Hughenden Irrigation Project

Fish Community Monitoring

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This report has been prepared by Australasian Fish Passage Services (AFPS).

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Please cite as: Marsden, T. (2021) Hughenden Irrigation Project, Fish Community Monitoring. Report to HIPCo, Australasian Fish Passage Services, 67pp.

Contents

1 Introduction

Australasian Fish Passage Services has been contracted by Jacobs to provide fish biology and passage advice for the Hughenden Irrigation Project (HIP). This study aims to:

- Identify the fish species in the upper Flinders River and their distribution
- Identify fish habitats and key refuge waterholes
- Identify the potential impacts of the dam works on these fish communities
- Provided recommendations moving forward through the business case

1.1 The Flinders River Catchment

The Flinders River rises in the Burra Range on the western slopes of the Great Dividing Range to the north-east of Hughenden in northwest Queensland. It descends 816m over its 1004 km course, flowing to the west past Hughenden, Richmond and Julia Creek before heading north to the coast, where it empties into the Gulf of Carpentaria 25 km west of Karumba (Wikipedia, 2021a) (Figure 1).

The Flinders River Catchment covers an area of 109,000 km^2 and is characterised by several river systems, wetlands, extensive floodplains and estuarine areas. A total of 36 tributaries flow into the Flinders River including the Cloncurry, Saxby, Corella, Dutton and Bynoe Rivers (Wikipedia 2021a), draining a large portion of central and western Queensland.

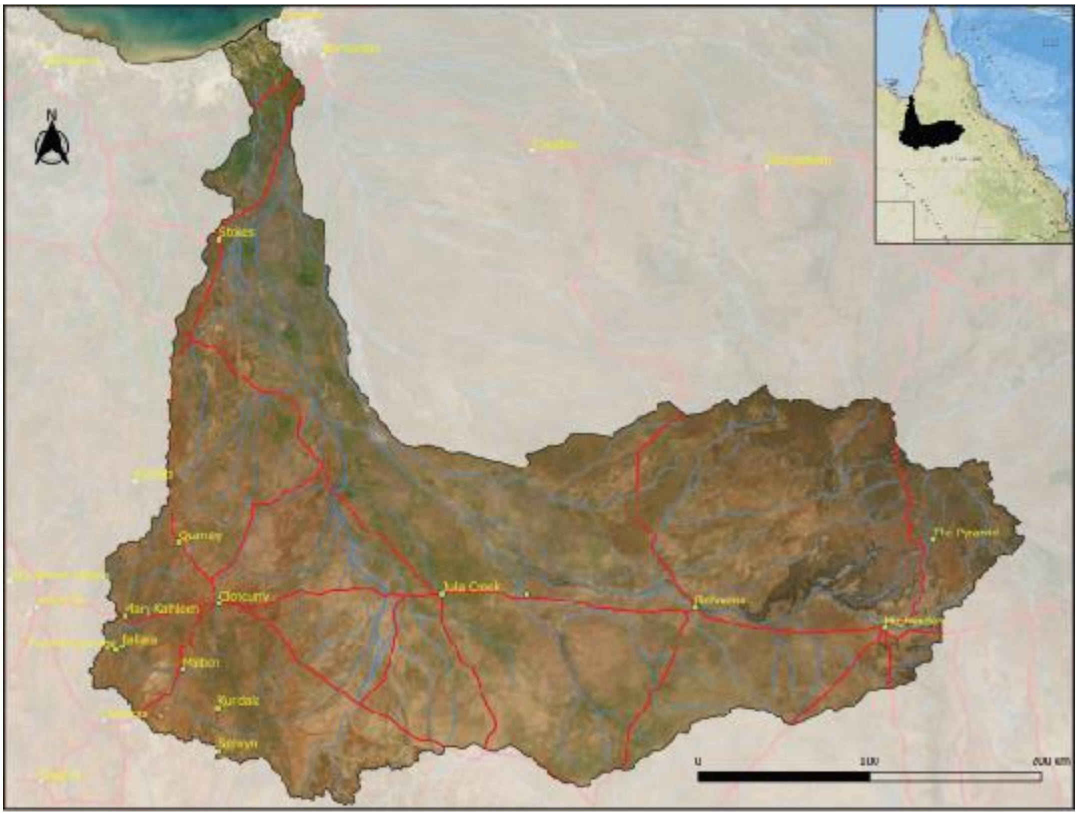

Figure 1. The extensive Flinders River Catchment in northwest Queensland.

1.2 Climate

The Flinders River Catchment has a hot semi-arid climate (Köppen type BSh) with temperatures at Hughenden ranging from a mean low of 8.8ºC in July to a mean high of 36.9 ºC in December (Wikipedia 2021b; BOM 2021).

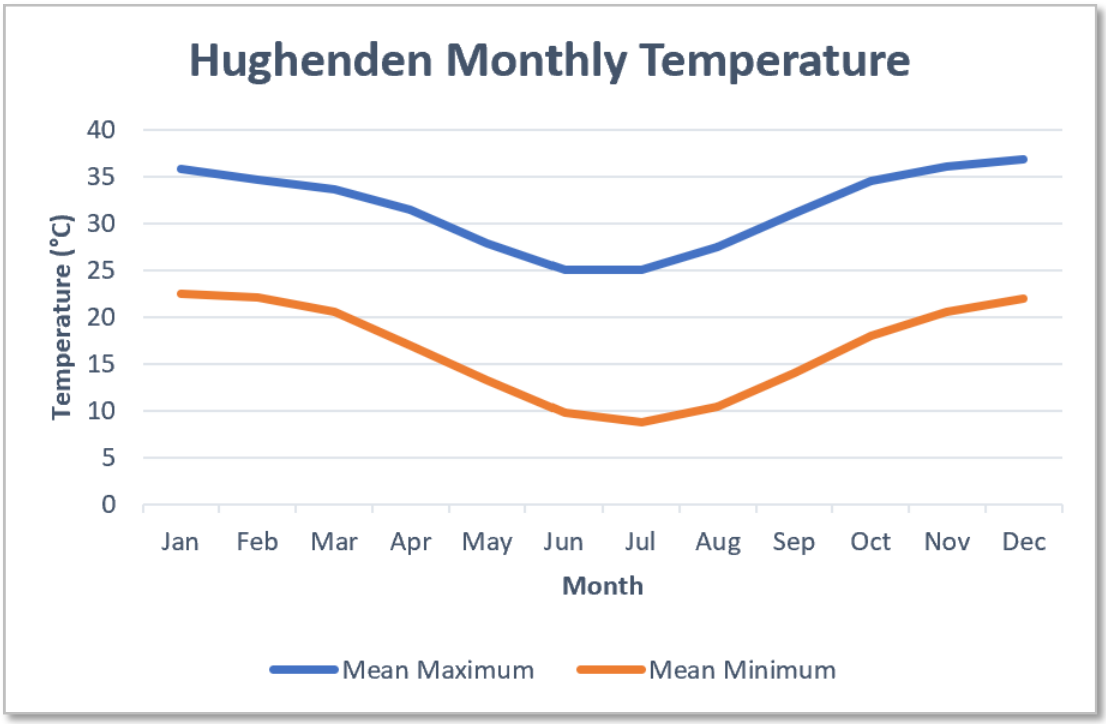

Figure 2. Historical mean minimum and mean maximum temperatures for Hughenden Post Office (data sourced from BOM 2021).

The Flinders River location in the tropical zone of Australia means that rainfall is influenced by the annual monsoon season. The mean average rainfall across the Flinders Catchment is 492 mm with 88% of this falling during the annual monsoon season over summer and early autumn (Dec-Mar) (CSIRO, 2013) (Figure 3). Across the catchment there is a significant rain gradient from the coast, which receives an average of 800 mm, to the inland areas which receive on average only 350 mm (CSIRO, 2013). During the dry season (May-Oct) rainfall is low, accounting for only about 12% of annual falls (CSIRO, 2013).

1.2.1 Hydrology

Annual flow patterns in the Flinders River closely correlate with rainfall, with flows typically commencing in November and flowing till approximately April (Figure 4). Evaporation in the catchment is very high, with 90% of rainfall lost to the atmosphere, and only 8% entering streamflow resulting in river discharges to the Gulf of Carpentaria of approximately 3,857 GL each year (CSIRO, 2013). Despite lower rainfall in the upper catchment, discharges from upstream reaches contribute significantly to river flows and consequent flooding in the downstream catchment (Ndehedehe, 2020).

During the dry season the majority of streams in the catchment stop flowing and are reduced to a series of isolated pools. Many of these pools are ephemeral or near permanent in nature, slowly drying up as water percolates or evaporates away. Very few waterholes are considered permanent in the upper catchment.

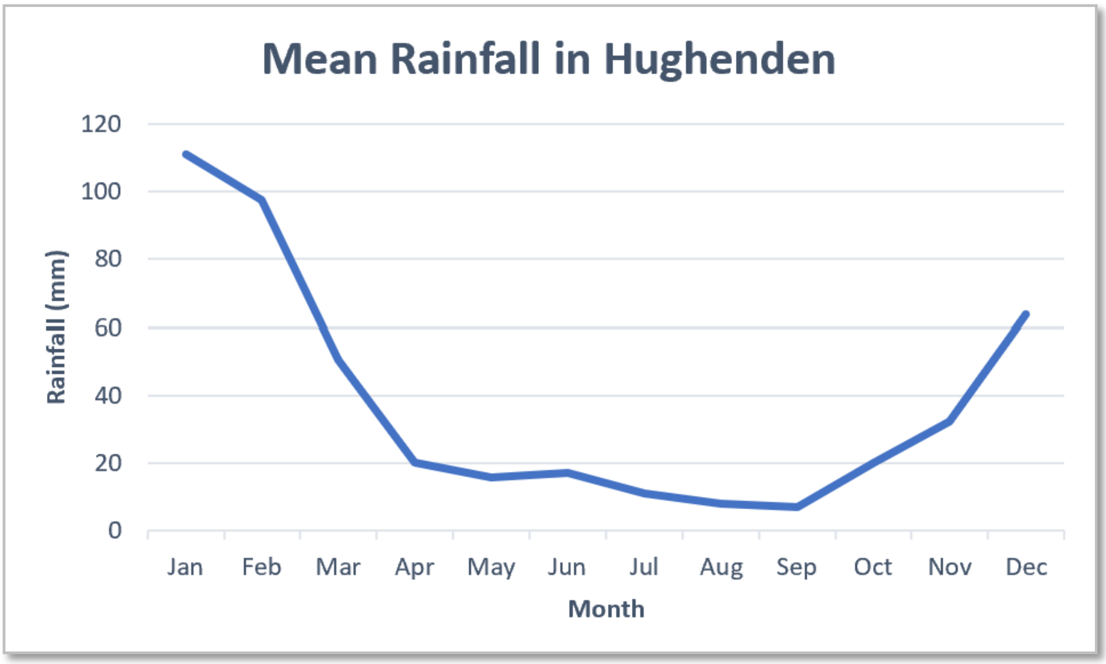

Figure 3. Historical (1887 to present) mean monthly rainfall at Hughenden (#30025). Data sourced from BOM 2021.

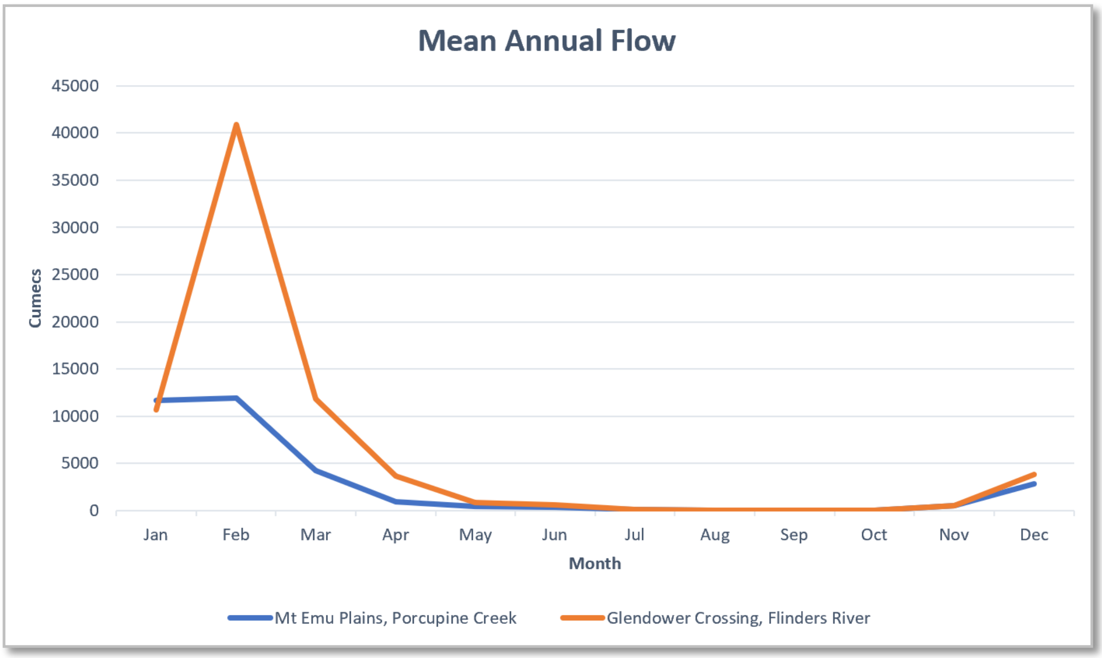

Figure 4. Historical mean annual flow at two stream gauges in the Upper Flinders Catchment: Mt Emu Plains Porcupine Creek (#915011A, 1971-present) and Glendower Crossing (#915015A, 2012 – present). Information sourced from [WMIP: Queensland Government (information.qld.gov.au)].

1.3 Bioregions

The Flinders Catchment spans three bioregions including the Einasleigh Uplands, Mitchell Grass Downs and the Gulf Plains.

The headwaters of the Flinders River lie within the Einasleigh Uplands bioregion. This bioregion is characterised by rugged hills and ranges of various altitudes formed on granites, acid volcanics, metamorphosed sediments and basalts (DERM 2009a). Eucalypt woodlands dominate the landscape that supports endemic species and specialised habitats (DERM 2009a). Ninety-three percent of the bioregion is grazed, with grazing pressure and excessive pasture utilisation resulting in more than 20% of the region having been cleared, notable changes to understorey species from perennial grasses to annual and exotic species, and soil erosion issues from ground cover reduction (Bastin & ACRIS Management Committee, Rangelands 2008a).

The Mitchell Grass Downs bioregion is characterised by rolling, largely treeless plains with cracking ‘black’ soils dominated by Mitchell grass (*Astrebla spp)* tussock grassland interspersed with gidgee Acacia (*Acacia cambagei*), herblands, eucalypt woodlands and isolated remnant plateaus (Sattler & Williams, 1999; Bastin & ACRIS Management Committee, Rangelands 2008b; DERM 2009). On the alluvial plains surrounding the Flinders River, Eucalypt communities are dominated by Coolabah (Eucalyptus coolabah) or River red gum (Eucalyptus camaldulensis) (DERM 2009). Ninety-six percent of the bioregion is under pastoral land use with high levels of pasture utilisation threatening the persistence and recovery of productive perennial grasses, including Mitchell grass, changing pasture composition, increasing woody weeds and increasing the cover of trees and shrubs in former grassland areas (Bastin & ACRIS Management Committee, Rangelands 2008b).

The Gulf Plains is characterised by flat grasslands, open woodlands of eucalypts, melaleuca and acacia, extensive alluvial plains and coastal areas (Bastin & ACRIS Management Committee, Rangelands 2008c; EHP 2015). Ninety-three percent of the bioregion is grazed, with pasture utilisation increasing in some areas threatening understorey species and increasing woody weeds (Bastin & ACRIS Management Committee, Rangelands 2008c). Despite this pressure, exceptionally little clearing of native vegetation has occurred within the bioregion, which possesses a high percentage of remnant vegetation (between 97.1 and 99.7%) (EHP 2015).

1.4 Land use

European settlement commenced in the Gulf region in the 1860’s, with the first land sale in August 1867 and the establishment of several cattle stations seeing the introduction of widespread cattle grazing in the region (Burke Shire Council n.d.). Today, land use in the Flinders Catchment is still dominated by grazing on native grassland pastures with small areas of nature conservation, residential, mining and irrigated cropping (Queensland Globe 2018).

1.4.1 Impacts on Fish Habitat

Grazing on savannah plains, altered fire regimes, alluvial and hard-rock mining and localised intensive agriculture, have all altered many processes and conditions in the region (Crowley & Garnett 2000; McDonald & Dawson, 2004; Brooks et al. 2008).

Many studies conducted in southeast Australia have shown large increases in erosion and sediment delivery as a result of land use changes following European settlement over the last 180 years ago (e.g., Prosser et al., 1994; Prosser and Winchester, 1996; Brooks and Brierley, 1997, 2000; Wasson et al., 1998; Scott, 2001; Pietsch et al. 2015). In a study by Caitcheon et al. (2012) that analysed concentrations of Caesium-137 to determine the erosion processes supplying sediment to three major rivers in Northern Australia (the Daly, Mitchell and Flinders), it was found that >90% of the sediment being transported along the rivers originated from subsoil erosion of gullies and stream banks.

Gully erosion is the process by which water carves new unstable channels into erodible regolith (unconsolidated soils) and is driven primarily by direct rainfall and infiltration-excess runoff (Shellberg 2011). In the nearby Mitchell catchment, the onset of significant gully erosion is closely correlated with the arrival of cattle (Shellberg et al., 2010; Pietsch et al. 2015), while evidence from gully fill sequences in the Normanby Catchment shows that similar changes in erosion rates accompanied settlement (Brooks et al. 2013).

Grazing impacts upon soil surface and perennial grass condition, particularly in riparian zones during the dry seasons, increases the potential for gully erosion as a result of reduced native grass cover, causes physical soil disturbance, concentrated runoff along cattle tracks, modified fire regimes, episodic drought and exotic weed and grass invasions (Shellberg 2011). Gully erosion causes severe land degradation, is a major component of contemporary sediment budgets, and is a major source of sediment pollution to aquatic ecosystems (Shellberg 2011).

Sediments deposited in waterways cause a reduction in channel depth, the infilling of refuge pools, and the creation of dynamic instream benches and temporal water quality issues. Collectively, this causes greater fragmentation of fish habitats, with fewer permanent refuge pools and greater distances between them.

1.5 Channel Morphology

The Flinders River is an ephemeral waterway that flows less than 30% of the time, reducing to a few refuge waterholes during the dry season (Petheram et al. 2013). The total length of major/named channels mapped in the Fitzroy River catchment is 29,927 km (Saynor et al. 2008).

In the Flinders River catchment, anabranching rivers predominate (77.77 %), with bedrock confined (13.22 %), meandering (2.63 %), bedrock channels (1.94 %) and low sinuosity rivers (1.50 %) also present (Saynor et al. 2008) (Table 1).

Within the Saego Dam project and Hughenden area, the river is primarily classified as wandering (Saynor et al. 2008).

Table 1. River types present in the Flinders Catchment (Saynor et al. 2008).

River Type	Characteristics
Anabranching	Multiple channels separated by ridges, islands and/or floodplain.
Meandering	Single channel with a sinuosity generally >1.5. Short sections of straighter channel with a sinuosity of >1.35 are included. Point bars usually well=developed on the inside of bends. Floodplain formed dominantly by lateral accretion and often consists of floodplain ridges.
Bedrock	Channel excavated into bedrock with essentially no floodplain.
Low sinuosity	Single channel with a sinuosity < 1.35. Floodplain usually has a well-developed natural levee with crevasses and splays.
Wandering	Usually gravel-bed but can be sand-bed. Intermediate form between meandering and braided rivers with islands and bars.

1.5.1 Groundwater Dependence

Jolly et al. (2013) performed an extensive review of the geology, hydrogeology and surface water - groundwater interactions in the Flinders catchment at six river and 17 waterhole sites in the Flinders catchment between May and December 2012. Groundwater, subject to reasonably long flow paths, having spent months to thousands of years in the sub surface environment, is characterised by specific major ion chemistry and naturally occurring isotopes (Jolly et al. 2013). These characteristics can be assessed in water samples to determine the likelihood of groundwater presence.

The majority of river and waterhole sites sampled by Jolly et al., had a nil or low likelihood of groundwater inflow. Only two sites, Fairlight Waterhole (within the Saego Dam project area) and the Flinders River Bridge site in Hughenden, had a high likelihood of groundwater inflow. Fairlight Waterhole was found to have high concentrations of ^{222}Rn in August but lower in October and December, and increases in $\delta^{2}H$, $\delta^{18}O$ and Cl^{-} between October and December, suggesting a reduction in groundwater inflow through the dry season. The Flinders River Bridge site, only sampled in May 2012, found high concentrations of ^{222}Rn, suggesting a high likelihood of groundwater inflow.

