrookensis* is known to occur in only one other location (Ellenbrook Nature Reserve). *W. nichollsi* is known from several scattered populations across the SWA and WAR bioregions, but taxonomic resolution is poor. Genetic analysis is required to confirm whether *W. nichollsi* specimens from the study area are the same species as specimens from other localities, or if they are restricted to Tutunup Wetland. Until better distributional data are available for *W. nichollsi* and the *Paroster* species to confirm their status as SREs, a precautionary approach is recommended in managing potential soil disturbance and altered hydroperiod in the wetland.

The 2020 survey recorded one microinvertebrate species that is a new record for Australia; the protist *Arcella* cf. *crenullata.* This is in addition to the protist *Difflugia* cf. *distenda*, collected in 2008, which is also a new record for Australia. The probability that these microinvertebrate species are restricted to the study area is considered low to negligible. Both are highly likely to be widely distributed in comparable habitats throughout Australia but have yet to be recorded due to historic under-sampling for microinvertebrates.

Overall, the study area is considered to support a high diversity of aquatic invertebrates, based on the DBCA reference ranges for wetland evaluation (Jones *et al*. 2009), relatively undisturbed seasonal wetlands sampled for the *Wetlands of the Swan Coastal Plain* study (Davis *et al.* 1993), and more disturbed wetlands in the region sampled by WRM (WRM 2006a-c, 2008a-b). While most species are common and widespread, the study area supports one listed conservation significant freshwater snail, and three potential SRE macroinvertebrates. The ecological value of the remnant aquatic habitat in the study area is considered to be high, based on the presence of these aquatic species, combined with the extensive local and regional-scale loss of seasonal wetland habitat to agriculture.

1 INTRODUCTION

1.1 Background

Iluka Resources Limited (Iluka) is undertaking baseline studies at their Tutunup Deposit located at Tutunup, approximately 195 km south of Perth and 17 km east of Busselton, in the South-West of Western Australia. The deposit is located at the base of the Whicher Scarp in the Vasse Wonnerup Estuary Catchment and the Abba River sub-catchment, in the South-West area of the Swan Coastal Plain Interim Biogeographic Regionalisation for Australia (IBRA) region (coded SWA). The Abba River is located approximately 6.6 km south west of the survey area, and the Ludlow River is approximately 1 km north east of the survey area.

Adjacent to the Tutunup Deposit is a small seasonal wetland that covers approximately 122.7 ha along Tutunup Road, and is hereafter referred to as "Tutunup Wetland" (Figure 1). To the north of Tutunup Road, the wetland occurs in Nature Reserve R4606, managed by the Department of Biodiversity and Attractions (DBCA), and to the south of Tutunup Road it is within privately owned property. Approximately half of the mapped area of Tutunup Wetland (~ 60 ha) falls within Iluka tenements, mostly on its eastern side south of the road, plus a small overlap with another Iluka tenement in the south-western corner.

The wetland is currently designated as part Conservation Category Wetland (CCW) and part Resource Enhancement Wetland (REW) under the Department of Biodiversity Conservation and Attractions (DBCA) management category codes (DBCA 2020). The total wetland area is approximately two-thirds CCW and one-third REW (Figure 1). Tutunup Wetland also supports an example of the Busselton Ironstone Threatened Ecological Community (TEC) (DBCA 2018). As such, the Environmental Protection Authority (EPA) environmental factors of *Inland Waters* and *Terrestrial Fauna* must be considered as part of an environmental impact assessment process for the Tutunup Project, in relation to the aquatic ecosystem values of Tutunup Wetland (see section 1.4).

In preparation for potential development of the Tutunup Deposit, Iluka requested Wetland Research & Management (WRM) to conduct a desktop review of literature and existing knowledge of the aquatic ecosystem of Tutunup Wetland, with consideration to the survey area, local and regional extent. The review was originally provided to Iluka as a separate stand-alone report (see WRM 2020), but is reproduced here (section 3) for completeness. The review determined that the only aquatic biology survey to have been conducted at Tutunup Wetland, was that by WRM (2008c) in August 2008. At the time the 2008 survey was requested, surface waters were limited to small, shallow, isolated water bodies, mainly beside former access tracks and in wheel ruts. The data generated by the 2008 survey effectively only captured a 'snap-shot' of the ecological condition and was considered insufficient for adequate characterisation of the aquatic ecosystem values of this wetland (WRM 2020). Iluka therefore commissioned WRM to undertake additional baseline sampling for aquatic fauna within the survey area in August 2020, to better ascertain the aquatic fauna species composition.

1.2 Study Purpose

The purpose of the 2020 aquatic biology survey was to provide additional baseline information on the ecological values of Tutunup Wetland in line with the Environmental Protection Agency (EPA) Environmental Factors Guidelines for Inland Waters and Terrestrial Fauna (EPA 2016a, 2018).

1.3 Study Objectives

The objectives for the current scope of works included:

- an aquatic biology field survey of Tutunup Wetland;
- provide a baseline aquatic biology survey report of the survey area including a summary of the findings and their local and regional significance, that is sufficient for Iluka to conduct an environmental impact assessment and to support State and Federal environmental approvals;
- supply an IBSA (Index of Biodiversity Surveys for Assessments) data package to Iluka;
- supply all spatial and field data generated as part of the scope of works to Iluka in suitable GIS mapping format (additional to IBSA).

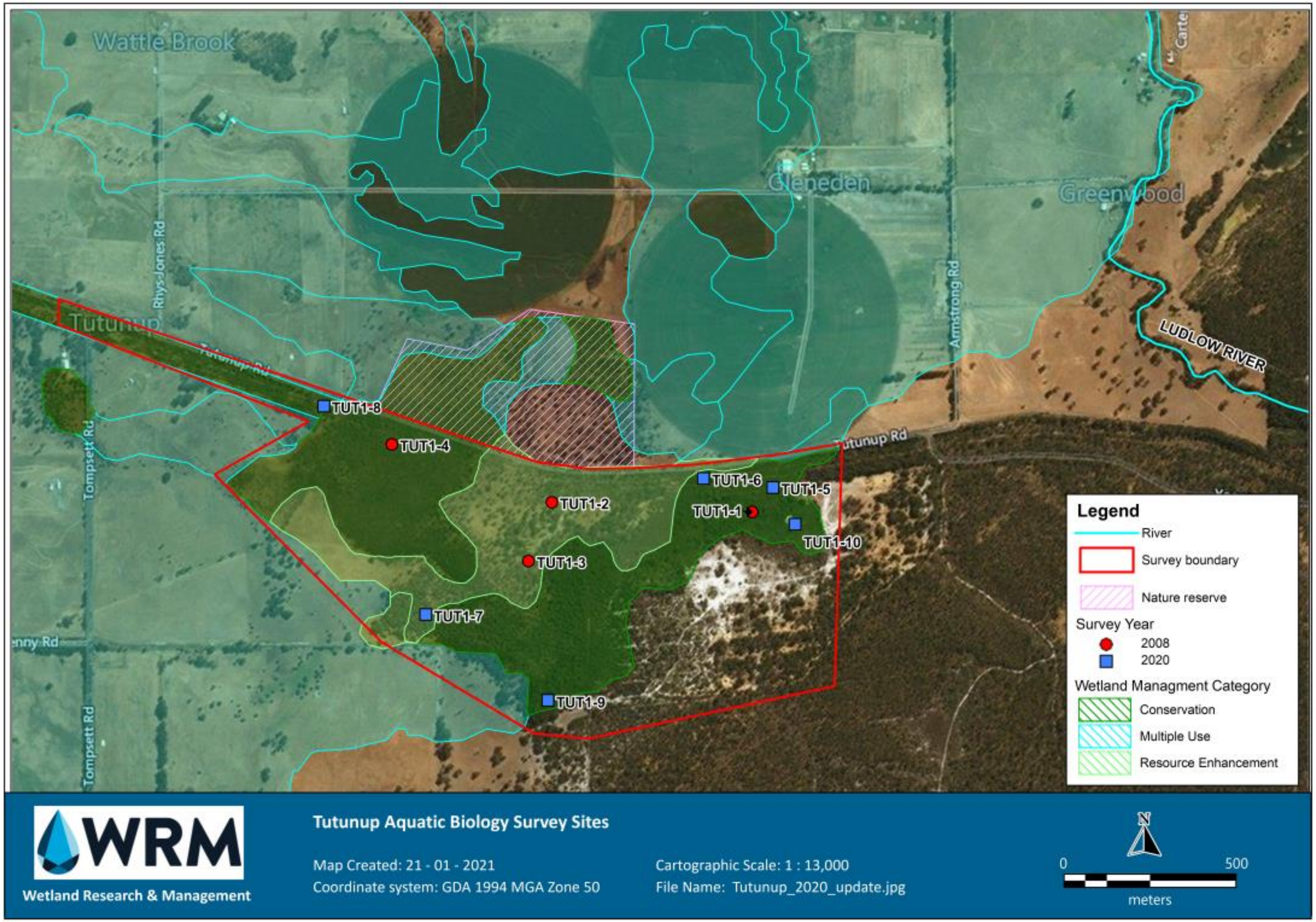

Figure 1. Tutunup Wetland survey area and sampling sites.

1.4 Legislation and Guidance

Environmental factors that must be considered as part of an environmental impact assessment (EIA) process, in relation to the aquatic ecosystem values of Tutunup Wetland, include *Inland Waters* and *Terrestrial Fauna*. Key environmental factors form the Western Australian Environmental Protection Authority (EPA)'s basis for the decision of whether a proposal's environmental impact is considered acceptable (EPA 2018).

For *Inland Waters*, this encompasses:

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

For *Terrestrial Fauna*, this encompasses:

"Terrestrial fauna are defined as animals living on land or using land (including aquatic systems) for all or part of their lives. Terrestrial fauna includes vertebrate (birds, mammals including bats, reptiles, amphibians, and freshwater fish) and invertebrate (arachnids, crustaceans, insects, molluscs and worms) groups." (EPA 2016a).

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 for 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 *Environmental Protection Act 1986* (EP Act) as a beneficial use or an ecosystem health condition. Aquatic fauna and flora and the ecological processes that support them are specifically listed in the *Inland Waters* Environmental Factor Guideline as one of the ecosystem health values that must be considered as part of the EIA process (EPA 2018). For the purposes of impact assessment for ecosystem health, the EPA focusses on impacts to significant ecosystems, which include (EPA 2018):

- Ramsar Wetlands of International Importance,
- Conservation category or Resource enhancement management wetlands mapped on the Swan Coastal Plain,
- wetlands listed in the Directory of Important Wetlands in Australia,
- wetlands protected by Environmental Protection Policies under the EP Act,
- wild rivers, as identified by the Australian Heritage Commission and DWER,
- wetland types which may be poorly represented in the conservation reserves system,
- springs and pools, particularly in arid areas,
- ecosystems which support significant flora, vegetation and fauna species or communities, including migratory waterbirds, bats, and subterranean fauna,
- ecosystems which support significant amenity, recreation and cultural values,
- saline lakes, estuaries and near shore ecosystems reliant on groundwater or surface water inputs, and
- downstream marine ecosystems.

The EPA's objective for the *Terrestrial Fauna* factor is "to protect terrestrial fauna so that biological diversity and ecological integrity are maintained" (EPA 2016a). EPA define ecological integrity as "the composition, structure, function and processes of ecosystems, and the natural range of variation of these elements". Considerations for EIA for the factor *Terrestrial Fauna* include, but are not necessarily limited to:

- application of the mitigation hierarchy to avoid or minimise impacts to terrestrial fauna, where possible,
- the terrestrial fauna affected by the proposal,
- the potential impacts and the activities that will cause them, including direct and indirect impacts,
- the implications of cumulative impacts,
- whether surveys and analyses have been undertaken to a standard consistent with EPA technical guidance,
- the scale at which impacts to terrestrial fauna are considered,
- the significance of the terrestrial fauna and the risk to those fauna,
- the current state of knowledge of the affected species/assemblages and the level of confidence underpinning the predicted residual impacts, and
- whether proposed management approaches are technically and practically feasible.

Despite the new updated Environmental Factor relating to Inland Waters (EPA 2018), there are still no prescriptive guidance statements at the state or Commonwealth level outlining surface water quality and aquatic fauna sampling design and methods. Therefore, the water quality and aquatic fauna baseline sampling employs methods and general approaches / rationale were consistent with the following:

- EPA Technical Guidance: Terrestrial vertebrate fauna surveys for environmental impact assessment (EPA 2020);
- EPA Technical Guidance: Sampling of short range endemic invertebrate fauna (EPA 2016b);
- the National Monitoring River Health Program (NRHP) Australia River Assessment Scheme (AusRivAS);
- Wetlands of the Swan Coastal Plain: wetland classification on the basis of water quality and invertebrate community data (Davis *et al.* 1993);
- Australian and New Zealand Guidelines for Fresh and Marine Water Quality (ANZG 2018), developed as art of the National Water Quality Management Strategy (NWQMS) (Australian Government 2018).

NWQMS provides authoritative guidance (*i.e.* a framework) on the management of water quality in Australia and New Zealand (ANZG 2018). To protect the community values of waterways (aquatic ecosystems and cultural and spiritual values), the Water Quality Management Framework (WQMF) applies a weight of evidence (WoE) process to collect, analyse and evaluate a combination of different qualitative, semi-quantitative or quantitative lines of evidence (LoE) to make an overall assessment of water quality and its associated management. Therefore, and in accordance with the WQMF (ANZG 2018) water quality (physical and chemical stressors and toxicants) and several aquatic fauna receptors, representative of different trophic groups (*i.e.* microinvertebrates, macroinvertebrates, and fish) were selected for survey, to characterise the aquatic biology of Tutunup Wetland.

2 STUDY AREA

2.1 Wetland Description

Tutunup Wetland covers an area of 122.7 ha located within a larger, seasonally inundated sumpland. V & C Semeniuk Research Group (1998) described the geomorphic setting as the Tutunup consanguineous wetland suite (Table 1). Tutunup Road and disused rail line divide what would once have been a large wetland, extending northward and westward. The wetland predominantly comprises waterlogged, peaty soils, shallowly inundated sedgelands and small areas of shallow open water, predominantly along former access tracks and rail embankment (WRM 2008). Soils and landforms of the study area have been described by Tille and Lantzke (1990) as winter wet flats and slight depressions with either shallow red brown sands and loams over ironstone (*i.e.* bog iron ore soils), or sandy grey brown duplex (Abba) and gradational (Busselton) soils.

Surface water presence at Tutunup Wetland is seasonal and dependent on winter rainfall patterns. Groundwater levels are close to the surface, with the average distance to the water table from the surface ranging from 1.8 m in February, to 0.3 m in August (WRM 2020). Groundwater levels appear to rise following the onset of the winter rainfall season in May, and fall as the rainfall tapers off again in spring (around October) (WRM 2020).

Table 1. Tutunup consanguineous wetlands suite and geomorphic setting described by V & C Semeniuk Group (1998).

Suite to which site belongs	UFI*	Geomorphic setting	Primary wetlands within the suite	Suite description	Suite stratigraphy
Tutunup Suite	836-43, 850-2	Contact between Blackwood Plateau and plain underlain by Bassendean Sand	Macroscale sump-lands and flood-plains.	Seepage from the Mesozoic sediments underlying the Blackwood Plateau at its contact with the plain, produce sumplands and floodplains on the low-lying areas between the alluvial fans.	Fine quartz sand with iron impregnations, ferricrete, and sandstone boulders.

* UFI = unique feature identifier from the *Geomorphic Wetlands, Swan Coastal Plain* (DBCA-019) dataset (DBCA 2020).

As noted in section 1.1, Tutunup Wetland is currently classified as part Conservation Category Wetland (CCW) and part Resource Enhancement Wetland (REW) under DBCA management categories (2020), defined as:

- Conservation Category Wetlands (CCW) – wetlands that support a high level of ecological attributes and functions (generally having intact vegetation and natural hydrological processes), or that have a reasonable level of functionality and are representative of wetland types that are rare or poorly protected.
- Resource Enhancement Wetlands (REW) – wetlands that have been modified (degraded) but still support substantial ecological attributes (wetland dependant vegetation covering more than 10%) and functions (hydrological properties that support wetland dependent vegetation and associated fauna), and have some potential to be restored to the Conservation management category. Typically, such wetlands still support some elements of the original native vegetation, and hydrological function.
- Multiple Use Wetlands (MUW) – wetlands that are assessed as possessing few remaining ecological attributes and functions. While such wetlands can still play an important role in regional or landscape ecosystem management, including water management, they are considered to have low

intrinsic ecological value. Typically, they have very little or no native vegetation remaining (less than 10%).

The Tutunup Wetland vegetation communities also classify as an example of the Shrublands on southern Swan Coastal Plain Ironstones (Busselton area) Threatened Ecological Community (Busselton Ironstone TEC) (DBCA 2018). Ironstone soils and the vegetation communities they support are associated with shallow seasonal inundation with fresh water and typically high groundwater levels, and thus are considered to be groundwater dependent ecosystems (GDEs) (English 1999, Meissner & English 2005). They are extremely restricted in coastal plain regions and as such, are of high conservation significance and listed as a Threatened Ecological Community (TEC) (Meissner & English 2005).

The DBCA is also custodian of the *Consanguineous Wetlands Suites* (DBCA-020) dataset (DBCA 2017). Consanguineous wetlands suites are areas containing a group of wetlands that are considered to have common or interrelated features, based on geomorphic setting, origin and water maintenance. Assessment of these groups provide a regional perspective on natural wetland groups, and can also be used to determine if certain individual wetland types are rare or unusual within consanguineous suites, and are therefore of greater conservation value.

A recovery plan was developed for ironstone communities of the Busselton Area, including those associated with Tutunup Wetland (see Meissner & English 2005), as much of the native vegetation of the sumpland has been cleared previously for agriculture and road and rail construction. Tutunup Road and a disused rail line divide what would once have been a large wetland, extending northward and westward. Remnant wetland vegetation is dominated by dense sedgelands on seasonally inundated soils and open to moderately dense myrtaceous shrublands on seasonally waterlogged soils. There is a fringe of scattered paperbarks (*Melaleuca* sp.) to the south.

2.2 Climate and Hydrology

Surface water at Tutunup Wetland is seasonal and dependent on winter rainfall patterns. The majority of annual rainfall occurs between May and September (Figure 2). Small, shallow, isolated water bodies, mainly beside former access tracks and along the rail line, were observed by WRM on the 18th August 2008, likely ponding on the underlying ironstone and impermeable heavy soils associated with the Busselton Ironstone TEC (Meissner & English 2005, WRM 2008c). There was no surface water present when the wetland was visited by WRM in November 2007 (WRM 2008c). Deeper areas of surface water were present in August 2020 in two man-made dams, approximately 1.5 m deep. The majority of the site consisted of small, isolated water bodies beside former tracks and along the railway line as observed in 2008.

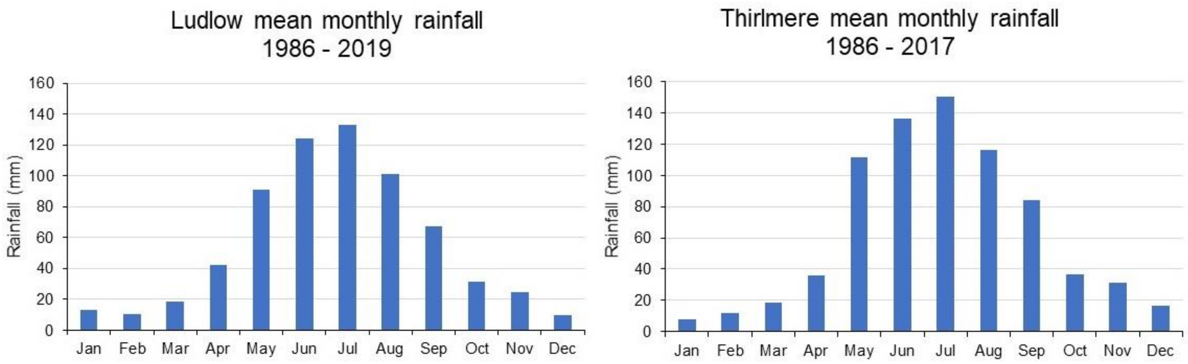

Figure 2. Mean monthly rainfall for the closest weather stations to Tutunup Wetland; (left) Ludlow, 10 km north-west of Tutunup, and (right) Thirlmere 18 km north of Tutunup. Data source: Bureau of Meteorology website (http://www.bom.gov.au/, accessed 6 October 2020).

Figure 3 shows the total monthly rainfall in the years 2007, 2008, 2019 and 2020 to the time of sampling. August 2008 was a relatively dry month (19 mm received at nearby Ludlow), compared to the August average (100 mm; Figure 2) following peak winter rainfall in July 2008 (161 mm). This may indicate that the wetland recedes relatively rapidly (*i.e.* within the space of a few weeks) following reduction in rainfall, as only small, shallow pools were present by 18th August 2008, despite a higher-than-average July rainfall. Rainfall in July 2020 was below average at 106 mm (average 131 mm), with only small, shallow pools present and sites sampled in 2008 were dry at the time of sampling in 2020.

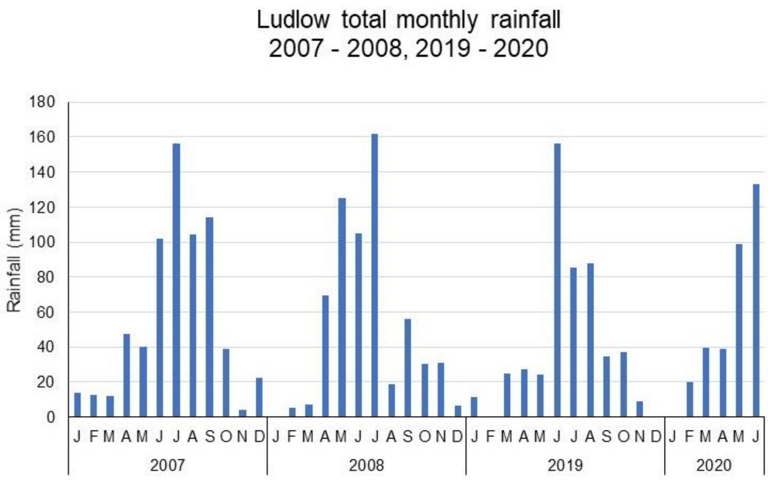

Figure 3. Total monthly rainfall for Ludlow weather station, 2007-08 and 2019-20. Data source: Bureau of Meteorology website (http://www.bom.gov.au/, accessed 6 October 2020).

Groundwater level data from Department of Water and Environmental Regulation (DWER) monitoring bore 61030067 in Tutunup Wetland is somewhat limited, with measurements for the last 10 years only taken from March to May, and September to November (Table 2). Groundwater levels at Tutunup Wetland are close to the surface, with the average distance to the water table from the surface ranging from 1.8 m in February, to 0.3 m in August (Table 2 & Figure 4). Groundwater levels appear to rise following the onset of the winter rainfall season in May, and fall as the rainfall tapers off again in spring (around October Figure 4).

Table 2. Distance (in meters) to the water table from ground level at Tutunup Wetland, 1984 – 2020. Blank cells = no data reported, n = total number of records. Data source: DWER Water Information Reporting website (http://wir.water.wa.gov.au/Pages/Water-Information-Reporting.aspx, accessed 2 July 2020).

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1984											0.5	
1985	1.9											
1987										0.8		
1988			1.7						0.0			
1989			1.4						0.4			
1990			1.6						0.1			
1991			1.6					0.2				
1992			1.6						0.3			
1993			1.6		1.4				0.3			
1994			1.7									1.2
1995			2.1									
1996					1.6					0.5		
1997					1.3							
1998				1.5							0.9	
1999				1.9								
2000					1.2					0.7		
2001				1.5						0.6		

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2002				1.7					0.3			
2003				1.7					0.4			
2004				1.8					0.5			
2005				1.4						0.4		
2006				1.7					0.7			
2007				1.0	1.6	1.6	0.7	0.3	0.1	0.4	0.9	
2008	0.4	1.8	1.9	1.9	1.8	1.0	0.7	0.2	0.8	0.8	1.0	1.2
2009	1.5	1.8	2.0	2.0	2.1	1.7	0.4	0.5	0.3	0.6	0.9	1.2
2010	1.5	1.9	2.1	1.9	1.7	1.3			0.7	1.1	1.2	
2011			2.0	2.2	2.0				0.4	0.9	1.0	
2012			2.2	2.2	1.9				0.7	0.9	1.1	
2013			2.1	2.1	1.3				0.1	0.7	1.0	
2014			2.0	2.1	1.7					0.7	1.0	
2015			1.9	1.4	0.9				0.4	1.0	1.2	
2016			1.7	1.1	1.1				0.4	0.5	0.9	
2017			1.8	1.7	1.6				0.7	0.6	1.0	
2018			1.9	1.9	1.9				0.4	0.6	0.9	
2019			1.7	1.7	1.5				0.7	0.8	1.0	
2020			1.7	1.7	1.6							
Mean	1.3	1.8	1.8	1.7	1.6	1.4	0.6	0.3	0.4	0.7	1.0	1.2
n	4	3	21	22	18	4	3	4	21	18	15	3

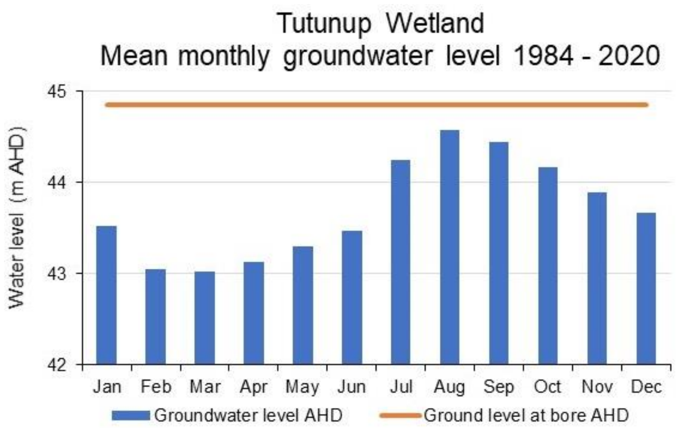

Figure 4. Average (mean) monthly groundwater levels at Tutunup Wetland, 1984 – 2020, at Department of Water and Environmental Regulation (DWER) monitoring bore 61030067. AHD = Australian Height Datum. Dataset from 1984 to 2020; all datapoints included. Data source: DWER Water Information Reporting website (http://wir.water.wa.gov.au/Pages/Water-Information-Reporting.aspx accessed 2 July 2020).

Figure 5 presents a time series (1987 - 2020) of groundwater levels at bore 61030067. As part of the desktop assessment by WRM (2020), recordings for the month with the minimum average groundwater level, March, were plotted to assess whether there had been a decline in the minimum level over time. It was assumed that the agricultural pivots located directly north of Tutunup Wetland (visible as part of the Multiple Use Wetland area, directly north of Tutunup Road, in Figure 1), use groundwater for irrigation. This usage may have a drawdown effect on the shallow groundwater that supports the GDE of Tutunup Wetland, particularly the Busselton Ironstone TEC. There appears to be an overall downward trend in groundwater level at this bore since 1987, though with some recovery from 2013 to 2020 (Figure 5). This suggests that groundwater drawdown in the vicinity of Tutunup Wetland is already occurring and appears to be associated with varying intensity of pivot use, as evidenced from Google-Earth imagery that shows a greening of the pivot areas in later years. However historic Google-Earth imagery is only available from 2005 onward.

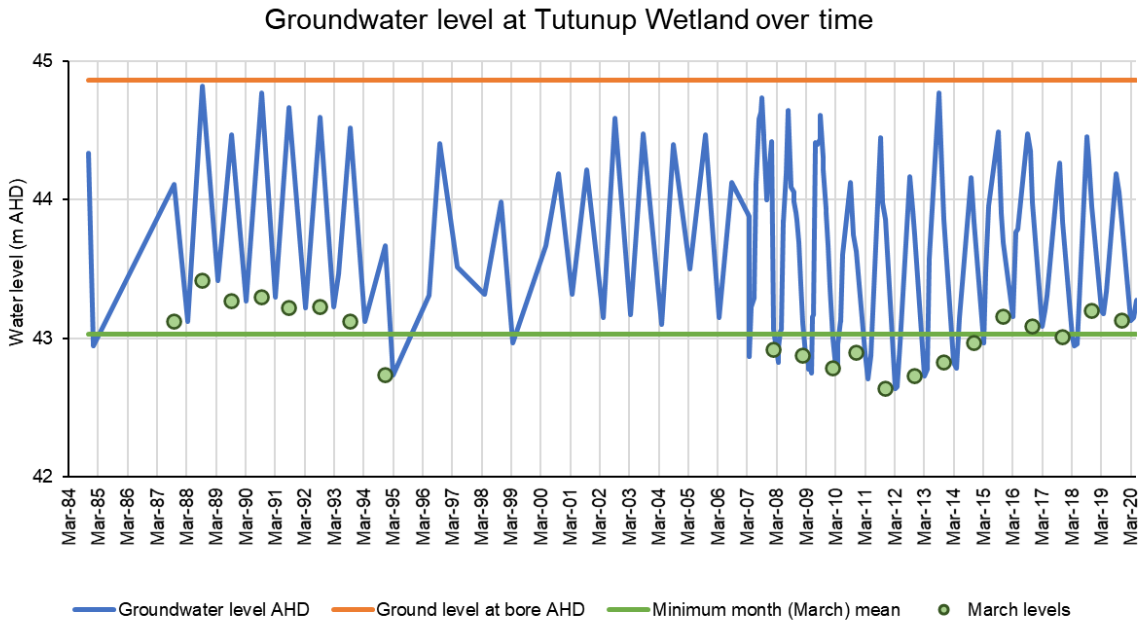

Figure 5. Groundwater levels time series at Tutunup Wetland, 1985 - 2020, at Department of Water and Environmental Regulation (DWER) monitoring bore 61030067. Levels for March, the month when groundwater is at minimum mean levels, are indicated, to assess whether groundwater levels have changed over time. All datapoints included. Data source: DWER Water Information Reporting website (http://wir.water. wa.gov.au/Pages/Water-Information-Reporting.aspx, accessed 2 July 2020).

3 DESKTOP REVIEW

3.1 Literature and Database Searches

Past aquatic and relevant aquatic biology survey reports were reviewed and sourced as part of the desktop review. This included relevant scientific reports and studies that have been undertaken on a local and regional scale, together with published and grey literature. For the purposes of the desktop assessment, the 'regional' extent in regards to the study area for Tutunup Wetland, was considered to be the area of the SWA IBRA region that intersects the South-West region as defined by the Department of Water and Environmental Regulation (DWER) regional boundaries (South-West SWA; Figure 6). Table 3 lists the databases searched to ascertain aquatic fauna distribution and significance of relevance to the desktop assessment.

Table 3. Databases searched.

Database	Description	Authority	Area of Search
Threatened and Priority Fauna List (DBCA 2019)	Reviewed on 23rd June 2020.	DBCA	40 km radius of study area
NatureMap	Search conducted by WRM on 2nd July 2020 (see Appendix 7).	DBCA and WAM	40 km radius of study area
Freshwater Fish Distribution in Western Australia	Search conducted by WRM on 23rd June 2020.	Department of Primary Industries and Regional Development (DPIRD)	All freshwater fish species
The Australian Faunal Directory (AFD)	Utilised in assessing taxonomic status and distribution of aquatic fauna.	Australian Biological Resources Study (ABRS; an initiative of DAWE)	All relevant species
Atlas of Living Australia (ALA 2020)	Search conducted by WRM on 24th June 2020. Utilised in assessing taxonomic status and distribution of aquatic fauna.	Collaborative project between academic, private and community groups	10 km radius of study area

3.2 Past Aquatic Fauna Surveys in the South-West SWA Region

The desktop review identified a number of studies with supporting literature and database records which were used to summarise current ecological values relevant to Tutunup and other South-West SWA wetlands (Table 4). However, the only relevant recent study found for the Tutunup Wetland local area / South-West SWA bioregion was the August 2008 survey by WRM (2008c) (Table 4).

Large scale studies of wetlands across southwestern WA have previously been undertaken with the funding of state agencies. Only three include invertebrate sampling in the SWA bioregion, and except for species distributions entered on NatureMap, the species datasets generated are only available for one study, that by Davies *et al*. (1993):

- Davies *et al*. (1993) conducted seasonal sampling for water quality, sediments, periphyton, macrophytes and invertebrates at 18 seasonal and 23 permanent wetlands, as part of the *Wetlands of Swan Coastal Plain* study funded by the Water Authority of Western Australia and the EPA. A large range of rural and semi-urban wetlands were surveyed in the SWA bioregion, with levels of disturbance ranging from relatively pristine to heavily disturbed. All were located over 100 km north of the current study area; *i.e.* between Lake Cooloongup (~154 km north of the current study area) and Gin Gin Brook (~260 km north of the current study area). Sampling was conducted in January 1989, November 1989 and November 1990. Macroinvertebrates and micro-crustacea (ostracods, copepods, cladocerans) were sampled using sweep nets. Davis *et al.* (1993) provide species occurrence (presence/absence) lists for each sampling occasion for each wetland.

- DBCA monitoring studies (https://www.dpaw.wa.gov.au/management/wetlands; Accessed 24 June 2020):
  - *Mapping Classification and Evaluation of Wetlands* (2006 - 2008), administered by the South West Catchments Council. DBCA surveyed water quality, phytoplankton, macroinvertebrates, waterbirds and vegetation at 25 wetlands between Mandurah and Augusta, including wetlands in the South-West SWA. The species datasets for the wetlands are not publicly available.
  - *Inland Aquatic Integrity Resource Condition Monitoring 2008* (DEC 2008a). The condition of 45 regionally significant wetlands throughout the state was surveyed in 2008, but only one wetland (the Vasse Estuary) within the South West SWA bioregion was included.

Online literature searches and queries fielded to DBCA Principal Research Scientist Adrian Pinder regarding monitoring project did not produce any additional relevant material for inclusion in this review. Similarly, enquiries to Dr Russell Shiel at University of Adelaide yielded no additional data on microinvertebrates in the south-west SWA bioregion.

The studies listed in Table 4 below cover a variety of freshwater ecosystems. While many freshwater aquatic fauna species have been recorded from both seasonal and permanent, lentic (still) and lotic (flowing) waters, there are distinct differences in assemblage composition, particularly among invertebrates, between these ecosystem types (see discussion in Box 1, below).

Box 1. Invertebrate fauna assemblage relationships to seasonality in aquatic ecosystems

Life history strategies of aquatic fauna are intrinsically linked to seasonality and predictability of flow regimes (Clifford 1966, Williams & Hynes 1977, Towns 1985, Boulton & Suter 1986, Boulton & Lake 1988, Boulton 1989, Bunn *et al.* 1989, Delucchi & Peckarsky 1989, Sheldon *et al.* 2002). Permanence of water has been found to be an overall determinant of the invertebrate fauna within aquatic ecosystems, such that streams and wetlands with seasonal or intermittent flows show distinctive communities compared to permanent waterbodies and flowing streams (ARL 1989; Storey *et al.* 1990). Species which inhabit ephemeral waterbodies must have strategies/adaptations to survive in systems which either dry out completely, or are reduced to a series of stagnant pools at certain times of the year. Despite these harsh environmental conditions, many invertebrates are found only in temporary waters (Bunn *et al.* 1989). In fact, Williams (1980) suggests that a number of invertebrates actually require a period of desiccation in order for further development to take place. In addition, biota are specifically adapted to the timing of drying and refilling cycles (Balla 1994).

Some species, including those that possess short maturation times, endure the dry season as terrestrial adult stages (*e.g.* mayflies, dragonflies, caddisflies and some beetles). Such species can be known as ‘temporary residents’ since they must reinvade each time a seasonal waterbody becomes inundated. Other species possess life history strategies which enable them to remain within a waterbody once surface waters have evaporated. Such taxa are known as ‘permanent’ residents. There are a number of strategies by which ‘permanent’ residents can survive in temporary environments. For example, some have drought-resistant stages such as spores, eggs, or larval stages (*e.g*. microcrustacea, shield shrimps, fairy shrimps, many species of nematode). Of the microinvertebrates, protozoans have cysts, rotifers have ephippia (resting eggs), cladocerans have diapausing eggs, copepods have nauplii (resistant early larval phase) and ostracods have resistant eggs. Most can survive extended periods of drought (Hairston *et al.* 1995). Other species are capable of burrowing into moist sediments, below stones, or into decomposing wood debris (*e.g*. nemerteans, oligochaetes, glossiphonid leeches, some species of chironomids, tabanids, and some mayflies). Many bivalves and gastropods are resistant to desiccation and those species which lack an operculum are able to seal their shells with a mucus plug, known as an epiphragm.

The relative success of each of the above strategies and subsequent recruitment and ecological succession will vary from year to year depending on a number of biotic (*e.g.* predator avoidance) and abiotic factors (*e.g*. weather - rainfall/evaporation). Therefore, large variations in community structure and composition may ensue in the absence of anthropogenic disturbance. Long-term temporal variation in aquatic communities has not been well-documented in Australia. In more permanent, less dynamic systems, temporal persistence may be high when assessed at family-level while at the same time, very low for individual species (Metzeling *et al.* 2002).

As such, any conservation significant fauna found in permanent or flowing seasonal ecosystems as part of these studies may not have suitable adaptations for survival in/re-colonisation of Tutunup Wetland which is seasonal, and therefore may not be expected to occur there. However, it was considered relevant to the desktop assessment to collate any records of lesser-known aquatic fauna, particularly microinvertebrates, in the South-West SWA bioregion, as published surveys of these fauna in this region are scarce and largely outdated (*i.e.* over a decade old), therefore their distributions and habitat preferences are not well documented.

The desktop assessment paid particular attention to microinvertebrate fauna, which have historically been under-studied in South-West SWA wetlands. Most aquatic fauna surveys of wetlands sample only macroinvertebrates and fish, despite the fact that microinvertebrates typically constitute around 45% of the total aquatic invertebrate fauna in any given wetland across Australia (Halse *et al.* 2002). The surveys conducted by WRM on behalf of Iluka between 2005 - 2008 at rural wetlands in this region, recorded several microinvertebrates that were previously unrecorded in the area, or were entirely new to science. This was despite the fact that wetland vegetation at all sites was assessed as moderately to heavily disturbed by historic pastoral practices. By contrast, the distributions and conservation status of other aquatic fauna groups, including macroinvertebrates, fish, crayfish, frogs, turtles and waterbirds, are comparatively well-understood, having been comprehensively studied for over 30 years through sampling conducted by the State's four main Universities (UWA, Murdoch, ECU & Curtin) and government environmental departments, to achieve what is currently known (WRM 2008c).

Table 4. Previous recent aquatic fauna surveys specific to the Tutunup Wetland local area / South-West SWA bioregion.

Field survey date	Report date	Report title	Author	Comments
Nov. 2005	Jan. 2006	Cloverdale Project: Baseline Aquatic Fauna and Water Quality Survey of the Ludlow River and Tiger Gully	WRM (2006a)	Potential relevant regional information
Nov. 2005	Jan. 2006	Elgin Project: Baseline Aquatic Fauna and Water Quality Survey of the Elgin Project Area	WRM (2006b)	
Nov. 2005	Apr. 2006	Burekup Project: Baseline Aquatic Biology and Water Quality Study	WRM (2006c)	
Nov. 2005	Apr. 2006	Tutunup South Project: Baseline Aquatic Biology and Water Quality Study	WRM (2006d)	
Jan. 2006	May 2006	Yoganup 215 Project: Baseline Aquatic Biology Study	WRM (2006e)	
Nov. 2007	Apr. 2008	Burekup, Tutunup South and Yoganup South Wetlands Aquatic Biology Surveys (Microinvertebrates)	WRM (2008a)	
Nov. 2007	Aug. 2008	Baseline Aquatic Biology Study of Gavin's Road Wetland	WRM (2008b)	
Aug. 2008	Nov. 2008	Tutunup Wetland Baseline Aquatic Biology Study	WRM (2008c)	Final report of aquatic biology survey of Tutunup Wetland including baseline data on macroinvertebrates, micro-invertebrates and tadpoles.
Nov. 2008	Apr. 2009	Collie River Values, including Henty Brook and Harris River	WRM (2009)	Potential relevant regional information
Nov. 2018	Apr. 2019	Bunbury Outer Ring Road Targeted Conservation Significant Aquatic Fauna Survey	WRM (2019)	Potential relevant regional information

Figure 6. Overview map of the Swan Coastal Plain (IBRA region code SWA) and the South-West region, with the locations of past aquatic fauna surveys indicated.

3.2.1 Tutunup Wetland Baseline Aquatic Biology Study - August 2008

Iluka contracted WRM to conduct a full wetland assessment of Tutunup Wetland study area in August 2008, to provide baseline data on aquatic macroinvertebrates, microinvertebrates (protists, rotifers, cladocerans, copepods and ostracods) and tadpoles (WRM 2008c). The invertebrate taxa ('species') recorded during the 2008 survey are provided in Appendices 4 and 5. Relatively low invertebrate diversity and abundance was recorded compared to the other rural wetlands surveyed in the region between 2005 and 2007 (see Table 4), though survey effort in 2008 was restricted by availability of surface water. At the time, it was considered that the study area may only support low diversity of aquatic fauna due to the highly seasonal nature of surface waters, which are unlikely to persist long enough for many freshwater species to complete their life-cycles (WRM 2008c).

A total of 10 microinvertebrate and 22 macroinvertebrate species were collected in samples taken from the small, shallow, isolated water bodies present in August 2008. Cyclopoid copepods were dominant among the microinvertebrate assemblage, while the amphipods *Austrochiltonia subtenuis* and *Perthia branchialis*, and in particular, several species of non-biting midge and mosquito larvae, were the most common species in the macroinvertebrate assemblage. It was assumed that the microinvertebrate and amphipod taxa that were present possess drought resistant eggs or resting stages that allow them to colonise temporary wetlands where the availability of surface water is unpredictable.

No microinvertebrate species listed for conservation significance at the state or Commonwealth level, were recorded. One species of protist found in the study area represented a new record for Australia, *Difflugia* cf *distenda*, however, this species is likely cosmopolitan, being previously undiscovered due to the low number of surveys done for microinvertebrates in Australia (WRM 2008c). Microinvertebrate expert Dr Russell Shiel, University of Adelaide, confirmed as of July 2020 that he has seen no further records of *Difflugia* cf *distenda* from Australian surveys. Further, Dr Shiel stated that he identified the specimen as 'cf', as it morphologically resembled *D. distenda*, but this Australian specimen may be genetically different to type specimen described from Switzerland.

One macroinvertebrate species listed for conservation significance was recorded; the freshwater snail *Glacidorbis occidentalis* (IUCN Vulnerable, DBCA Priority 3). The status and distribution of *G. occidentalis* is discussed further in section 3.3.1 below. Additionally, four specimens of a new species of epigean[1] dytiscid beetle, genus *Paroster*, were collected from the study area. Specimens were confirmed by Dr Chris Watts at the South Australian Museum as being new to science. As the species has not previously been recorded, the extent of its current distribution in south west WA is unknown. As of July 2020, it does not appear that the *Paroster* specimens from the study area have been formally described as a species, as literature and database searches of the University of Western Australia library OneSearch, the Western Australian and South Australian Museum websites, ALA, and AFD failed to find any record of a 'new' (*i.e.* post 2008 description) *Paroster* from surface waters in south west WA.

Tadpoles of the moaning frog *Helioporus eyrie*, the slender tree frog *Litoria adelaidensis* and the froglet *Crinia* sp. were abundant during August 2008. Froglets were too small to be positively identified but, based on known distributions, were most likely to be quacking froglets *Crinia georgiana*, squelching froglets *Crinia insignifera* and/or Glauert's froglet *Crinia glauerti*. All of the frog species positively identified, and the three possible *Crinia* species, are widespread and abundant throughout south west WA.

WRM (2008c) concluded that the occurrence of conservation-listed *G. occidentalis*, the good condition of remnant wetland vegetation and the presence of the Busselton Ironstone TEC, conferred a high conservation significance on Tutunup Wetland.

[1] Epigean = above ground and/or surface water species; in contrast to groundwater species.

3.2.2 Surveys of Other Seasonal Wetlands

Between 2005 and 2008, Iluka also contracted WRM to conduct aquatic ecosystem field surveys in other rural wetlands in the South-West SWA bioregion (Table 4). The methodologies were similar to WRM (2008), though additional fauna groups, such as fish, crayfish and waterbirds were also surveyed. More recently, in 2018, WRM has conducted targeted surveys for species of conservation significance in seasonal wetlands on behalf of Main Roads WA for the Bunbury Outer Ring Road infrastructure project, also located in the region. Key findings and species of interest identified in these studies are discussed below.

3.2.2.1 Tutunup South Project - November 2005

In November 2005, WRM sampled two coloured acidic MUW category seasonal wetlands within the Tutunup South mine footprint (approximately 10 km south-west of the current study area), and a seasonally-flowing creek along the south-east edge of the Tutunup South project area (WRM 2006c). Sampling included microinvertebrates, macroinvertebrates, fish, crayfish, frogs and waterbirds. The three survey sites had a more diverse and abundant micro- and macroinvertebrate assemblage compared to the Tutunup Wetland study area; a total of 98 invertebrate species, including 28 microinvertebrate and 70 macroinvertebrate species. Both wetland sites recorded 54 species, while the creekline recorded 37 species (microinvertebrates were virtually absent from the creek). Three microinvertebrate species collected from the wetlands were new to science, the notommatid rotifer *Cephalodella* n. sp., the cyclopoid copepod *Paracyclops* n. sp., and an indeterminate difflugiid rhizopod. Although these species have not previously been collected, they are likely to be more widely distributed in comparable habitats throughout Australia that historically have been under-sampled (Dr R. Shiel, University of Adelaide, pers. comm).

Also of scientific interest, was the synthemistid dragonfly *Archeosynthemis leachii*, being an uncommon endemic species with a restricted distribution in the southwest of Western Australia (WA). *A. leachii* was recorded from the seasonally-flowing creek site. *A. leachii* is likely a Gondwanan[2] relict species of insect, with specimens of synthemistid dragonflies evident in fossil records dating back to the early Permian period, approximately 290 million years ago (Chessman 1995). In south west WA, Gondwanan species are believed to be particularly at risk because they have specialised requirements and habitats that are usually topographically restricted and vulnerable to disturbance and fragmentation (Main 1996). Relict fauna has survived from an age that was typically more humid and wetter, with a less markedly seasonal climate than prevails today. As the climate became drier and environments fire-prone, relict fauna were increasingly restricted to specialised micro-habitats in damp, wetter regions (Main 1996).