The assessment by Jolly et al. indicated that overall, it is unlikely that the dry season persistence of waterholes in the upper Flinders is related to groundwater. However, it is possible that parafluvial groundwater (surface water that infiltrates fluvial sediments in the river channel often resurfacing downstream), could support waterholes throughout the dry season, and was recommended for further investigation.

The limited influence of groundwater on the permanence of instream waterholes within the Flinders River has a significant impact on the persistence of fish communities within the stream, as waterhole permanence is greatly influenced by the strength of the wet season and the volume of river flows.

1.5.2 Climate Change Impacts

Climate change is affecting aquatic environments all over the world. In Australia, droughts are becoming more common and lasting for longer periods of time, while wet seasons are becoming less predictable and less wet, but with more frequent large and often catastrophic flood events. As a result, connectivity for fish is greatly reduced, as flows are of shorter duration. During dry times permanent aquatic habitat becomes increasingly important, having to support the aquatic community until the rain returns. As a result of the shorter connectivity times, it is thought that there is potential that refuge pools may become separated by too great a distance for some species to move between during the shorter wet season flow periods.

Into the future, it is refuge habitats that need to be protected to ensure that fish communities survive through droughts, to provide new recruits when waterway connectivity returns. Without the protection of these refuges, recruitment for the upper Flinders catchment will have to occur from pools further downstream, increasing the distance that migrants have to travel within a wet season.

Protecting refuge pools can occur by ensuring that during the wet season connectivity is restored, riparian vegetation is present and in good condition, providing shade during hot conditions to reduce evaporation, and fencing can also be used to keep cattle out of important habitats.

2 Fish Communities

Several fish community surveys have been conducted within the Flinders River catchment, finding 42 species of freshwater fish (Table 2). In the upper Flinders River, records reviewed by Pusey et al. (2017), indicate that 24 species are potentially present near/within the study area. Twenty of these species, including Barramundi, Sawfish, Fork-tailed catfish, Long-tom, Mullet and Nurseryfish are diadromous, meaning that they migrate between freshwater and the sea at some point in their life cycle. The remaining 22 species are potamodromous migrating wholly within freshwater including Rainbowfish, Giant glassfish, Sleepy cod and Sooty grunter.

Many of the species in the Flinders River are endemic to and/or rare in the Southern Gulf region, including the Carpentaria catfish, Giant glassfish, Golden goby, Gulf grunter, Northern trout gudgeon, Nurseryfish, Papuan river sprat, River gar, Salmon catfish, Saltpan sole, Sawfish, Small mouth catfish, Speckled goby, Spotted scat and the Tadpole goby. The Sawfish (Pristis pristis), once historically widespread, is now extinct or severely depleted in numbers in much of its former range, including in the Gulf of Carpentaria. The species is listed as 'vulnerable' and migratory under the EPBC Act and Critically Endangered under the International Union for Conservation of Nature (IUCN) threatened species list.

Table 2. Fish species present and predicted (*) in the Flinders River Catchment, the upper Flinders Catchment (^), their migration characteristics and size range at migration. Information sourced from Hogan and Vallance (2005), Renfree and Marsden (2006), Pusey, Kennard and Arthington (2004), Allen, Midgley and Allan (2002), www.fishbase.org, O'Brien et al. (2010) and Pusey et al (2017).

SPECIES	MIGRATION CHARACTERISTICS					
	Seasonal Movement	**Flows**	**Spawning**	**Colonisation**	**Dispersal**	**Size Range (mm)**
DIADROMOUS SPECIES						
Barramundi ^ *Later calcarifer*	All (Sum/Spr)	Low-Mod	✓	✓		100-1200
Crimson-tipped flathead gudgeon *Butis butis*	?	?				20-150
Diamond mullet *Liza alata*	?	Low-Mod				80-500
Forktailed catfish ^ *Neoarius graeffei*	All	All	✓	✓	✓	100-600
Freshwater anchovy ^ *Thryssa scratchleyi*	?	?				50-400
Freshwater sole ^ *Brachirus selmheimi*	?	?				30-150
Freshwater stingray *Himantura chaophyra*	All	All				?
Golden goby ^ *Glossobobius aureus*	All	Low			✓	20-140
Freshwater longtom *Strongylura krefftii*	Sum, Aut	Low-Mod		✓	✓	100-500
Nurseryfish *Kurtus gulliveri*	?	?			✓	100-500
Papuan river sprat *Clupeoides cf. papuensis*	?	?				20-80
Salmon catfish	All	All	✓	✓	✓	100-500

Arius leptaspis						
Saltpan sole ^ *Brachirus salinarum*	?	?				30-150
Small-eyed sleeper *Prionobutis microps*	?	Low-Mod				40-200
Small-mouthed catfish *Cinetodus froggatti*	?	Low-Mod				100-450
Speckled goby *Redigobius bikolanus*	?	?				15-40
Spotted scat *Scatophagus argus*	Sum/Spr	Low-Mod				20-300
River garfish *Zenarchopterus spp*	?	?				80-230
Tadpole goby *Chlamydogobius ranunculus*	?	Low				15-40
Sawfish * *Pristus sp.*	?	?	✓	✓	✓	400-2000+
POTAMODROMOUS SPECIES						
Archerfish ^ *Toxotes chatareus*	Sum, Spr	Mod		✓	✓	100-500
Banded grunter ^ *Amniataba percoides*	Sum, Win, Spr	Low-Mod			✓	40-180
Berney's catfish ^ *Arius berneyi*	?	Low-Mod				100-450
Black banded gudgeon ^ *Oxyeleotris selheimi*	?	?				100-550
Black catfish *Neosilurus ater*	Sum, Spr	High	✓			100-450
Bony herring ^ *Nematalosa erebi*	All	All	✓		✓	50-320
Carpentaria catfish^ *Arius paucus*	?	?				100-1000
Eastern rainbowfish ^ *Melanotaenia splendida*	All	All		✓	✓	30-140
Giant glassfish ^ *Parambassis gulliveri*	?	?				20-250
Gulf grunter ^ *Scortum ogilbyi*	?	?				80-400
Hyrtyl's tandan ^ *Neosilurus hyrtlii*	Sum, Spr	High	✓			100-400
Mouth almighty ^ *Glossamia aprion*	All	All	✓		✓	30-100
Northern trout gudgeon ^ *Mogurnda*	Sum, Spr	Low-Mod			✓	30-120
Northwest glassfish ^ *Ambassis sp.*	?	All				20-50
Rendahl's tandan ^ *Porochilus rendahli*	Sum	High	✓			80-240
Reticulated glassfish ^ *Ambassis macleayi*	All	All				30-90
Sleepy cod ^ *Oxyeleotris lineolate*	Sum	Mod			✓	80-450
Spangled perch ^ *Leiopotherapon unicolor*	All	All	✓		✓	50-300
Square blotch goby ^ *Glossogobius sp. C*	?	Low-Mod				30-100
Toothless catfish ^ *Anodontiglanis dahli*	Sum	Low/ High				100-400
Unidentified goby *Glossogobius sp.*	?	?				-
Unidentified catfish *Porochilus sp.*	?	?				-

3 Methods

3.1 Site selection

To determine the effects that construction of the weir and dam will have on the upper Flinders River system, sampling was undertaken both within the zone where construction will be undertaken as well as in habitats upstream and downstream of the proposed construction area. Sampling in this manner provides a comprehensive guide to the fish communities currently present in the system, as well as changes in the system as a result of the construction of the dam and weir in the project area.

To identify suitable sampling locations, satellite imagery was examined to identify potential waterholes that maintained water over the dry season. From this desktop evaluation, 28 sites were identified as possibly maintaining waterholes throughout the dry season, with sufficient water to sustain fish populations (Figure 5).

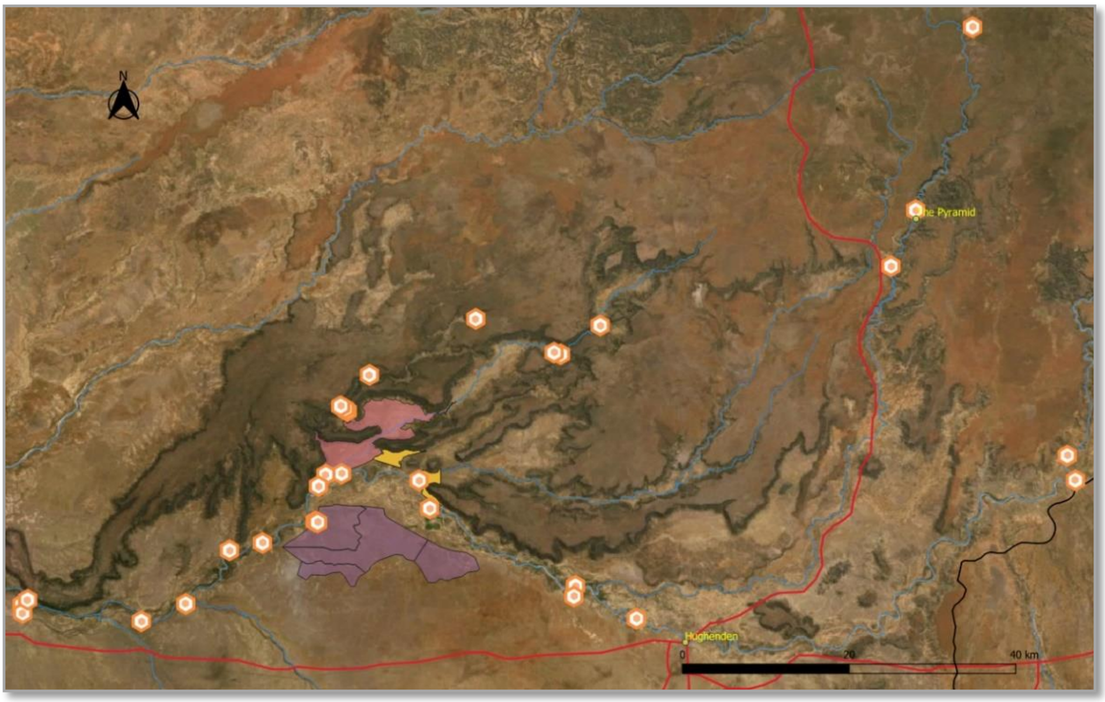

Figure 5. Potential sampling sites within the upper Flinders River catchment identified via remote imagery.

Once on-site a drone was deployed to confirm the presence of water and its location within the watercourse in relation to the access points available. This enabled field teams to identify exactly where water was within the watercourse, as the location of remaining waterbodies varies from season to season.

Through this site selection methodology, fourteen sites were sampled where sufficient water remained to maintain fish populations (Figure 5). The sites included both instream and off stream waterholes at varying distances from and within the development area (Table 3).

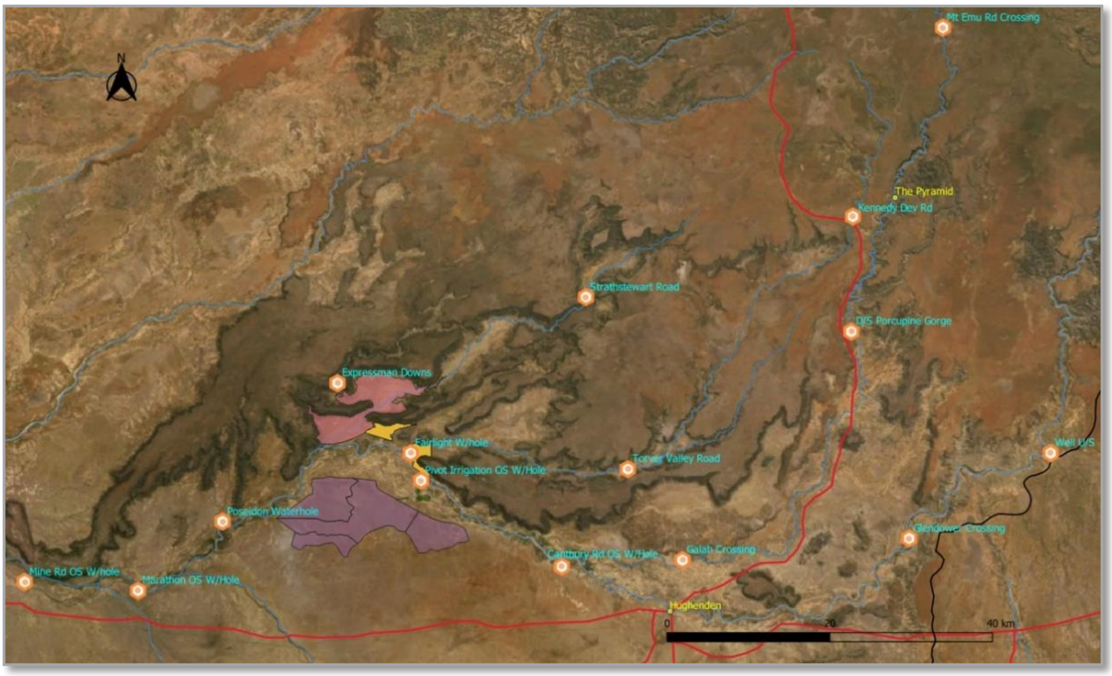

Figure 5. Sites sampled during the current study and the inundation area from the proposed works shown in blue.

Table 3. Site Overview (* indicates sites where eDNA was sampled).

Site	Site Code	Stream	Type	Stream Type	distance from Flinders River	Approx. distance from recruitment site	Distance from dam zone
Mine Road *	MR	Flinders River	Off-stream waterhole	Near permanent	11.5 km	Recruitment site	D/S
Marathon Station *	MS	Flinders River	Off-stream waterhole	Permanent	6.5	Recruitment site	D/S
Poseidon *	P	Flinders River	Riverine waterhole	Ephemeral	-	10 km – Marathon (D/S)	D/S
Expressman Downs *	ED	Stewart Creek	Riverine waterhole	Ephemeral	20 km	50 km – Marathon (D/S) 34 km – Fairlight (U/S)	Within
Fairlight Waterhole *	FC	Betts Gorge Creek	Riverine Waterhole	Permanent	0 km	44 km – Marathon (D/S)	Within
Strathstewart Road	SR	Jones Valley Creek	Riverine waterhole	Ephemeral	50 km	80 km – Marathon (D/S) 64 km – Fairlight (U/S)	U/S
Torver Valley Road	TV	Betts Gorge Creek	Riverine waterhole	Ephemeral	30 km	30 km – Fairlight (D/S)	U/S
Kennedy Dev. Road	KD	White Cliffs Gorge Creek	Riverine waterhole	Ephemeral	90 km	90 km – Fairlight (D/S)	U/S
Cantebury Road *	CR	Flinders River	Off-stream oxbow waterhole	Near permanent	0 km	27 km – Fairlight (D/S) 70 km – Marathon (D/S)	U/S
Galah Crossing *	GC	Porcupine Creek	Riverine waterhole	Ephemeral	16 km	47 km – Fairlight (D/S0 90 km – Marathon (D/S)	U/S
D/S Porcupine Gorge *	DP	Porcupine Creek	Riverine waterhole	Ephemeral	80 km	110 km – Fairlight (D/S) 153 km – Marathon (D/S)	U/S
Mt Emu Road Crossing	ME	Porcupine Creek	Riverine waterhole	Permanent	140 km	170 km – Fairlight (D/S) 213 km – Marathon (D/S)	U/S
Glendower Crossing	GL	Flinders River	Riverine waterhole	Ephemeral	-	98 km – Fairlight (D/S) 140 km – Marathon (D/S)	U/S
Well Upstream *	WU	Flinders River	Riverine waterhole	Near permanent	-	128 km – Fairlight (D/S) 170 km – Marathon (D/S)	U/S

3.2 Sampling Methods

3.2.1 Electrofishing

The sampling consisted of boat and/or backpack electrofishing at all sites. Boat electrofishing was undertaken using Hypnos II, a 3.7m vessel which operates a Smith-Root 2.55 GPP electrofisher unit, two boom arms with 4 dropper anode arrays and two 20 dropper cathode arrays. An operator and single dip-netter were employed during sampling activities on Hypnos II. Electrofishing was carried out in 300 second replicates or ‘shots’ during which immobilised fish were netted and placed in a live well to recover. Any fish observed, positively identified but not collected, were also recorded and included in fish counts. If a site was too small for a 300 second shot, then all habitats within the site were sampled and the effort levels was recorded.

Backpack electrofishing uses the same principles as boat electrofishing, but on a smaller scale using a Smith Root Model 12b electrofisher. This method was used in shallow, wadable pools and where it was unsuitable for boating. Electricity is provided from batteries then transferred into the water, as a pulsed DC waveform, via a backpack unit which is carried by the operator, with portable electrodes. Immobilised fish are dip-netted from the water by an assistant and placed in a bucket of water to recover. Any fish observed, positively identified but not collected, were also recorded and included in any fish counts. Backpack electrofishing was carried out in 300 second replicates or "shots", unless the site was too small for a 300 second shot, then all habitats within the site were sampled and the effort levels recorded.

All fish captured during electrofishing surveys were identified to species level, counted and measured to the nearest millimetre (fork length for forked-tailed species, total length for all other species). Fish were then released into the area from which they were captured. When more than 50 individuals of a single species were captured in any single sample, randomised sub-samples of 50 fish were measured and the remainder counted. All fish recorded on datasheets were given a six-letter code consisting of the first three letters of the genus name in the first three letters of their species name. These codes are outlined in Appendix 2.

3.2.2 Environmental DNA

Environmental DNA (eDNA) samples were collected at 10 sites to determine the presence of the sawfish (Pristis sp.). The ray species has been previously noted as potentially present within the project area (Brizga 2020). Sample sites were chosen based on the potential for suitable sawfish habitat, being sites with larger and deeper water bodies. Five samples were collected throughout each site, along with a distilled water control sample. The five samples consisted of 500ml of surface waters collected at least 20 - 50 m apart throughout the waterhole added to a sample jar containing a preservative solution. Contamination is a critical issue for sampling and as such new jars and gloves were used at every site. Once collected, jars were sealed and labelled with location, site, sample number and date. Photographic records, stream and habitat information were recorded for each site. Samples and data were sent to James Cook University, Townsville for analysis.

4 Results

Sampling of all sites captured a total of 4784 individuals representing 21 species of fish. Of this total, 581 fish were caught and measured with the rest being observed as affected by the electrofisher.

The most widespread species was Spangled perch (*Leiopotherapon unicolor*) being present at 12 sites, with the next most widespread species being Eastern rainbowfish (*Melanotaenia splendida*) present at 10 sites, Bony bream (*Nematalosa erebi*) present at 9 sites and Banded grunter (*Amniataba percoides*) present at 8 sites (Table 4).

The most abundant species across all sites was the Bony bream making up 44.82% of the total catch, followed by Eastern rainbow fish at 27.09%, Spangled perch at 17.83% and Ambassid species (*Ambassis sp.*) at 3.99% (Figure 6).

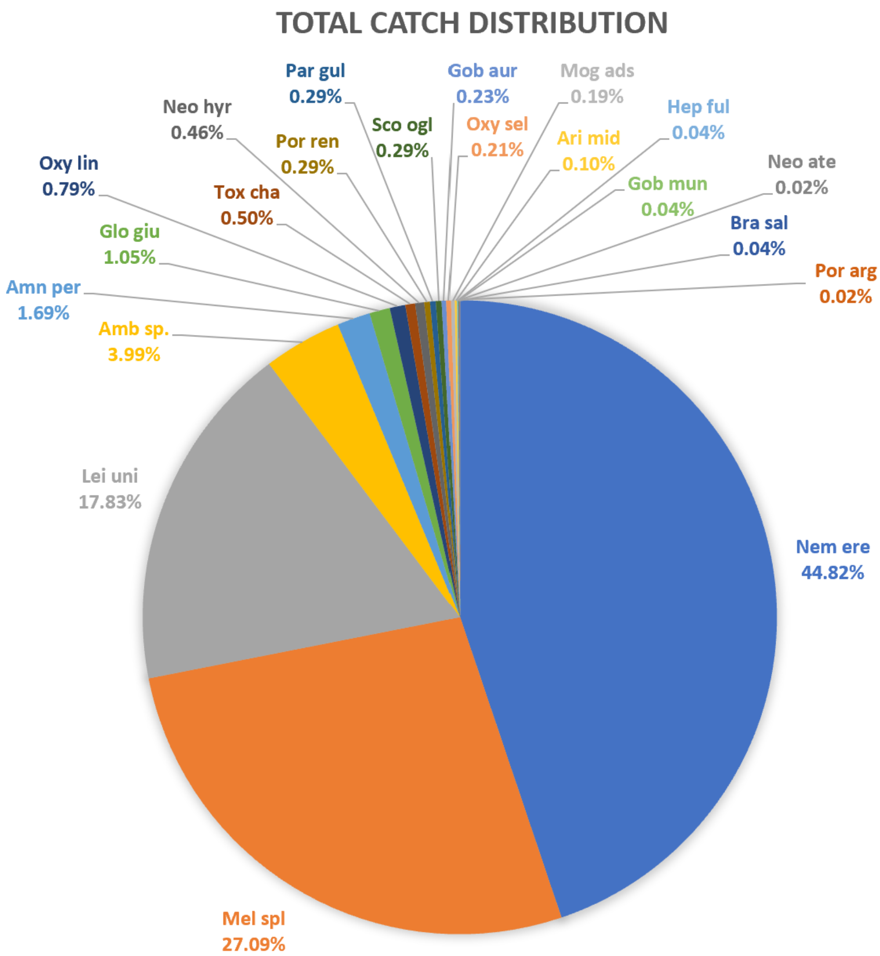

Figure 6. Total catch distribution across from all 14 sites.