Habitats that support a number of Gondwanan species are considered important and unique elements of the SWA bioregion; *i.e.* they have significant conservation value (Main 1996). They are typically more sensitive to current land management practices (*e.g.* frequent controlled burns) than are "later-evolved species" (Hopper *et al.* 1996, Main 1996). Relict fauna tend not to be well-represented in current reserves (Trayler *et al.* 1996). However, further surveys throughout south west WA may find Gondwanan species are more widespread in distribution, and may be more tolerant of environmental disturbance, than previously suspected. *A. leachii* likely has a preference for lotic (flowing water) habitats, rather than the lentic (still water) conditions present at Tutunup Wetland.

[2]Gondwanan: relict species from the southern super-continent Gondwana that existed from approximately 144 to 195 million years ago and included what is now Australia, Africa, Antarctica, South America, India, New Zealand and Madagascar.

Fish and crayfish were not recorded from the Tutunup South wetland sites. The frogs *Crinia insignifera* and *Litoria adelaidensis*, and waterbird *Threskiornis spinicollis* (straw-necked ibis), were the only vertebrate species recorded from the wetland sites, and none are listed at the state of Commonwealth level for conservation significance.

3.2.2.2 Elgin Wetlands - November 2005

Similar to the Tutunup South Project (section 3.2.2.1), WRM (2006a) sampled Elgin Wetlands, four seasonal wetlands approximately 18 km north of the current study area, on behalf of Iluka, in November 2005. Sampling again included aquatic microinvertebrate, macroinvertebrate, fish, crayfish, frogs and waterbirds. Results were similar to the Tutunup South study; a combined total of 93 micro- and macroinvertebrate species were detected. Seven taxa recorded were endemic to south west WA, and only one of these, the lepadellid rotifer *Lepadella oblonga*, was considered rare within south west WA. Though rarely collected *L. oblonga* is not listed as conservation significant. No fish, crayfish or frogs were detected, and all waterbirds species observed were common throughout south west WA.

3.2.2.3 Burekup Wetlands - November 2005

WRM (2006b) sampled two coloured, acidic wetlands and three flowing brook sites in the Burekup area, approximately 40 km north/north-east of the current study area, in November 2005, on behalf of Iluka. A combined total of 65 micro- and macroinvertebrate species were collected from the Burekup wetlands. Of scientific interest was the collection of a number of microinvertebrates from one of the wetland sites; the rotifers *Dicranophoroides caudatus* and *Filinia* cf. *passa*, which were the first records of these species in (WA), and an indeterminate Difflugiidae rhizopod, which was likely a species new to science, but additional specimens are required to confirm the taxonomy. No fish, crayfish or frogs were detected, and all waterbirds species observed were common throughout south west WA.

3.2.2.4 Microinvertebrate communities of south-west SWA Wetlands – November 2007

WRM (2008a) conducted targeted sampling for microinvertebrates in five coloured, acidic wetlands (two at Burekup, two at Tutunup South and one at Yoganup South) in November 2007, on behalf of Iluka. At least 83 species of microinvertebrate were identified during the 2007 surveys, yielding several new or potentially new species. Specimens of protists *Centropyxis* and *Difflugia*, collected from Yoganup South and Tutunup South, were unidentifiable from published keys and undoubtedly new. The cladocerans *Alona* cf. *rectangula novaezealandiae*, *Alona* sp. (valves with hexagons) and *Ceriodaphnia* sp., collected from Burekup, were also likely to be new species.

3.2.2.5 Bunbury Outer Ring Road targeted conservation significant aquatic fauna surveys – August 2018

WRM was contracted by consultants GHD, on behalf of Main Roads WA, to survey several wetlands in the Bunbury Outer Ring Road (BORR) investigation area, to determine the occurrence of any aquatic fauna considered to be of conservation significance (WRM 2019). These wetlands are located approximately 30km north of the current study area. One black-stripe minnow (*Galaxiella nigrostriata*), which is listed as Endangered by the DBCA (DBCA 2019) and Lower Risk/Near Threatened on the IUCN Redlist of Threatened Species (IUCN 2020), was recorded at one of the surveyed wetlands in November 2018. No other aquatic species listed as conservation significant were recorded from seasonal wetland habitats during this survey.

3.2.3 Surveys of Permanent Wetlands and Lotic Ecosystems

3.2.3.1 Cloverdale - January 2006

WRM (2006d) sampled perennial (permanent and semi-permanent) pool sites on the Ludlow and Capel Rivers, located approximately 4 to 8 km north of the current study area, in November 2005 and January 2006, for the Iluka Cloverdale project area. Microinvertebrates were not sampled on this survey. The survey recorded 135 macroinvertebrate species, three native freshwater crayfish species (smooth marron *Cherax cainii*, gilgies *C. quinquecarinatus* and koonacs *C. preissii*), three species of native fish (western minnow *Galaxias occidentalis*, nightfish *Bostockia porosa*, western pygmy perch *Nannoperca vitta*), and one introduced species of fish (gambusia *Gambusia holbrooki*). Of the fish and crayfish species, the only species with potential to occur in Tutunup Wetland are gilgies and koonacs, as these are the only species that can withstand seasonal drying. Gilgies and koonacs are common in many seasonal and ephemeral waterbodies throughout south west WA. While fish may opportunistically invade seasonal waterbodies that connect to permanent rivers and wetlands during winter, there appears to be no such seasonal connectivity for Tutunup Wetland.

Only one species listed as conservation significant was recorded; Carter's freshwater mussel *Westralunio carteri*, which was collected from the Capel River. *W. carteri* is listed as Vulnerable on both the IUCN Red List of Threatened Species (IUCN 2020) and the DBCA Threatened and Priority Fauna List (DBCA 2019). *W. carteri* is highly unlikely to occur in Tutunup Wetland, as it is restricted to permanent/semi-permanent stream and riverine habitats (Klunzinger 2012). There were also anecdotal (local landowner) that adults and larvae of conservation listed pouched lamprey *Geotria australis* (Priority 1, DBCA 2019) were seasonally common within the surveyed area. The chance that lamprey occur in Tutunup Wetland is considered negligible, as this species is anadromous (enters rivers from the ocean to spawn).

3.2.3.2 Yoganup 215 - January 2006

WRM (2006e) sampled two permanent artificial wetlands and the Ludlow River, approximately 1-2 km north-east of the current study area, in January 2006, for the Iluka Yoganup 215 project area. Of scientific interest was the collection of a cladoceran, *Alona* n. sp. from one of the artificial permanent wetlands, which was a species new to science. The collection of the water mite *Gretacarus* sp. from one of the wetlands was of scientific interest, being rarely recorded in south west WA. Also of scientific interest was the dragonfly nymph *Archaeosynthemis macrostigma*, recorded from the Ludlow River. *A. macrostigma*, like *A. leachii* (discussed above in section 3.2.2.1) is of scientific interest as it is also likely a Gondwanan relict species. Two native fish (western pygmy perch and nightfish) and two native crayfish species (smooth marron and gilgies) were also recorded.

3.2.3.3 Gavin's Road Wetland - November 2007

WRM (2008b) sampled the coloured, acidic MUW wetland at Gavin's Road, approximately 18 km north-east of the current study area, on behalf of Iluka in November 2007. At the time of sampling, surface water was present in one farm dam, one small shallow waterbody and an agricultural drainage channel that traversed the wetland. The microinvertebrate assemblage of this wetland was highly diverse, compared to the Tutunup Wetland sampling in August 2008, with 30 species recorded. One microinvertebrate species new to science was recorded, a notommatid rotifera of the genus *Notommata* n. sp. Additionally, 42 species of macroinvertebrates were recorded at Gavin's Road in November 2007, though none are listed as conservation significant. Smooth marron (*Cherax cainii*) were present in the drainage channel, and as marron are restricted to permanent waterbodies, it was considered that water likely persists here throughout the summer period (WRM 2008b). The greater diversity of aquatic invertebrates at Gavin's Road, compared to Tutunup Wetland in 2008, was considered due to greater persistence, depth and extent of water at Gavin's Road.

3.2.3.4 Collie River Values Assessment, including Henty Brook and Harris River

WRM (2009), on behalf of the Department of Water (now DWER), undertook sampling for macroinvertebrates, crayfish and fish in the Collie River, Harris River and Henty Brook, approximately 50 - 60 km north-east of the current survey area). Sampling was conducted in November 2008. The study reorded 129 macroinvertebrate species, two native freshwater crayfish species (marron and gilgies), five native freshwater fish species (western minnow, western pygmy perch, nightfish, freshwater cobbler *Tandanus bostocki* and Swan River gobies *Pseudogobius olorum*) and three introduced fish species (gambusia and redfin perch *Perca fluviatilis* and rainbow trout *Oncorhynchus mykiss*).

Only one conservation listed species was recorded, *Westralunio carteri* (Priority 3, DBCA 2019) which was collected from the Collie River and Henty Brook. A number of Gondwanan relict macroinvertebrate species were also collected, including the gripopterygid stonefly *Newmanoperla exigua*, the telephlebiid damselfly *Austroaeschna anacantha* and three dragonflies; the synthemistid *Austrosynthemis cyanitincta*, the oxygastrid *Hesperocordulia berthoudi* and the gomphid *Austrogomphus collaris*.

3.3 Occurrence of Conservation-Significant Aquatic Fauna in Tutunup Wetland

All aquatic fauna listed by DBCA as conservation significant (DBCA 2019), known to occur in the South-West region, were assessed for likelihood of occurrence in Tutunup Wetland study area (Table 5). Reference was made to the IUCN Red List of Threatened Species (IUCN 2020) as well as the DBCA Threatened and Priority Fauna List (provides listings for both the state *Biodiversity Conservation Act 2016* and the Commonwealth *Environment Protection and Conservation Act 1999*) (DBCA 2019). Reference was also made to other south west WA studies, as well as databases such as ALA, NatureMap, The Australian Faunal Directory, and in-house WRM database for distribution and occurrence information for all aquatic and wetland-associated terrestrial species.

Only one species listed as conservation significant was confirmed to occur in the current study area; the freshwater snail *Glacidorbis occidentalis* (DBCA Priority 3[3]) (Plate 1) (WRM 2008c). The desktop review identified one additional listed species listed with the potential to occur in the study area; the black-stripe minnow *Galaxiella nigrostriata* (EPBCA Act and DBCA Endangered). The study area is within the known distribution of this species and contains its preferred habitat. These species, their statuses and likelihood of occurrence in the study area are discussed in sections 3.3.1 and 3.3.2, below.

3.3.1 Freshwater Snail *Glacidorbis occidentalis*

A single specimen of the freshwater snail, *Glacidorbis occidentalis*, was recorded from the REW within the current study area in 2008 (WRM 2008c). *G. occidentalis* was not found at the other wetlands surveyed by WRM around that time period (Table 4). *G. occidentalis* is the only representative of this genus in WA, and is considered an indicator of fresh, temporary waterbodies (Bunn & Stoddart 1983, Bunn *et al.* 1989). This minute snail (shell diameter < 2 mm) is known to occur in both lentic and lotic habitats (Bunn & Stoddart 1983, Bunn *et al.* 1989, WRM 2008c). Adults survive dry periods by burrowing into sediment, sealing their shell with an operculum, and emerge after the first winter flows. It is widespread throughout the northern jarrah forest and has also been collected from the Warren bioregion and peat wetlands on the southern coast (Bunn *et al.* 1989). However, it usually occurs only in low numbers in scattered populations (Bunn *et al.* 1989), and because of this, it is currently listed at state level as Priority 3 on the

[3] DBCA Priority 3 species = poorly known species that are known from several locations, and the species does not appear to be under imminent threat, or from few but widespread locations with either large population size or significant remaining areas of apparently suitable habitat, much of it not under imminent threat. Species may be included if they are comparatively well known from several locations but do not meet adequacy of survey requirements and known threatening processes exist that could affect them. Such species are in need of further survey.

DBCA Threatened and Priority Fauna List (DBCA 2019), and as Vulnerable on the IUCN Redlist (IUCN 2020). *G. occidentalis* is also a Gondwanan relict species (Bunn & Stoddart 1983).

Plate 1. Freshwater snail *Glacidorbis occidentalis* (Ponder *et al.* 2016).

3.3.2 Black-Stripe Minnow *Galaxiella nigrostriata*

The black-stripe minnow (*Galaxiella nigrostriata*; Plate 2) is currently listed as Endangered at both the Commonwealth level (EPBC Act) and state level (DBCA 2019), and as IUCN Lower Risk/Near Threatened (IUCN 2020). This species is endemic to south west WA and rare throughout its distribution. Its main distribution lies within 100 km of the coast, between Albany and Augusta, with isolated populations known from further north, including Lake Chandala (Gingin), Melaleuca Park (Perth), and wetlands within the Kemerton Nature Reserve (Bunbury) (Morgan *et al*. 1998, Allen *et al.* 2002, Smith *et al*. 2002). The black-stripe minnow is restricted to shallow, tannin stained, ephemeral pools and are most common in waterbodies of peat flats (Morgan & Gill 2000). They are capable of aestivating (burrowing) into soils to survive dry summers and will appear in pools within hours following first rains. Interestingly, it does not have any specific anatomical, physiological, or behavioural adaptations to aid aestivation, and presumably survives within moist soils or crayfish burrows that contain water through the dry season.

Plate 2. Black-stripe minnow *Galaxiella nigrostriata* (photo WRM).

Most fish only live for one year, dying shortly after spawning (Morgan *et al*. 2011). It is thought that the populations in the SWA bioregion are remnants of a once wider distribution (Morgan *et al.* 1998, Smith *et al*. 2002), suggesting that the loss of habitat caused by urban and rural development during the previous hundred years has had a significant impact on this species. As such, their biggest threat is loss of suitable habitat through clearing/infilling for urbanisation and rural development.

The location of Tutunup Wetland is within the known distribution range of black-stripe minnow, and WRM recently found previously-undiscovered populations of black-stripe minnow in targeted surveys of seasonal wetlands on the SWA bioregion for the proposed BORR project, approximately 30 km north of the current study area (WRM 2019). Based on the desktop review, this species was considered to have only low-moderate chance of occurrence in the study area, given the short hydroperiod of the wetland, coupled with the (likely) shallow depth of soils over sheet ironstone that would restrict burrowing into soils to survive drying. While a soil depth of only 4 cm appears sufficient for this species, the soil temperature and moisture content must be maintained over summer by cool groundwater input, as *G. nigrostriata* is intolerant of temperatures > 26°C, with a preferred temperature of around 14.5°C (Smith *et al*. 2002).

Table 5. Conservation-significant aquatic fauna listed under the *Biodiveristy Conservation Act 2016*, with distributions within south west WA, and the likelihood of their occurrence at Tutunup Wetland, based on desktop review (WRM 2020).

Scientific name	Common name	WA conservation listing (DBCA 2019)	Likelihood of occurrence at Tutunup Wetland
Cherax tenuimanus	Margaret River hairy marron	Critically Endangered	No chance of occurrence. Restricted to Margaret River, > 50 km south-west of Tutunup Wetland (Department of the Environment 2020a).
Engaewa pseudoreducta	Margaret River burrowing crayfish	Critically Endangered	Highly unlikely to occur. Restricted to south-west of Busselton, > 30km of Tutunup Wetland (DEC 2008b).
Engaewa reducta	Dunsborough burrowing crayfish	Endangered	Highly unlikely to occur. Restricted to west of Busselton, > 30km of Tutunup Wetland (DEC 2008b).
Daphnia occidentalis	Water flea (Karri forests)	Priority 3	Highly unlikely to occur. Known only from a vegetated roadside swamp near Northcliffe, > 120 km south-east of Tutunup Wetland (DBCA NatureMap, latest record 1990; species described by Benzie 1986).
Glacidorbis occidentalis	Freshwater snail	Priority 3	Known to occur. Recorded from REW area of Tutunup Wetland in 2008 (WRM 2008c).
Westralunio carteri	Carter's freshwater mussel	Vulnerable	Highly unlikely to occur. Closest record ~ 8 km north of Tutunup Wetland (WRM 2006a). Restricted to permanent and seasonal stream and riverine habitats (Klunzinger 2012).
Galaxiella munda	mud minnow, western dwarf galaxias	Vulnerable	Unlikely to occur. Closest record ~ 20 km south-east of Tutunup Wetland. Prefer relatively undisturbed, permanent stream habitats (Department of Water and Environmental Regulation 2020a). Have occasionally been recorded from ponds, swamps and roadside drains (Gomon and Bray 2020).
Galaxiella nigrostriata	black-stripe minnow, black-striped dwarf galaxias	Endangered	Low-moderate chance of occurrence based on desktop review. Closest confirmed record 30 km north of Tutunup Wetland (WRM 2019), and other recent records of populations ~70 km to the south (Department of Water and Environmental Regulation 2020b). Known to inhabit seasonal, vegetated wetlands (Galeotti 2013).
Geotria australis	pouched lamprey	Priority 1	Highly unlikely to occur. Closest record ~ 12 km north of Tutunup Wetland, from Capel River (Morgan, Gill & Potter 1998). Restricted to riverine habitats with marine connections (Department of Water and Environmental Regulation 2020c).
Lepidogalaxias salamandroides	salamanderfish	Endangered	Unlikely to occur. Closest record ~ 20 km west of Tutunup Wetland (DBCA NatureMap, 2017 record). Known to inhabit seasonal, acidic, coastal heath wetlands (Department of Water and Environmental Regulation 2020d).
Nannatherina balstoni	Balston's pygmy perch	Vulnerable	No chance of occurrence. Closest record > 30 km south west of Tutunup Wetland (DBCA NatureMap, 1996 record). Likely restricted to near-coastal permanent/semi-permanent stream, riverine and wetland habitats (Department of Water and Environmental Regulation 2020e).
Geocrinia alba	white-bellied frog	Critically Endangered	Highly unlikely to occur. Restricted to Margaret River/Augusta area, > 50km south-west of Tutunup (Department of the Environment 2020b).
Geocrinia vitellina	orange-bellied frog	Vulnerable	Highly unlikely to occur. Restricted to ~6 km^2 area near the Blackwood River, > 50km south-west of Tutunup Wetland (Department of the Environment 2020c).

3.4 Candidate Priority Aquatic Fauna of South West WA

Pennifold (2018), under the direction of DBCA, developed a protocol for assessing how many freshwater invertebrates from the entire south west of WA, a broad area defined as west of a line between Shark Bay and Cape Arid, may be candidates for listing on the Western Australian Priority Fauna list, and provided an overview on a selection of those species for listing. Using DBCA records, a search was conducted to find species which only occurred west of a line between Shark Bay and Cape Arid, with restricted distributions. This process yielded a set of 49 aquatic invertebrate species, determined to be candidates for listing as priority species in need of further investigation. Out of this list, WRM identified a subset of nine species with habitat preferences that might include conditions similar to the current study area (*i.e.* were not restricted to permanent or lotic water), and were not restricted to one location (*i.e.* short-range endemic). These species are listed, along with their known distribution and nearest record of occurrence to the Tutunup Wetland study area, in Table 6.

Table 6. A subset of the candidate Priority aquatic fauna identified by Pennifold (2018), with habitat preferences that might include conditions similar to Tutunup Wetland, and their known distribution and nearest record to Tutunup Wetland.

Scientific name	Common name	Known distribution	Nearest record to Tutunup Wetland
Boeckella bispinosa	Copepod	10 known populations, distribution range from Perth to Frankland in WA, but has also been collected from Tasmania.	100 km to the east (DBCA NatureMap, record from 2008).
Boeckella geniculata	Copepod	7 known populations, distribution range from Perth to Northcliffe.	130 km to the south-east (DBCA NatureMap, record from 1998).
Sternopriscus wattsi	Diving beetle	Known from several records in both lentic and lotic seasonal habitats, from Nannup are to Albany area.	32 km to the south/south-east (DBCA NatureMap, record from 2006).
Rhantus simulans	Diving beetle	Widely distributed, from Perth to Albany, but rarely collected (six records).	100 km to the south-east (DBCA NatureMap, record from 2010).
Hygrobia wattsi	Screech beetle	Known from only 5 records between Collie and Albany.	46 km to the south (DBCA NatureMap, record from 1999).
Notonecta handlirschii	Backswimmer bug	Widely distributed, from Perth to Albany, but collections have been declining with few records since 1988.	140 km to the south-east (DBCA NatureMap, record from 2006).
Nannophya occidentalis	Western pygmyfly dragonfly	Large distribution range extending from Kalbarri to Esperance, but is rarely collected.	70 km to the north-east (DBCA NatureMap, record from 2006).
Lectrides sp. AV1	Long-horned caddisfly	Large distribution range extending from Northam area to Lake Muir area, but is rarely collected and considered at risk of extinction by Sutcliffe (2003).	140 km to the south-east (DBCA NatureMap, record from 1998).
Plectrocnemia eximia	Tube-making caddisfly	Distribution range from Harvey area to Albany area, but is rarely collected and considered worthy of IUCN listing by Sutcliffe (2003).	75 km to the north-east (ALA 2020, record from 1978).

4 IDENTIFIED KNOWLEDGE GAPS

A number of knowledge gaps are identified as part of the desktop review and priority issues are listed below:

1. Tutunup Wetland has only been sampled once, over a decade ago.

The conditions at the time of the WRM (2008c) sampling were not ideal for thorough sampling to occur, with only shallow, small waterbodies present. Additional species that inhabit the wetland may have evaded detection at that time, as rainfall conditions that particular year may have been unfavourable for colonisation of temporary residents or the emergence of permanent residents, or the maturation and dispersal of temporary residents may have occurred prior to sampling taking place, as the waterbodies receded. Timing of sampling presents a particular challenge for seasonal wetlands, due to the inherent uncertainties around the presence and persistence of surface water. Surface water presence is dependent on variables that are challenging to predict or characterise, such as climate patterns, underlying geology and surrounding landscape/catchment.

2. No comprehensive, coordinated sampling programs for temporary wetlands of the South-West SWA bioregion.

There have been no comprehensive, coordinated sampling programs for aquatic fauna in Tutunup Wetland, or other temporary wetlands of the South-West SWA bioregion. Most of the data collated for the current review are from once-off surveys conducted more than a decade ago. Once-off sampling of macro- and microinvertebrates from any wetland will produce a species list which is effectively a 'snapshot' of that time and place. Therefore, re-sampling the same wetland at another time will likely produce a different set of taxa. The microfauna, in particular, are cued to natural cycles, with emergence from resting stages dependent on the cues to which they are 'tuned', such as day-length, water chemistry, temperature, algal exudates or prey hormones. The speed of these species' replacements can be in hours or days (cf. Tan & Shiel 1993, for billabong microfauna in eastern Australia). Thus, it follows that the species recorded from Tutunup Wetland on the August 2008 sampling date would be progressively replaced by another suite of species over the following week(s) and season(s). Most of these species which are likely to be known taxa, but a proportion of which (commonly 10 - 15%) are likely to be unknown. There is insufficient historical information on Western Australian microinvertebrates to provide precise numbers, however in eastern Australia, Shiel *et al.* (1998) reported > 500 taxa of microinvertebrates from ca. 100 ephemeral wetlands sampled once, with > 300 species of rotifers and microcrustaceans recorded from one of these sites sampled intensively over an extended time frame. Evidence from southwest WA suggests that comparable diversity is present in WA wetlands (Pinder *et al.* 2005).