Species abundance was greatest at Fairlight Waterhole with 16 species recorded, followed by Poseidon Waterhole, Mine Road and Marathon Waterhole with 13, 12 and 11 species respectively. The lowest abundance was found at the Cantebury Road site where no fish were captured, and only one species was located at three of the sites sampled: Strathstewart Road, Torver Valley and Kennedy Development Road (Table 4).

Table 4. List of fish species captured at each of the sites of sampled in the Flinders River catchment.

Common Name	Scientific Name	Site and Location from Development Zone													
		Downstream			Within		Upstream								
		MR	MS	P	ED	FW	SR	TV	KD	CR	GC	DP	ME	GL	WU
Ambassis species	*Ambassis sp.*	✓	✓	✓	✓	✓					✓				
Archerfish	*Toxotes chatareus*	✓		✓	✓	✓									
Banded grunter	*Amniataba percoides*	✓	✓	✓	✓	✓						✓	✓		✓
Black banded gudgeon	*Oxyeleotris selheimi*	✓	✓			✓									
Black catfish	*Neosilurus ater*		✓												
Bony bream	*Nematalosa erebi*	✓	✓	✓	✓	✓					✓	✓	✓		✓
Eastern rainbowfish	*Melanotaenia splendida*	✓	✓	✓	✓	✓		✓			✓	✓	✓	✓	
Giant glassfish	*Parambassis gulliveri*	✓	✓	✓		✓									
Golden flathead goby	*Glossogobius aureus*					✓									
Gulf grunter	*Scortum ogilbyi*	✓		✓		✓									
Hyrtl's tandan	*Neosilurus hyrtli*		✓			✓					✓	✓	✓		✓
Rendahl's catfish	*Porochilus rendahli*	✓		✓		✓									
Saltpan sole	*Brachirus salinarum*			✓											
Silver catfish	*Porochilus argenteus*					✓									
Silver cobbler	*Neoarius midgleyi*		✓	✓											
Sleepy cod	*Oxyeleotris lineolate*	✓	✓	✓		✓					✓				
Sooty grunter	*Hephaestus fulignosus*					✓									
Northern purple spotted gudgeon	*Mogurnda mogurnda*												✓	✓	
Spangled perch	*Leiopotherapon unicolor*	✓	✓	✓	✓	✓	✓		✓		✓	✓	✓	✓	✓
Square blotch goby	*Glossogobius munroi*					✓									
Tank goby	*Glossogobius giuris*	✓		✓	✓						✓				
TOTAL SPECIES		12	11	13	7	16	1	1	1	0	7	5	6	3	4

4.1 Mine Road

The Mine Road site was the most downstream site sampled. It is an off-stream waterhole located within an anabranch channel that runs parallel to the main Flinders River channel. The site collects water from channels draining the plains to the south and east. The waterway connects to the main Flinders River channel via an 11.5 km anabranch channel (Figure 7). The site also connects to the Marathon Station site to the east during flooding flows.

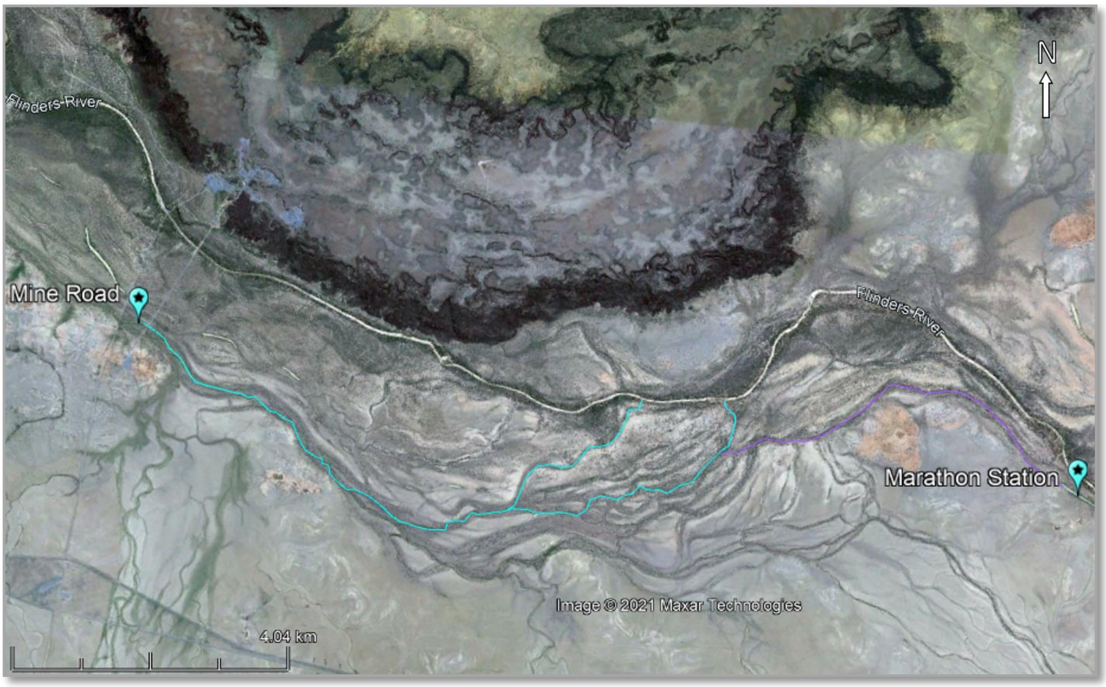

Figure 7. Mine Road site and it's connection to the main Flinders River channel (blue) and the Marathon Waterhole site (purple).

The Mine Road waterhole is long and narrow, with intermittent riparian vegetation, instream woody debris and aquatic macrophytes in the shallow margins (Figure 8).

Figure 8. Mine Road waterhole.

Twelve species were captured at the Mines Road site, with Bony bream (*Nematalosa erebi*) dominating the catch (70.3%) with Ambassid species (6.9%), Sooty grunter (*Hephaestus fulignosus*) (3.3%), Giant glassfish, Archerfish (*Toxotes chatareus*), and Eastern rainbowfish each representing 3.3% the next most abundant species (Figure 9).

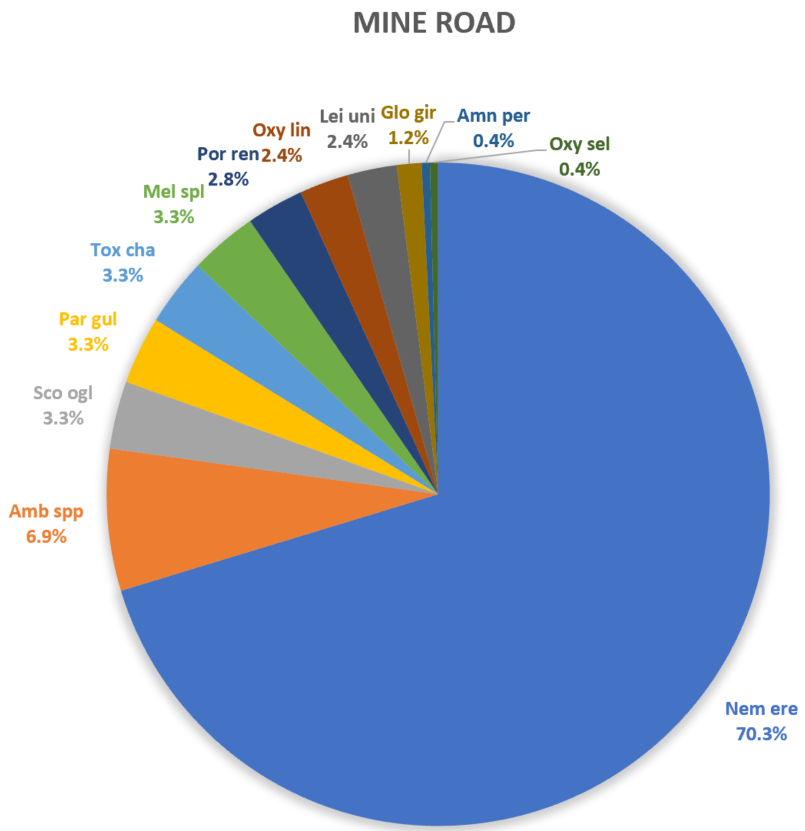

Figure 9. Mine Road species distribution.

Sizes of the species captured ranged from a 34 mm Bony bream to a 245 mm Giant glassfish (*Parambassis gulliveri*) (Figure 10 and 11). The average lengths for all species were less than 150 mm.

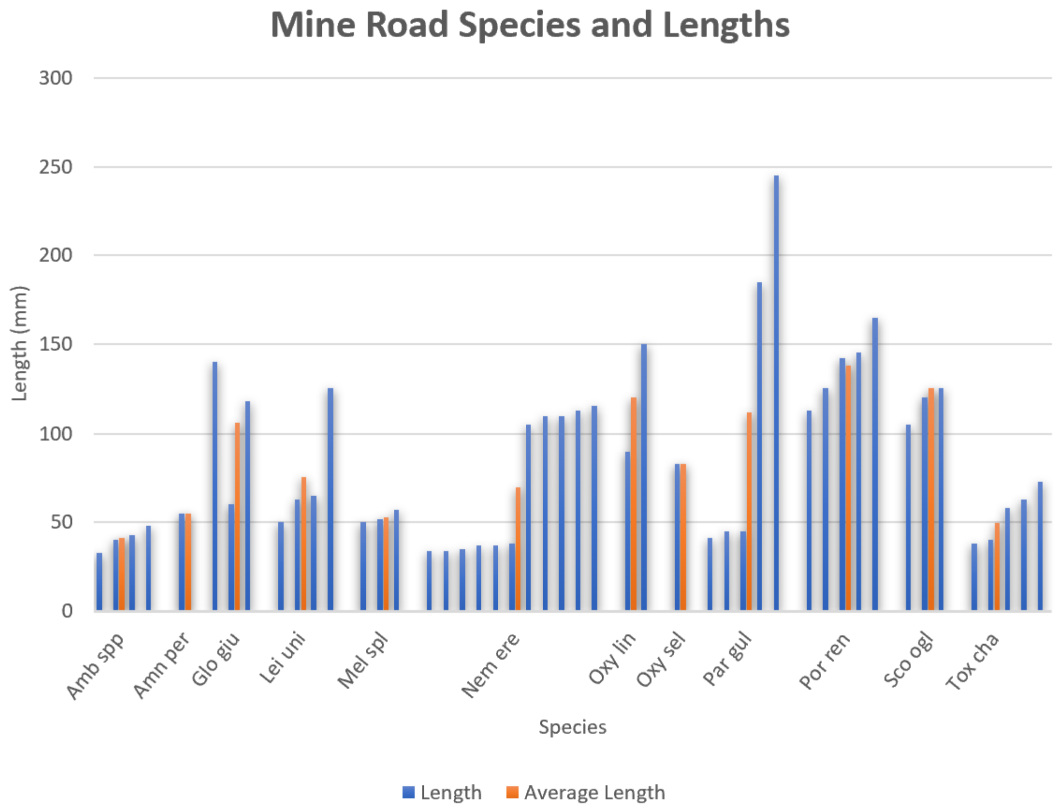

Figure 10. Size ranges of fish captured and average length for each species.

Figure 11. Giant glassfish (*Parambassis gulliveri*) captured at the Mine Road site.

4.2 Marathon Station

Marathon Station is an off-stream waterhole located within an anabranch channel that runs parallel to the main Flinders River channel. It collects water from channels draining the plains to the south and east, and connects to the main Flinders River channel via a 6.5 km long channel during high flows (Figure 12)

Figure 12. Marathon Station waterhole and its connection to the Flinders River (in blue).

The site is a large permanent waterhole with deep water, good riparian vegetation, woody debris, bedrock outcrops and macrophytes in the shallow margins (Figure 13. Marathon Station waterhole.).

Figure 13. Marathon Station waterhole.

Eleven species were captured at Marathon Station with Eastern rainbowfish dominating the catch (62.9%) (Figure 14). Bony bream (10%), Ambassis sp. (10%) and Banded Grunter (7.4%) were the next most numerous species. A freshwater crocodile (~1.5 m long) was also observed in the waterhole.

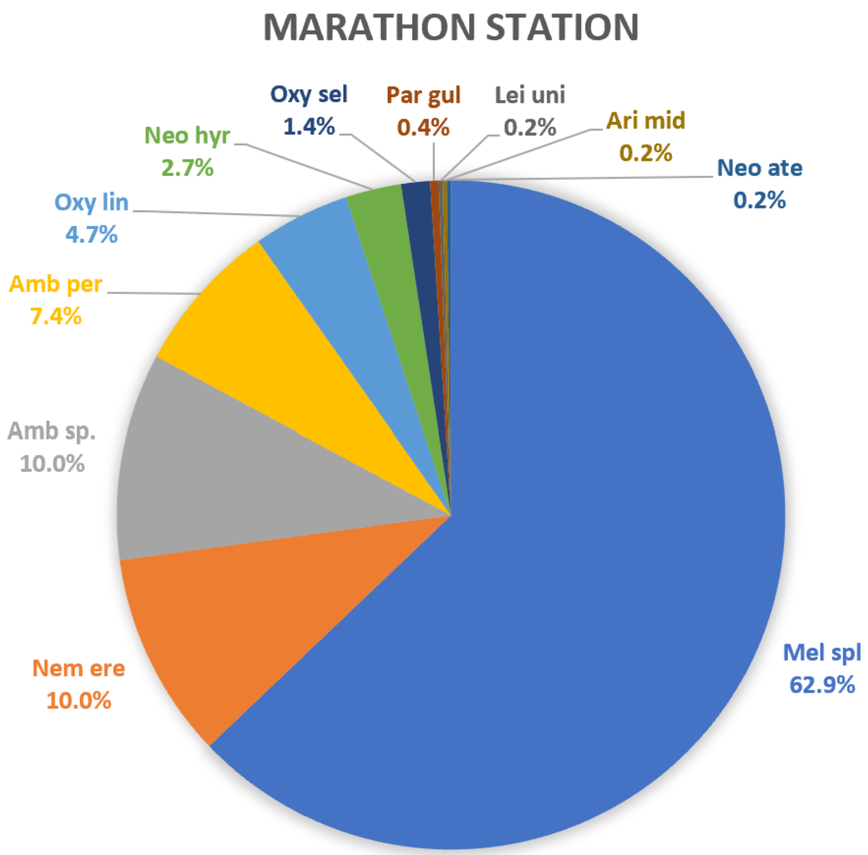

Figure 14. Marathon Waterhole species distribution.

Fish sizes ranged from a 22 mm Eastern rainbowfish to a 399 mm Black banded gudgeon (*Oxyeleotris selheimi*), with average lengths for the small bodied species well below 100 mm (Figures 15 and 16).

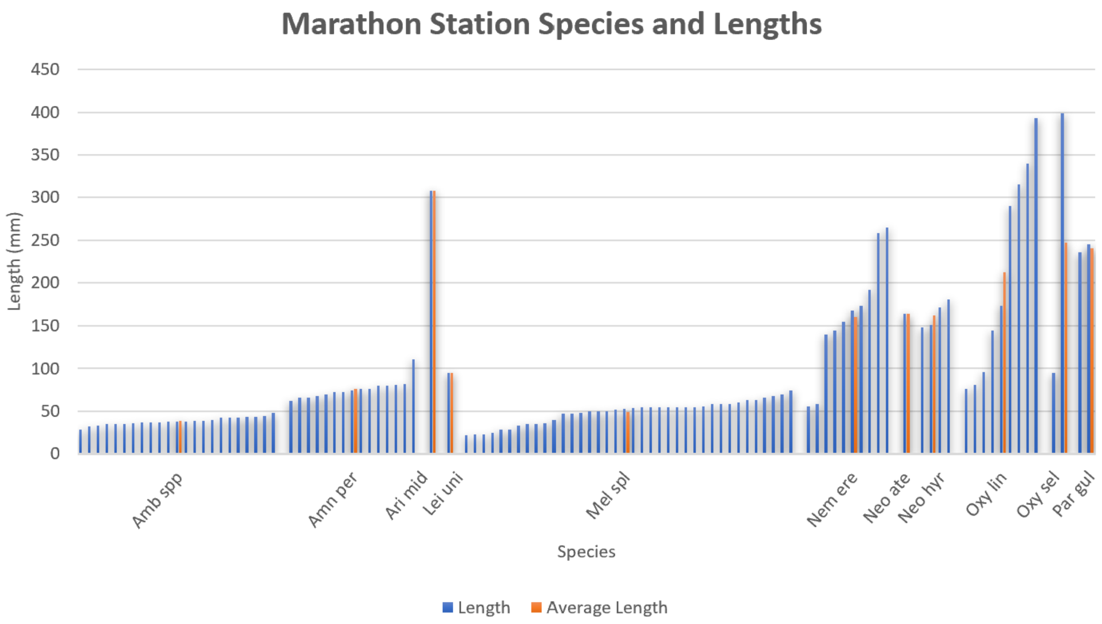

Figure 15. Size ranges of fish captured at Marathon Station and average length for each species.

Figure 16. Black banded gudgeon captured at Marathon Station.

4.3 Poseidon Waterhole

Poseidon Waterhole is located on the Flinders River approximately 70 km downstream of Hughenden. The waterhole is sited on a large bend and is characterised by a steep outer right bank, vegetated banks, woody debris and a sandy substrate (Figure 17).

Figure 17. Poseidon Waterhole on the Flinders River.

A total of 13 species were captured at Poseidon Waterhole, the catch dominated by Bony bream (83%). Eastern rainbowfish (7.4%), Tank goby (*Glossogobius giuris*) (3.3%) and Archerfish were the next most numerous species (Figure 18). It was the only site where the Freshwater sole (*Brachirus salinarum*) was observed.

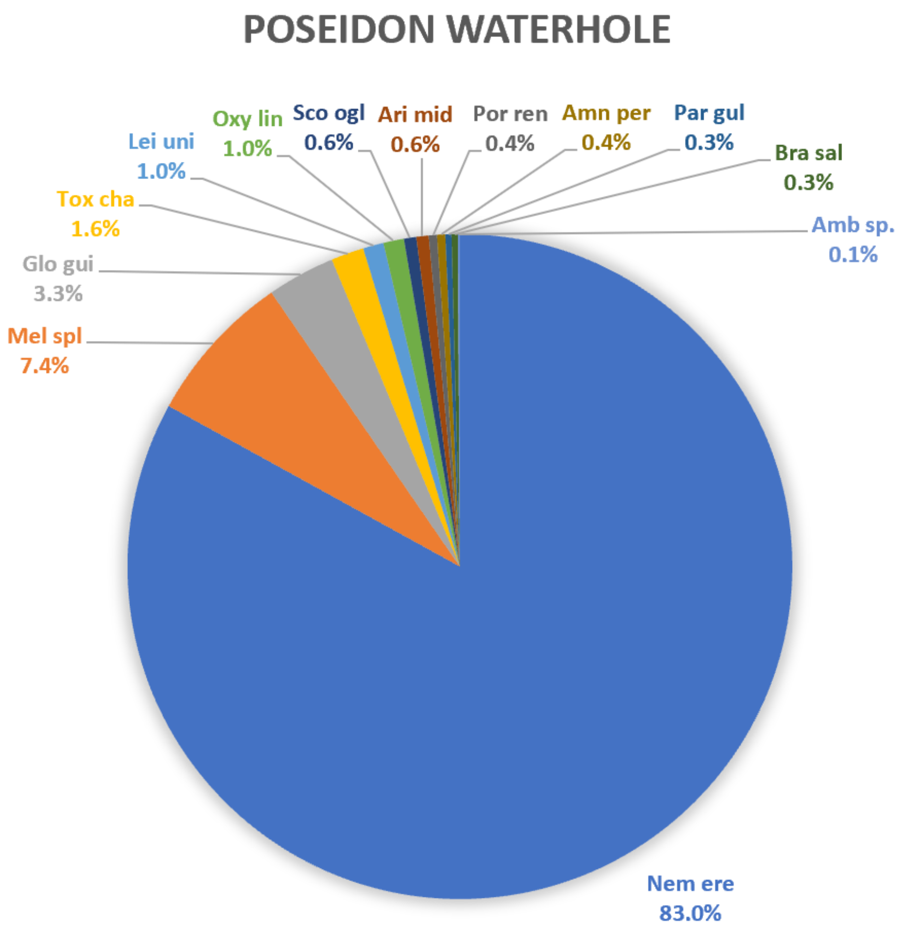

Figure 18. Poseidon Waterhole species distribution.

Fish captured at Poseidon waterhole ranged from a 38 mm Ambassis to a 627 mm Silver cobbler (*Neoarius midgleyi*) (Figure 19 and Figure 20). Despite this large size range, more than 75% of the catch was less than 200 mm.

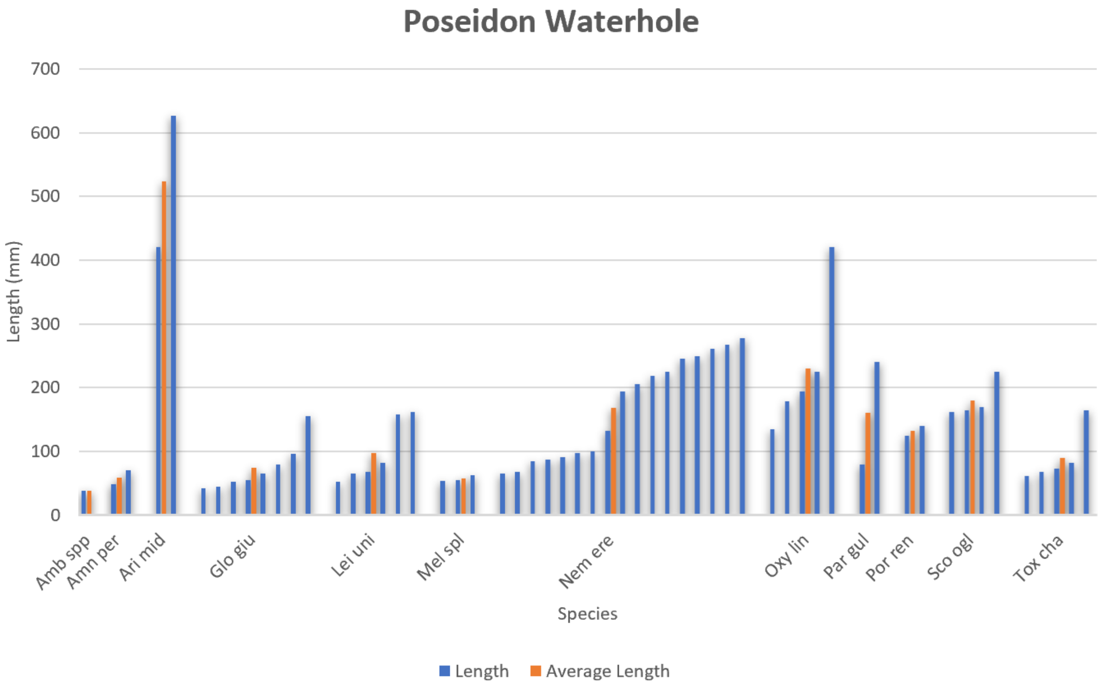

Figure 19. Size ranges of fish captured at Poseidon Waterhole and average length for each species.

Figure 20. Large Silver cobbler captured at Poseidon Waterhole.

4.4 Expressman Downs, Stewart Creek

Expressman Downs is located on Stewart Creek, a tributary to the Flinders River, that will be on the edge of the dam impoundment following construction. The site is approximately 20 km upstream of the convergence with the Flinders River. The site consisted of a large, shallow pool with intermittent riparian vegetation and a rock and boulder substrate (Figure 21).

Figure 21. Expressman Downs Waterhole, Stewart Creek.

A total of seven species were captured at the Expressman Downs site, dominated by Bony bream (56.4%), Spangled perch (20.6%), Ambassid species (7.5%) and Tank Goby (4%) (Figure 22).

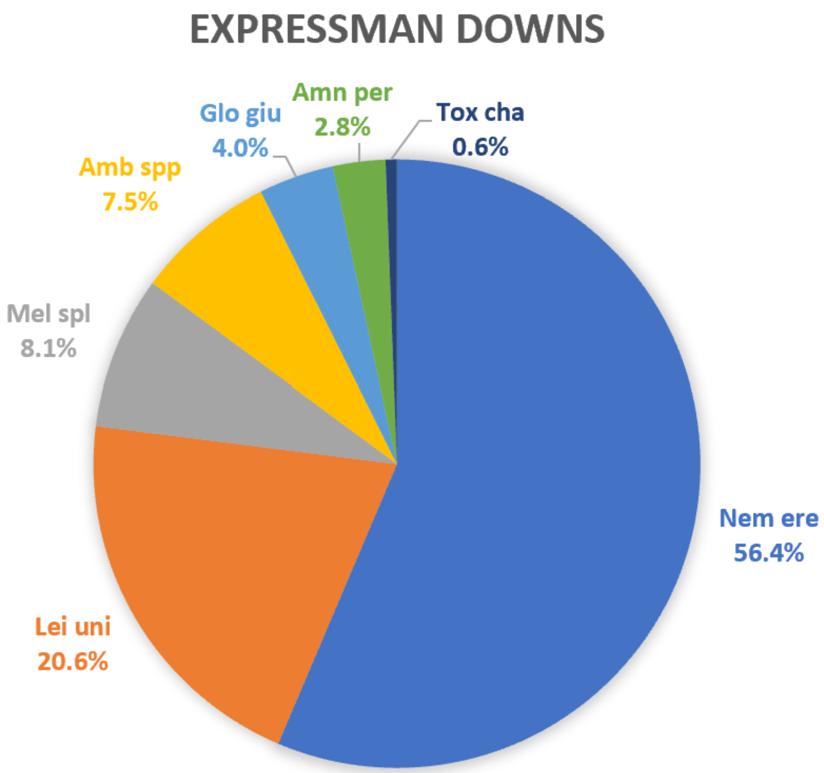

Figure 22. Expressman Downs species distribution.

The size of the fishes captured ranged from a 28 mm Ambassis sp. to a 168 mm Tank goby (Figure 23 and Figure 24). The majority of fish had average lengths under 100 mm, with only three Tank gobies and one Sleepy cod (*Oxyeleotris lineolate*) over this size.

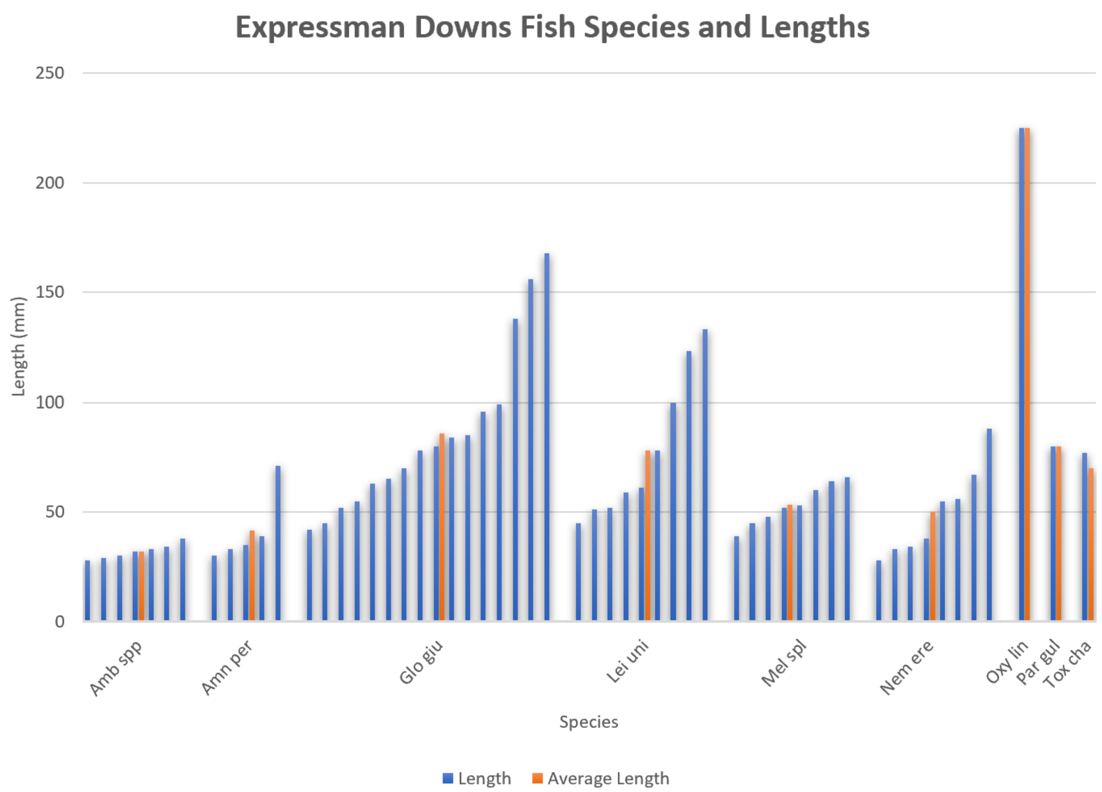

Figure 23. Size ranges of fish captured at Expressman Downs and average length for each species.

Figure 24. Ambassid species captured at Expressman Downs.

4.5 Fairlight Waterhole, Betts Gorge Creek

Fairlight Waterhole is located at the convergence of Betts Gorge Creek (locally known as Fairlight Creek) and the Flinders River. A large sand plug separates the waterhole from the river, effectively damming the water within Betts Gorge Creek when flows recede. The waterhole is characterised by steep vegetated banks, overhanging vegetation and instream debris (Figure 25).

Figure 25. Fairlight Waterhole, Betts Gorge Creek.

Sixteen species were captured at Fairlight Waterhole. The catch was dominated by Bony bream (61%), Eastern rainbowfish (25.5%) and Spangled perch (6.2%) (Figure 26). Fairlight waterhole was the only site where a Square blotch goby (*Glossogobius munroi*) was captured (Figure 28).

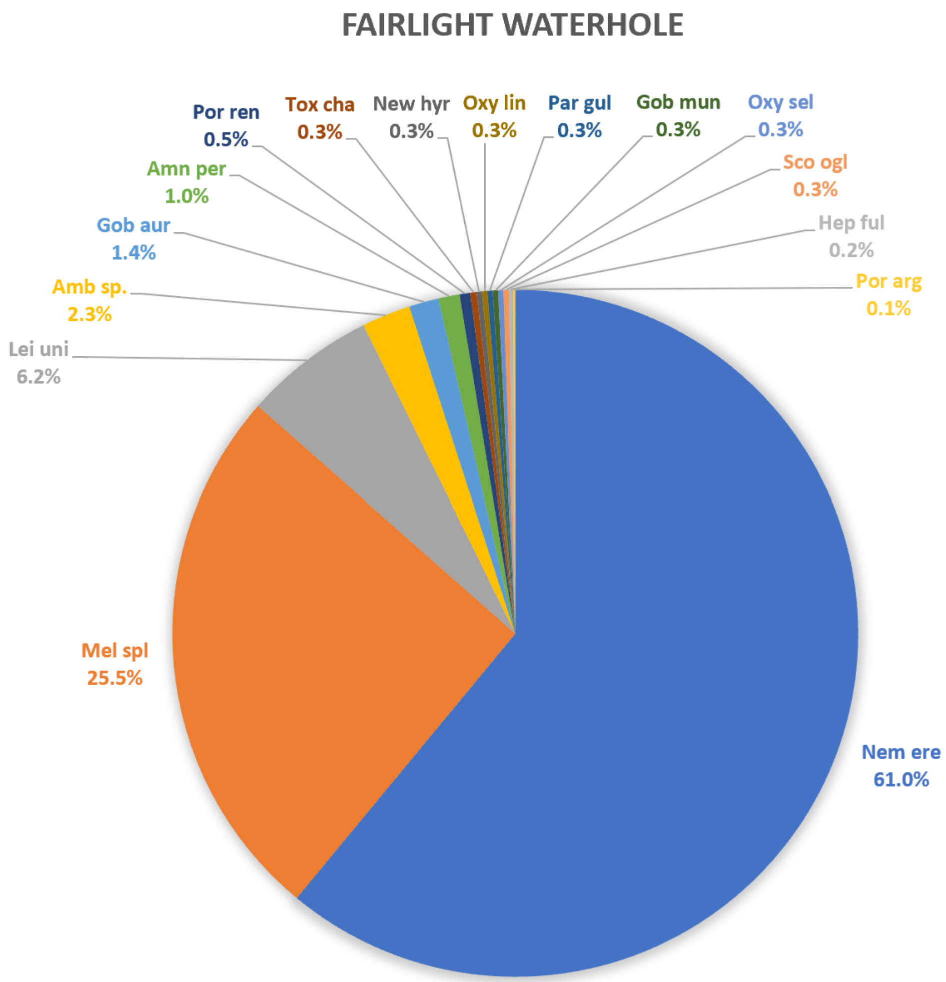

Figure 26. Fairlight Waterhole species distribution.

The average lengths of the various species caught in the waterhole were less than 170 mm (Figure 27). The largest fish caught was a Bony bream at 255 mm and the smallest were two Rainbowfish at 22 mm.

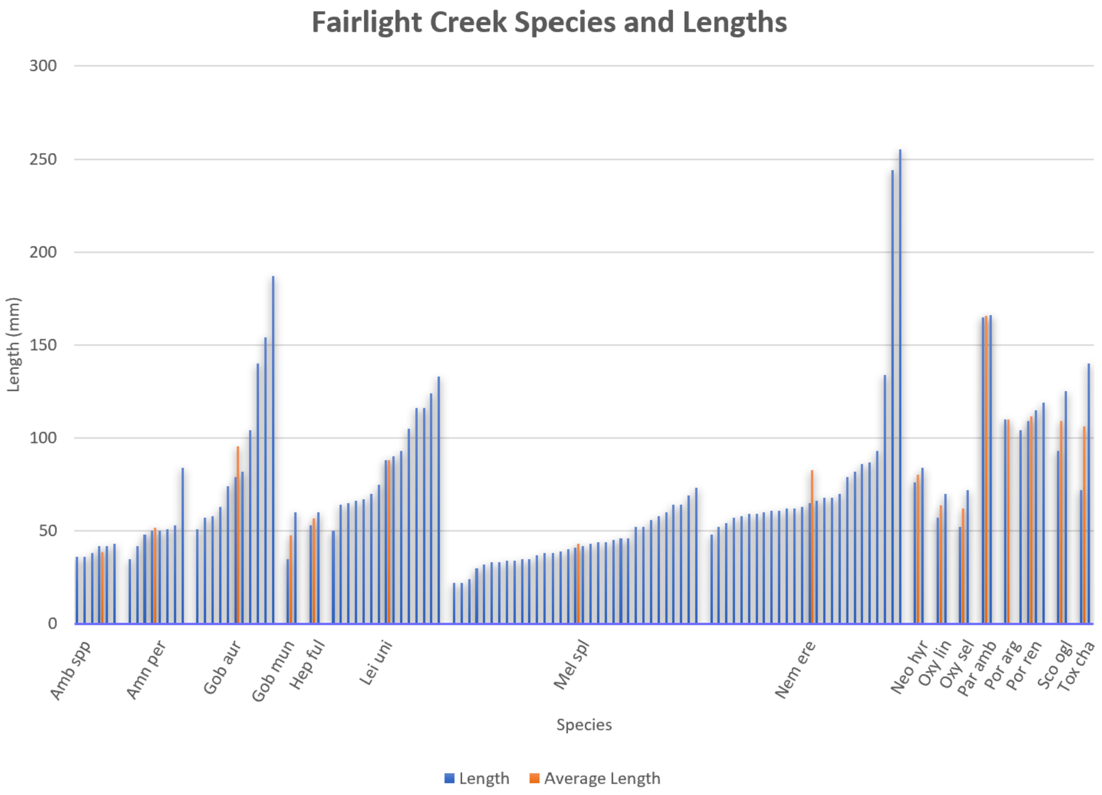

Figure 27. Size ranges of fish captured at Fairlight Waterhole and average length for each species.

Figure 28. A Square blotch goby (*Glossogobius munroi*)) captured at Fairlight Waterhole.

4.6 Strathstewart Road, Jones Valley Creek

The Strathstewart Road site is located on Jones Valley Creek, a tributary of Stewart Creek which flows into the Flinders River. The site is approximately 50 km upstream of the convergence with the Flinders River. The waterhole is characterised by sparse riparian vegetation dominated by weeds and was heavily impacted by cattle grazing (Figure 29). The substrate of the waterhole was primarily black mud with areas of sand and gravel.

Figure 29. Strathstewart Road, Back Gully Creek.

Only one species, Spangled Perch was located at the Strathstewart Road site. Thirteen individuals were captured ranging in size from 25 to 50 mm, with an average length of 38.8 mm (Figure 30).

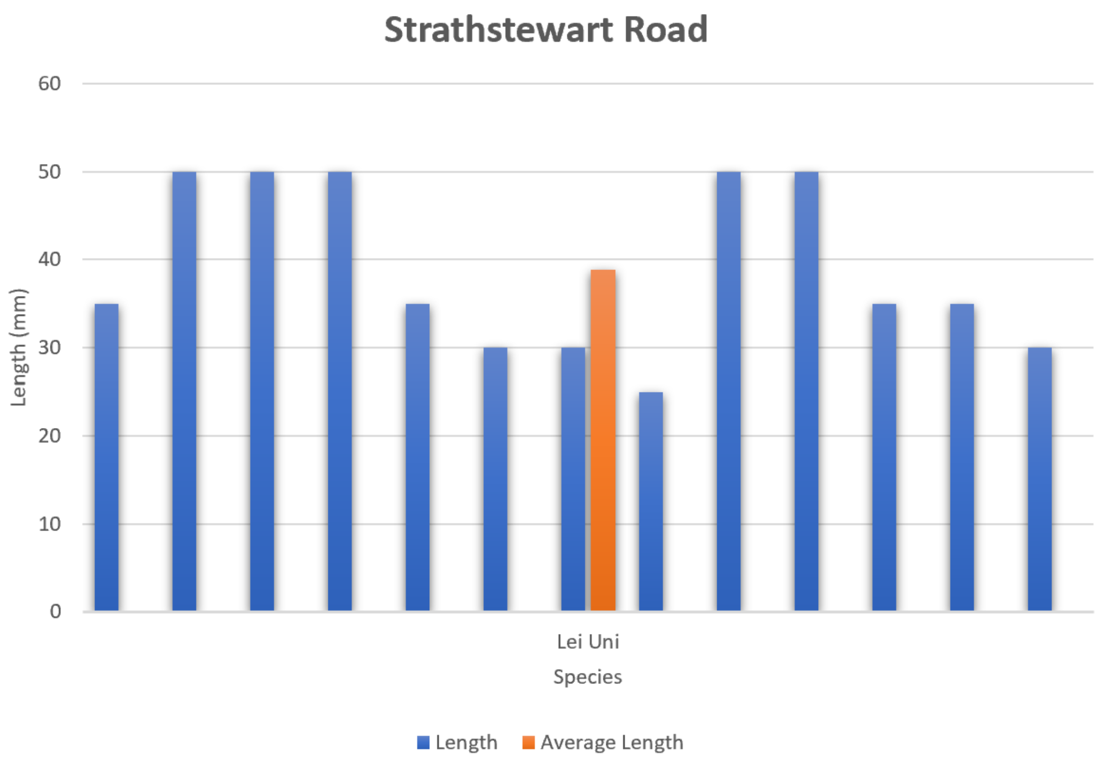

Figure 30. Size ranges of fish captured at Strathstewart Road and average length the species.

4.7 Torver Valley Road, Betts Gorge Creek

The Torver Valley Road site is located on Betts Gorge Creek approximately 30 km upstream from the convergence with the Flinders River. Only small shallow pools remained in the creek which was characterised by intermittent riparian vegetation and a sand/gravel substrate (Figure 31).

Figure 31. Torver Valley Road site on Betts Gorge Creek.

Only one species was found at the site, Eastern rainbowfish, which ranged in size from 15 mm to 57 mm, with an average length of 39.55 mm (Figure 32 and Figure 33).