3. Past aquatic invertebrate surveys restricted to November or January

The majority of the aquatic invertebrate surveys discussed in this review, were conducted in November or January, which is towards the end of the spring season/well into summer. Many taxa may have completed their life cycles earlier in the year (*i.e.* August to October, following peak winter rainfall in July) and left the respective wetlands. Therefore, the sampling of wetlands in November and January may have resulted in lower diversities than sampling earlier in the spring season could have yielded, and some species may not have been detected.

4. Paucity of published data

There is a distinct lack of published data on aquatic fauna available for the South-West SWA bioregion, and sampling of seasonal wetlands in the South-West SWA that are comparable to Tutunup Wetland, does not appear to have occurred for over a decade. As such, very little information exists for the majority of aquatic invertebrate taxa in terms of their hydro-ecological relationships that would be required to help predict potential impacts of future hydrological change or disturbance by development. Replicated sampling across multiple years would be required, and would also be useful in characterising the ecology of seasonal wetlands. The implementation of comprehensive, coordinated sampling programs, and the publishing/uploading of data and records from once-off surveys, is limited by funding and resource constraints across industry, university, government and community sectors, and the hesitancy of some project proponents (including private industry and government) to share potentially sensitive information relating to developments. It is likely there are a range of studies on comparable wetlands in the South-West SWA, which are in 'grey literature' and unpublished reports and which are not publicly available.

5. Ecological values used to assign conservation significance for wetlands are based on species-level identifications

Most ecological values used to assign conservation significance for wetlands are based on species-level identifications, as individual species are listed under various legislation and policies, and not families or genera. As immature/damaged specimens of potentially significant species have been previously collected and identified to broad family groups for many records listed in NatureMap and ALA, it is likely the conservation significance of macroinvertebrate and microinvertebrate assemblages, and in turn individual species has been underestimated due to taxonomic resolution and data deficiency. This limits the ability to determine conservation significance of the individual wetlands and underestimates the actual conservation significance of associated habitats and assemblages.

5 FIELD SURVEY

Based on the literature available for the desktop review (section 3), and the fact that Tutunup Wetland has only been previously sampled once, in August 2008, Iluka requested WRM conduct an aquatic biology survey in 2020, to provide more comprehensive baseline data. Surveys were undertaken in winter 2020, between 4th and 5th of August.

5.1 Sampling Sites

Comprehensive sampling for water quality and aquatic fauna was undertaken at four sites (TUT1-5 to TUT1-8) in 2020 (Table 7 & Figure 1). Although sites sampled in August 2008 (TUT1-1 to 1-4; Table 7 & Figure 1) were targeted for the 2020 survey, none were inundated with water in August 2020. The area sampled within each site in 2020 was also broadened compared to 2008, to include all available aquatic habitats, with the aim of maximising the number of species collected. In addition, opportunistic sampling was conducted at two artificial dams; Tut1-9 (water quality only) and TUT1-10 (crayfish only), where surface waters were comparatively deep (Table 7 & Figure 1). The dam sites were sampled, respectively, to provide additional data on quality of surface waters with longer residence times, and as a potential refuge habitat for freshwater crayfish. Photographs of sites are provided in Appendix 1.

Table 7. Site locations for the 2008 and 2020 surveys.

Site Code	MGA GDA94 Zone 50		Description	Survey Date
	Easting	Northing		
TUT1-1	368478	6273791	Very shallow (max. depth 0.15 m) water beside wheel ruts within CCW	18/08/2008
TUT1-2	367898	6273811	Inundated sedges, very shallow (max. depth 0.05 m), within REW	18/08/2008
TUT1-3	367833	3273641	Inundated sedges, shallow (max. depth 0.25), within REW	18/08/2008
TUT1-4	367433	6273971	Small open waterbody (max. depth 0.5 m) within CCW	18/08/2008
TUT1-5	368537	6273862	Very shallow (max. depth 0.2 m) inundated vehicle access track, fringing vegetation within CCW	04/08/2020
TUT1-6	368336	6273885	Small, shallow (max. depth 0.4 m) open water body, vegetated on the edges, on boundary of CCW and REW	04/08/2020
TUT1-7	367538	6273483	Shallow (max. depth 0.3 m) inundated vegetation within REW	04/08/2020
TUT1-8	367235	6274078	Very shallow (max. depth 0.2 m) inundated vehicle access track, fringing vegetation within CCW	04/08/2020
TUT1-9	367895	6273241	Deep (max. depth 1.5 m) artificial dam within CCW	05/08/2020
TUT1-10	368603	6273757	Deep (max. depth 1.75 m) artificial dam within CCW	04/08/2020

5.2 Sampling Methods

5.2.1 Water Quality

Sites TUT1-5 to 1-9 were sampled for water quality. *In situ* spot measurements of dissolved oxygen, pH, temperature and turbidity were made using hand-held Wissenschaftlich-Technische-Werkstätten (WTW) field meters, at a depth of approximately 10 cm below the water surface. Undisturbed water samples were also collected from each of these sites for laboratory analyses of conductivity, major ions, alkalinity, dissolved metals, nitrogen, phosphorus and total suspended solids. All samples were collected from a depth of approximately 15 cm below the water surface. Samples for analysis of nitrogen, phosphorus and dissolved metals were filtered in the field through 0.45 µm Millipore nitrocellulose filters, with samplers wearing nitrile gloves at all times. 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 ChemCentre, Bentley, WA (a NATA accredited laboratory). All water quality variables measured are summarised in Table 8.

Values for dissolved metals and nitrate were compared against ANZG (2018) default guidelines values (DGVs) for toxicants for 95%, 90% and 80% species protection (Appendix 2). Use of DGVs for 99% species protection was not considered appropriate, as based on field surveys in 2008 and 2020, the wetland is considered to be slightly to moderately disturbed by historic agricultural practices and groundwater abstraction. For stressors, such as conductivity, pH, dissolved oxygen, temperature and turbidity, which typically display naturally high variability, ANZG (2018) recommend the use of local DGVs where available, or development of site-specific GVs. Where neither local DGVs nor site-specific GVs are available, as is the case for the Tutunup Wetland, ANZG (2018) recommend use of regional DGVs reported in ANZECC/ARMCANZ (2000), which are designed to protect at least 95% of species (Appendix 2). These have been applied for the current report.

Table 8. Water quality parameters measured, indicating units of measurement.

Parameter	Units	Parameter	Units
General		***Dissolved metals/metalloids***	
Electrical conductivity (EC)	µS/cm	Silver (Ag)	mg/L
pH	pH units	Aluminium (Al)	mg/L
Dissolved oxygen (DO)	% saturation	Arsenic (As)	mg/L
Dissolved oxygen (DO)	mg/L	Boron (B)	mg/L
Water temperature	°C	Barium (Ba)	mg/L
Total suspended solids (TSS)	mg/L	Beryllium (Be)	mg/L
		Bismuth (Bi)	mg/L
Ionic composition - Major ions		Cadmium (Cd)	mg/L
Sodium (Na)	mg/L	Cobalt (Co)	mg/L
Potassium (K)	mg/L	Chromium (Cr)	mg/L
Calcium (Ca)	mg/L	Copper (Cu)	mg/L
Magnesium (Mg)	mg/L	Iron (Fe)	mg/L
Chloride (Cl)	mg/L	Lithium (Li)	mg/L
Carbonate (CO_3)	mg/L	Manganese (Mn)	mg/L
Hydrogen carbonate (HCO_3)	mg/L	Molybdenum (Mo)	mg/L
Sulfate (SO_4)	mg/L	Nickel (Ni)	mg/L
Hardness (as $CaCO_3$)	mg/L	Lead (Pb)	mg/L
Alaklinity (as $CaCO_3$)	mg/L	Antimony (Sb)	mg/L
		Selenium (Se)	mg/L
Nutrients - Nitrogen and Phosphorus		Uranium (U)	mg/L
Nitrate (N_NO_3) mg/L	mg/L	Vanadium (V)	mg/L
Nitrite (N_NO_2)	mg/L	Zinc (Zn)	mg/L
Soluble Reactive Phosphorus (P_SR)	mg/L		
Total Nitrogen (total N)	mg/L		
Total Phosphorus (total P)	mg/L		

5.2.2 Aquatic Habitat Evaluation and Characterisation

Qualitative visual observations of habitat characteristics were made at the five sites sampled for water quality (TUT1-5 to TUT1-9), to assist in interpreting any patterns in species assemblages. 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. General observations regarding the condition of wetland habitat and disturbance were made, with site photographs taken.

5.2.3 Aquatic Invertebrates

Sites TUT1-5 to TUT1-8 were sampled for aquatic invertebrates. Aquatic invertebrate sampling methods were in accord with those used by WRM (2008c) for the 2008 survey, and for all previous aquatic biology studies by WRM in the region (see Table 4). These methods have been extensively employed for numerous other wetland assessments conducted on behalf of the DBCA and the Department of Water and Environment (DWER) (refer Halse & Storey 1996, Storey & Humphrey 1996, Halse *et al*. 2000, Storey & Humphrey 1996, Storey *et al*. 1993, 2004a,b, Lynas *et al*. 2006, WRM 2018). Dr Russell Shiel (University of Adelaide), one of Australia's foremost experts on microinvertebrates, was consulted on specific techniques for sampling microinvertebrates. Dr Shiel also performed all microinvertebrate identifications for the current study and 2008 study, and for all previous surveys conducted by WRM for Iluka, allowing direct comparison of taxonomy across surveys. Dr Shiel provided advice on conservation significance. Dr Adrian Pinder (DEC Woodvale) and Dr Ralf Meisterfeld (Institut for Biology II, Aachen, Germany) provided further advice on biogeography and taxonomy of microfauna for the 2008 study (WRM 2008c). *Paroster* (Coleoptera: Dytiscidae) specimens collected in 2008 and 2020 were sent to Dr Chris Watts (South Australian Museum) for confirmation. The late Dr Don Edward (University of Western Australia) was contracted for identifications of all Chironomidae (Diptera) collected in 2008, and Dr Edward's vouchered specimens used for in-house confirmation of specimens recorded in 2020.

5.2.3.1 Microinvertebrates

Microinvertebrates (zooplankton) were collected from the water column using a 53 µm mesh plankton net to sweep over a standard 20 m x 0.3 m area within each site in order to provide a semi-quantitative measure of richness and abundance. Samples were preserved in 70% ethanol for laboratory enumeration and identification. External specialist taxonomist, Dr Russell Shiel (University of Adelaide) was sub-contracted to assist with microinvertebrate taxonomy. Microinvertebrate 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 and enumerated to total sample density (cells/ml). Where specific names could not be assigned, vouchers were established. These vouchers are held by Dr Shiel at The University of Adelaide.

5.2.3.2 Macroinvertebrates

A 250 µm Freshwater Biological Association (FBA) 'D' frame style dip net was used to selectively collect benthic macroinvertebrates, and involved kick-sweep sampling over an equivalent 50 m x 0.3 m area within each site in order to provide a semi-quantitative measure of richness and abundance. All mesohabitats 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, leaf litter and other debris, with any large coarse material (*i.e.* leaves, roots etc.), carefully washed in the sieve to remove attached fauna and discarded. Samples were then transferred to a 1L polypropylene container and preserved in 70% ethanol for laboratory enumeration and identification.

In the laboratory, each sample was sorted into different size fractions (1 mm, 500 µm and 250 µm) by washing through a series of sieves. Each size fraction was then sorted under high-power microscope to remove a maximum of 40 specimens of each family (or sub-family for Chironomidae). All specimens were identified to the lowest taxonomic level practicable (typically species or genus) and enumerated to $\log_{10}$ scale abundance per sample for all fractions combined (*i.e*. 1 = 1 individual, 2 = 2 - 10 individuals, 3 = 11 - 100 individuals, 4 = 101 - 1,000 individuals, *etc*.). In-house expertise was used to identify invertebrate taxa using available published keys and through reference to the established voucher collections held by WRM. Taxa that could not be identified to species level generally were assigned a voucher number and lodged in the WRM voucher collection.

5.2.4 Fish and Crayfish

Sites TUT1-5 to TUT1-8 and TUT1-10 were sampled for fish and crayfish. It was originally intended to use a range of sampling methods to survey for fish and crayfish, including dip nets, box traps, fyke nets, and visual observation. However, surface water across much of the wetland was too shallow to deploy box traps and fyke nets. Fine mesh dip nets (500 mm x 500 mm opening x 450 mm deep and 3 mm mesh) were used, by sweeping through the water to encompass a variety of different habitat types. In deeper water at TUT1-2 and TUT1-10, five fine mesh box traps (21 H x 47 W x 60 L cm, 3 mm mesh, mesh slit opening) were set overnight as water levels were deep enough to enable them to be fully submerged. Traps were baited with a mix of cat biscuits and chicken pellets. Two fyke nets comprising a double 10 m leader/wing (7 mm mesh, 1.5 m drop) and a 5 m hooped net (75 cm diam. semi-circular opening, 10 mm mesh) were also set at these sites overnight. Fyke nets were set at a 45^{o} angle to the water edge to create a complete barrier to fish passage at each location. A floating platform was placed at the cod-end (closest to the bank) to provide an air space for any freshwater turtles, or water rats if present.

All species were identified in the field, measured by standard length[4] (SL mm, for fish) or carapace length (CL mm, for crayfish) and then released alive. Any introduced species collected (*i.e.* yabbies *Cherax destructor*) were euthanised humanely on site in an ice slurry in an esky. Fish nomenclature followed that of Allen *et al.* (2002).

5.3 Sampling Permits

This study was conducted under Fisheries Licence EXEM 3407 (Instruments of Exemption to the Fish Resources Management Act 1994 for Scientific Research Purposes), and DBCA Licence 27000282 (Reg 27; 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.

5.4 Data Analysis

All data collected were entered into Excel spreadsheets. Other data generated as part of the project included:

- an IBSA data package in line with 'Instructions – IBSA Data Packages' (EPA 2018);
- all field data and spatial data captured in a suitable format (GIS shapefiles, Excel spreadsheets, *etc.*).

5.4.1 Assessment of Conservation Status of Fauna

Conservation significant species were identified as those:

- listed under the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act),
- listed under the WA *Biodiversity Conservation Act 2016* (BC Act) as Threatened or Priority species, as listed on the DBCA Threatened and Priority Fauna List (DBCA 2019),

[4] Standard length (SL) = tip of the snout to the posterior end of the last vertebra (*i.e.* this measurement excludes the length of the caudal fin). Carapace length (CL) = anterior tip of the rostrum to the posterior median edge of the carapace.

- listed as Near Threatened, Vulnerable, Endangered or Critically Endangered on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (IUCN 2020),
- listed on the Australian Society for Fish Biology Conservation List (ASFB 2019),
- potential or known short range endemic (SRE) freshwater invertebrate species, that have naturally small distributions of less than 10,000 km^2 (after Harvey 2002), as described by the EPA (2016c) for the purposes of environmental impact assessment, and/or
- stygofauna (groundwater) species that are also potential or known short range endemic (SRE) species, as described by the EPA (2016b) for the purposes of environmental impact assessment.

As discussed in sections 3.1 and 4, few aquatic invertebrates, and microinvertebrates in particular, are formally listed under the EPBC Act or BC Act as conservation significant due to data deficiency. Because of this, the current report includes specific mention of Candidate Priority species as listed by Pennifold (2018) (see section 3.4 above), and species of scientific interest, being rare or restricted in distribution, rarely collected, or new to science, but not formally listed as conservation significant. For some species the fact they are rarely collected or new to science, may merely reflect under-sampling, but for others there is the potential of shortrange endemism, which puts them at higher risk from disturbance and makes them more vulnerable to extinction. Known distributions and occurrence of species of scientific interest were determined from The Australian Faunal Directory (AFD), The Australian National Insect Collection Database, the Atlas of Living Australia (ALA), the WRM in-house database, and, for microinvertebrates, the database of Dr Russel Shiel (University of Adelaide).

5.4.2 Data Descriptives

Community summaries were derived for number of taxa (*i.e*. taxa richness) and number of specimens per sample (*i.e*. taxa abundance). In this context, "taxa" includes invertebrate specimens which could not be identified to species level, due to unresolved taxonomy and/or immaturity of specimens. Therefore, the total microinvertebrate and macroinvertebrate taxa richness is likely greater than reported here. The original intent for data analysis was to source data for other similar wetlands to demonstrate similarity/dissimilarity between the Tutunup Wetland fauna and that of other rural wetlands within the region. However, no available recent data were identified for other such wetlands in the region (see section 3). Therefore micro- and macroinvertebrate taxa richness data was compared with that for other seasonal wetlands in the region previously surveyed by WRM in November 2005 and 2007 (section 3.2). WRM data included that for Burekup B3 and B9, Elgin E1, E3, and E4, Gavin's Road, Tutunup South T1 and T2, and Yoganup South Y7 and Y8 (WRM 2006a-c, 2008a-b). This was in acknowledgement that these wetlands were considered to be more disturbed than the current study area and differ in aquatic fauna habitat, and therefore likely to differ in invertebrate species composition.

Combined taxa richness for all micro- and macroinvertebrates was also categorised according to the reference ranges used by DBCA for wetland biodiversity monitoring (see Cale *et al*. 2010). Again, this is in acknowledgement that these reference ranges are not specific to the SWA bioregion, but were developed for monitoring wetlands in increasingly salinised landscapes. The ranges were first used by Sim *et al.* (2008) as part of a trial of national indicators of wetland condition and originally developed by Jones *et al*. (2009). The reference ranges were developed using the 25th and 75th percentile richness values from wetlands surveyed by DBCA that were categorised by DBCA as near natural:

	Taxonomic Richness Reference Range		
	High condition	Medium condition	Low condition
Micro- + Macroinvertebrate taxa richness:	> 54	27 - 54	< 27
Macroinvertebrate taxa richness:	> 35	18 - 35	< 18

The reference ranges are intended to be applied at a single point in time. Ideally this point in time should correspond to peak water level in winter-spring.

5.4.3 Multivariate Analyses

Multivariate clustering and ordination 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 highlight the similarity/dissimilarity in the overall microinvertebrate and macroinvertebrate community assemblages between the study area and the other regional wetlands previously surveyed by WRM (2006a-e, 2008a-c), for which there were available data. Hierarchical clustering and non-metric Multi-Dimensional Scaling (nMDS) ordination were based on Bray-Curtis similarity measures for taxa abundance ($\log_{10}$ class) data. nMDS plots were constructed to visualise differences between wetlands. Similarity Profile Analysis (SIMPROF) was undertaken within the cluster analysis to test for significant differences among wetlands. SIMPROF analysis examines whether the similarities observed in the data (Bray-Curtis similarities) are smaller or larger than those expected by chance. Significant SIMPROF groupings were overlain on the ordination plots.

6 RESULTS AND DISCUSSION

6.1 Water Quality

Water quality data for the previous (2008) and current (2020) survey are summarised in **Error! Reference source not found.**, and the laboratory report for 2020 samples provided in Appendix 3.

Values for water quality parameters indicated high spatial variability, likely reflecting different residence time of surface waters, and possible variability in geology across the wetland. Waters appeared highly tannin-stained, with generally acidic pH (pH 5.66 - 6.66), low alkalinity (3 - 16 mg/L, as $CaCO_3$) and conductivity (229 - 605 μS/cm), and variable turbidity (1 - 39 NTU) and dissolved oxygen (10.8 - 126.0% saturation). Conductivity in the artificial dam at TUT1-9 (1,930 μS/cm), and pH in inundated vehicle track at TUT1-8 (pH 7.3) close to Tutunup Road, was also relatively high compared to all other sites sampled. The higher conductivity at TUT1-9 was possibly due to enhanced accumulation of salts by evaporation over a period of years/decades in the dam, though it may also reflect upwelling of saline groundwater at this location. The higher pH at TUT1-8 was likely due to lower abundance of humic substances (gilven) at this particular location, given that coloured (tannin-stained wetlands) such as Tutunup Wetland, tend to have naturally lower pH (< 6.5) due to dissolved humic substances that originate from breakdown of plant material (Davies *et al.* 1993). The pH of temporary wetlands varies seasonally, decreasing as the wetland dries, and as primary productivity declines, and increasing again as the wetland fills (Davis *et al.* 1993). Comprehensive component analysis of water samples taken in 2020 indicated ionic composition at all sites was dominated by sodium cations (Na^+) cations and chloride anions (Cl^-), with magnesium cations (Mg^{2+}) and sulfate anions (SO_4^{2-}) subdominant.

Conductivity at TUT1-9 exceeded the ANZG (2018) DGV in 2020, while pH and dissolved oxygen exceeded DGVs at the majority of sites on occasions (Table 9). Exceedance of DGVs for stressors, such as conductivity, pH and dissolved oxygen, was not unexpected. ANZG (2018) note that it was not possible to develop universal DGVs for stressors that apply equally to all regions of Australia, and recommend that site-specific guidelines are developed wherever possible.

For parameters sampled in both years, the main differences between 2008 and 2020 sampling occasions, were the generally lower conductivity (except TUT1-9) and dissolved oxygen for sites sampled in August 2020 (typically ≤ 385μS/cm EC; ≤ 81%DO), compared to sites sampled in August 2008 (600 - 605 μS/cm EC; 120 - 124% DO) (Table 9).

Background levels of dissolved aluminium exceeded the 80% species protection DGV (0.15 mg/L) at three of the five sites sampled in 2020 (TUT1-5 to 1-7) and exceeded the 90% species protection DGV (0.08 mg/L) at TUT1-9 (Table 9). Note that ANZG (2018) provide only a low reliability DGV for aluminium at pH < 6.5 (Appendix 2). Relatively high background levels of dissolved iron were recorded at TUT-2 (2.6 mg/L) compared to DGV (0.7 mg/L), though ANZG (2018) do not provide a DGV for iron. The reason for the elevated aluminium and iron is unknown, though comparatively high levels of aluminium and iron are commonly associated with acidic wetlands (Sammut & Lines-Kelly 2000), and elevated iron, at least, may merely be indicative of the ironstone soils of the study area. Elevated concentrations of aluminium have previously been recorded for some Burekup (0.094 - 0.17 mg/L) and Elgin wetlands (0.14 - 0.41 mg/L) (WRM 2006a-b). It is not known if the elevated aluminium reflects local geology or is due to input from agricultural sources such as fertiliser runoff.

Concentrations of other dissolved metals were low, and nitrogen and phosphorus concentrations well within concentrations expected for south-west wetlands in rural and semi-rural catchments of the SWA bioregion (Davis *et al*. 1993).

Table 9. Surface water quality data measured in conjunction with aquatic fauna sampling in August 2008 (TUT1-1 to 1-4) and August 2020 (TUT1-5 to 1-9). Highlighting indicates exceedance of ANZG (2018) default guideline value (DGV) for various levels of species protection. Refer Appendix 2 tables for DGVs.