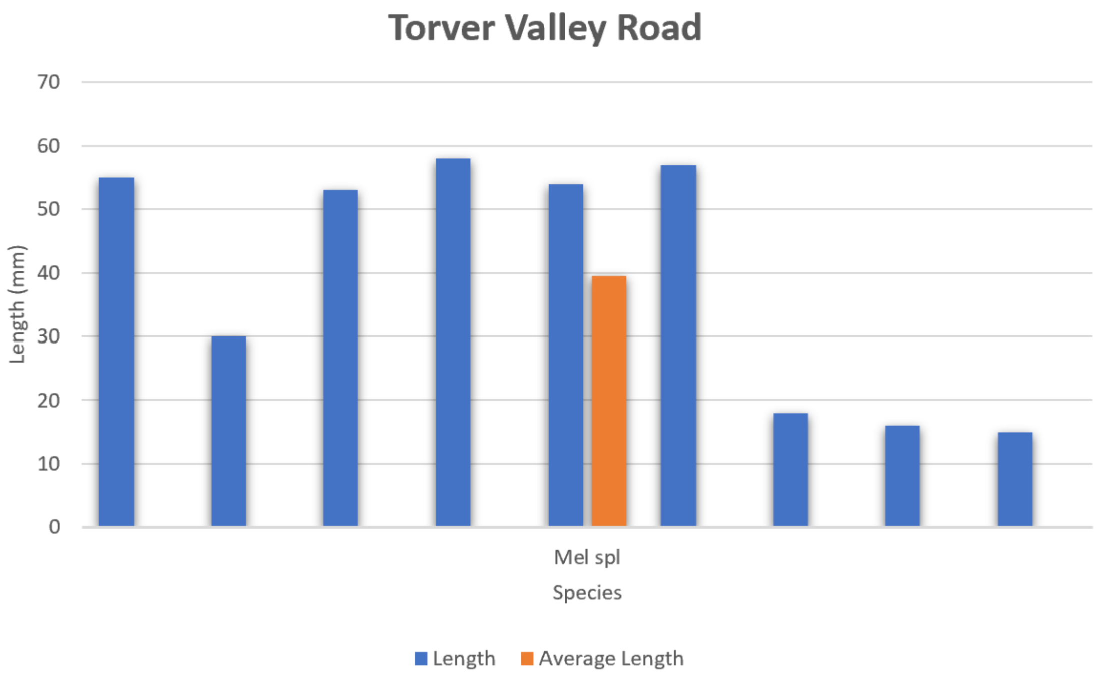

Figure 32. Size ranges and average length of Eastern rainbowfish captured at Torver Valley Road.

Figure 33. Eastern rainbowfish (Mel spl) captured at the Torver Road site.

4.8 Kennedy Development Road, White Cliff Gorge Creek

White Cliff Gorge Creek flows into Betts Gorge Creek which enters the Flinders River at the development site. The site is approximately 90 km upstream of the convergence with the Flinders River. The sampling site consisted of several pools, where the waterway flowed through a small sandstone and basalt gorge with good riparian vegetation and rocky outcrops (Figure 34).

Figure 34. Kennedy Development Road, White Cliff Gorge Creek.

Only one species, the Spangled Perch was captured and observed at the site. The size range for this species was from 42 -140 mm, with an average length of 76.75 mm (Figure 35).

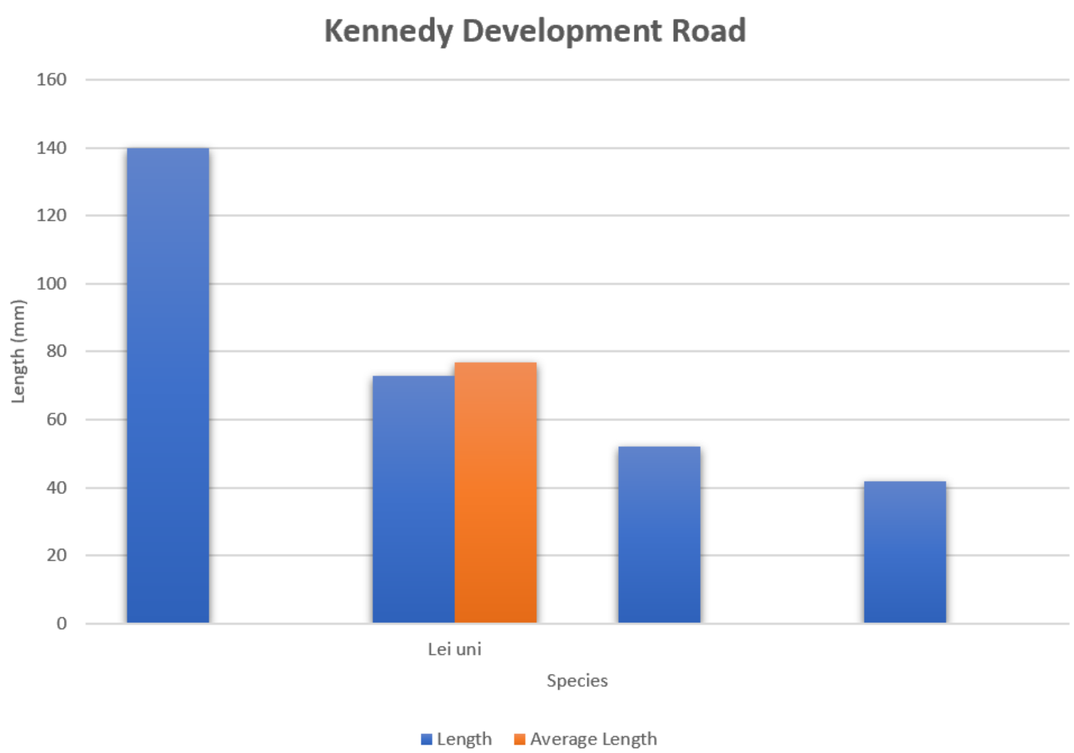

Figure 35. Length of fish captured at Kennedy Development Road and the average length.

4.9 Cantebury Road

The Cantebury Road site is an oxbow lake which has formed as the Flinders River has carved a straighter channel cutting off the meander that is now the lake (Figure 36).

Figure 36. Cantebury Road Waterhole close to the main Flinders River channel.

The site is a substantial waterhole with fringing vegetation and aquatic macrophytes (Figure 37). Despite offering good fish habitat, no fish were captured nor observed in the waterhole.

Figure 37. Cantebury Road Waterhole.

4.10 Galah Creek Crossing, Porcupine Creek

The Galah Crossing site is located on Porcupine Creek, approximately 16 km upstream of the convergence with the Flinders River. The site is characterised by a high, steep right bank covered in good riparian vegetation, and a lower grassy left bank permitting cattle access to the creek (Figure 38). The waterhole had some woody debris and overhanging vegetation with depths up to 2 m. Substrate was coarse sand and mud.

Figure 38. Galah Creek Crossing, Porcupine Creek.

A total of seven species were detected at the Galah Crossing site. The catch was dominated by Bony bream (62.3%), Eastern rainbowfish (17.9%), Ambassid spp. (10.3%) and Spangled perch (8.5%) (Figure 39).

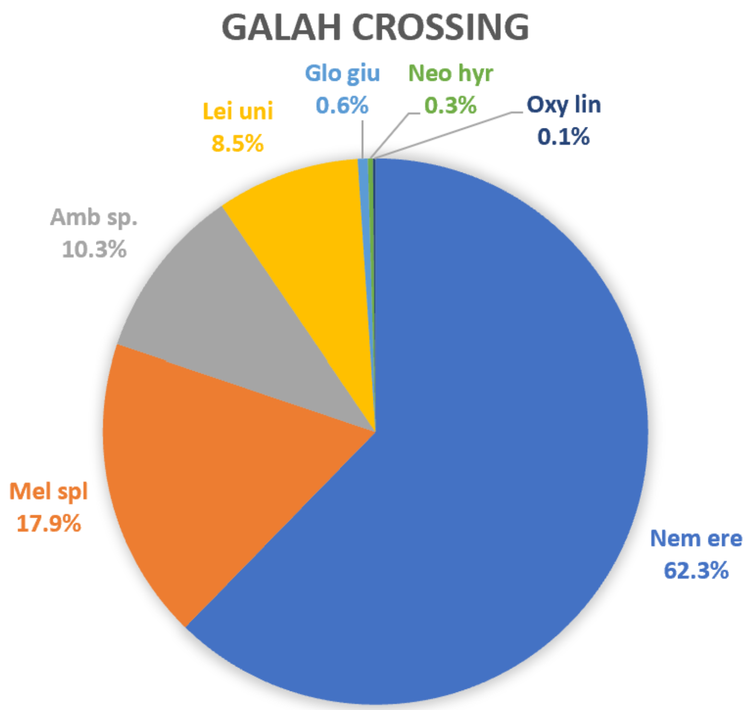

Figure 39. Galah Crossing species distribution.

Lengths of the fishes captured ranged from a 35 mm Eastern rainbowfish to a 180 mm Bony bream, with average lengths for all species except Hyrtl's tandan (*Neosilurus hyrtli*) below 100 mm (Figure 40 and Figure 41).

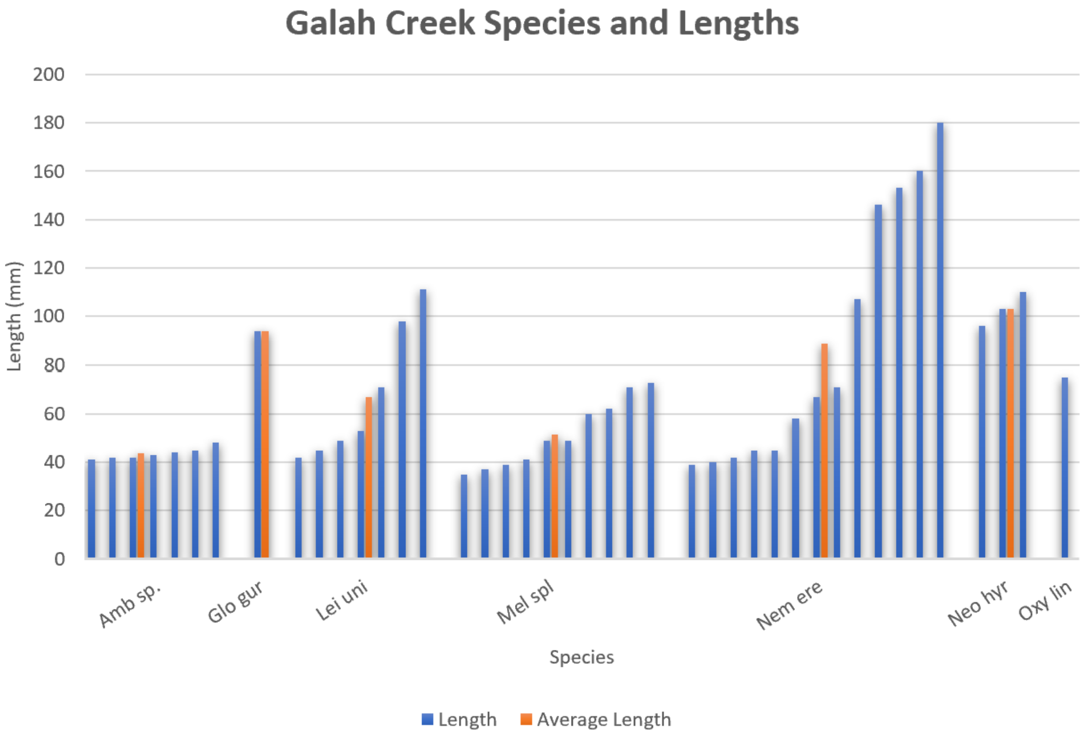

Figure 40. Length of fish captured at Galah Creek Crossing and species average length.

Figure 41. Hyrtl's tandan captured at Galah Crossing.

4.11 Porcupine Creek, Downstream of Porcupine Gorge

The Porcupine Creek site was located downstream of the famous Porcupine Gorge and approximately 500 m downstream of the National Park boundary. The site is approximately 80 km upstream of the convergence with the Flinders River. The site consisted of several large pools confined by sandstone bedrock and sand benches (Figure 42). Some riparian vegetation was present on the banks and sand benches, and water levels were dropping.

Figure 42. Porcupine Creek Site, downstream of Porcupine Gorge.

Five species were observed at Porcupine Creek site, the dominant species being Eastern rainbowfish (56%) and Spangled perch (38%) (Figure 43).

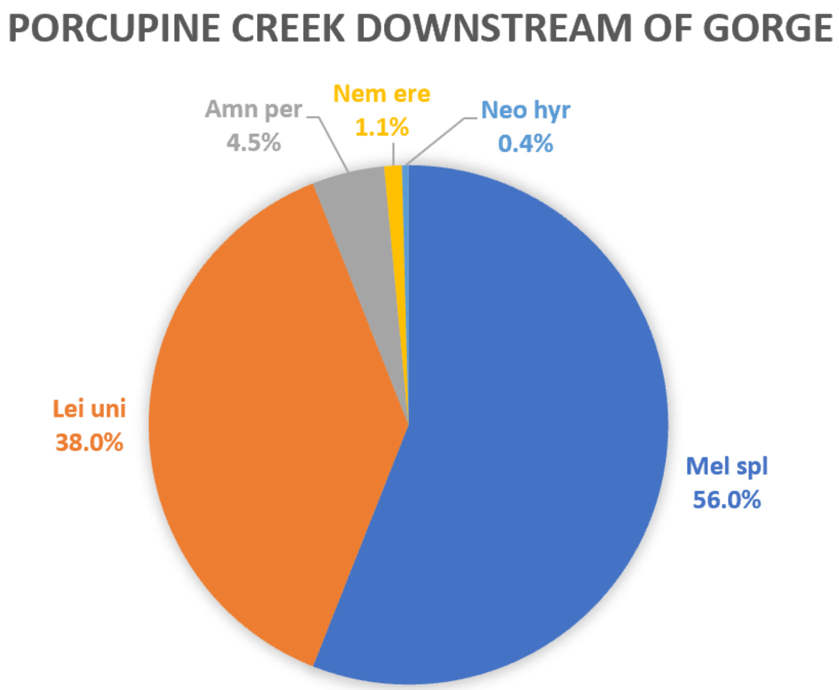

Figure 43. Porcupine Creek Downstream of Gorge species distribution.

4.12 Mount Emu Road Crossing

The Mount Emu Road Crossing on Porcupine Creek is the most upstream site in the survey area at approximately 140 km upstream of the convergence with the Flinders River. The site is a wide, shallow waterhole dominated by a sand and gravel substrate, with intermittent riparian vegetation and some instream macrophytes and woody debris (Figure 44).

Figure 44. Mt Emu Road Crossing, Porcupine Creek

A total of 53 fish were captured with another 333 observed representing six species, with Eastern rainbowfish (67.3%) and Spangled perch (28%) dominating the catch (Figure 45).

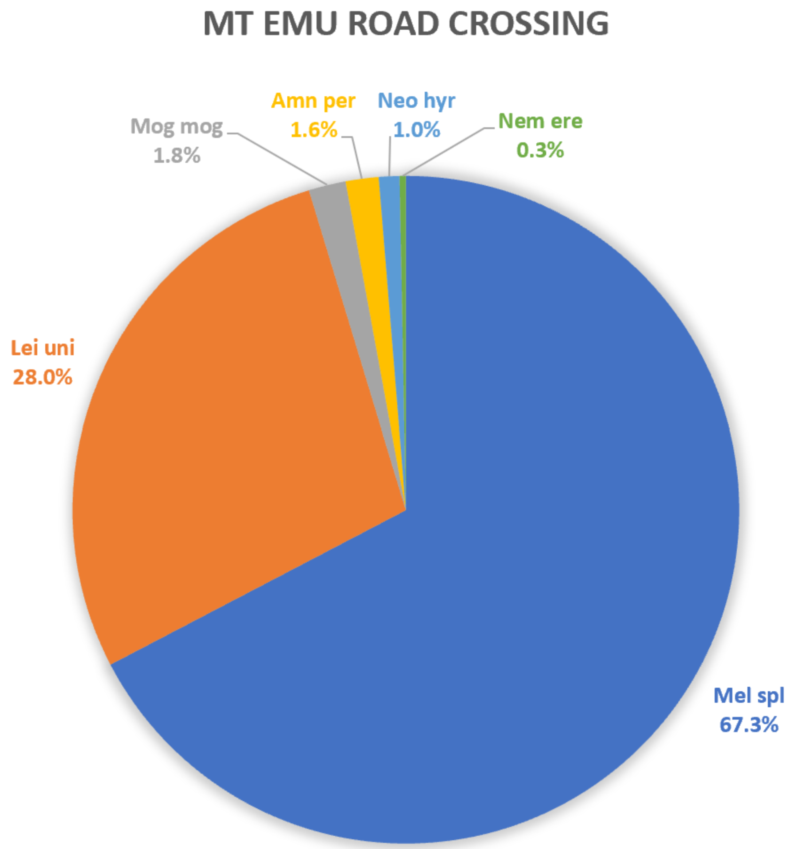

Figure 45. Mt Emu Road Crossing species distribution.

Fish sizes ranged from a 22 mm Eastern rainbowfish to a 148 mm Spangled perch with the average across all species below 100 mm (Figure 46 and Figure 47).

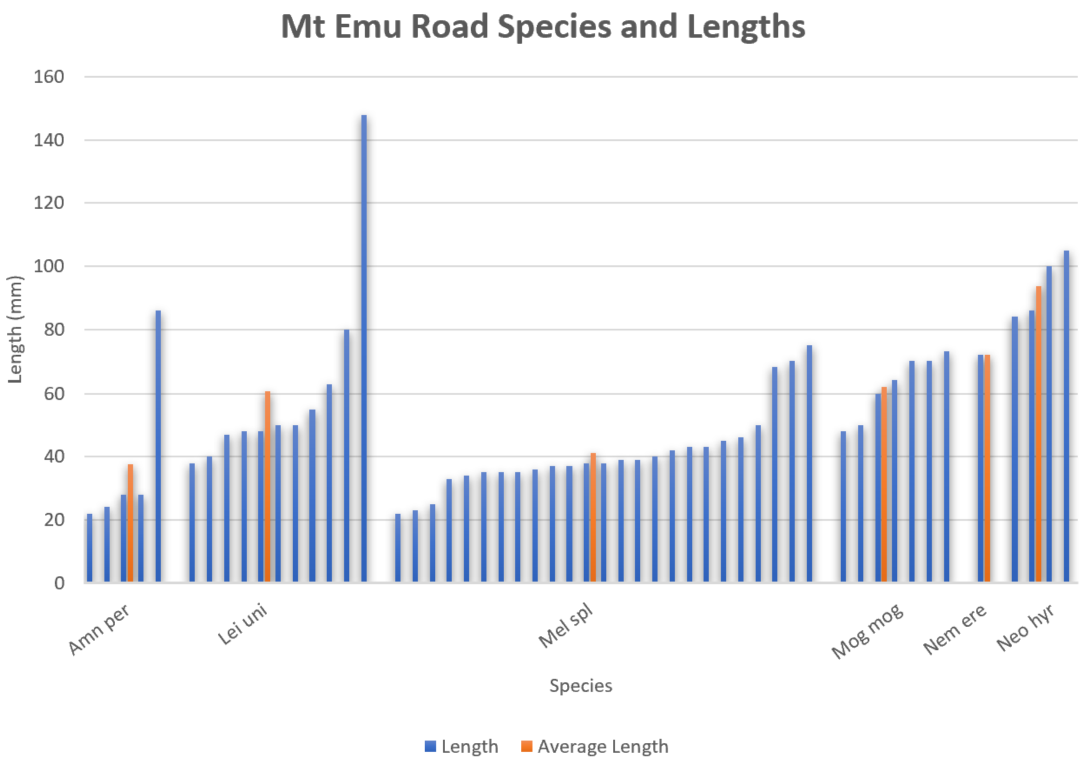

Figure 46. Length and average length of fishes captured at Mt Emu Road Crossing.

Figure 47. Banded grunter captured at Mt Emu Road Crossing.

4.13 Glendower Crossing

Glendower Crossing is approximately 30 km downstream of the Well Upstream site on the Flinders River. The site is adjacent to the Glentor-Prairie Road Crossing and consisted of three small scour pools immediately downstream of the causeway (Figure 48).

Figure 48. Glendower Crossing site, adjacent to the Glentor-Prairie Road causeway.

The catch was dominated by Eastern rainbowfish (65.7%) and Spangled perch (33.7%), with two specimens of Northern purple-spotted gudgeons (*Mogurnda mogurnda)* caught (Figure 49).

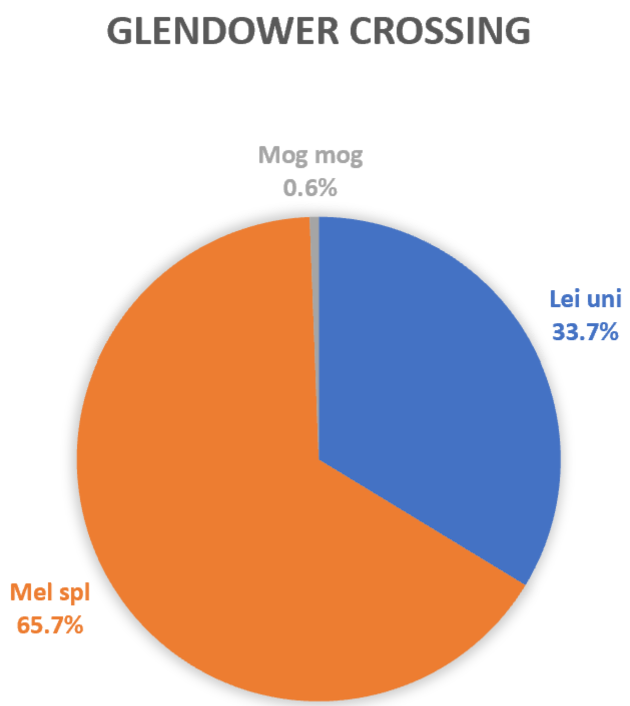

Figure 49. Glendower Crossing species distribution.

The fishes captured ranged in size from a 28 mm Eastern rainbowfish to a 97 mm Spangled perch with average lengths less than 70 mm (Figure 50 and Figure 51).

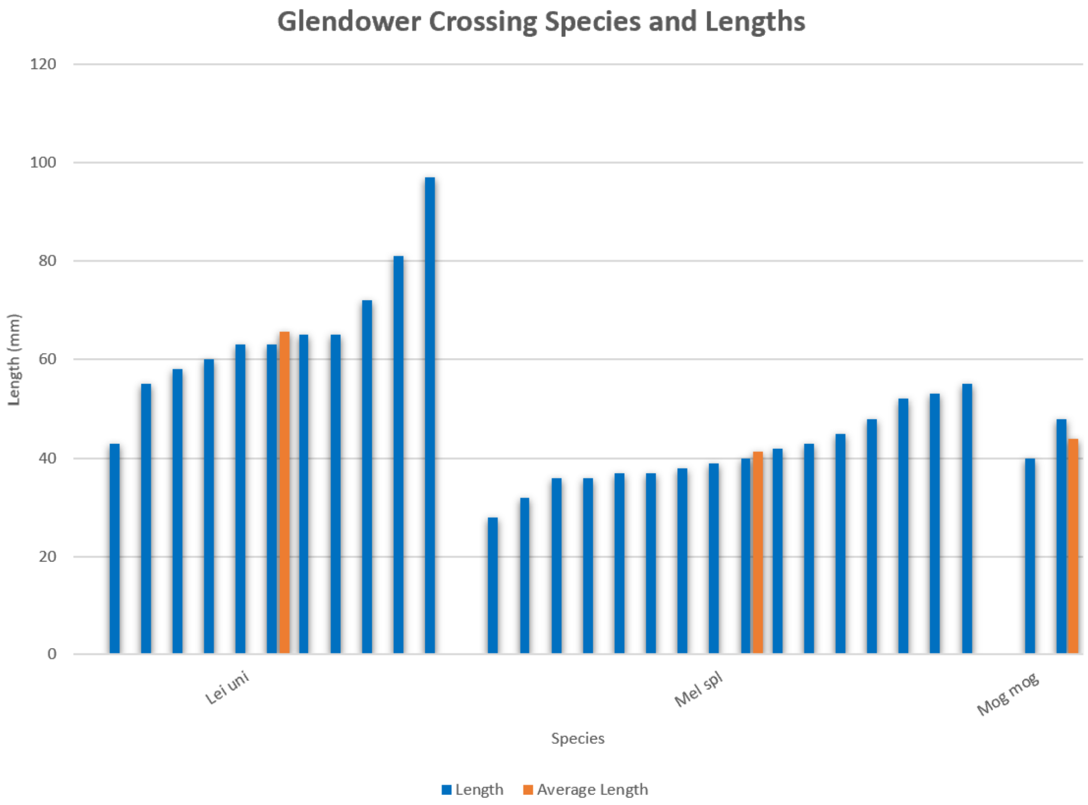

Figure 50. Lengths and average length of fishes captured at Glendower Crossing.

Figure 51. Northern purple spotted gudgeon (*Mogurnda mogurnda*) captured at Glendower Crossing.

4.14 Well Upstream

The Well Upstream site is located on Glendower Station and forms the most upstream site sampled on the Flinders River. The site consisted of a river reach confined by sandstone outcrops, with fish habitat dominated by rocky undercut banks, occasional woody debris and a sandy substrate (Figure 52).

Figure 52. Well Upstream site, Flinders River.

Despite the good quality fish habitat, only four species were detected at this site. The catch was dominated by Bony bream (79%), with some Spangled perch (20%) and one specimen each of Hyrtl's tandan and Banded grunter (Figure 53).

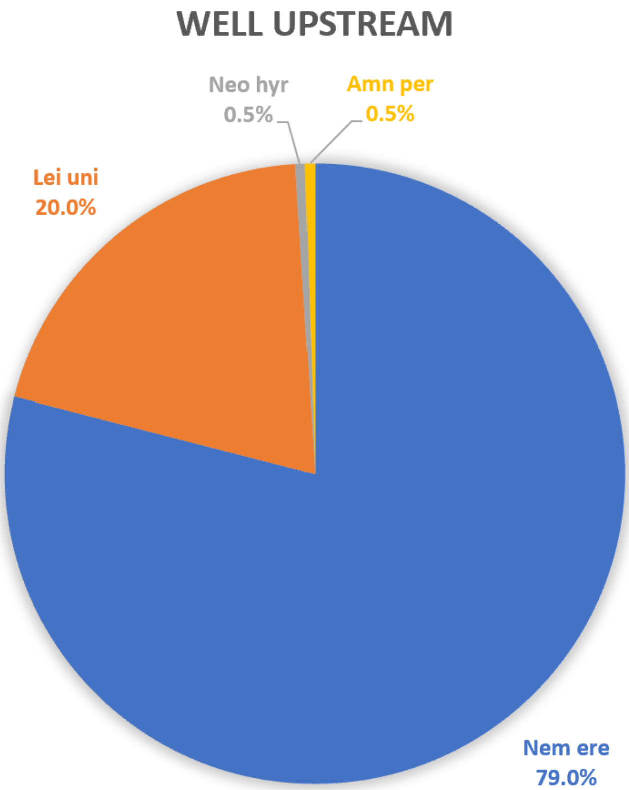

Figure 53. Well Upstream species distribution.

The sizes of the fishes captured ranged from a 46 mm Spangled perch to a 236 mm Bony bream (Figure 54 and Figure 55). The average length of Bony bream was 153 mm, while the average length for the other three species was below 85 mm.

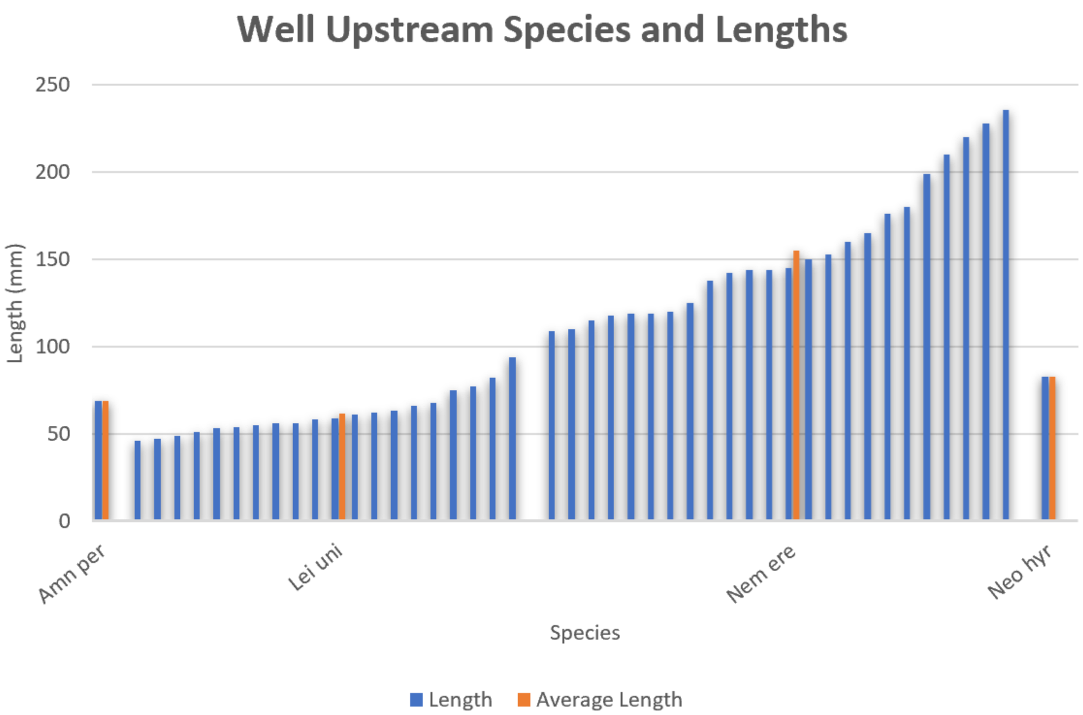

Figure 54. Length and average length of fishes captured at Glendower Crossing.

Figure 55. Spangled perch captured at the Well Upstream site.

4.15 Sawfish

Based on the assessment criteria to consider a positive detection of both target species, there was no presence of P. pristis eDNA at any of the surveyed sites. All field and extraction control samples were verified to be devoid of the target species eDNA by qPCR. See Attachment 1: Environmental DNA survey of large tooth sawfish in the Flinders River area – June 2021 for further information.

5 Discussion

A total of 21 species were captured across the 14 sites during fish community monitoring in June 2021. Of these 21 species, only two species (Saltpan sole and Golden flathead goby) were diadromous (migrating between the freshwater and the sea), while the remaining 19 were potamodromous (migrate wholly within freshwater). Past studies conducted in the Flinders Catchment detected a total of 42 species (see Table 2) with 20 diadromous and 22 potamodromous species throughout the catchment.

A review by Pusey et al. (2017) which assessed all published and unpublished survey data, museum records and WildNet data, indicated that 24 species had been previously detected in the upper Flinders River. Of these 24 previously recorded species, 17 were detected during this sampling with an additional four species detected during that had not been previously recorded. These four species include the Sooty grunter (*Hephaestus fuliginosus*), black catfish (*Neosilurus ater*), Hyrtl's tandan (*Neosilurus hyrtli*) and the Silver catfish (*Porochilus argenteus*). All of these four species have been captured in parts of the lower Flinders River, but not in the upper catchment (Atlas of Living Australia 2021).

Within the upper catchment, it is to be expected that fewer species are to be found, particularly diadromous species that migrate between fresh and salt waters. The study area sits between approximately 665 km ATMD (Mine Road Site) and 878 km ATMD (Mt Emu Road Site). This constitutes a great distance for diadromous species to migrate, and therefore their presence is generally limited in river reaches this far from the estuary, with potamodromous fishes dominating.

The migration of diadromous species is greatly affected by instream barriers. The current low number of migration barriers within the Flinders River catchment allows for generally free movement of diadromous species throughout the river system. The low numbers of diadromous species present in the sampling is indicative of the distance that the sites are from the ocean, with diadromous species generally reducing in number the further upstream you travel. The construction of any new dam or weir in this section of the river will have a limited effect on diadromous species.

5.1 Biological

The fish communities of the upper Flinders River catchment have low species diversity in comparison to the lower parts of the river system and other river systems. This follows the general pattern of the distribution of fish species within a river system but is also as a result of the limited fish habitat that occurs within the river system over the dry season, with substantial distances occurring between refuge waterholes.

The fish communities differ between off stream and instream habitats as well as downriver and upriver habitats, but species diversity and abundance is generally reflective of the persistence of waterholes in each of these habitat areas.

5.1.1 Instream Fish Communities

Instream fish communities showed the greatest diversity of species where permanent habitat was available, but also had the lowest species diversity in areas where waterhole persistence was low. Fairlight Waterhole, an instream waterhole on Betts Gorge Creek had the greatest species diversity and abundances with 16 species, 121 fish captured and 691 observed. As has been previously discussed, this waterhole is groundwater fed during some parts of the year, and this may help it to persist over the dry season. For this reason, it forms an important refuge pool for fish during the dry season, before wet season rains restore connectivity and permit dispersal from this population of recruits.

Other instream waterholes including Poseidon Waterhole on the Flinders River and Galah Crossing on Porcupine Creek, had 13 species and seven species, respectively. Abundances at Poseidon Waterhole were 57 fish captured and 648 observed and at Galah Crossing, 38 fish captured and 633 observed. At both sites, Bony bream and Eastern rainbowfish dominated the catch.

At the remaining eight instream waterholes, much lower species diversity and fish abundances were recorded. This would appear to be a reflection of the size and permanence of the waterholes over the dry season. The majority of the waterholes sampled are considered ephemeral, with only seasonal permanence and water levels that drop very quickly after the cessation of flows. At three of the instream sites, Fairlight Waterhole, Galah Crossing and Mt Emu crossing, there is a high confidence that the waterholes are Groundwater Dependent Ecosystems (GDE) (Jolly et al. 2013; Queensland Globe 2018). Queensland Globe (2018) also indicated that there is a moderate to high confidence that waterholes just upstream of the Well Upstream site are also GDE. Therefore, it can be concluded that both the size and permanence of the waterhole effect the fish community.

In general, fish species diversity at instream sites decreased the further away from permanent waterholes a site was found. This indicates that fish species in instream habitats are highly dependent on these permanent refuge habitats to supply new recruits to more ephemeral habitats upstream and downstream.

5.1.2 Off-Channel Fish Communities

Three off-stream waterholes were sampled for the project including Mine Road, Marathon Waterhole and Cantebury Waterhole. Both Mine Road and Marathon Waterhole recorded high species abundances with 12 and 11 species respectively. Catch and observation data was greater at Marathon Waterhole with 188 fish captured and 378 observed compared to Mine Road with 44 fish captured and 202 observed. This catch data is in line with the size of the fish habitat at Marathon which was a much larger and deeper waterhole that offered greater permanence over the dry season.

Cantebury Waterhole offered great fish habitat with deep water (> 2 m), fringing riparian vegetation, aquatic macrophytes and snags. The waterhole was popular with aquatic bird species including ducks and grebes. However, no fish were detected in the waterhole during sampling. Historical satellite imagery reveals that the waterhole completely dried out during the 2013 dry season. It is hypothesised that the waterhole has not connected back up with the main Flinders River channel during a flood since this time, and as a result, no new recruits have been able to migrate into the habitat.

Off-channel fish habitat will be affected in the areas downstream of the new development, as potentially reduced flows within the river system may affect the period of connection these habitats have to the main river channel. As can be seen at the Cantebury waterhole, while habitats may refill from local run-off, if they have dried out previously and do not reconnect to the main river channel, no fish are able to recruit back into the habitat. As such, it will be critically important to ensure that flows in the river are able to reconnect these habitats on a regular basis after the construction of any instream regulating structures.

5.1.3 Refuge Habitats

From the results it is evident that the larger, more permanent waterholes support both greater fish numbers and species diversity. Marathon and Fairlight Waterholes appear to be the two most permanent refuge habitats sampled. It is also possible that Galah Crossing and Mt Emu Crossing afford refuge habitats over some dry seasons. These refuge habitats are extremely important as they maintain fish populations over the dry season, supplying new recruits to the system when connectivity is restored during the wet season.

Any development that occurs within the river system should ensure that the permanence and connectivity of these refuge habitats is maintained to ensure that the pools remain as refuge habitats and that fish within these refuge habitats are able to migrate and repopulate ephemeral areas of the river system. Even though fish may die in these ephemeral habitats, it is important that the transfer of fish within the system is maintained as there are likely to be many other species reliant on this yearly transport of food into their habitat.

Opportunity also exists within the development area to create more permanent refuge habitats within the upper Flinders River system. This can assist in maintaining fish populations within the refuge habitats at a higher level than currently exists and allow greater expansion into the ephemeral habitats of the upstream catchment because of the greater density of fish held within these refuges. Refuge habitats could be maintained within both the weir and dam footprints, and potentially also in the delivery channel from the weir to the dam.

5.1.4 Community Change with Distance Upstream

There was a distinct change in the fish communities at sites further upstream, and further away from the permanent refuge pools (Figure 56) that support recruitment within the river (Marathon Station or Fairlight Waterhole).

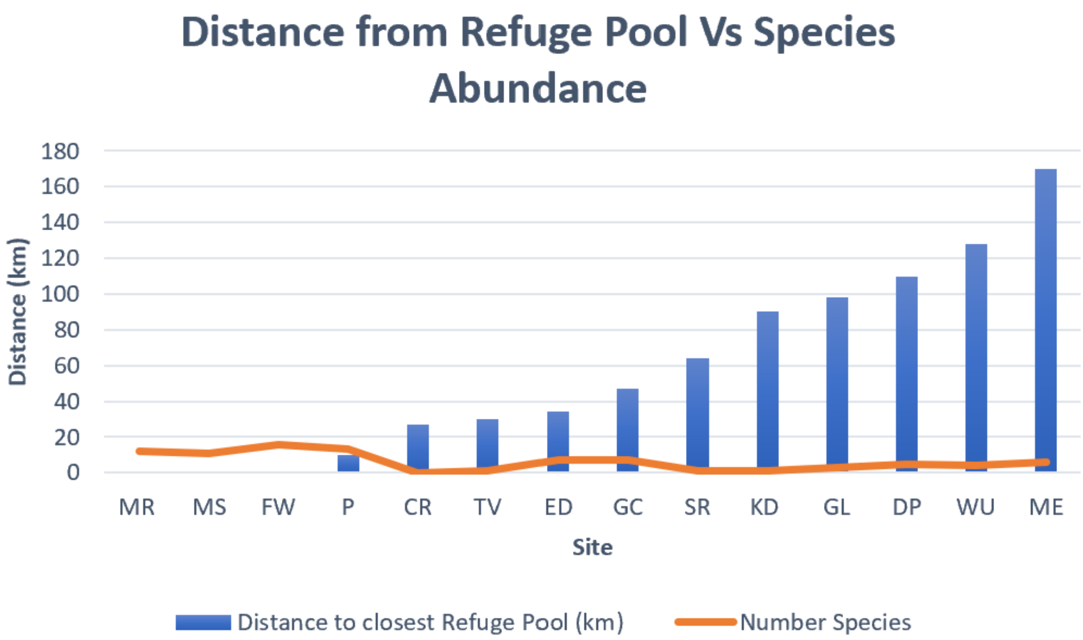

Figure 56. Distance from refuge pool Vs species abundance.

Excluding Cantebury Waterhole which had no fish community, there were nine sites located greater than 30 km away from a refuge pool (Marathon or Fairlight Waterhole). Across these nine sites, a total of 10 species were detected. The most abundant of these species was the Spangled perch found at eight of the nine sites, followed by Eastern rainbowfish at six sites, Bony bream at five sites and Banded grunter and Hyrtl's tandan at four sites. (Figure 58).

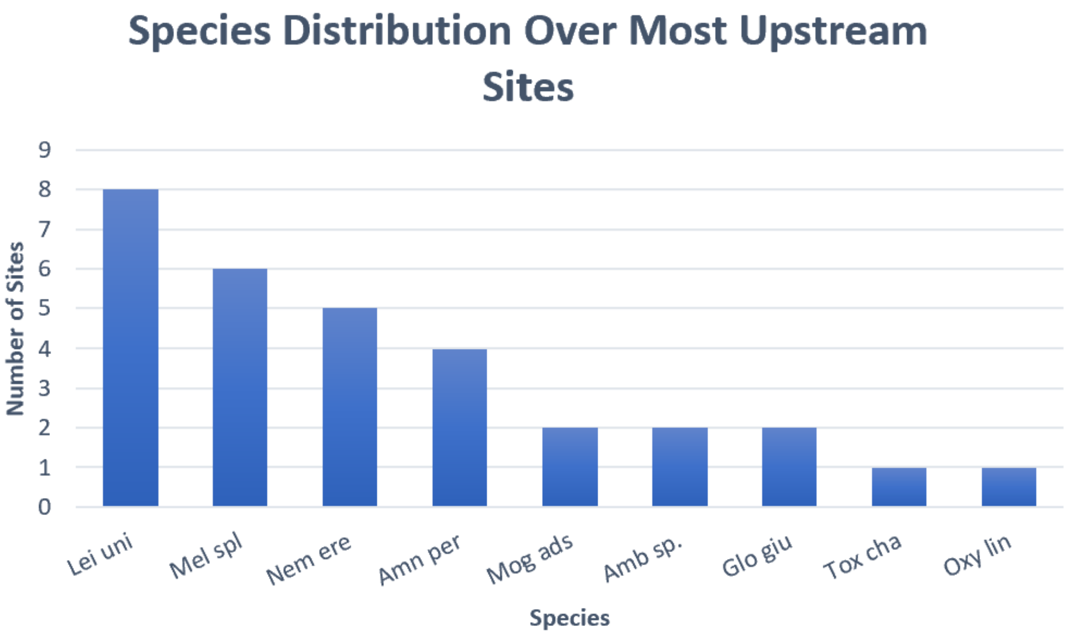

Figure 57. Species distribution over the most upstream nine sites.