☐ > default 95% DGV (and/or lower for DO and pH), ☐ > default 90% DGV, ☐ > default 80% DGV

Tutunup Wetland surface water quality									
Parameter	TUT1-1	TUT1-2	TUT1-3	TUT1-4	TUT1-5	TUT1-6	TUT1-7	TUT1-8	TUT1-9
Date	18/08/2008	18/08/2008	18/08/2008	18/08/2008	04/08/2020	04/08/2020	04/08/2020	04/08/2020	05/08/2020
Time (hrs)	10:00	11:00	13:00	12:00	13:00	14:30	12:00	14:00	08:15
Max. water depth (m)	0.15	0.05	0.25	0.50	0.2	0.4	0.3	0.2	1.5
pH (pH units) (field)	6.01	6.00	6.12	6.14	6.66	6.56	5.66	7.30	6.36
DO (%) (field)	124.0	123.0	120.0	121.0	77.4	81.0	36.2	126.0	10.8
DO (mg/L) (field)	11.0	11.0	10.5	10.5	7.55	7.91	3.82	11.76	1.25
Temp. (°C) (field)	18.0	19.11	19.5	19.5	15.00	17.00	12.60	17.00	12.10
EC (μS/cm) (field)	604	600	600	605	229	240	385	345	1930
Turbidity (NTU) (field)	-	-	-	-	39.22	31.61	1.0	1.14	5.79
TDS_calc (mg/L)	-	-	-	-	150	130	210	200	1100
TSS (mg/L)	-	-	-	-	63	16	4	<1	85
Alkalinity (as $CaCO_3$)	-	-	-	-	6	16	6	7	3
Hardness (as $CaCO_3$)	-	-	-	-	25	20	39	36	160
Ca (mg/L)	-	-	-	-	1.7	2.3	2.2	2.8	10.7
K (mg/L)	-	-	-	-	3.3	3.2	1.9	4.3	12.7
Mg (mg/L)	-	-	-	-	5.1	3.5	8.2	7	32.2
Na (mg/L)	-	-	-	-	33	36	55.8	45.5	309
HCO_3 (mg/L)	-	-	-	-	7	19	7	8	4
Cl (mg/L)	-	-	-	-	67	60	99	96	588
S_SO_4 (mg/L)	-	-	-	-	12.5	4.8	26.1	17.2	73.5
CO_3 (mg/L)	-	-	-	-	<1	<1	<1	<1	<1
N_NH_3 (mg/L)	-	-	-	-	<0.01	<0.01	0.02	<0.01	0.02
N_NO_2 (mg/L)	-	-	-	-	0.01	<0.01	<0.01	<0.01	<0.01
N_NO_3 (mg/L)	-	-	-	-	0.01	0.03	0.02	<0.01	0.02

Tutunup Wetland surface water quality									
Parameter	TUT1-1	TUT1-2	TUT1-3	TUT1-4	TUT1-5	TUT1-6	TUT1-7	TUT1-8	TUT1-9
Date	18/08/2008	18/08/2008	18/08/2008	18/08/2008	04/08/2020	04/08/2020	04/08/2020	04/08/2020	05/08/2020
Time (hrs)	10:00	11:00	13:00	12:00	13:00	14:30	12:00	14:00	08:15
N_NOx (mg/L)	-	-	-	-	0.02	0.03	0.02	<0.01	0.02
N_total (mg/L)	-	-	-	-	1.1	1.1	1.4	0.5	1.9
P_total (mg/L)	-	-	-	-	0.012	0.023	0.053	0.019	0.034
P_SR (mg/L)	-	-	-	-	<0.01	<0.01	0.02	<0.01	0.01
Ag (mg/L)	-	-	-	-	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Al (mg/L)	-	-	-	-	0.23	0.19	0.72	0.035	0.14
As (mg/L)	-	-	-	-	<0.001	<0.001	<0.001	<0.001	<0.001
B (mg/L)	-	-	-	-	0.03	0.02	0.02	0.02	0.04
Ba (mg/L)	-	-	-	-	0.023	0.017	0.012	0.023	0.058
Be (mg/L)	-	-	-	-	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Bi (mg/L)	-	-	-	-	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Co (mg/L)	-	-	-	-	0.0003	0.0003	0.0005	<0.0001	0.0033
Cr (mg/L)	-	-	-	-	0.0006	0.001	0.0014	<0.0005	<0.0005
Cu (mg/L)	-	-	-	-	0.0008	0.0019	0.0009	0.0007	0.0017
Fe (mg/L)	-	-	-	-	0.82	2.6	0.92	0.059	0.82
Li (mg/L)	-	-	-	-	0.0014	0.001	0.0019	0.0041	0.0075
Mn (mg/L)	-	-	-	-	0.016	0.012	0.029	0.006	0.065
Mo (mg/L)	-	-	-	-	<0.001	<0.001	<0.001	<0.001	<0.001
Ni (mg/L)	-	-	-	-	0.001	0.002	0.001	<0.001	0.002
Pb (mg/L)	-	-	-	-	0.0002	0.0003	0.0004	<0.0001	<0.0001
Se (mg/L)	-	-	-	-	<0.001	<0.001	<0.001	<0.001	<0.001
U (mg/L)	-	-	-	-	0.0002	0.0001	0.0002	<0.0001	<0.0001
V (mg/L)	-	-	-	-	<0.005	<0.005	<0.005	<0.005	<0.005
Zn (mg/L)	-	-	-	-	0.007	0.009	0.008	0.002	0.016

6.2 Aquatic Habitat Evaluation and Characteristics

As described by WRM (2008), much of the natural aquatic habitat of the REW within the study area, and of the REW and MUW adjacent the study area (Figure 1), has been disturbed or lost due to vegetation clearing for agriculture and/or road and rail construction. Based on visual observation, there appeared to be little change in habitat, other than the greater spatial extent of surface waters in August 2020, compared to August 2008. Remnant aquatic habitat and wetland vegetation was dominated by dense sedgelands on seasonally inundated soils and open to moderately dense myrtaceous shrublands on seasonally waterlogged soils (see photos Appendix 1). In 2020, as in 2008, the overall condition of aquatic habitats within the CCW areas was considered to be slightly to moderately disturbed, with fringing paperbarks, healthy remnant thickets of myrtaceous shrubs, shallowly inundated sedges and seasonally dry open waters still offering a good diversity of aquatic habitats. Habitats sampled at TUT1-5 and TUT1-8 were highly disturbed as these were immediately adjacent to, and within, vehicle access tracks, and as such, cleared of vegetation (Appendix 1). Similarly, the artificial dam site TUT1-9 was also classified as highly disturbed.

The wetland vegetation appears more structurally complex than at the other regional wetlands surveyed by WRM between 2005 and 2007 (section 3.2.2). Burekup and Elgin wetlands, in particular, are heavily degraded by clearing and cattle grazing and have no remnant understorey vegetation. At Tutunup Wetland, there is also the potential to improve or restore habitat linkages with other remnant vegetation units to the east and south-east.

All sites sampled in 2020 were dominated by sand substrates (Table 10). Habitat diversity was high, with most sites supporting five of the eight broad habitat types (Table 11). Lowest habitat diversity was at the dam site TUT1-9, where water depth was approximately 1.5 m and wetted width approximately 12 m. Average water depth at other sites was 0.2 - 0.4 m (Table 9).

Table 10. Substrate composition; percentage cover by bedrock, boulders, cobbles, pebbles, gravel, sand, silt and clay at each site in August 2020.

Site	Bedrock (%)	Boulder (%)	Cobble (%)	Pebble (%)	Gravel (%)	Sand (%)	Silt (%)	Clay (%)
TUT1-5	0	0	0	10	10	80	0	0
TUT1-6	0	2	3	15	15	50	0	0
TUT1-7	0	0	0	0	0	100	0	0
TUT1-8	0	0	10	10	30	50	0	0
TUT1-9	0	0	0	0	0	100	0	0

Table 11. Aquatic habitat diversity as a percentage cover at each site in August 2020.

Site	Mineral (%)	Emergent Vegetation (%)	Submerged Vegetation (%)	Floating Vegetation (%)	Algae (%)	Detritus (%)	Trailing Vegetation (%)	Large Woody Debris (%)	Habitat Diversity Score
TUT1-5	25	10	0	0	0	15	40	10	5
TUT1-6	25	30	0	0	0	10	30	5	5
TUT1-7	15	40	10	0	10	10	10	5	7
TUT1-8	25	30	0	0	0	10	30	5	5
TUT1-9	45	0	0	0	0	50	0	5	3

6.3 Microinvertebrates

6.3.1 Species Richness and Taxonomic Composition

A systematic list of all microinvertebrate taxa recorded in August 2008 and August 2020 is provided in Appendix 4.

A total of 21 microinvertebrate taxa were recorded from the four sites sampled in 2020, compared with 10 microinvertebrate taxa recorded from the four sites sampled in 2008. Combined microinvertebrate taxa richness for 2008 and 2020 was 27. Microinvertebrates comprised 44% of all invertebrates (*i.e*. microinvertebrates and macroinvertebrates) recorded for the study area. This was in accordance with the findings of Halse *et al*. (2002) that microinvertebrates typically constitute around 45% of the total aquatic invertebrate fauna in Australian wetlands.

Spatial and temporal variability in microinvertebrate species richness in the current study area was high, similar to results for other seasonal wetlands in the region surveyed by WRM in 2005 and 2007 (section 3.2.2). Relatively low taxa richness was recorded at TUT1-7 (4 taxa) within the REW area, and at TUT1-5 (6 taxa) and TUT1-8 (7 taxa) within the CCW areas. Notably higher species richness was recorded at TUT1-6 (18 taxa) on the boundary of REW and CCW areas (Appendix 4). It was not possible to examine between-site variability in taxa richness for the 2008 samples, as all four samples collected in 2008 were combined into one composite sample in the field, rather than processed as individual sites. Six taxa recorded in 2008 were not recorded in 2020, and 17 taxa recorded in 2020 were not recorded in 2008. Singletons (*i.e.* those taxa recorded from only one site) constituted 66% of all microinvertebrates recorded for the study area.

Combined taxa richness for 2008 and 2020 (27) was within the range for other regional wetlands similarly surveyed by WRM over two occasions, *i.e.* Tutunup South wetlands T1 (20) and T2 (36), Yoganup South Y7 (38) and Y8 (30), and Burekup B3 (39) (section 3.2.2). Taxa richness in 2020 (21) was also notably higher than recorded for the three more disturbed Elgin wetlands which were surveyed on one occasion; E1 (15), E3 (14) and E5 (5) (section 3.2.2). The lower richness at the Elgin wetlands was not unexpected as these wetlands are largely devoid of native vegetation and as such have low aquatic habitat diversity.

Rotifera were the most abundant group of microinvertebrates present in the study area in 2020, with six taxa, dominated by the families Arcellidae and Centropyxidae. The next most abundant groups were, Copepoda and Protista, each represented by five taxa (Table 12). By contrast, in 2008, when there was little surface water, cyclopoid copepods were the most abundant group present (Table 12).

Table 12. Summary of higher-order microinvertebrate taxa composition from the study area; *n* = number of sites sampled. Refer Appendix 4 for full taxa list.

Microinvertebrates		Number of Taxa	
Scientific name	Common name	2020 (*n* = 4)	2008 (*n* = 4)
Protista	Protists	5	1
Rotifera	Rotifers	6	1
Cladocera	Water fleas	2	1
Copepoda	Copepods	5	5
Ostracoda	Seed shrimp	3	2
Total taxa richness		**21**	**10**

One of the few other studies of coloured acidic wetlands is that by Pusey and Edwards (1990), who surveyed micro-crustacea (copepods, ostracods & cladocerans) at eight relatively undisturbed, seasonal wetlands on peat flats in the Warren (WAR) IBRA region, on the south coast. Pusey and Edwards (1990) recorded similar micro-crustacea species richness to that recorded for the current study area; *i.e.* between one and six species of Ostracoda, three to four species of Copepoda and two to five species of Cladocera. Identification of other taxonomic groups such as Rotifera and Protista that typically dominate south-west microinvertebrate communities, was not undertaken as part of the study by Pusey and Edwards (1990).

However, micro-crustacea taxa richness was low compared to the proportional range documented by Davies *et al.* (1993) for seasonal wetlands to the north of the South-West SWA bioregion (see section 3). In these wetlands, micro-crustacea appear to constitute approximately 19 to 35% of all invertebrates (micro- and macroinvertebrates). This includes the only coloured, acidic seasonal wetland sampled by Davies *et al*. (1993); Banganup Lake (21%), within Harry Waring Nature Reserve in the city of Cockburn. In contrast, micro-crustacea constituted 12% of all invertebrates collected from the current study area. The lower proportional representation of micro-invertebrates was likely due to the very short hydroperiod. Davis *et al*. (1993) also note that coloured wetlands, such as the current study area, support a distinctive composition of both invertebrate and algal communities to those of other wetlands, yet are not typically considered in wetland management plans.

Multivariate analyses were undertaken to illustrate the differences in microinvertebrate taxa assemblages between the study area and the other more disturbed wetlands sampled by WRM. Results for both cluster and nMDS ordination analyses (Figure 7) showed similar patterns, with a separation of samples into four broad groups; i) 2020 study area samples, ii) 2008 study area sample, iii) Burekup and Elgin, and iv) all other wetlands. As expected, there was a clear separation between the 2008 and 2020 samples for the study area, due to the limited habitat availability in 2008, and resultant low species richness and abundance.

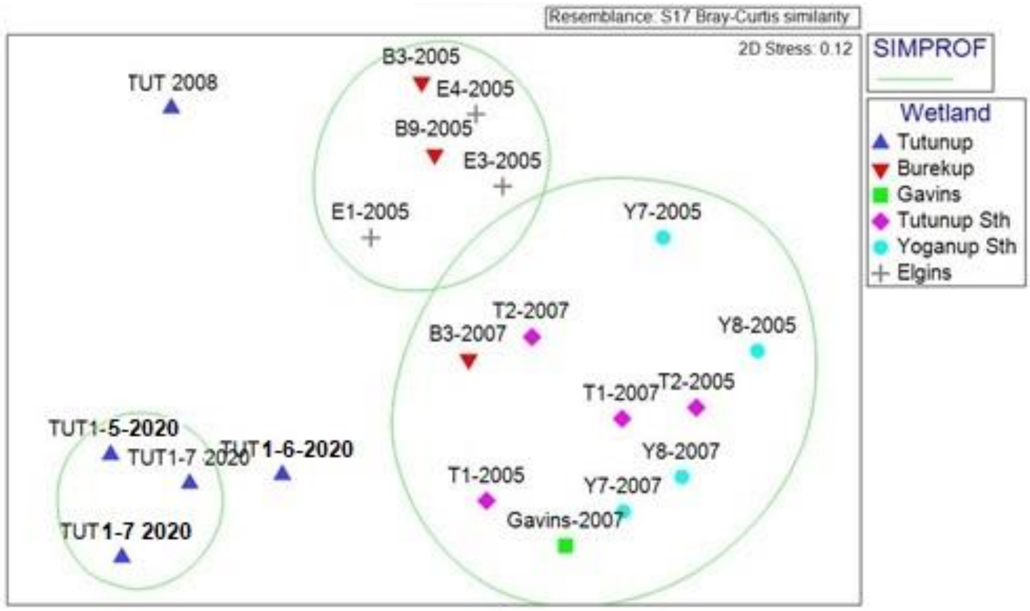

Figure 7. nMDS plot of microinvertebrate taxa assemblage data (log_{10} abundance) collected from the study area (Tutunup) and other regional wetlands. Samples grouped within green circles are based on significant clusters as determined using SIMPROF cluster analysis.

Average similarity (Bray-Curtis) in taxa assemblages between the study area and the other wetlands was also low (< 10%). Contributing most to these differences was the greater abundance of the two species of rotifer, *Keratella slacki* and *Euchlanis dilatata*, two species of copepod, *Australcyclops australis* and *Metacyclops superincidentis,* and immature copepods (copepodites and nauplii) in the study area

(SIMPER). Together, these species accounted for approximately 41% of the total variation between the study area and the other wetlands. This variation was attributed to a number of factors, including naturally high variability in microinvertebrate populations, differences in hydroperiod among the wetlands, and greater diversity of aquatic habitats in the current study area, compared to most of the other wetlands. Most of the above species are known to occur in disturbed as well as undisturbed wetlands, but *E. dilatate*, at least, is sensitive to contaminants and higher water temperatures (> 20 - 26°C) (Wenjie *et al*. 2019). However, further surveys throughout the South-West SWA bioregion are required to confirm its efficacy as an indicator of disturbance.

6.3.2 Microinvertebrate Species of Conservation Significance

None of the microinvertebrate species recorded from the study area in 2008 and 2020 are formally listed as conservation significant, or likely rare or restricted in distribution. The majority were common, ubiquitous species, with distributions extending throughout Australasia or the world (*i.e*. cosmopolitan species).

6.3.3 Microinvertebrate Species of Scientific Interest

Two Protista species of scientific interest were recorded, being new records for Australia; *Arcella* cf. *crenullata* collected in 2020, and *Difflugia* cf. *distenda*, collected in 2008. *A.* cf. *crenullata* was collected from TUT1-2 in 2020 and has not previously been recorded from Australia. D. cf. *distenda* which was recorded during 2008, was not recorded during 2020. The 2008 record was the first recording of this species in Australia, but it likely occurs more widely across Australia (see section 3.2.1). It is likely this species still occurs within the study area, but was not collected in 2020 due to low abundance, or to the stage in seasonal succession and composition of the microinvertebrate community when sampling occurred.

The fact that *A.* cf. *crenullata* and *D.* cf. *distenda* have rarely been collected does not necessarily mean they rare or restricted in distribution and therefore of conservation significance. As shown by the desktop review (section 3.2.2), it is not uncommon or unexpected to find species that are new to science or new records for WA due to the low level of research. It is probable that these species are more widely distributed in comparable habitats throughout Australia but have yet to be recorded (Dr R. Shiel, University of Adelaide, pers. comm).

6.4 Macroinvertebrates

6.4.1 Species Richness and Taxonomic Composition

A systematic list of all macroinvertebrate taxa recorded in August 2008 and August 2020 is provided in Appendix 5.

A total of 48 macroinvertebrate taxa were recorded from the four sites sampled in August 2020, compared with 22 macroinvertebrate taxa from the four sites sampled in 2008. Combined macroinvertebrate taxa richness for 2008 and 2020 was 59. Between-site variability in taxa richness was relatively low compared to that for microinvertebrates, with macroinvertebrate taxa richness ranging from 15 at TUT1-7 within the REW area, to 20 at TUT1-5, 21 at TUT1-8 and 26 at TUT1-6, all within CCW areas (Appendix 5). As for microinvertebrates, it was not possible to examine between-site variability in macroinvertebrate taxa richness in 2008, as all four samples collected for the 2008 study were combined into one composite sample in the field, rather than processed as individual sites. Eleven of the macroinvertebrate taxa recorded in 2008 were not recorded in 2020, and 37 taxa recorded in 2020 were not recorded in 2008.

The macroinvertebrate taxa richness for Tutunup Wetland in 2020 (48) was slightly higher than the regional wetlands sampled on only one occasion by WRM; Gavins Road (42), Tutunup South T1 (31) and T2 (28), Yoganup South Y7 (28) and Y8 (46), Burekup B3 (18) and B9 (42), and Elgin E1 (23), E3 (18) and E4 (11). However, the macroinvertebrate taxa richness for Tutunup Wetland in 2008 was lower than most other wetlands sampled. Combined macroinvertebrate taxa richness for 2008 and 2020 (59) was similar to that for each of the three most taxa-rich (62 - 68 taxa) seasonal wetlands sampled by Davies *et al.* (1993) in January 1989, November 1989 and November 1990. Combined taxa richness for Tutunup Wetland was also higher than the only coloured acidic wetland included in the study by Davies *et al*. (1993); Banganup Lake (21 taxa). The full range in macroinvertebrate taxa richness for seasonal wetlands reported by Davies *et al*. (1993) was 21 - 68.

Singletons constituted a relatively high proportion (56%) of all macroinvertebrates recorded from the study area. This was consistent with the more disturbed regional wetlands sampled by WRM (average 40%). A high proportion of singletons is quite common in seasonal and permanent freshwater systems in WA (see Pinder *et al*. 2005). The proportion of singletons is expected to be relatively higher in ephemeral wetlands, such as the current study area, due to the high turnover of species as natural succession progresses during the cycle of wetland filling and drying. Davis *et al*. (1993) found singletons constituted 24.1% of all taxa recorded, but don't provide percentages for micro- versus macroinvertebrates, or for seasonal versus permanent wetlands. However, they do note that coloured wetlands had a high proportion of rare (singleton) species and high species richness, as was found for the current study area.

Data for 2020 show the macroinvertebrate assemblage and the total invertebrate assemblage to be characterised by a high diversity of taxa, based on the reference range developed by Jones *et al.* (2008) (see section 5.4.2). Insecta were the dominant group, as is common in most lentic and lotic waters across WA, with Diptera and Coleoptera the best represented taxa in both 2008 and 2020 (Table 13). Relatively high diversity (> 35 taxa) of macroinvertebrate taxa was also recorded for the more disturbed Tutunup South, Gavin's Road, Yoganup South Y8 and Burekup B9 wetlands. Davies *et al*. (1993) similarly found highest species richness in disturbed wetlands which were moderately eutrophic, and in coloured wetlands. Nutrient enrichment, and associated higher primary productivity, is one possible explanation for the high invertebrate diversity in these wetlands (Davies 1993). The source of enrichment is usually input from surrounding rural and urban lands, and abundant vegetation in less disturbed wetlands.

Table 13. Summary of higher-order macroinvertebrate taxa composition from the study area. *n* = number of sites sampled. Refer Appendix 5 for full taxa list.

Macroinvertebrates		Number of Taxa	
Scientific name	Common name	2020 (*n* = 4)	2008 (*n* = 1)
Mollusca	Freshwater snails	1	1
Oligochaeta	Aquatic worms	1	1
Amphipoda	Amphipods	3	2
Acarina	Water mites	2	0
Collembola	Springtails	3	0
Hemiptera	True bugs	2	1
Coleoptera	Aquatic beetles	18	6
Diptera	Two-winged flies	18	10
Odonata	Dragonflies & damselflies	0	1
	Total taxa richness	**48**	**22**

The most common species were non-biting midge larvae from the sub-families Chironominae and Orthocladiinae, and mosquito larvae of the genus *Culex*. Orders such as Odonata (dragonfly and damselflies) and Trichoptera (caddisflies) which are normally associated with less disturbed ecosystems were not recorded in 2020, and only one immature damselfly was recorded in 2008. Although the aquatic habitats were considered to be slightly to moderately disturbed, this level of disturbance was not expected to result in complete loss of these orders. Odonates and trichopterans were recorded in low abundance in nearby Tutunup South in November 2005 (WRM 2006c), and four species of odonate and one trichopteran were collected from the more disturbed Burekup wetlands in November 2005 (WRM 2006b). Adults of these species are known to be 'temporary residents', reinvading seasonal waterbodies when they become inundated (WRM 2006c). It is probable that the study area does not remain inundated for long enough for these species to recolonise and complete their breeding and development phases.