These species are well known for their incredible ability to migrate quickly and over large distances, having both endurance and burst swimming ability. Throughout inland

Australia, Spangled perch, Rainbowfish, Bony bream and Neosilurid catfish are regularly observed attempting to migrate past barriers during short duration flow events within these systems (Figure 58 and Figure 59). They demonstrate incredible swimming ability to negotiate these barriers, often coming fully out of the water to cross shallow streams over barriers. This ability has meant that these species are some of the most widespread species within central Australia. Their presence within the ephemeral sections of the Flinders River is a result of strong migratory instincts.

Figure 58. Catfish attempting to migrate past a barrier in central Australia (ABC News Brendan Esposito).

Figure 59. Spangled perch attempting to cross a flooded road in inland Australia (Photo: Australian Geographic, Reece Pedler)

The average length of the four most widespread species at the upstream sites varied with increasing distance from a refuge pool (Figure 59). Average lengths for these species were greatest in the larger refuge pools provided at Marathon Station, Poseidon Waterhole and Well Upstream, and were lowest at the small ephemeral instream sites. This data may be indicative of adults successfully migrating over the wet season to permanent waterholes or choosing to remain where habitat and food supplies are ample. Whereas juveniles from these refuge habitats are attempting dispersal migration throughout the system but are not making it to permanent waterholes, and become isolated in ephemeral habitats, such as those sampled.

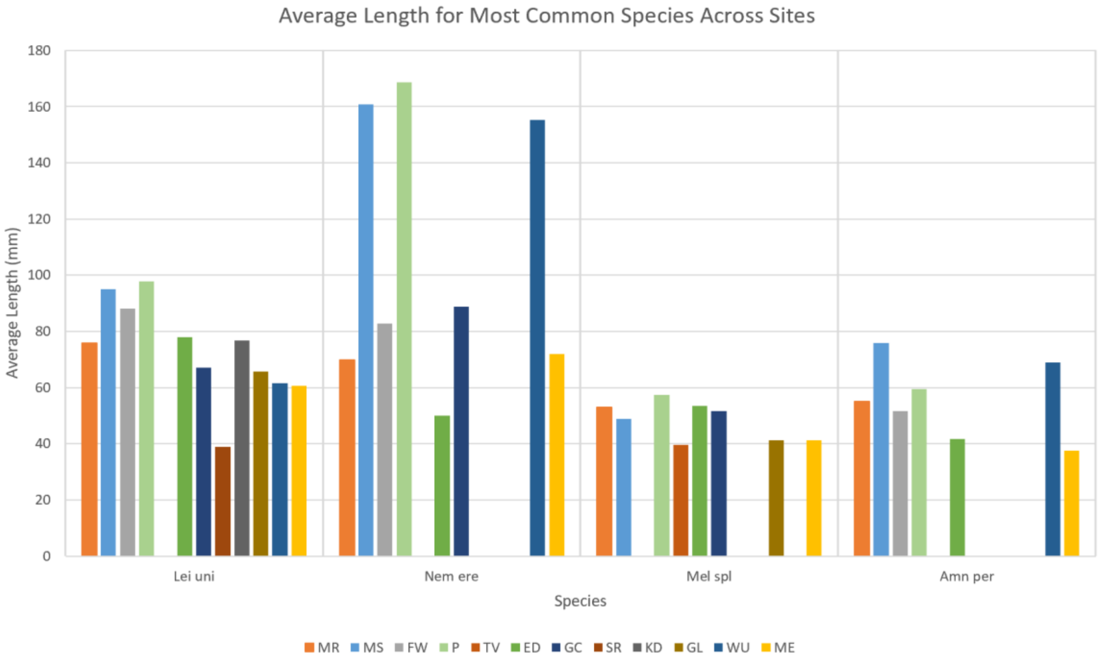

Figure 60. Average lengths of the four most widespread species at upstream sites.

5.1.5 Sawfish

The presence of sawfish in the Flinders River in the vicinity of the project area was considered uncertain. While no previous studies have identified sawfish in the area, the CSIRO Waterhole Ecology report from 2013 placed a siting point for sawfish in the vicinity of the project area (potentially a community siting) (Waltham et. al. 2013). To definitively identify if sawfish are present in the upper catchment, sites were sampled with eDNA techniques to detect sawfish DNA. The species is uncommon and difficult to catch with traditional fishing methods and eDNA sampling provides the best methodology of establishing the presence of the species. This technique collected DNA from water samples in the sampling sites and compared it to known DNA samples for sawfish from other sites in northern Australia.

From the ten sites sampled for sawfish DNA, no sites were detected as having sawfish present (See Attachment 1).

Previous fish surveys place sawfish captures in the lower Flinders Catchment, with no scientific survey records of sawfish captured in the upper Flinders River. The lack of sawfish in the upper catchment is likely due to the large distance from the sea of the sites in the upper Flinders River. These sites are well beyond the known upper limit for the penetration of sawfish into a catchment. In addition, it is likely that the scarcity of suitable habitat, and long distances between habitats, may also play a role in the lack of sawfish in these reaches with little or no physiochemical cues encouraging the sawfish to migrate further upstream.

5.2 Short-Term Construction Impacts

During the construction period, there are several impacts that affect fish communities that need to be considered and addressed with suitable remediation actions.

5.2.1 Period of construction

The construction should have a staged program that will minimise the time that construction is underway within the waterways. Timing for construction of all works should coincide with the dry season and all construction works should be completed prior to flows at the commencement of the wet season, that generally occur late November to early December.

Where the period of construction in any of the waterways spans across the wet season, then temporary fish passage structures should be provided within any temporary structure.

5.2.2 Temporary fish passage

Where freshwater waterways will be blocked across the wet season, temporary fish passage must be provided with suitable fish passage characteristics. This could include ensuring that any road crossing structures have suitable cross-section areas to maintain low velocity zones, are built in pools with no velocity (Figure 61) or have fish passage devices such as baffles installed to create low velocity zones suitable for the passage of fish through of the structure.

Figure 61. Temporary pipe culverts with low velocity provide passage during construction.

5.2.3 Site clean-up and rehabilitation

All works within the waterway should return the waterway bed and banks adjacent to the development footprints to a stable natural profile within five days of the completion of the construction. Contractors should ensure that the waterway bed retains a natural substrate consistent with the size and materials of the natural bed in the waterway. Banks should be profiled similar to adjacent banks in a manner that will allow the rapid re-establishment of native vegetation and cover.

5.2.4 Water Quality

Impacts on water quality should be minimised by undertaking the works to standards such as those set out in the current version of the Transport and Main Roads Specifications MRTS52 Erosion and Sediment Control documentation (IECA 2019).

5.2.5 Fish Salvage

It is important to ensure that any fish habitats in waterways adjacent to the development are protected and conditions are maintained throughout the construction process. The project should endeavour to ensure that development works do not isolate any section of the waterway from the main body of water to prevent the requirement for fish salvage to be undertaken. Given the location of the works, it is unlikely that this will occur, however, in the event that fish become trapped by the works, the project team should undertake fish salvage in accordance with the Fisheries Queensland Fish Salvage Guidelines (available at www.daf.qld.gov.au).

5.3 Long-Term Effects on Fish

After the construction of the project is completed, there are likely to be several long-term effects to fish communities within the Flinders River system. It is essential that mitigation measures are put in place to ameliorate the impact that these effects may have on fish communities.

5.3.1 Migration Pathways

Currently, the waterways within the development area connect every wet season permitting the free migration of the entire fish community both upstream and downstream. If the project goes ahead, any waterway barriers constructed will impact this migration.

The current proposal indicates that there will be a dam built across Stewart Creek, a diversion channel across Back Valley Creek and a weir built on the Flinders River. These structures will limit the migration pathways of fish within the waterways and will require amelioration of fish passage to maintain fish populations and attain approval.

5.3.2 Limitation to Fish Migrations

Due to the ephemeral nature of the stream, fish migrations in the Flinders River are limited by the flow conditions that occur and the large accumulation of sand in the bed that has reduced the number of permanent refuge holes. Flows generally only occur during the wet season and last for around 3 to 4 months, before the river dries back firstly to a series of isolated waterholes and then further to a small number of refuge waterholes.

The short duration of flow during the wet season limits the distance that fish are able to move from refuge waterholes to repopulate the rest of the river system. Data collected during field sampling indicates that a limited number of species (Bony bream, Spangled perch, Rainbowfish and Hyrtyl's tandan) have the migration characteristics for endurance and speed swimming that enable them to cover the distances required to spread throughout the river system. These species are therefore the most widely distributed fish species within the river system.

In effect, the distance between refuge waterholes in the short duration of flow within the system is likely to limit the distribution of many of the other species in this catchment. It would be likely that if more permanent refuge habitats were available in the upper catchment, that there would be a greater distribution of the other species in these areas, as this part of the river system is similar to many other areas where they are found.

5.3.2.1 Flinders River Weir

Once the Flinders River Weir is constructed, free upstream and downstream migration will be affected as will be natural downstream larval dispersal.

An upstream fishway specifically designed for the migrating fish community of the upper Flinders River, flow regimes found in the river and the structural configuration of the weir is recommended to ensure that upstream fish passage is available anytime that there are flows in the river. This will allow fish to access the upstream reaches of the Flinders River and its tributaries including Betts Gorge Creek and Porcupine Creek.

With the construction of the Flinders River Weir across the waterway, and the offtake channel diverting water to the dam impoundment, fish larvae moving downstream with river flows will inevitably end up being diverted with the water. This diversion reduces natural downstream dispersal and increases recruitment within the new dam impoundment.

Downstream migrations are critically important within the Flinders River and may be impacted by the weir structure in three ways:

1. Larvae may be diverted away from the river via the inlet channel for the dam.
2. Larvae may be damaged when passing over/though the weir structure.
3. Floating larvae may sink and die within the slow flowing waters within the weir pool.

For these reasons it is essential that fish larvae are preserved through the implementation of suitable mitigation measures. For larvae that are diverted from the river, this can be achieved by ensuring that diversion channels are screened, that any destination water bodies into which they are taken are maintained across the dry season and that access is provided back to the main river in the following seasons. To ensure the safety of larvae that pass over or through the weir structure, it is essential to ensure that ogee crests are smooth and have no steps, and any gated structures are overshot, with both structure types discharging into a significant depth of water downstream to ensure that fish survive passage through the structure. If undershot gates are used, they should only be used in a fully open or fully closed scenario to minimise larval fish deaths from impact and pressure changes encountered as the fish pass under the gates. To ensure the survival of fish larvae that enter the weir pool upstream, modelling should be undertaken to determine suitable weir operating strategies to maintain minimum water velocities in the weir pool upstream. This will ensure that the larvae remain in suspension through the weir pool and do not sink to the bottom and die.

As the weir is a significant structure across the Flinders River, consideration should be given to maintaining a pool of water within the weir pool across the dry season to improve refuge habitat conditions within the Flinders River. Fairlight Waterhole on Betts Gorge Creek which is located just upstream from the proposed weir site, provides refuge habitat for a wide range of fish species in this section of the river, will be drowned out by the weir. Ensuring that an area of water larger than the Fairlight Waterhole remains behind the new weir would provide the opportunity to increase refuge habitat in this section of the Flinders River. This would allow a greater biomass of fish to develop in the refuge hole, and more species to maintain populations, enabling fish from this refuge to push into other sections of the river from the enlarged and more permanent refuge habitat.

5.3.2.2 Gravity-fed Channel

The gravity fed channel that connects the Flinders River Weir to the Saego Dam may become an important fish habitat, as well as a linkage between the weir and dam. To ensure the maximum survival of fish entering and leaving Saego Dam via this channel, it is essential to ensure that in-channel flow velocities are suitable for both upstream migrating fish exiting the dam, and downstream drifting larvae leaving the Flinders River and entering the dam.

It would also be important to ensure that any headworks structures that are placed within the channel allow for the free movement of fish in both an upstream and downstream direction. This could be achieved by ensuring that any headworks include no undershot gates, limited head loss and a low velocity zone of no greater than 0.3 m/s through the structure and against the banks. Consideration could also be given to screening the inlet of this channel to ensure that larvae are not able to enter the channel out of the Flinders River. However, as many fish will be exiting Saego Dam via the channel, being attracted to the flows from the Flinders River and Betts Creek Gorge into the dam, it is essential that these fish are able to leave the channel and enter the Flinders River, which could be impeded by screens.

After flows have ceased in the Flinders River and the dam level has dropped to below the level of the connecting channel, it is essential that any fish left within the channel are protected. This may be achieved by ensuring that the channel has a suitable gradient that prevents the accumulation of water in the channel after flows cease, or through the maintenance of refuge habitat within the channel that provides fish habitat over the dry season. In all cases, a fish salvage plan will be required to ensure that any fish that are in danger of dying within the channel can be actively relocated to suitable refuge habitats.

The construction of the gravity-fed channel across Back Valley Creek (Green stream) will pose a barrier to fish passage. Upstream and downstream migration over the barrier can be provided by constructing the proposed emergency spillway with a continuous slope no greater than 1:20. For fish migrating up the spillway, exit conditions must be safe, which will entail ensuring that velocities flowing down the gravity-fed channel remain low and do not sweep fish away and into the dam impoundment.

Fish migrating into Back Valley Creek from the spillway or gravity-fed channel, must be provided with adequate fish habitat, so that when the water level in the stream falls below the gravity-fed channel invert height, they have a refuge pool for the dry season.

5.3.2.3 Saego Dam

Saego Dam, although located on a smaller waterway (Stewart Creek), will still need to be maintained as a migration corridor into and out of the dam along the spillway alignment, as sampling has indicated that there are significant migratory fish populations within the upstream catchment.

The dam construction will need to take into consideration several factors in preserving fish populations within Stewart Creek and the broader Flinders River catchment. These include:

1. Providing access for fish into Stewart Creek and the upper catchment above the dam.
2. Providing safe passage for fish out of Saego Dam during flooding flows.
3. Maintaining refuge habitat within the impoundment footprint to preserve the fish community already found within Stewart Creek.

Fish passage into the dam will be required to allow fish to access the upper catchment as currently occurs within the system. This can be achieved in a number of ways, including the construction of a fishway on the outlet of the dam, or by allowing fish to pass into the dam via the connecting channel to the Flinders River weir. As the structure is quite large, construction of a fishway on the main dam wall will be a significant challenge, so consideration of a small fishway on the outlet spillway or enhancing fish passage into the dam from the connecting channel to the Flinders River Weir, may provide a more robust and successful option.

The passage of fish out of the dam during overtopping releases will need to ensure that a safe migration pathway is provided for downstream migrating fish. This can be achieved by ensuring that any spillway has a smooth ogee crest arrangement, discharging into a tailwater pool with sufficient depth. Any spillway should not contain dissipation steps as these negatively impact downstream migrating fish, causing strike injuries and death. Other options may include a downstream lock arrangement, that consists of a gate in the impoundment linked to a pipe that discharges into the tailwater pool of the spillway.

As Saego dam will create new fish habitat within Stewart Creek when the dam fills up, it is essential to have a suitable operating plan for the impoundment to ensure that fish are not stranded at the end of the dry season. As fish communities already exist within Stewart Creek and there will also be fish moving from the Flinders River via the connecting channel into Saego dam, the population of fish in the impoundment is likely to be quite large. It will be incumbent upon the operator of the structure to ensure the safety of this fish community by implementing a management plan to leave water in the impoundment throughout the dry season. This could be achieved via operations within the dam ceasing at a certain level of drawdown, or through the construction of a dedicated refuge pondage within the dam from which no water could be drawn. Any refuge habitat in the dam would need to be quite significant with a size greater than 10 ha in surface area and a minimum depth of 3 m when drawdown of the dam ceases recommended. These dimensions will need to be modelled to ensure that they are able to always maintain a minimum depth of 1.5 m at the end of the dry season.

5.3.3 Increased Refuge Habitat

The construction of the weir, dam and gravity-fed diversion channel will lead to the creation of new fish habitat within both the Flinders River, Stewart Creek and Back Valley Creek. The structures will create aquatic habitats and encourage macrophyte growth that will change the water chemistry of both impoundments. Fish will naturally be attracted to the new habitats as flows carry organic cues that suggest the presence of organic growth (Leonard 2012). Several studies around the world have found that different migratory species recognise and are attracted to river waters that possess distinct odorants (Leonard 2012, James et al. 2007; Sola & Tosi 1993). The distinct earthy-musty odour comes from different substances produced by bacteria, fungi and algae (Sola & Tosi 1993). Once the impoundment pools are created, these odorants will naturally form, attracting fish to migrate towards them to seek refuge.

This phenomenon has been observed in north Queensland on a newly constructed dam near Weipa. At this location, the existing stream only contained two species of freshwater fish prior to dam construction, whereas once completed, several previously unrecorded diadromous species were recorded entering the impoundment via the spillway fishway (pers comm A. Berghuis). These fish were drawn to the newly created conditions within the impoundment, which were significantly different from the previous stream condition.

While this could be seen to be problematic from an operational perspective, it would be wise to embrace the opportunity for the dam, channel and weir pool to afford and increase the amount of permanent refuge habitat available in the upper Flinders River, as fish will take advantage of the extra habitat created within the impoundments regardless of operator preference.

Refuge habitat can be achieved by ensuring that a dead storage level for each impoundment is set that maintains ample water and habitat for the fish community. This does not necessarily require a reduction in yield from the impoundments but may be being provided through the construction of specific refuge habitats at the lowest levels of the dam, below which water would not be drawn for operational purposes.

5.3.4 Reduction of Over Channel Flows and the Drying of Refuge Pools

Extraction of water from the Flinders River via the Flinders River Weir for storage within Saego Dam will impact the volume of water that is passed downstream of the project and, will in turn, affect the flooding of the downstream floodplain. Any reduction in flooding may have flow on effects for the off-channel fish habitats downstream from the project area, due to a reduction in peak water levels across the floodplain, as well as the duration in which peak flows maintain connectivity to off-channel water bodies.

Downstream of the project area there are several significant off-channel fish habitats including Marathon waterhole, that have been identified as providing refuge habitat for fish across the dry seasons. These habitats are critically important for maintaining fish populations within the river system as they provide a stable, permanent waterhole in which fish can reach maturity, and from which new recruits are able to expand into the rest of the river system when these habitats are reconnected to the main river channel.

Ensuring that water extraction has a minimal impact on the connection of these water bodies to the main river system will be critical to ensuring ongoing recruitment from these habitats into other areas of the catchment. This can be achieved by ensuring that peak floods still reach heights suitable to connect these habitats, and that the duration for which they are connected enables fish to enter and exit. In this way, the connectivity of these off-channel habitats to the main river system will be maintained, which will in turn ensure fish communities can continue to utilise the off-channel habitats as a base from which to spread to other parts of the system.

It is recommended that further investigation be conducted into the reduction of over channel flows, to determine how the refuge waterholes will be impacted both spatially and temporally. Modelling should be undertaken of various flow scenarios associated with the Flinders River Weir and extraction of water into Saego Dam to ensure that suitable conditions can continue to be achieved to maintain the condition of off-channel habitats. Ensuring suitable flow conditions are maintained will negate the need to implement diversion structures to connect these habitats in reduced flow scenarios.

5.3.5 Runoff from Irrigation Areas

Runoff from the new irrigation area to the south of the Flinders River threatens the health of both the instream and off-stream fish communities. Flows from the irrigation area drain into channels that feed the anabranches of the Flinders River where important refuge habitats including Marathon and Mine Road Waterholes are located (Figure 62).

Increased nutrient and sediment runoff from the irrigated cropping area that flows into these off-stream waterholes will have a negative effect on aquatic water quality, especially if the waterholes are no longer receiving typical over channel flows that provide annual flushing.

Managing runoff through the implementation of treatment trains at obvious pollution source points, could have a beneficial outcome for the aquatic community. Treatment trains utilise a combination of treatments including swales, sediment ponds, infiltration trenches and wetlands to filter pollutants such as sediments, nutrients and herbicides from runoff water, before it enters the off-stream habitats.

It is recommended that further research into the implementation and placement of treatment trains is carried out as part of the EIS to improve water quality of flows entering the Flinders River and its off-stream habitats.