Multivariate analysis was again undertaken to highlight the similarities/dissimilarities in macroinvertebrate taxa assemblages between the study area and other wetlands surveyed (Figure 8). The nMDS ordination (Figure 8) for macroinvertebrate communities showed similar patterns to microinvertebrate communities, with the separation of samples into four broad groups; i) study area 2020, ii) study area 2008, iii) Yoganup South, and iv) all other wetlands. SIMPROF also indicated there were significant differences in macroinvertebrate assemblages among the Burekup, Elgin, Gavin's Road and Tutunup South wetlands (Figure 5). Assemblages in the study area were most similar to those of Gavin's Road and Tutunup South wetlands, possibly due to the less disturbed condition of these wetlands (more remnant vegetation) compared to the other wetlands analysed. Within the study area, the clear separation between 2008 and 2020 samples reflected the considerably lower species richness and abundance in 2008, due to limited habitat availability at the time of sampling. Average similarity (Bray-Curtis) in taxa assemblages between the study area and other wetlands was only 10%, indicative of high spatial and (likely) temporal variability, not uncommon in seasonal and ephemeral waterbodies. The difference was due to differences in abundance of a large number of taxa. Species contributing to the variation were the mosquitoes *Culex* spp. and chironomids *Tanytarsus* spp. and Orthocladinae sp. V76, which tended to be more abundant in the study area, and the chironomid *Chironomus alternans*, water boatmen *Micronecta robusta*, aquatic beetle *Limbodessus inornatus*, and backswimmers *Sigara* sp. and Anisops, which tended to be more abundant at other wetlands. However, no single species or group of taxa could be viewed as indicative of the study area, or the level of disturbance among the wetlands analysed.

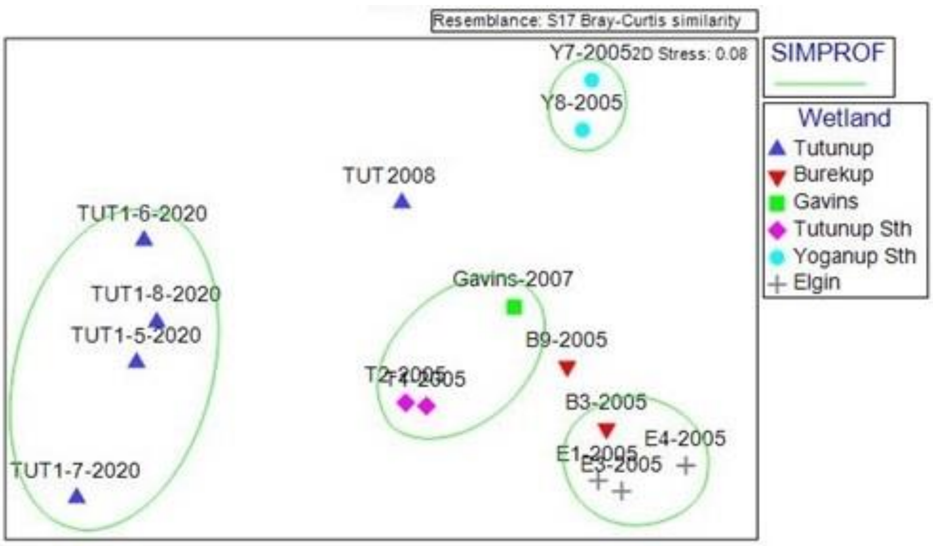

Figure 8. nMDS plot of macroinvertebrate taxa assemblage data (log_{10} abundance) collected from the study area (Tutunup) and other regional wetlands. Samples grouped within green circles are based on significant clusters as determined using SIMPROF cluster analysis.

6.4.2 Listed Conservation Significant Macroinvertebrate Species

The majority of macroinvertebrate taxa recorded were common, ubiquitous species, with distributions extending across south-western Australia, Australasia, and the world (*i.e*. cosmopolitan species). Only one species formally listed as conservation significant was recorded, the tiny freshwater snail *Glacidorbis occidentals*, as discussed below. No other conservation significant macroinvertebrate species were recorded during the baseline surveys, and none are likely to occur. This includes candidate Priority species identified in the desktop review as having the potential to occur (see Table 6 section 3.4).

6.4.2.1 Freshwater Snail *Glacidorbis occidentalis*

The only listed conservation significant macroinvertebrate species known to occur, or potentially occurring in the study area is the minute freshwater snail *Glacidorbis occidentalis* (DBCA Priority 3, IUCN Vulnerable). A single individual *G. occidentalis* was recorded from the REW within the current study area in 2008, but was not recorded in 2020, despite the greater availability of inundated habitat, and greater sampling effort. Adult *G. occidentalis* survive dry periods by burrowing into moist soils and are likely still present, but not recorded in 2020 due to their characteristically low abundance. The kick-sweep net sampling method used for the 2008 and 2020 surveys is considered an effective and efficient method, widely used for sampling macroinvertebrate, including *G. occidentalis*, yet *G. occidentalis* has only ever been recorded in low numbers (one or two specimens) across the south-west (Bunn & Stoddart 1983, Bunn *et al*. 1989, WRM unpubl. data).

The low abundance and scattered populations make this species highly vulnerable to physical disturbance of soils, and to alterations to hydroperiod that result in either permanent inundation or prolonged drying. The species restriction to temporary waters also puts it at high risk of extinction from climate change.

6.4.3 Potential SRE Macroinvertebrate Species

Two potential SRE macroinvertebrate species were recorded during the 2020 field survey, in addition to the new species of epigean diving beetle, *Paroster* sp. Nov., recorded in 2008. These were the stygal amphipod *Wesniphargus nichollsi*, and another epigean *Paroster* species, *P. ellenbrookensis*.

No other known or potential macroinvertebrate SRE species were recorded during the baseline surveys, and none are likely to occur.

6.4.3.1 Stygal Amphipod *Wesniphargus nichollsi*

Wesniphargus nichollsi (family Neoniphargidae) is a stygobitic (groundwater dependent) south-west endemic and potential SRE, known only from a few scattered coastal populations within a < 700 km^2 area, from near Muchea, 230 km north of the study area, to Witchcliffe 54 km south-west of the study area, and inland across the northern jarrah forest 100 km north east of the study area (Bradbury & Williams 1997, WRM, unpubl. data). *W. nichollsi* was recorded from TUT1-5 and TUT1-6 in the CCW area of the current study area in 2020, but was not recorded in 2008. The taxonomy of the genus *Wesniphargus* is poorly resolved. Genetic analysis is required to confirm whether specimens from the current study area are the same species as specimens from other south west localities, and thereby confirm the extent of their distribution and whether or not they are SREs. Given that short-range endemicity is high in many stygobitic amphipods (see Hose *et al*. 2015), the likelihood that specimens recorded from the study area are also short-range endemic is considered to be high.

The presence of *W. nichollsi* is considered indicative of groundwater dependent ecosystems (GDEs) as this amphipod requires strong connectivity between ground and surface waters in order to frequent surface waters in search of food. Temporal and spatial variability in their abundance in northern jarrah forest streams appears strongly linked to rainfall patterns (WRM unpubl. data). It is not understood if epigean and hyporheic[5] habitats are critical for long-term survival of *W. nichollsi* (see Knott 1986). Knott (1986) considered some stygal species should more appropriately be considered as groundwater species that have re-invaded surface waters, and now only take refuge in interstitial waters of bed substrates as a short-term strategy to survive seasonal dry periods. The same may be true of at least some stygal amphipods, such as *W. nichollsi*, and if so, increases their vulnerability to physical disturbance, sedimentation and drying.

6.4.3.2 Diving Beetles *Paroster* Species

P. ellenbrookensis was recorded in 2020 from TUT1-5 and TUT1-8 in CCW areas, but was not recorded in 2008. This epigean species is known only from one other location, Ellenbrook Nature Reserve, 215 km to the north-north-east of the study area (Pennifold 2018). This reserve is a shallow, peaty swamp that dries out in summer. The collection of specimens in the current study area constitutes a significant range expansion for the species, with the species now appearing to be more widespread, but in scattered populations with low abundances. However, as the known distribution is still < 10,000 km^2, it is considered here as a potential SRE, as defined by Harvey (2002).

Four specimens of a new species of epigean *Paroster*, *Paroster* sp. nov., were collected from the REW within the study area in 2008, but were not recorded in 2020. This was not unexpected as turnover of macroinvertebrate species in ephemeral wetlands can be considerable. Rates of colonisation and survival of different invertebrate species are dependent on stochastic factors such as antecedent rainfall, temperature, predator-prey relationships *etc*. The specimens of *Paroster* sp. nov. collected in 2008 were confirmed by Dr Chris Watts at the South Australian Museum as being new to science (section 3.2.1). As the species has not previously been recorded, the extent of its current distribution in south west WA and short range endemicity is unknown. As of July 2020, the *Paroster* sp. nov. specimens from the study area have not been formally described and named (C. Watts, pers. comm.).

Epigean species of *Paroster* appear to be highly specialised in exploiting ephemeral and seasonal water bodies, and are often the dominant species in particularly shallow, ephemeral aquatic habitats (Watts & Leys 2008). Their preferred habitat is temporary creeks, swamps, inundated vegetation, and puddles on granite outcrops that form in winter-spring, but are dry by summer (Watts & Leys). They have a short breeding cycle, and the adults are winged, though there is no published information on their dispersal capabilities. There is little published literature on the degree of short-range endemicity in epigean species of *Paroster*, but it is known to be high among subterranean species of the genus. Short range endemicity is also likely in epigean *Paroster* as they too are highly adapted to specific environments and appear to be rare across their range (see Toussaint *et al.* 2014). Further research across the south-west is required to determine the full distribution of *P. ellenbrookensis* and *Paroster* sp. nov. across south west WA, and thereby confirm their status as SREs.

[5] The hyporheic zone is the saturated interstitial spaces below the stream bed, and where present, can act as an ecotone between the surface water and hypogean (subterranean) environments.

6.5 Fish & Crayfish

6.5.1 Fish

No fish species were recorded in either 2008 or 2020, despite the greater sampling effort for fish in 2020. Although the study area is within the known range of several native freshwater fish species (see section 3.2.2), the likelihood that fish occur is considered negligible for most of these species. This is based on the results of the field surveys, the ephemerality of surface water in the study area, and the lack of connectivity to more permanent water sources that would provide refugia during dry periods.

One possible exception identified by the desktop review as having a low to moderate chance of occurring in the study area, was the black-stripe minnow *Galaxiella nigrostriata* (Endangered, DBCA 2019) (section 3.3.2). This species occurs in ephemeral and seasonal waters, and the study area is within the known range. However, based on combined field observations from 2008 and 2020, it is now considered highly unlikely that black-stripe minnows occur in the study area. Suitable habitat is restricted by the very short persistence time and shallow depth of surface waters, coupled with the (likely) limited spatial extent of cool soils that are sufficiently deep for fish to aestivate in over the summer.

6.5.2 Crayfish

Two species of freshwater crayfish were recorded from the study area; the endemic koonac (*Cherax preissii*) from TUT1-6, and the introduced yabby (*Cherax destructor*) from the dam at TUT1-9 (Appendix 6). Koonacs are not listed as conservation significant. Koonacs occupy a wide range of freshwater habitats but are most prevalent in seasonally inundated wetlands (Morgan *et al*. 2011). The current study area is within the known range for this species. The only other native crayfish to occur in SWA ephemeral wetlands and with a known range that overlaps the study area, is the gilgie (*C. quinquecarinatus*) (section 3.2.2). Gilgies have a breeding strategy that allow them to survive in other temporary waters, *i.e*. multiple spawning events, short brood and gonadal recovery period (see Beatty *et al*. 2005). The apparent absence of gilgies from the study area is therefore assumed due to the very short hydroperiod, that is too short to enable successful recruitment in this species.

The introduced yabby is extremely adaptable to site conditions and, like the koonac, is capable of burrowing down to the water table to survive dry conditions (Morrissy *et al.* 1984). Yabbies are also more tolerant than native species of extremes in temperature, hypoxia, and salinity (Morrissy *et al.* 1984, Holdich and Lowery 1988). Since its introduction, the yabby has proven to be a successful invasive species and is spread throughout much of the southwest of the state (Lynas *et al.* 2004, 2007). It has an aggressive nature and the potential for the yabby to out-compete native species has been well documented (Lynas *et al*. 2004, 2006, 2007). Due to their considerable negative impact on native freshwater species there are restrictions on the farming and movement of yabbies in WA.

7 SUMMARY AND CONCLUSIONS

Findings from a recent desktop review and baseline field survey for aquatic biology of "Tutunup Wetland" are presented in this report. The wetland conservation category of Tutunup Wetland, as mapped by DBCA, is part Conservation, and part Resource Enhancement (total wetland area is approximately two-thirds CCW and one-third REW). Tutunup Wetland also supports an example of the Busselton Ironstone TEC. For the purposes of the desktop review, the 'regional' extent in regards to the study area for Tutunup Wetland, was considered to be the area of the SWA IBRA that intersects the South-West region as defined by DWER regional boundaries (South-West SWA).

The desktop review indicated the aquatic biota of Tutunup Wetland has only been surveyed once, in August 2008 by WRM (2008c), and that the aquatic biota of seasonal wetlands in general has been historically understudied in WA. This is especially so for microinvertebrate (zooplankton) communities in coloured, acidic wetlands like Tutunup Wetland. Few aquatic invertebrates, and microinvertebrates in particular, are formally listed under the Commonwealth EPBC Act or state BC Act as conservation significant due to data deficiency.

The 2008 field survey recorded one conservation listed species, the minute freshwater snail *Glacidorbis occidentalis* (DBCA Priority 3), and one SRE species, the epigean diving beetle *Paroster* sp. Nov. from the study area. Though not formally listed as conservation significant, *Paroster* sp. Nov. is new to science, and known only from the study area, and as such, considered restricted in distribution. The 2020 desktop review indicated low-moderate likelihood of one other conservation listed species occurring in the study area; the black-stripe minnow *Galaxiella nigrostriata* (EPBC Endangered, DBCA Endangered).

Water quality, microinvertebrates, macroinvertebrates, fish and crayfish were therefore re-surveyed in August 2020, to provide additional baseline data to that recorded in 2008. The study area for the 2008 and 2020 surveys predominantly comprised seasonally waterlogged, peaty soils, shallowly inundated sedgelands, fringing paperbarks, and small areas of shallow open water, predominantly along access tracks and rail embankment. Four sites were targeted in 2020, with additional water quality sampling at one farm dam (TUT1-9), and additional crayfish sampling at another farm dam (TUT1-10), both within CCW areas. The 2020 survey extends the total area surveyed in 2008, with better representation of aquatic habitats that were mostly dry in 2008, and limited to small, shallow, isolated pools and channels, mainly beside former access tracks and along the rail line. As hydroperiod is a known determinant of aquatic fauna composition, the 2020 survey was expected to record additional species to those recorded in 2008.

Water quality sampling found surface waters to be sodium-chloride dominated, highly coloured (visually tannin-stained), generally acidic (pH 5.66 - 6.66), and with low alkalinity (3 - 16 mg/L, as $CaCO_3$) and conductivity (229 - 605 µS/cm). Conductivity in one farm dam (TUT1-9; 1,930 µS/cm) within the CCW was considerably higher than that of the surrounding wetland. Background levels of dissolved aluminium exceeded the ANZG (2018) 80% species protection DGV at three sites in 2020, and exceeded the 90% species protection DGV in the farm dam (TUT1-9); noting that ANZG (2018) provide only a low reliability DGV for aluminium at pH < 6.5. There have been previous records of elevated aluminium concentrations in other rural wetlands in the region (WRM 2006a,b), though it is not known if this reflects local geology or input from agricultural sources.

Despite the greater spatial extent of inundated habitat and greater sampling effort in 2020, conservation listed species *G. occidentalis* and *Galaxiella nigrostriata* were not recorded. *G. occidentalis* is likely still present in the wetland, but not collected in 2020 due to the species characteristically low abundance. *G. occidentalis* is considered highly vulnerable to physical disturbance of soils, alterations to hydroperiod, and to climate change. *G. occidentalis* occurs widely throughout the SWA and WAR (Warren) bioregions, but has a fragmented distribution. As the species is not restricted to the study area, disturbance to the local population would not result in direct loss of regional biodiversity.

Based on observations from the 2008 and 2020 field surveys, *G. nigrostriata* is considered highly unlikely to occur in the study area. Though the study area is within the known range for this species, suitable habitat is limited by the very short persistence time and shallow depth of surface waters, coupled with the (likely) limited spatial extent of cool soils that are sufficiently deep for this minnow to aestivate in over the summer.

Paroster sp. nov., recorded in 2008, was not recorded in 2020, though two other potential SRE macroinvertebrates were present; the epigean *Paroster ellenbrookensis* (TUT1-5, TUT1-8), and the stygobitic amphipod, *Wesniphargus nichollsi* (TUT1-5, TUT1-6). None are formally listed as conservation significant. *P. ellenbrookensis* is known only from one other location, Ellenbrook Nature Reserve, 215 km NNE of the study area. As *P. ellenbrookensis* occurs outside the study area, disturbance to the local population would not result in direct loss of regional biodiversity. *W. nichollsi* occurs at several localities in the SWA and WAR regions, outside the study area, though total distribution covers a relatively small (< 700 km^2) area, and populations are fragmented. The taxonomic resolution of species of the genus *Wesniphargus* is poorly resolved. Genetic analysis is required to confirm whether *W. nichollsi* specimens from the study area are the same species as specimens from other localities in regions, or if they are restricted to Tutunup Wetland. The presence of *W. nichollsi* is indicative of surface expression of groundwater at the locations collected. Until better distributional data are available for *W. nichollsi* and the *Paroster* species, a precautionary approach to managing potential disturbance is recommended.

Not unexpectedly, the 2020 survey recorded another microinvertebrate species that is a new record for Australia; the protist *Arcella* cf. *crenullata.* This is in addition to the protist *Difflugia* cf. *distenda*, collected in 2008, which is also a new record for Australia. The probability that these microinvertebrate species are restricted to the study area is considered low to negligible, and as such risk to regional populations is considered to be negligible. Both species are likely widely distributed in comparable habitats throughout Australia but have yet to be recorded due to historic under-sampling for microinvertebrates (Dr Russel Shiel, University of Adelaide, pers. comm).

Overall, the study area is considered to support a high diversity of aquatic invertebrates (27 microinvertebrate and 59 macroinvertebrate taxa), based on the reference ranges originally developed by DBCA for wetland evaluation (Jones *et al*. 2009). Diversity was also high relative to the only available data for other seasonal wetlands. This included more disturbed wetlands within the South-West WA region, sampled by WRM between 2005 and 2007 (WRM 2006a-c, 2008a-b), and a range of seasonal wetlands sampled by DBCA to north of the region for the large-scale *Wetlands of the Swan Coastal Plain* study (Davis *et al.* 1993).

In addition to the TEC, the regional ecological value of the remnant aquatic habitat in the study area is considered to be high, given the extensive clearing of seasonal wetlands at the local and regional-scale for agriculture. The remnant wetland vegetation is likely to provide at least some of the energy, as food sources, that drives many aquatic processes, as well as providing food, shade and shelter for both terrestrial and aquatic fauna. The remnant vegetation still offers a good diversity of aquatic habitats (*i.e.* shrub thickets, fringing paperbarks, shallowly-inundated sedges and seasonally dry open waters) both within each of the CCW and REW areas, and across these areas combined.

REFERENCES

Allen GR, Midgley SH, Allen M (2002) *Field Guide to the Freshwater Fishes of Australia.* Western Australian Museum, Perth WA.

Aquatic Research Laboratory (ARL) (1989) Stream fauna study – Canning Reservoir Catchment; Lower Canning River Catchment; Serpentine/Gooralong Brook Catchments; Dirk Brook Catchment; North Dandalup Pipehead Dam Catchment. Results and Recommendations 1988. Unpublished report to the Water Authority of Western Australia by the Aquatic Research Laboratory, the University of Western Australia. ARL015. June 1989.

ANZECC/ARMCANZ (2000) Australian and New Zealand Guidelines for Fresh and Marine Water Quality. Australia and New Zealand Environment and Conservation Council and the Agriculture and Resource Management Council of Australia and New Zealand. Paper No. 4. Canberra.

ANZG (2018) Australian and New Zealand Guidelines for Fresh and Marine Water Quality. Australian and New Zealand Governments and Australian state and territory governments, Canberra ACT, Australia. https://www.waterquality.gov.au/guidelines/anz-fresh-marine. Accessed 21 January 2021

ASFB (2018) Conservation status of Australian Fishes. Australian Society for Fish Biology Newsletter **46**: 142-144.

ALA (2020) Atlas of Living Australia http://www.ala.org.au. Accessed Wed, 24 Jun 2020.

Australian Government (2018) Charter: National Water Quality Management Strategy, Department of Agriculture and Water Resources, Canberra, March. CC BY 3.0.

Balla S (1994) Wetlands of the Swan Coastal Plain Volume 1. Their Nature and Management. Water Authority of Western Australia and the Western Australian Department of Environmental Protection.

Beatty SJ, Morgan DL, Gill H (2005) Life history and reproductive biology of the gilgie, *Cherax quinquecarinatus*, a freshwater crayfish endemic to southwestern Australia. *Journal of Crustacean Biology* **25**(2): 251-262. DOI: 10.1651/C-2518.

Benzie JAH (1986) Daphnia occidentalis, New Species (Cladocera: Daphniidae) from Western Australia: New Evidence on the Evolution of the North American *D. ambigua-D. middendorffiana* Group. *Journal of Crustacean Biology* **6**(2): 232-245.

Bouck GR (1980) Etiology of Gas Bubble Disease. *Transactions of the American Fisheries Society* **109**: 703-707.

Boulton AJ, Suter PJ (1986). Ecology of temporary streams – an Australian perspective. [In] DeDecker P, Williams WD (eds). *Limnology in Australia*. CSIRO, Melbourne. Pp 313-329.

Boulton AJ, Suter PJ (1986) Ecology of temporary streams – an Australian perspective. [In] DeDecker P, Williams WD (eds). *Limnology in Australia*. CSIRO, Melbourne. Pp 313-329.

Boulton AJ, Lake PS (1988). Australian temporary streams – some ecological characteristics. *Verhandlungen Internationale Vereinigung Limnologie* **23**: 1380-1383.

Boulton AJ (1989) Over-summering refuges of aquatic macroinvertebrates in two intermittent streams in central Victoria. *Transactions of the Royal Society of Society of South Australia* **113**: 23-34.

Bradbury JH, Williams WD (1997) The amphipod (Crustacea) stygofauna of Australia: description of new taxa (Melitidae, Neoniphargidae, Paramelitidae), and a synopsis of known species. *Records of the Australian Museum* **49**: 249-341. ISSN 0067-1975.

Bunn SE, Stoddart JA (1983) A new species of the prosobranch gastropod *Glacidorbis* and its implications for the biogeography of south-western Australia. Records of the *Western Australian Museum* **11**(1): 49-57.

Bunn SE, Davies PM, Edward DHD (1989) The association of *Glacidorbis occidentalis* Bunn & Stoddart (Gastropoda: ?Hydrobiidae) with intermittently-flowing forest streams in south-western Australia. *Journal of the Malacology Society of Australia* **10**: 25-34.

Butler B, Doudoroff P, Shumway DL (1970) Dissolved oxygen requirements of freshwater fishes. FAO Fisheries Technical Paper No. 86, 291 pp.

Cale D, Lyons M, McCormick C, Pinder A, Walker C (2010) State Salinity Strategy Wetland Biodiversity Monitoring Report: Lake Eganu 1998 to 2007. Science Division, Department of Environment and Conservation, Perth. April 2010.

Chessman BC (1995) Rapid assessment of rivers using macroinvertebrates: a procedure based on habitat specific sampling, family level identification and a biotic index. *Australasian Journal of Ecology* **20**: 122-129.

Clarke KR, Gorley RN (2014) PRIMER v7: User manual/Tutorial, Primer E: Plymouth. Plymouth Marine Laboratory, Plymouth, UK.

Clifford H (1966) The ecology of invertebrates in an intermittent stream. *Investigations of Indiana Lakes and Streams* **7:** 57-98.

Connolly NM, Crossland MR, Pearson RG (2004) Effect of low dissolved oxygen on survival, emergence, and drift of tropical stream macroinvertebrates. *Journal of the North American Benthological Society* **23:** 251-270.

Creagh S, Davies PM, Chandler L, Lynas J, Shiell G (2004) Baseline Aquatic Ecosystem Surveys of Brooks in the Cameron Corridor Area. Report ST 13/03. Streamtec Pty Ltd.

Davis J, Rosich R, Bradley J, Growns J, Schmidt L, Cheal F (1993) Wetlands of the Swan Coastal Plain Volume 6: Wetland Classification on the Basis of Water Quality and Invertebrate Community Data. Water Authority of Western Australia, January 1993.

DBCA (2018) List of Threatened Ecological Communities. Department of Biodiversity, Conservation and Attractions, Species and Communities Branch, Parks and Wildlife Service. 28 June 2018. https://www.dpaw.wa.gov.au/plants-and-animals/threatened-species-and-communities/wa-s-threatened-ecological-communities. Accessed 21 January 2021.

DBCA (2019) Threatened and priority fauna list. Department of Biodiversity, Conservation and Attractions, Species and Communities Branch, Parks and Wildlife Service. 3 January 2019. https://www.dpaw.wa.gov.au/plants-and-animals/threatened-species-and-communities/threatened-animals. Accessed 21 January 2021.

DBCA (2020) Geomorphic Wetlands, Swan Coastal Plain (BCA-019) dataset. Department of Biodiversity and Attractions, 17 January 2020. https://catalogue.data.wa.gov.au/dataset/geomorphic-wetlands-swan-coastal-plain. Accessed 21 January 2021.

DEC (2008) Methodology: Rapid Indicator Assessment of Western Australian Inland Aquatic Ecosystems. Department of Environment and Conservation, Perth, Western Australia. November 2008.

DEC (2008b) Dunsborough Burrowing Crayfish, Margaret River Burrowing Crayfish and Walpole Burrowing Crayfish Recovery Plan 2007 – 2016. Wildlife Management Program No. 41. Department of Environment and Conservation, Species and Communities Branch, Bentley, Western Australia. https://www.dpaw.wa.gov.au/plants-and-animals/threatened-species-and-communities/threatened-animals# recoveryplans. Accessed 24 June 2020.

Delucchi CM, Peckarsky BL (1989) Life History patterns of insects in an intermittent and a permanent stream. *Journal of the North American Benthological Society* **8**: 308-321.

Department of the Environment (2020a) *Cherax tenuimanus* in Species Profile and Threats Database, Department of the Environment, Canberra. Available from: http://www.environment.gov.au/sprat. Accessed Wed, 24 Jun 2020.

Department of the Environment (2020b) *Geocrinia alba* in Species Profile and Threats Database, Department of the Environment, Canberra. Available from: http://www.environment.gov.au/sprat. Accessed Wed, 24 Jun 2020.

Department of the Environment (2020c) *Geocrinia vitellina* in Species Profile and Threats Database, Department of the Environment, Canberra. Available from: http://www.environment.gov.au/sprat. Accessed Wed, 24 Jun 2020.

Department of Environment Sport and Territories, Land and Water Resources – Research and Development Corporation, Commonwealth Environment Protection Authority (1994) National River Processes and Management Program: Monitoring River Health Initiative. River Bioassessment Manual Version 1.0. February 1994.

Department of Water and Environmental Regulation (2020a) Western mud minnow – *Galaxiella munda*. Available from: https://rivers.dwer.wa.gov.au/species/galaxiella-munda/. Accessed Wed, 24 Jun 2020.

Department of Water and Environmental Regulation (2020b) Black-stripe minnow – *Galaxiella 47igrostriatal*. Available from: https://rivers.dwer.wa.gov.au/species/galaxiella-nigrostriata/. Accessed Wed, 24 Jun 2020.

Department of Water and Environmental Regulation (2020c) Pouched lamprey – *Geotria australis*. Available from: https://rivers.dwer.wa.gov.au/species/geotria-australis/. Accessed Wed, 24 Jun 2020.

Department of Water and Environmental Regulation (2020d) Salamanderfish – *Lepidogalaxias salamandroides*. Available from: https://rivers.dwer.wa.gov.au/species/lepidogalaxias-salamandroides/. Accessed Wed, 24 Jun 2020.

Department of Water and Environmental Regulation (2020e) Balston's pygmy perch – *Nannatherina balstoni*. Available from: https://rivers.dwer.wa.gov.au/species/nannatherina-balstoni/. Accessed Wed, 24 Jun 2020.

English V (1999) Shrubland Association on Southern Swan Coastal Plain Ironstone (Busselton Area) (Southern Ironstone Association). Interim Recovery Plain No. 4, 1992-2002. Department of Conservation and Environment Western Australian Threatened Species and Communities Unit, Wanneroo. September 1999.

EPA (2002) Position Statement Number 3: Terrestrial biological surveys as an element of biodiversity protection. Environmental Protection Authority.

EPA (2009) EPA Guidance Statement Number 20: Sampling of short range endemic invertebrate fauna for environmental impact assessment in Western Australia. Environmental Protection Authority, May 2009.

EPA (2016a) Environmental Factor Guideline: Terrestrial Fauna. Environmental Protection Authority, Western Australia. 13 December 2016.

EPA (2016b) Technical Guide: Sampling methods for Subterranean fauna. Environmental Protection Authority. December 2016.

EPA (2016c) Technical Guide: Sampling of short range endemic invertebrate fauna. Environmental Protection Authority. December 2016.

EPA (2018) Environmental Factor Guideline: Inland Waters. Environmental Protection Authority, Western Australia. 27 June 2018.

EPA (2020) Technical Guide: Terrestrial Vertebrate fauna surveys for environmental impact assessment. Environmental Protection Authority. June 2020.

Flint N, Crossland MR, Pearson RG (2014) Sublethal effects of fluctuating hypoxia on juvenile tropical Australian freshwater fish. *Marine and Freshwater Research* **66**: 293-304.

Galeotti DM (2013) Metapopulation theory explains black-stripe minnow (Pisces: Galaxiidae, *Galaxiella 48igrostriatal*) distribution in seasonal wetlands in south-western Western Australia. Available from: https://ro.ecu.edu.au/cgi/viewcontent.cgi?referer=https://www.google.com/&httpsredir=1&article=1709&context=theses. Accessed Wed, 24 Jun 2020

Gomon MF, Bray DJ (2020) Western Dwarf Galaxias, *Galaxiella munda* McDowall 1978. Fishes of Australia website, Museums Victoria and OzFishNet. Available from: https://fishesofaustralia.net.au/home/species/3683#moreinfo. Accessed Tues, 21 July 2020

Hairston NG Jnr, Van Brunt RA, Kearns CM, Engstrom DR (1995) Age and survivorship of diapausing eggs in a sediment egg bank. *Ecology* **76**: 1706-1711.

Halse SA, Storey AW (1996) Aquatic invertebrate surveys and water quality of Perth Airport swamps. Unpublished report to the Perth Airport Authority, 23pp.

Halse SA, Cale DJ, Jasinska EJ, Shiel RJ (2002) Monitoring change in aquatic invertebrate biodiversity: sample size, faunal elements and analytical methods. *Aquatic Ecology* **36**: 395-410.

Halse SA, Shiel RJ, Storey AW, Edward DHD, Lansbury I, Cale DJ, Harvey MS (2000) Aquatic invertebrates and waterbirds of wetlands and rivers of the southern Carnarvon Basin, Western Australia. *Records of the Western Australian Museum, Supplement* **61**: 217-267.

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

Holdich DM, Lowery RS (eds) (1988) Freshwater crayfish: biology, management and exploitation. Croom Helm London, UK.

Hopper SD, Harvey MS, Chappill JA, Main AR, Main BY (1996) The Western Australian biota as Gondwanan Heritage – a review. [In] Hopper SD, Chappill JA, Harvey MS, George AS (eds). *Past, Present and Future of the Western Australian Biota*. Chipping Norton: Surrey Beatty & Sons. Pp 1-46.

Horwitz P, Rogan R, Halse S, Davis J, Sommer B (2009) Wetland invertebrate richness and endemism on the Swan Coastal Plain, Western Australia. *Marine and Freshwater Research* **60**(10): 1006–1020.

Hose GC, J Sreekanth, Barron O, Pollino C (2015) Stygofauna in Australian Groundwater Systems: Extent of knowledge. CSIRO, Australia. July 2015.

IUCN (2020). The IUCN red list of threatened species. Version 2020-1. Available from: http://www.iucnredlist.org. Accessed 14 December 2020.

Jones SM, Pinder AM, Sim LL, Halse SA (2009) Evaluating the Conservation Significance of Basin Wetlands within the Avon Natural Resource Management Region: Stage Three Assessment Method. Prepared for the Avon Catchment Council by the Department of Environment and Conservation, Perth. May 2009.

Karanovic T (2002). Subterranean Copepoda from arid Western Australia. *Crustaceana Monographs 3*. Koniklijke Brill Academic Publishers. Netherlands.

Klunzinger MW (2012) Ecology, life history and conservation status of *Westralunio carteri* Iredale 1934, an endemic freshwater mussel of south-western Australia. PHD Thesis. Murdoch University, Perth Western Australia.

Knott B (1986) Isopoda: Phreatoicidea. [*In*] L Botosaneanu (ed.) Stygofauna Mundi. Brill-Backhuys, Leiden. Pp. 486-492.

Lynas J, Lindhjem P, Storey A, Knott B (2004). Is the yabby *Cherax destructor* (Parastacidae) an ecological threat in Western Australia? *Freshwater Crayfish* **14**: 37-44.

Lynas J, Storey A, Shiel R (2006) Buntine-Marchagee Natural Diversity Recovery Catchment (BMRC), Wetland Invertebrate Fauna Monitoring: 2005. Unpublished report to the Department of Conservation and Land Management, Mid-west Regional Office. School of Animal Biology, The University of western Australia, Nedlands. January 2006.

Lynas J, Storey A, Knott B. (2007) Aggressive interactions between three species of freshwater crayfish of the genus *Cherax* (Decapoda: Parastacidae). *Marine and Freshwater Behaviour and Physiology* **40**(2): 105-116.

Main BY (1996) Microcosmic biogeography: trapdoor spiders in a time warp at Durokoppin. [In] Hopper SD, Chappill JA, Harvey MS, George AS (eds). *Past, Present and Future of the Western Australian Biota*. Chipping Norton: Surrey Beatty & Sons. Pp 163 – 171.

McFarlane D, George R, Ruprecht J, Charles S, Hodgson G (2020) Runoff and groundwater responses to climate change in South West Australia. *Journal of the Royal Society of Western Australia* **103**: 9–27.

Meissner R, English V (2005) Shrubland Association on Southern Swan Coastal Plain Ironstone (Busselton Area) (Southern Ironstone Association) Interim Recovery Plan 2005-2010. Department of Conservation and Land Management Interim Recovery Plan No. 215, March 2005.

Metzeling, L, Chessman BC, Hardwick RA, Wong V (2003) Rapid assessment of rivers using macroinvertebrates: the role of experience, and comparisons with quantitative methods. *Hydrobiologia* **510**: 39-52.

Morgan DL, Gill HS, Potter IC (1998) Distribution, identification and biology of freshwater fishes in south-western Australia. Records of the Western Australian Museum. No. 56. Available from: http://museum.wa.gov.au/sites/default/files/1.%20Morgan,%20Gill,%20Potter%20.pdf. Accessed Wed, 24 Jun 2020.

Morgan DL, Gill HS (2000) Fish associations within the different inland habitats of lower south-western Australia. *Records of the Western Australian Museum* **20**: 31-37.

Morgan DL, Beatty SJ, Klunzinger MW, Allen MG, Burnham QF (2011) *A field guide to freshwater fishes, crayfishes and mussels of southwestern Australia*. SERCUL and Freshwater Fish Group, Perth WA.

Morrissy N, Caputi N, House R (1984) Tolerance of marron (*Cherax tenuimanus*) to hypoxia in relation to aquaculture. *Aquaculture* 41: 61-74.

Pennifold M (2018) Identifying Priority Species Within the South-western Australian Aquatic Invertebrate Fauna. Department of Biodiversity, Conservation and Attractions, Perth. Available from: https://library.dbca.wa.gov.au /static/FullTextFiles/072273.pdf. Accessed Wed, 1 July 2020.

Pinder AM, Halse SA, McRae JM, Shiel RJ (2005) Occurrence of aquatic invertebrates of the wheatbelt region of Western Australia in relation to salinity. *Hydrobiologia* **543**: 1-24.

Ponder WF, Hallan A, Shea M, Clark SA (2016) Australian Freshwater Molluscs. Available from: http://keys.lucidcentral.org/keys/v3/freshwater_molluscs/. Accessed Thu, 25 Jun 2020.

Pusey B, Edward D (1990) Limnology of the southern acid peat flats, South-western Australia. *Journal of the Royal Society of Western Australia* **73** (2): 29-46.

Sammut J, Lines-Kelly R (2000) An Introduction to Acid Sulfate Soils. Environment Australia, Canberra.

Semeniuk C, Semeniuk V (2011) A comprehensive classification of inland wetlands of Western Australia using the geomorphic-hydrologic approach. *Journal of the Royal Society of Western Australia* **94**(3): 449–464.

Shiel RJ, Green JD, Nielsen DL (1998) Floodplain biodiversity: why are there so many species? *Hydrobiologia* **387**: 39-46.

Sheldon F, Boulton AJ, Puckridge JT (2002) Conservation value of variable connectivity: aquatic invertebrate assemblages of channel and floodplain habitats of a central Australian arid-zone river, Cooper Creek. *Biological Conservation* **103**: 13-31.

Sim L, Nowicki A, Pinder A, Prideaux C, Cale D, Coote M (2008) Trialling a framework and indicators for wetland extent, distribution and condition in Western Australia, Department of Environment and Conservation, Perth, Western Australia.

Smith KD, Knott B, Jasinska EJ (2002) Biology of the Black-stripe minnow *Galaxiella nigrostriata*, (Galaxiidae) in an acidic, black-water lake in Melaleuca Park near Perth, Western Australia. *Records of the Western Australian Museum* **21**: 277-284 (2002). DOI: 10.18195/issn.0312-3162.21(3).2002.277-284

Storey AW, Bunn SE, Davies PM, Edward DH (1990) Classification of the macroinvertebrate fauna of two river systems on southwestern Australia in relation to physical and chemical parameters. *Regulated Rivers* **5**: 217-232.

Storey A.W. & Humphrey C. (1996). Quality Assurance/Quality Control in Rapid Bioassessment Projects with Preliminary Guidelines for Implementation in the Australian Monitoring River Health Initiative. Unpublished Report to the Land & Water Resources Research & Development Corporation, Canberra, ACT, Aust. Pp. 29.

Storey AW, Shiel RJ, Lynas J (2004a) Buntine-Marchagee Natural Diversity Recovery Catchment: Wetland Invertebrate Fauna Monitoring: November 2003. Data analysis and interpretation. Unpublished report by the School of Animal Biology, The University of Western Australia to the Department of Conservation and Land Management, Mid-West Regional Office. Pp. 41.

Storey AW, Shiel RJ, Lynas J (2004b) Buntine-Marchagee Natural Diversity Recovery Catchment (BMRC), Wetland Invertebrate Fauna Monitoring: August 2004. Unpublished report to the Department of Conservation and Land Management, Mid-west Regional Office. School of Animal Biology, The University of Western Australia, Nedlands. December 2004.

Storey A.W, Vervest RM, Pearson GB, Halse SA (1993) Wetlands of the Swan Coastal Plain Volume 7. Waterbird Usage pf Wetlands on the Swan Coastal Plain. Environmental Protection Authority and Water Authority of Western Australia.

Sutcliffe K (2003) *The conservation status of aquatic insects in south-western Australia.* PhD, Murdoch University, Perth, Western Australia.

Tan LW, Shiel RJ (1993) Responses of billabong rotifer communities to inundation. *Hydrobiologia* **255/256**: 361-369.

Tille P, Lantzke N (1990) Land Resources of Busselton-Margaret River-Augusta. Busselton-Dunsborough Map. Western Australian Department of Agriculture. Perth.

Toussaint EFA, Condamine FL, Hawlitschek O, Watters CH, porch N, Hendrich L, Balke M (2014) Unveiling the diversification dynamics of Australasian predaceous diving beetles in the Cenozoic. *Systematic Biology* **64**(1): 3-24.

Towns DR (1985) Limnological characteristics of a South Australian intermittent stream, Brown Hill Creek. *Australian Journal of Marine and Freshwater Research* **36**: 821-837.

Trayler KM, Davis JA, Horwitz P, Morgan D (1996) Aquatic fauna of the Warren bioregion, south-west Western Australia: does reservation guarantee preservation? *Journal of the Royal Society of Western Australia* **79**: 281-291.

V & C Semeniuk Research Group (1998) Evaluation of Wetlands on the Southern Swan Coastal Plain. Report to the Water & Rivers Commission, Perth. June 1998

Wenjie L, Binxia L, Cuijuan N (2019) Effects of temperature on life history strategy of the rotifer *Euchlanis dilatate*. *Zoological Science* **36**(1): 52-57.

Western Australian Planning Commission (WAPC) (2005) Guideline for determination of wetland buffer requirements. Western Australian Planning Commission, Perth, Western Australia.

Williams WD, Hynes HBN (1977). The ecology of temporary streams II: general remarks on temporary streams. *Internationale Revue der gesamten Hydrobiologie* **62**: 53-61.

Watts CHS, Leys R (2008) Review of the epigean species of Paroster SHARP, 1882, with descriptions of three new species, and phylogeny based on DNA sequence data of two mitochondrial genes. *Spixiana* **78**: 9-36.

WRM (2006a) Elgin Project: Baseline Aquatic Biology and Water Quality Study. Unpublished report by Wetland Research & Management to Iluka Resources Ltd. January 2006.

WRM (2006b) Burekup Project: Baseline Aquatic Biology and Water Quality Study. Unpublished report by Wetland Research & Management to Iluka Resources Ltd. January 2006.

WRM (2006c) Tutunup Project: Baseline Aquatic Biology and Water Quality Study. Unpublished report by Wetland Research & Management to Iluka Resources Ltd. January 2006.

WRM (2006d) Cloverdale Project: Baseline Aquatic Biology and Water Quality Study of the Ludlow River and Tiger Gully. Unpublished DRAFT report by Wetland Research & Management to Iluka Resources Ltd. January 2006.

WRM (2006e) Yoganup 215 Project: Baseline Aquatic Biology Study. Unpublished DRAFT report by Wetland Research & Management to Iluka Resources Ltd. February 2006.

WRM (2008a) Aquatic Microinvertebrate Communities of South-west Wetlands. Unpublished report by Wetland Research & Management to Iluka Resources Ltd. August 2008.

WRM (2008b) South-west Wetlands Assessments: Baseline Aquatic Biology Study of Gavin's Road Wetland, November 2007. Unpublished report by Wetland Research & Management to Iluka Resources Ltd. August 2008.

WRM (2008c) Tutunup Wetland: Baseline Aquatic Biology Study. Unpublished report by Wetland Research & Management to Iluka Resources Ltd. November 2008.

WRM (2009) Collie River Ecological Values Assessment 2008. Unpublished report to the Department of Water, Perth, by Wetland Research & Management. April 2009.

WRM (2018) Perth Airport Macroinvertebrate Study. Spring 2017. Unpublished report by Wetland Research & Management to Perth Airport Pty. Ltd. February 2018

WRM (2019) Bunbury Outer Ring Road Northern and Central Investigation Area: Targeted Conservation Significant Aquatic Fauna Survey 2018. Unpublished final report by Wetland Research & Management to BORR Team Pty Ltd. April 2019. Available from: http://www.epa.wa.gov.au/sites/default/files/Referral_Documentation/Appendix%20F%20%20BORR%20Northern%20and%20Central%20Section%20Targeted%20Cons%20Sig%20Aquatic%20Fauna%20Survey%20%28WRM%202019%29.pdf. Accessed Wed, 24 Jun 2020.

WRM (2020) Tutunup Wetland Aquatic Ecosystem values Desktop Assessment. Unpublished draft report by Wetland Research and Management for Iluka Resources Limited. July 2020.

York Main B (1996) Terrestrial invertebrates of the south-west Australian forests: the role of relict species and habitats in reserve design. Journal *of the Royal Society of Western Australia* **79**:277-280.

APPENDICES

Appendix 1. Site photographs from August 2020

TUT1-5 Shallow inundated vehicle access track within REW

TUT1-6 Shallow water body on boundary of CCW and REW

TUT1-7 Very shallow inundated paperbark woodland within REW

TUT1-8 Shallow inundated vehicle access track, within CCW

TUT1-9 Artificial dam and surrounding vegetation within CCW

Appendix 2. ANZG (2018) default guideline values for protection of aquatic systems in south west Western Australia

Table A2-1. Default guidelines for physical and chemical stressors for south-west Australia for slightly disturbed ecosystems, based on ANZECC/ARMCANZ 2000 guidelines. ANZG (2018) note that more specific regional guidelines are yet to be developed. recommend that stakeholders and managers site-specific guidelines for stressors wherever possible. There are currently no regional guidelines specific to the south west coast. Guidelines used for the current study are highlighted yellow.

Ecosystem type	TP	FRP	TN	N-NO_X	N-NH_4^+	DO[b]	pH
	(ug/L)	(ug/L)	(ug/L)	(ug/L)	(ug/L)	(% sat.)	
Upland river[a]	20	10	450	200	60	90	6.5 – 8.0
Lowland river[a]	65	40	1200	150	80	80 – 120	6.5 – 8.0
Freshwater lakes & reservoirs	10	5	350	10	10	90	6.5 – 8.0
Wetlands	60	30	1500	100	40	90 – 120	7.0 [c] – 8.5[c]

a = all values derived during base river flow conditions not storm events.

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

c = in highly coloured wetlands (gilven > 52 g_{440}/m) pH typically ranges 4.5 – 6.5.

Table A2-2. Ranges for default guideline values for conductivity (EC, salinity) and turbidity indicative of South West Australia, as reported in ANZECC/ARMCANZ (2000). Values reflect high site-specific and regional variability. Explanatory notes provide detail on specific variability issues for ecosystem types. Note, ANZG (2018) recommend development of regional or site-specific guidelines for stressors wherever possible. There are currently no regional guidelines specific to the south west coast. Guidelines used for the current study are highlighted yellow.

Ecosystem type	Salinity (µs/cm)	Explanatory notes
Upland & lowland Rivers	120 – 300	Conductivity in upland streams will vary depending on catchment geology. Values at the lower end of the range are typically found in upland rivers, with higher values found in lowland rivers. Lower conductivity values are often observed following seasonal rainfall.
Lakes, reservoirs & wetlands	30 – 1500	Values at the lower end of the range are observed during seasonal rainfall events. Values even higher than1500 µs/cm are often in saltwater lakes and marshes. Wetlands typically have conductivity values in the range 500-1500 µs/cm over winter. Higher values (> 3000 µs/cm) are often measured in wetlands in summer due to evaporative water loss.
	Turbidity (NTU)	
Upland & lowland Rivers	10 – 20	Turbidity and SPM are highly variable and dependent on seasonal rainfall runoff. These values representative of base river flow in lowland rivers
Lakes, reservoirs & wetlands	10 – 100	Most deep lakes and reservoirs have low turbidity. However, shallow lakes and reservoirs may have slightly higher turbidity naturally due to wind-induced resuspension of sediments. Lakes and reservoirs in catchments with highly dispersible soils will have high turbidity. Wetlands vary greatly in turbidity depending upon the general condition of the catchment or river system draining into the wetland and the water level in the wetland.