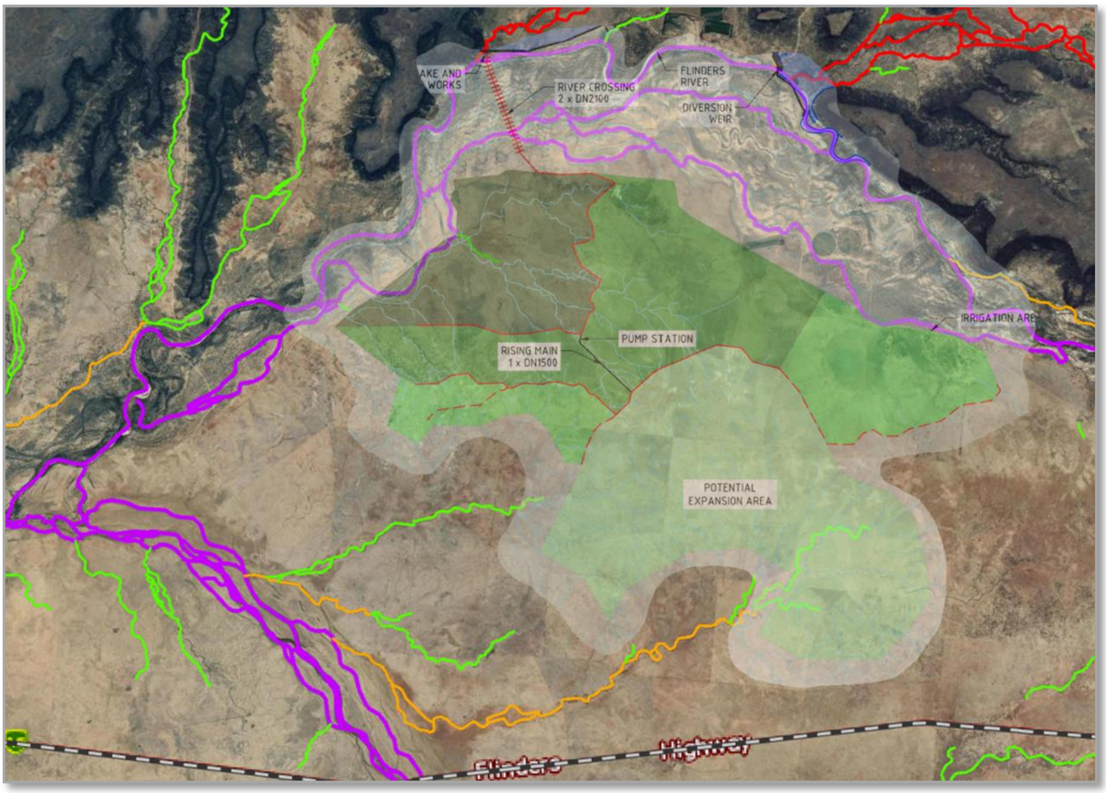

Figure 62. The irrigation area (green) in relation to the Queensland Waterways for Waterway Barrier Works mapping.

6 Recommendations

It is recommended that the following monitoring be carried out as part of the future EIS process:

- Identify and map permanent refuge pools in the greater development area and implement a conservation and management program for each.
- Ascertain the distance fish move from the identified refuge pools during a wet season and determine if new flow regimes will affect ability to reach refuge habitats.
- Study the current and proposed future over channel flows to identify connection impacts to identified off-stream refuge pools.
- Assess the instream pool drying cycles/permanence with proposed future flow regime.
- Assess the permanence of fish habitat in Porcupine Gorge NP.
- Assessment of impact of downstream barriers on migration to project area.
- Study the larval fish load throughout the wet season to determine suitable tactics for preventing the loss of juveniles from the river system.

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8 Attachment 1. Sawfish eDNA results.

Environmental DNA survey of largetooth sawfish in the Flinders River area – June 2021

Prepared by Cecilia Villacorta-Rath and Damien Burrows

Report No. 21/43

August 2021

Environmental DNA survey of largetooth sawfish in the Flinders River area – June 2021

A report for Australasian Fish Passage Services

Report No. 21/43

August 2021

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Information should be cited as:
Villacorta-Rath C, Burrows D (2021). Environmental DNA survey of largetooth sawfish n the Flinders River area – June 2021. Centre for Tropical Water & Aquatic Ecosystem Research (TropWATER), James Cook University, Townsville, 15 pp. TropWATER Report 21/43.

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EXECUTIVE SUMMARY

Environmental DNA (eDNA) methods are increasingly applied to detection of freshwater species with equal or more efficiency than traditional methods. Australasian Fish Passage Services engaged the Centre for Tropical Water and Aquatic Ecosystem Research (TropWATER) at James Cook University to conduct environmental DNA (eDNA) analysis of water samples collected at the Flinders River area, Queensland. A total of ten sites were sampled by Australasian Fish Passage Services staff. At each site, five replicate water samples were collected. Environmental DNA from the water samples was isolated at a dedicated eDNA laboratory at James Cook University and DNA extracts were screened for presence of largetooth sawfish, *Pristis pristis*, using species-specific quantitative PCR (qPCR). No presence of *P. pristis* eDNA was detected, which indicates that the target species is likely not to have been present at the sampling sites for up to one week prior to the sampling event.

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1 INTRODUCTION

Sawfishes (family Pristidae), a family of shark-like rays, are considered among the most threatened marine fishes in the world (Harrison and Dulvy 2014). The Global Sawfish Conservation Strategy states the need for capacity building towards effective sawfish conservation and management (Harrison and Dulvy 2014), however, information of the contemporary distribution of sawfish is limited, hindering conservation initiatives (Dulvy et al. 2016). Traditional sawfish survey methods include visual observations, fishing using gillnets or lines, traditional ecological knowledge, and public encounter data (Simpfendorfer et al. 2016). Of these, the most useful method for compiling important biological data has been capture with gillnets and line fishing, however, fishing becomes logistically difficult in remote and sparsely populated regions where sawfish are known to occur (Simpfendorfer et al. 2016). Largetooth sawfish (*Pristis pristis*) occurs in freshwater systems in northern Australia. This species historical distribution includes coastal waters of 75 countries, however, it has experienced a 61% decline in geographic range (Dulvy et al. 2016). There is an urgent need to apply less laborious field methods to detect presence of this threatened species in order to determine the current extent of its distribution.

The study of environmental DNA (eDNA) provides a significant opportunity for non-invasive detection of aquatic species. This technique is based on the principle that all organisms shed genetic material into their environment via physiological processes (Jerde et al. 2011). Capture and extraction of DNA from environmental samples (i.e. water, soil, snow, etc.), followed by a targeted polymerase chain reaction (PCR) allows for species detection with high confidence (Ficetola et al. 2008). Therefore eDNA analysis provides a mean of species detection that does not require to physical capture, or sighting of the organisms (Thomsen et al. 2012). Environmental DNA analysis has been successfully applied to sawfishes around the world (Cooper et al. 2021; Lehman et al. 2020; Simpfendorfer et al. 2016).

Largetooth sawfish inhabits freshwater systems and has been historically found in sections of the Mitchell and Gilbert Rivers >300km inland. Weir development along large rivers threaten the species, since sawfishes do not use fish passages. Currently, there is a proposal for irrigation development in the Flinders River, where it is unknown how far downstream sawfish occur. The aim of the present work was to determine presence of largetooth sawfish eDNA across ten sites in the Flinders River area, sampled during June 2021.

2 METHODOLOGY

2.1 Field collection of eDNA samples

Environmental DNA kits were shipped to the Australasian Fish Passage Services prior to fieldwork, including materials for eDNA sample collection and a field protocol. Field collection was carried out by the Australasian Fish Passage Services staff using the eDNA kits and following TropWATER's eDNA collection protocol. Water samples for eDNA analysis were directly collected and preserved from ten field sites in the Flinders River are during June 2021 (Table 2.1). At each site, five replicate 300 mL samples were collected using a clean collection jar (500 mL capacity) and decanted into another jar (500 mL capacity) containing 100 mL of Longmire's preservative solution (Longmire, Maltbie, and Baker 1997). At every site, a field blank was also taken to ensure that the process of sample collection did not introduce contamination. The field blank consisted of decanting 300 mL of laboratory-grade water into a jar containing 100 mL of preservative solution

Table 2.1 Sampling sites for the direct water collection and preservation of samples in the Flinders River area for *P. pristis* eDNA detection during June 2021

Location	Site name	No. field samples	Collection date
Flinders River	Glendower	5	23/06/2021
	Galah crossing	5	24/06/2021
	Mine Road offstream waterhole	5	26/06/2021
	Poseidon waterhole	5	27/06/2021
	Pivot irrigation offstream waterhole	5	27/06/2021
Porcupine Creek	Porcupine Gorge downstream	5	24/06/2021
Fairlight waterhole - Fairlight Creek	Fairlight Creek at Flinders River junction	5	25/06/2021
Marathon Station	Flinders offstream waterhole	5	26/06/2021
Canterbury Road	Offstream waterhole	5	26/06/2021
Stewart Creek	Expressman Downs	5	27/06/2021

2.2 Environmental DNA extractions

Before extraction commenced, bench top surfaces and floor in dedicated eDNA laboratory were decontaminated with 10% *v/v* bleach, as per standard operating procedure. Upon returning to the laboratory, the exterior of all sampling jars was washed with 2% decon solution and blot dried. Each field replicate and controls were then aliquoted into five DNA LoBind (Eppendorf®) Falcon tubes of 50 mL capacity, each containing 20 mL of sample (100 mL total). Environmental DNA was extracted from samples using a glycogen-aided ethanol precipitation method (Edmunds and Burrows 2020) at the JCU-TropWATER dedicated eDNA laboratory. Briefly, each 20 mL sample was mixed with 5 µL glycogen (20 mg/mL), 5 mL NaCl (5 M) and 20 mL isopropanol, vortexed and incubated at 4°C overnight. Tubes were then centrifuged at 6,750 g for 10 min, the supernatant was discarded and the pellet was resuspended in 120 µL lysis buffer. The resuspended pellet from all five aliquots belonging to each field replicate were then pooled into a 2 mL DNA LoBind tube, constituting a total of 600 µL lysis buffer per field replicate. Samples were then kept at -20°C overnight. Subsequently, samples were thawed, vortexed at maximum speed for 30 sec and incubated at 50°C for five hours. At the end of this period, samples were allowed to come to room temperature, 1,200 µL PEG-NaCl buffer and 1 µL glycogen was added and samples were stored at 4°C overnight. Following this, tubes were centrifuged at 14,000 g for 30 min, and the pellet was washed twice with 70% ethanol. The pellet was air-dried and 100 µL TE buffer was added. Finally, a DNA purification step was performed using the DNeasy PowerClean Pro Clean up kit (Qiagen Pty. Ltd.) following the manufacturer's protocol and samples were eluted in 100 µL elution buffer. For each eDNA extraction batch, a negative extraction control was added to each batch of eDNA extractions to ensure that no contamination was introduced during laboratory procedures (Goldberg et al. 2016).

2.3 Detection of species-specific eDNA by quantitative PCR (qPCR)

Detection of largetooth sawfish was performed using species-specific qPCR assays developed by (Cooper et al. 2021). The assay was designed to detect a short segment of the *12S* rRNA mitochondrial gene of *P. pristis* (179 bp) with an LOD at 1.25 DNA copies per reaction. The *12S* segment was chosen based on adequate interspecific sequence divergences and intraspecific sequence similarity using reference sequences in Geneious 10.2.6 software. The *P. pristis* 12S assay uses forward primer 5'-GTGCCTCAGACCCACCTAGA-3', reverse primer 5'-CATCATACTGTTCGTTTTTTCTTAGGAG-3', and probe 5'-VIC- AAATGAACTAACCTTCAATACG-MGBNFQ-3'.

qPCR assays were set-up using the EzMate™ 401 Automated Pipetting System (Arise Biotech) and run on a QuantStudio™ 5 Real-Time PCR System (Thermo Fisher Scientific Australia Pty Ltd) in white 384-well plates sealed with optical films (Thermo Fisher Scientific Australia Pty Ltd). Eight technical replicates of each sample were used per species, representing 48% of the total available DNA elution volume (Fig. 2.1). Additionally, three no template control (NTC) samples, consisting of laboratory-grade water were included in each plate. Each qPCR assay consisted of 6 µL of template DNA and 14 µL of master mix (10 µL of Taqman™ Environmental Master Mix 2.0; 0.06 µL forward primer at 100 µM; 0.06 µL reverse primer at 100 µM; 0.05 µL Taqman™ probe at 100 uM; 1 µL bovine serum albumin [BSA]; and 2.83 µL laboratory-grade water). Thermal cycling conditions were as follows: initial denaturation at 95°C for 10 min, followed by 55 cycles of 95°C for 15 sec and 60°C for 1 min.

The qPCR plate was analysed with a common fluorescence threshold (0.2) using QuantStudio™ Design and Analysis Software (version 1.4.2; Thermo Fisher Scientific Australia Pty Ltd) before export and subsequent analyses in Microsoft Excel. Samples for which the amplification curve crossed the common fluorescence threshold within 45 cycles were sequenced at Australian Genome Research Facility (AGRF) to determine if they were true detections.

A field site was considered to be positive for target detection if at least one of the total technical qPCR replicates for that site met the following criteria: 1) Sanger sequence data showed >98% pairwise identity with the target species genetic sequences from GenBank, and 2) corresponding field blank and extraction blank were not contaminated.

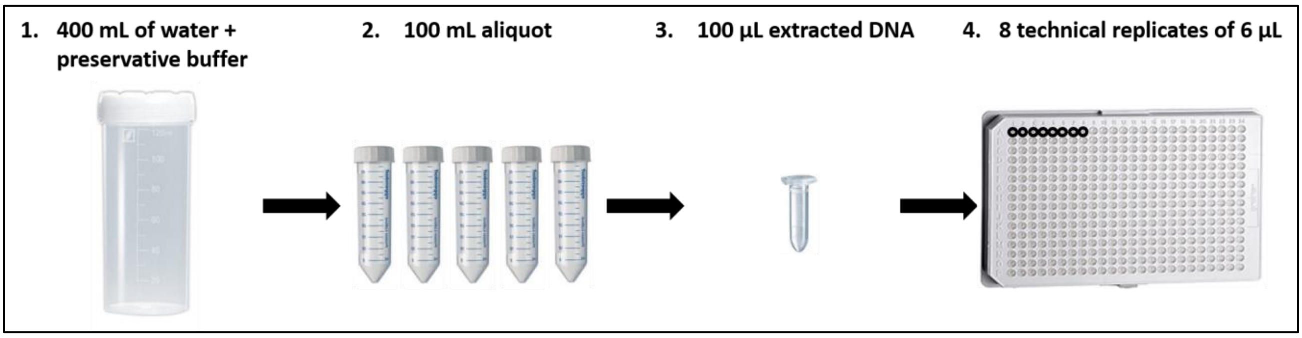

Figure 2.1 Field sample consisting of 400 mL of water and preservative solution, out of which 100 mL were concentrated into 100 µL of DNA eluted in laboratory-grade water and 48 µL loaded into a qPCR plate for each of the target species detection

3 RESULTS

3.1 Screening of field samples for target species eDNA

Based on the assessment criteria to consider a positive detection of both target species, there was no presence of *P. pristis* eDNA at any of the surveyed sites (Table 3.1). All field and extraction control samples were verified to be devoid of the target species eDNA by qPCR.

Table 3.1 Summary of *P. pristis* eDNA analysis from ten field sites at the Flinders River area

Location	Site name	Field samples			qPCR analysis		
		# field samples	# positive field samples	% positive detections	# technical replicates	# positive technical replicates	% positive detections
Flinders River	Glendower	5	0	0	40	0	0
	Galah crossing	5	0	0	40	0	0
	Mine Road offstream waterhole	5	0	0	40	0	0
	Poseidon waterhole	5	0	0	40	0	0
	Pivot irrigation offstream waterhole	5	0	0	40	0	0
Porcupine Creek	Porcupine Gorge downstream	5	0	0	40	0	0
Fairlight waterhole - Fairlight Creek	Fairlight Creek at Flinders River junction	5	0	0	40	0	0
Marathon Station	Flinders offstream waterhole	5	0	0	40	0	0
Canterbury Road	Offstream waterhole	5	0	0	40	0	0
Stewart Creek	Expressman Downs	5	0	0	40	0	0

4 DISCUSSION

The eDNA technique is increasingly being recognised as a sensitive tool for detection of cryptic species (Dejean et al. 2012). During June 2021, Australasian Fish Passage Services staff collected water samples for eDNA analysis across ten sites in the Flinders River area for *P. pristis* eDNA detection.

In the present field survey, *P. pristis* eDNA was not detected from any of the field sites. Because eDNA analysis often involves detection of species that occur at low abundance, determining an appropriate sampling effort is crucial in order to avoid false negative detections and obtain reliable eDNA results (Furlan et al. 2019). Researchers generally suggest filtering large volumes of water (> 1 L) to detect species that occur at low abundance (Sepulveda et al. 2019). However, field studies consistently report that water filtration in the presence of high suspended sediment load is a tedious, time-intensive task (Raemy and Ursenbacher 2018; Wilson et al. 2018). In a new study, Cooper et al. (*in review*) determined that precipitating 100 mL of water was more efficient at recovering the largetooth sawfish eDNA than filtering 1 L of water through a 0.45 µm filter pore size. Filtering 1 L of water with a high-suspended sediment load though a 0.45 µm filter would be a lengthy process and it reality, field staff would be able to filter a much smaller volume. In which case, water precipitation would outperform filtration.

In the present study, 100 mL of water from five replicate samples at each site was processed (total of 500 mL at each site). TropWATER has previously determined that precipitating eDNA from 100 mL replicates can provide high resolution for species detection in a running stream in tropical Australia (Villacorta-Rath et al. in press). By using two rainforest frogs as model species, five replicate 100 mL water samples had enough power to detect the target species eDNA 22 km downstream from the lower limit of the population's distribution. Additionally, in a recent eDNA survey of *E. irwini* eDNA in the lower Burdekin area carried out by TropWATER, the species eDNA was detected at sites where there has not been scientific evidence of its presence for several decades (unpublished data). The field and laboratory methods followed in that survey were the same as the ones followed in the present study (i.e. amount of water sampled from the source, volume of water processed for eDNA analysis and eDNA assay). Finally, it has been demonstrated that increasing qPCR replication, from three to six technical replicates (12 µL to 24 µL eDNA extract), can also improve detection (Feist et al. 2018). In order to maximize detection, in the present study, eight technical qPCR replicates were screened, each consisting of 6 µL of extracted eDNA (total of 48 µL eDNA extract).

In flowing streams, eDNA detectability not only depends on shedding rates and population abundance of the target organism, but also on physical processes such as eDNA transport, retention, resuspension and decay (Barnes, Turner, and Turner 2016). Recent studies increasingly show that eDNA transport distance and retention in a system are highly influenced by water discharge and physical-chemical characteristics of a stream (i.e. susbtrate type) (Shogren et al. 2017; 2019). Additionally, eDNA decay is mainly driven by the biotic (i.e. bacterial community) and abiotic factors (e.g., temperature, pH, UV) surrounding it (Nielsen et al. 2017). Studies on several taxa show that eDNA decays rapidly and can be reliably detected up to eight days after been shed (Villacorta-Rath et al. 2020; Eichmiller, Best, and Sorensen 2016; Sassoubre et al. 2016). Therefore, any positive eDNA detection found in the present study means that the target species has likely occupied the area up to one week prior to sample collection.

Therefore, the amount of water processed for the present study, the number of replicate samples and qPCR technical replicates screened at each site, the number of sites surveyed and the distance between them provide strong resolution to detect the target species eDNA if present at the sampling area. Based on this, we can conclude that there has not been *P. pristis* presence at the sampling sites for up to one week prior to the sampling event.

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9 Attachment 2 - Fish Sampling Codes

Common Name	Scientific Name	Code
Ambassid species	*Ambassis sp.*	Amb sp.
Archerfish	*Toxotes chatareus*	Tox cha
Banded grunter	*Amniataba percoides*	Amn per
Black banded gudgeon	*Oxyeleotris selheimi*	Oxy sel
Black catfish	*Neosilurus ater*	Neo ate
Bony bream	*Nematalosa erebi*	Nem ere
Eastern rainbowfish	*Melanotaenia splendida*	Mel spl
Giant glassfish	*Parambassis gulliveri*	Par gul
Golden flathead goby	*Glossogobius aureus*	Gob aur
Gulf grunter	*Scortum ogilbyi*	Sco ogl
Hyrtyl's tandan	*Neosilurus hyrtli*	Neo hyr
Rendahl's catfish	*Porochilus rendahli*	Por ren
Saltpan sole	*Brachirus salinarum*	Bra sal
Silver catfish	*Porochilus argenteus*	Por arg
Silver cobbler	*Neoarius midgleyi*	Ari mid
Sleepy cod	*Oxyeleotris lineolate*	Oxy lin
Sooty grunter	*Hephaestus fulignosus*	Hep ful
Northern purple spotted gudgeon	*Mogurnda mogurnda*	Mog ads
Spangled perch	*Leiopotherapon unicolor*	Lei uni
Square blotch goby	*Glossogobius munroi*	Gob mun
Tank goby	*Glossogobius giuris*	Glo giu