Table A2-3. Summary of default surface water quality guidelines for freshwater ecosystems. Refer Australian Government *Water Quality Australia* website (http://www.waterquality.gov.au) for revised (2018) procedures for reporting against the default guidelines. Guidelines used for the current study are highlighted yellow.

Analyte		ANZG (2018) Water Quality Guidelines			
		Freshwater Ecosystems			
		Level of species protection			
		99%	95%	90%	80%
Al (mg/L) (pH>6.5)	T	0.027	0.055	0.08	0.15
Al (mg/L) (pH<6.5)	T	ID	*0.0008	ID	ID
Ag (mg/l)	T	0.00002	0.00005	0.0001	0.0002
As (III) (mg/L)	T	0.001	0.024	0.094	0.36
As (V) (mg/L)	T	0.0008	0.013	0.042	0.14
As-total (mg/L)	T	NP	NP	NP	NP
B (mg/L)	T	0.09	0.37	0.68	1.30
Ba (mg/L)	T	NP	NP	NP	NP
Be (mg/L)	T	ID	ID	ID	ID
Bi (mg/L)	T	ID	ID	ID	ID
Cd (mg/L)	H,T	0.00006	0.0002	0.0004	0.0008
Chlorine (mg/L)	T	0.0004	0.003	0.006	0.013
Co (mg/L)	T	ID	NP	ID	ID
Cr (III) mg/L	H,T	ID	ID	ID	ID
Cr (VI) (mg/L)	T	0.00001	0.001	0.006	0.04
Cr-total (mg/L)	T	NP	NP	NP	NP
Cu (mg/L)	T	0.0001	0.0014	0.0018	0.0025
Fe (mg/L)	T	ID	ID	ID	ID
Hg-inorganic (mg/L)	B,T	0.00006	0.0006	0.0019	0.0054
Li (mg/l)		ID	ID	ID	ID
Mn (mg/L)	T	1.2	1.9	2.5	3.6
Mo (mg/L)	T	ID	ID	ID	ID
N-NH_3 (mg/L) (pH 8)	T	0.32	0.9	1.43	2.3
N-NO_3 (mg/L)	N,T	1.1	2.1	3.1	5.4
Ni (mg/L)	H,T	0.008	0.011	0.013	0.017
Pb (mg/L)	H,T	0.001	0.0034	0.0056	0.0094
Se-total (mg/L)	B,T	0.005	0.011	0.018	0.034
U (mg/L)	T	NP	NP	NP	NP
V (mg/L)	T	ID	ID	ID	ID
Zn(mg/L)	H,T	0.024	0.06	0.12	0.25

Notes:

* = ANZG (2018) provide only a low reliability guideline for aluminium at pH < 6.5, which is based on the value reported b ANZECC/ARMCANZ (2000).

B = metals for which bioaccumulation and secondary poisoning effects on aquatic biota should be considered.

E = default guideline for freshwater ecosystems is for protection against effects of eutrophication.

H = default guideline for freshwater ecosystems should be adjusted for site-specific water hardness using algorithms in Warne *et al.* (2018), as specified by ANZG (2018).

ID = insufficient data to derive a default guideline at the time of ANZG (2018) publication.

N = default guideline shown for N-NO_3 is the Grading concentration for nitrate as a toxicant derived from the species NOEC values and recommended for compliance assessment based on the annual median concentrations.

NP = not provided in the guidelines.

T = default guideline for freshwater ecosystems is for protection against effects of direct toxicity (either chronic or acute).

Appendix 3. Laboratory report for water quality samples.

Refer to embedded file '20S0627_R0.pdf' below, for laboratory report on water quality for 2020 samples.

Note, equivalent site codes for laboratory sample codes are as follows:

Site Code	Laboratory (Client ID) Code
TUT1-5	TUT1-1
TUT1-6	TUT1-2
TUT1-7	TUT1-3
TUT1-9	TUT1-3A
TUT1-8	TUT1-4

20S0627_R0.pdf

Appendix 4. Microinvertebrate taxa

Table A4-1. Microinvertebrate taxa collected in August 2008 and 2020. Values are number (N) of cells per unit volume of sample (see section 2.4.3 for sub-sampling method); [juv.] = juvenile specimen.

Phylum/Class	Family	Lowest Taxon	2008	2020			
				TUT1-5	TUT1-6	TUT1-7	TUT1-8
PROTISTA							
Rhizopoda	**Arcellidae**	*Arcella cf. crenulata*			1		
		Arcella discoides			1		
	Centropyxidae	*Centropyxis cf. constricta*			1		
		Centropyxis ecornis			1		
	Difflugiidae	*Difflugia cf. distenda*	1				
		Difflugia lacustris			1		
ROTIFERA							
Monogononta	**Branchionidae**	*Keratella procurva*			197		
		Keratella slacki		168	1	176	1
	Epiphanidae	*Epiphanes brachionus*			1		
	Euchlanidae	*Euchlanis dilatata*			1		13
	Lecanidae	*Lecane bulla*			1		
	Notomatidae	*Cephalodella gibba*					1
	Trichocercidae	*Trichocerca bicristata*	1				
CLADOCERA	**Chydoridae**	*cf. Pleuroxus* sp. [juv.]					
	Daphnidae	*Ceriodaphnia* spp. [juv.]			1		
	Ilycryptidae	*Ilyocryptus* spp. [juv.]			1		
COPEPODA							
Calanoida		*Boeckella* sp.	1				
Cyclopoida	**Cyclopidae**	*Australocyclops australis*		4		2	
		Metacyclops superincidentis		6	1		1
		Metacyclops sp. a	3				
		Metacyclops sp. b	1				
		Copepodites		4	1	10	40
		Nauplii	8	13	2	12	26
Harpacticoida		indet. spp. [juv.]	1		1		
CLADOCERA	**Chydoridae**	cf. *Pleuroxus* sp. [juv.]	1				
OSTRACODA		cf. *Alboa* sp.	1	1			
		Cypretta sp.	1		1		
		cf. Ilyocypris sp.			1		1
		N encountered in settled volume	19	196	215	200	83
		N cells (of 576)	576	237	3.5	1	576
		Proportion (%) of settled volume	100	41.2	0.6	0.2	100
		Taxa richness	**10**	**6**	**18**	**4**	**7**

Appendix 5. Macroinvertebrate taxa

Table A5-1. Macroinvertebrate taxa collected in August 2008 and 2020. Values are log_{10} abundance categories, where 1= 1 individual, 2 = 2-10 individuals, 3 = 11-100, 4 = 101-1000, and so on; L = larva, F = female, P = pupa, imm./dam. = immature or damaged specimen.

Phylum/Class/Order	Family	Lowest taxon	2008	2020			
				TUT1-5	TUT1-6	TUT1-7	TUT1-8
MOLLUSCA							
Gastropoda							
	Glacidorbidae	*Glacidorbis occidentalis*	1	0	0	0	0
Hygrophila	**Planorbidae**	*Glyptophysa* sp.	0	0	0	1	0
ANNELIDA							
Oligochaeta		Oligochaeta spp.	1	0	2	0	0
ARTHROPODA							
Malacostraca							
Amphipoda		Amphipoda sp. (imm./dam.)	0	0	0	1	0
	Ceinidae	*Austrochiltonia subtenuis*	3	0	0	0	0
	Neoniphargidae	*Wesniphargus nichollsi*	0	3	2	0	0
	Perthiidae	*Perthia acutitelson*	0	0	0	1	2
		Perthia branchialis	4	0	0	0	0
Arachnida							
Mesostigmata		Mesostigmata sp.	0	1	0	0	0
Trombidiformes	**Hydryphantidae**	Tartarithyas sp.	0	0	0	0	1
Collembola							
Entomobryomorpha		Entomobryoidea sp.	0	1	3	1	0
Poduromorpha		Poduroidea sp.	0	0	2	0	0
Symphypleona		Symphypleona spp.	0	0	2	0	0
Insecta							
Zygoptera		Zygoptera spp. (imm.)	2	0	0	0	0
Diptera	**Ceratopogonidae**	Ceratopogonidae spp. (P)	2	0	0	0	1
	Chironomidae	Chironomidae spp. (P)	1	0	1	0	1
	Chironominae						
	Chironomini	*Chironomus tepperi*	0	2	0	2	0
		Chironomous aff. *alternans* (V24)	2	0	0	0	0
		Cladopelma curtivalva	0	0	1	0	0
		Omisus sp.	0	2	0	2	2
	Tanytarsini	*Tanytarsus* sp. (V6)	3	3	3	0	3
	Orthocladiinae	*Botryocladius petrophilus*	0	2	0	0	2
		Anzacladius sp. nr. *numbat* (V31)	1	0	2	0	2
		Corynoneura sp. (V49)	0	1	2	0	2
		Orthocladiinae sp. (V52)	0	1	0	2	0
		Orthocladiinae sp. (V76)	0	2	2	3	2
		Orthocladiinae sp. (VSC8)	1	0	0	0	0
		Paralimnophyes pullulus (V42)	2	0	0	1	0
		Paramerina levidensis (V1)	3	3	0	0	2
	Tanypodinae	Pentaneurini genus C	0	2	0	0	0
		Procladius paludicola (VCD1)	1	0	0	0	1
	Culicidae	Culicidae spp. (P)	0	3	2	2	3
		Anopheles spp.	4	0	2	0	0
		Culex spp.	0	3	2	2	3
Hemiptera	**Notonectidae**	*Anisops* spp. (F)	1	0	0	0	1
	Veliidae	Veliidae spp. (imm/dam.)	0	0	0	2	0
Coleoptera	**Dytiscidae**	*Allodessus bistrigatus*	0	1	0	1	0
		Antiporus femoralis	1	0	0	0	0

Phylum/Class/Order	Family	Lowest taxon	2008	2020			
				TUT1-5	TUT1-6	TUT1-7	TUT1-8
		Antiporus gilberti	0	0	1	0	0
		Antiporus sp (L)	0	1	2	0	0
		Bidessini sp. (L)	0	0	0	0	1
		Carabhydrus sp. (L)	0	0	1	0	0
		Copelatus sp. (L)	1	0	2	2	2
		Limbodessus shuckardii	0	0	1	0	0
		Megaporus solidus	1	0	0	0	0
		Necterosoma darwini	0	0	0	3	0
		Paroster ellenbrookensis	0	2	0	0	2
		Paroster sp. (L)	0	0	1	0	0
		Paroster sp. nov.	2	0	0	0	0
		Platynectes sp. (L)	0	1	2	0	2
		Rhantus sp. (L)	0	0	0	0	1
		Sternopriscus brownii	2	0	0	0	0
		Sternopriscus marginatus	0	0	1	0	0
	Haliplidae	*Haliplus gibbus/fuscatus* (F)	0	0	1	0	0
	Hydrophilidae	*Berosus approximans*	0	0	2	0	0
		Paracymus pygmaeus	0	1	0	0	0
		Paracymus sp. (L)	0	2	2	0	1
	Scirtidae	Scirtidae spp. (L)	2	0	1	0	0
		Taxa richness	**22**	**20**	**26**	**15**	**21**

Appendix 6. Freshwater crayfish data

Table A6-1. Freshwater crayfish collected in 2020.

Site	Date	Species name	Common name	CL (mm)	Sex
TUT1-6	04/08/2020	*Cherax preissii*	Koonac	92	M
TUT1-6	04/08/2020	*Cherax preissii*	Koonac	60	M
TUT1-6	04/08/2020	*Cherax preissii*	Koonac	40	F
TUT1-9	05/08/2020	*Cherax destructor*	Yabby	65	M
TUT1-9	05/08/2020	*Cherax destructor*	Yabby	72	M
TUT1-9	05/08/2020	*Cherax destructor*	Yabby	59	M
TUT1-9	05/08/2020	*Cherax destructor*	Yabby	59	M
TUT1-9	05/08/2020	*Cherax destructor*	Yabby	57	F
TUT1-9	05/08/2020	*Cherax destructor*	Yabby	50	F
TUT1-9	05/08/2020	*Cherax destructor*	Yabby	50	M

Appendix 7. DBCA NatureMap Invertebrate Database Search Results

DBCA NatureMap database aquatic invertebrate records within search radiuses 40 km, 15 km and 5 km of Tutunup Wetland. Conservation-listed fauna are indicated adjacent to taxa name in **red bold**; VU = Vulnerable, CR = Critically Endangered.

Invertebrate type	Taxa name	Radius from Tutunup Wetland		
		40 km	15 km	5 km
Platyhelminthes (flatworms)	Turbellaria sp.	Y		
	Temnocephaloidea sp.	Y	Y	Y
Nematoda (round worms)	Nematoda sp.	Y		
Mollusca (bivalves)	Sphaeriidae sp.	Y		
	Hyriidae sp.	Y	Y	
	Westralunio carteri **VU**	Y		
Mollusca (snails)	Lymnaeidae sp.	Y		
	Physidae sp.	Y		
	Planorbidae sp.	Y	Y	
	Ancylini sp.	Y	Y	
Oligochaeta (segmented worms)	Oligochaeta sp.	Y	Y	Y
	Enchytraeidae sp.	Y		
	Naididae sp.	Y		
	Phreodrilidae sp.	Y	Y	
	Insulodrilus bifidus	Y	Y	
Hirudinea (leeches)	Hirudinidae sp.	Y	Y	Y
Acarina (water mites)	Acarina sp.	Y	Y	Y
	Arrenuridae sp.	Y	Y	
	Aturidae sp.	Y	Y	
	Hydrachnidae sp.	Y		
	Hydryphantidae sp.	Y		
	Limnesiidae sp.	Y		
	Oxidae sp.	Y		
	Oxidus gracilis	Y		
Cladocera (water fleas)	Cladocera (non-daphniidae)	Y		
	Cladocera (unident.)	Y		
Ostracoda (seed shrimp)	Ostracoda (unident.)	Y	Y	
Copepoda	Copepoda sp.	Y	Y	
	Calanoida sp.	Y		
Parastacidae (freshwater crayfish)	Parastacidae sp.	Y	Y	Y
	Cherax sp.	Y		
	Cherax cainii	Y		
	Cherax destructor	Y	Y	
	Cherax preissii	Y	Y	
	Cherax quinquecarinatus	Y	Y	Y
	Cherax tenuimanus **CR**	Y		
	Engaewa pseudoreducta **CR**	Y		
	Engaewa reducta **CR**	Y		
Palaemonidae (freshwater prawns)	Palaemonidae sp.	Y	Y	
Amphipoda (sideswimmers)	Neoniphargidae sp.	Y	Y	
	Paramelitidae sp.	Y		
	Perthiidae sp.	Y	Y	
Isopoda	Phreatoicidae sp.	Y	Y	

Invertebrate type	Taxa name	Radius from Tutunup Wetland		
		40 km	15 km	5 km
Diptera (two-winged flies)	Athericidae sp.	Y	Y	
	Ceratopogonidae sp.	Y	Y	Y
	Culex australicus	Y		
	Culicidae sp.	Y	Y	
	Empididae sp.	Y		
	Muscidae sp.	Y		
	Psychodidae sp.	Y		
	Simuliidae sp.	Y	Y	Y
	Stratiomyidae sp.	Y		
	Tabanidae sp.	Y	Y	
	Tipulidae sp.	Y	Y	Y
Chironomidae (non-biting midges)	*Alotanypus dalyupensis*	Y		
	Chironominae sp.	Y	Y	Y
	Chironomus aff. *alternans* (V24)	Y	Y	
	Chironomus tepperi	Y	Y	
	Cladopelma curtivalva	Y		
	Cladotanytarsus sp. A	Y		
	Corynoneura sp. (V49)	Y	Y	
	Cricotopus 'brevicornis'	Y		
	Cricotopus 'parbicinctus'	Y	Y	
	Cryptochironomus aff *griseidorsum*	Y		
	Dicrotendipes sp. A (V47)	Y	Y	
	Gymnometriocnemus sp. 1 (V44)	Y		
	Gymnometriocnemus spp. (not V44 or V45)	Y		
	Harrisius sp.	Y		
	Harrisius sp. A (SAP)	Y		
	Harrisius sp. B (SFM)	Y	Y	
	Kiefferulus intertinctus	Y		
	Limnophyes vestitus (V41)	Y	Y	
	Nanocladius sp.2 (V71)	Y		
	Orthocladiinae SO3 sp. A (SAP)	Y	Y	
	Orthocladiinae sp.	Y		Y
	Orthocladiinae sp. 5 (SFM)	Y		
	Paracladopelma M1 [SFM)	Y		
	Parahyborrhynchus convexiuculus	Y		
	Parakiefferiella sp. S1	Y		
	Parakiefferiella variegatus	Y	Y	
	Paralimnophyes pullulus (V42)	Y	Y	
	Paramerina levidensis	Y	Y	
	Pentaneurini genus (V20)	Y	Y	
	Polypedilum nr. *convexum* (SAP)	Y	Y	
	Polypedilum watsoni	Y	Y	
	Procladius paludicola	Y		
	Rheotanytarsus flabellatus	Y		
	Rheotanytarsus juliae	Y		
	Rheotanytarsus sp.	Y		
	Rheotanytarsus sp. (SFM)	Y		
	Rheotanytarsus trivittatus	Y		

Invertebrate type	Taxa name	Radius from Tutunup Wetland		
		40 km	15 km	5 km
	Rheotanytarsus underwoodi	Y		
	Riethia (V5)	Y	Y	
	Skusella/"V12 ex-WA" (Cranston)	Y		
	Stempellina sp. 1 (SFM)	Y		
	Stictocladius occidentalis	Y		
	Tanypodinae sp.	Y	Y	Y
	Tanytarsus aff *manleyensis*	Y		
	Tanytarsus b1	Y		
	Tanytarsus fuscithorax/semibarbitarsus	Y		
	Tanytarsus nr K5	Y	Y	
	Tanytarsus palmatus	Y	Y	
	Thienemanniella sp. (V19) (SAP)	Y		
Coleoptera (beetles)	Dytiscidae sp.	Y	Y	Y
	Antiporus occidentalis	Y	Y	
	Antiporus sp.	Y	Y	
	Batrachomatus nannup	Y		
	Batrachomatus sp.	Y		
	Exocelina ater	Y		
	Lancetes lanceolatus	Y		
	Limbodessus inornatus	Y	Y	
	Limbodessus shuckhardi	Y		
	Necterosoma sp.	Y		
	Platynectes aenescens	Y		
	Platynectes decempuntatus var *polygrammus*	Y	Y	
	Platynectes sp.	Y	Y	
	Rhantus suturalis	Y		
	Sternopriscus browni	Y	Y	
	Sternopriscus marginatus	Y		
	Sternopriscus minimus	Y		
	Sternopriscus sp.	Y	Y	
	Sternopriscus wattsi	Y		
	Uvarus pictipes	Y	Y	
	Gyrinidae sp.	Y	Y	
	Macrogyrus angustatus	Y		
	Macrogyrus sp.	Y		
	Heteroceridae sp.	Y		
	Hydraenidae sp.	Y	Y	
	Hydraena sp.	Y		
	Hydrochus australis	Y		
	Hydrophilidae sp.	Y	Y	
	Berosus discolor	Y		
	Berosus munitipennis	Y		
	Helochares tenuistriatus	Y		
	Hydrophilus triangulans	Y		
	Limnoxenus sp.	Y		
	Limnoxenus zelandicus	Y		
	Paracymus spenceri	Y	Y	

Invertebrate type	Taxa name	Radius from Tutunup Wetland		
		40 km	15 km	5 km
	Limnichidae sp.	Y		
	Scirtidae sp.	Y	Y	Y
Ephemeroptera (mayflies)	Baetidae sp.	Y	Y	
	Cloeon sp. 2 (SFM)	Y		
	Offadens soror (ex genus 1 WA sp. 1)	Y		
	Caenidae sp.	Y	Y	
	Tasmanocoenis tillyardi	Y		
	Leptophlebiidae sp.	Y	Y	Y
	Bibulmena kadjina	Y		
	Leptophlebiid genus S sp. AV1	Y	Y	
	Neboissophlebia occidentalis	Y		
	Nousia sp. AV16	Y	Y	
	Nyungara bunni	Y		
Hemiptera (true bugs)	Corixidae sp.	Y	Y	
	Gelastocoridae sp.	Y	Y	
	Hydrometridae sp.	Y		
	Micronecta robusta	Y	Y	
	Notonectidae sp.	Y	Y	Y
	Anisops sp.	Y		
	Veliidae sp.	Y	Y	
	Microvelia sp.	Y		
Megaloptera (alderflies)	Megaloptera sp.	Y		
	Corydalidae sp.	Y		
	Archichauliodes sp.	Y		
Odonata (dragonflies & damselflies)	Aeshnidae sp.	Y	Y	Y
	Austroaeschna anacantha	Y		
	Hesperocordulia berthoudi	Y		
	Coenagrionidae sp.	Y	Y	
	Corduliidae sp.	Y	Y	
	Hemicordulia sp.	Y	Y	
	Gomphidae sp.	Y	Y	
	Austrogomphus lateralis	Y		
	Lestidae sp.	Y	Y	
	Megapodagrionidae sp.	Y	Y	
	Archiargiolestes pusillus	Y		
	Miniargiolestes minimus	Y		
	Libellulidae sp.	Y	Y	
	Protoneuridae sp.	Y	Y	
	Synthemistidae sp.	Y	Y	Y
	Archaeosynthemis occidentalis	Y		
	Archaeosynthemis spiniger	Y		
	Austrosynthemis cyanitincta	Y		
	Telephlebiidae sp.	Y	Y	Y
Plecoptera (stoneflies)	Gripopterygidae sp.	Y	Y	Y
	Leptoperla australica	Y	Y	
	Leptoperla sp.1 (nsp)	Y		
	Newmanoperla exigua	Y	Y	
	Newmanoperla sp.	Y		

Invertebrate type	Taxa name	Radius from Tutunup Wetland		
		40 km	15 km	5 km
	Riekoperla occidentalis	Y		
Trichoptera (caddisflies)	Ecnomidae sp.	Y	Y	
	Ecnomina E group sp. 5	Y		
	Ecnomus sp.	Y		
	Hydroptilidae sp.	Y	Y	
	Acritoptila globosa	Y		
	Acritoptila margaretae	Y		
	Hellyethira sp.	Y		
	Oxyethira sp.	Y		
	Hydrobiosidae sp.	Y	Y	
	Hydropsychidae sp.	Y		
	Leptoceridae sp.	Y	Y	Y
	Lectrides parilis	Y		
	Leptoc Genus A sp. AV1	Y		
	Notalina nr. sp. AV14	Y		
	Notalina sp.	Y		
	Notalina sp. AV15 (PSW)	Y		
	Notalina sp. AV16 (SFM)	Y		
	Notoperata sp. AV4 (SFM)	Y		
	Notoperata tenax	Y		
	Oecetis sp.	Y		
	Atriplectides dubius	Y		
	Triplectides australis	Y		
	Triplectides sp. AV1 (SFM)	Y		
	Triplectides sp. AV21 (SFM)	Y	Y	
	Philopotamidae sp.	Y	Y	Y