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Determining the distribution of platypuses & short-finned eels in the Moorabool River subcatchment

Prepared for:
Jackson Cass

Moorabool Catchment Landcare Group
PO Box 239
Ballan, VIC 3342
Australia

Prepared by:
Josh Griffiths, Sarah Licul and Dr Andrew Weeks

EnviroDNA
293 Royal Parade
Parkville, VIC 3052
Australia

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Project team

Title	Name
Project Manager	Josh Griffiths
Project Consultant	Sarah Licul
Project Supervisor	Dr. Andrew Weeks

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27/06/2021	1.0	Final Report_Draft	JG	JC
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Abbreviations

Abbreviations	Description
eDNA	environmental DNA
MCLG	Moorabool Catchment Landcare Group
CCMA	Corangamite Catchment Management Authority
ISC	Index of Stream Condition 2010
qPCR	quantitative Polymerase Chain Reaction

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Background

The platypus (*Ornithorhynchus anatinus*) is a semi-aquatic mammal that inhabits a variety of freshwater habitats throughout eastern Australia (Grant 1992). The species was recently listed as “Near Threatened” by the IUCN (Woinarski *et al.* 2014; Woinarski and Burbidge 2016) and “Vulnerable” in Victoria in recognition of mounting evidence of population declines and localised extinctions throughout its range (Bino *et al.* 2020). Difficulties in assessing platypus populations and lack of historical data have hampered studies to quantify the impacts of various threats. However, the platypus populations are vulnerable to a number of potential threats including drought, altered flow regimes, changes to surrounding catchment area due to agriculture or urbanisation, removal of riparian vegetation, habitat fragmentation, poor water quality, and predation from invasive predators (Grant and Temple-Smith 1998, 2003).

The Moorabool Catchment has been significantly modified due to widespread land clearing for agriculture, presence of invasive species (e.g. willows), poor water quality, and reduced river flows due to water extraction and diversion as well as climate change. The Moorabool River is estimated to receive only 10% of its natural flow (ref) and was rated as Moderate to Very Poor by the last Index of Stream Condition assessment in 2010 (Department of Environment and Primary Industries 2010). All of these factors can be expected to have impacts on platypus populations (and other aquatic dependent species), however little contemporary information exists on the current distribution or abundance of platypuses in the area. Platypus populations in other similar areas in Victoria (i.e. agricultural areas of the Wimmera, Corangamite and Campaspe Catchments) are considered under serious stress although quantifying declines is limited by lack of rigorous historical data (Serena *et al.* 2002; Griffiths *et al.* 2018; Griffiths and Weeks 2018; Griffiths *et al.* 2019; Griffiths and Licul 2020)

This project aimed to address the lack of data by using environmental DNA to investigate the current distribution of platypuses throughout the Moorabool Catchment while engaging the local community in conservation issues. Available data is limited to anecdotal reports of platypus sightings and no systematic surveys have previously been undertaken. Environmental DNA (eDNA) is a non-invasive sampling technique that detects genetic material from a target species secreted into its surrounding environment (water). Quantitative comparisons with traditional sampling methods already indicate that eDNA methods are superior in terms of sensitivity and cost efficiency, particularly for scarce, elusive or cryptic species (Biggs *et al.* 2015; Smart *et al.* 2015), including platypuses (Lugg *et al.* 2018; Weeks *et al.* 2015), enabling effective detection at low densities. As part of the project, we also used the eDNA samples to investigate the distribution of short-finned eels (*Anguilla australis*). The project aimed to identify declines where possible, provide a comprehensive baseline for future monitoring and direct management actions to improve the long term viability of platypus populations.

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Methods

Investigating platypus distribution.

Eighteen survey sites were selected in collaboration with Moorabool Catchment Landcare Group (MCLG) to include a variety of waterways and habitat and provide good spatial coverage throughout the region (Figure 1, Appendix 1). Sampling sites were selected with consideration of known previous distribution of platypuses, recent sightings, and accessibility of sites along the waterways. Historical data on platypus distribution was collated from online databases (www.ala.org.au, www.vba.vic.gov.au, www.platypusSPOT.org).

The current occurrence of platypuses and short-fin eel at each site was determined using environmental DNA techniques. Water sampling was undertaken during May 2021 to target the juvenile dispersal period for platypuses in Victoria (Grant 2007) and ensure adequate surface water availability. Water samples were collected by volunteers from the MCLG and local residents following detailed instructions and demonstration of correct sampling techniques by EnviroDNA. At each site, water samples were collected in duplicate by passing up to 400 ml water (average 200 ml) through a 0.22 µm filter (Sterivex). Filtration was undertaken on site to reduce DNA degradation during transport of whole water samples (Yamanaka et al. 2016). Clean sampling protocols were employed to minimise contamination including new sampling equipment at each site, not entering water, and taking care not to transfer soil, water or vegetation between sites. Filters were stored on ice for a maximum of 48 hrs before being transported to the laboratory for processing.

DNA was extracted from the filters using a commercially available DNA extraction kit (Qiagen DNeasy Blood and Tissue Kit). Real-time quantitative Polymerase Chain Reaction (qPCR) assays were used to amplify the target DNA, using species-specific markers targeting a small region of the mitochondrial DNA, previously developed and assessed for specificity and sensitivity by EnviroDNA (e.g. Lugg *et al.* 2018; Weeks *et al.* 2015). Assays were performed in triplicate on each sample. Negative controls were included for the DNA extraction and qPCR steps. At least two positive PCR's (out of six or nine assays undertaken for the site) were required to classify the site as positive for the presence of platypus.

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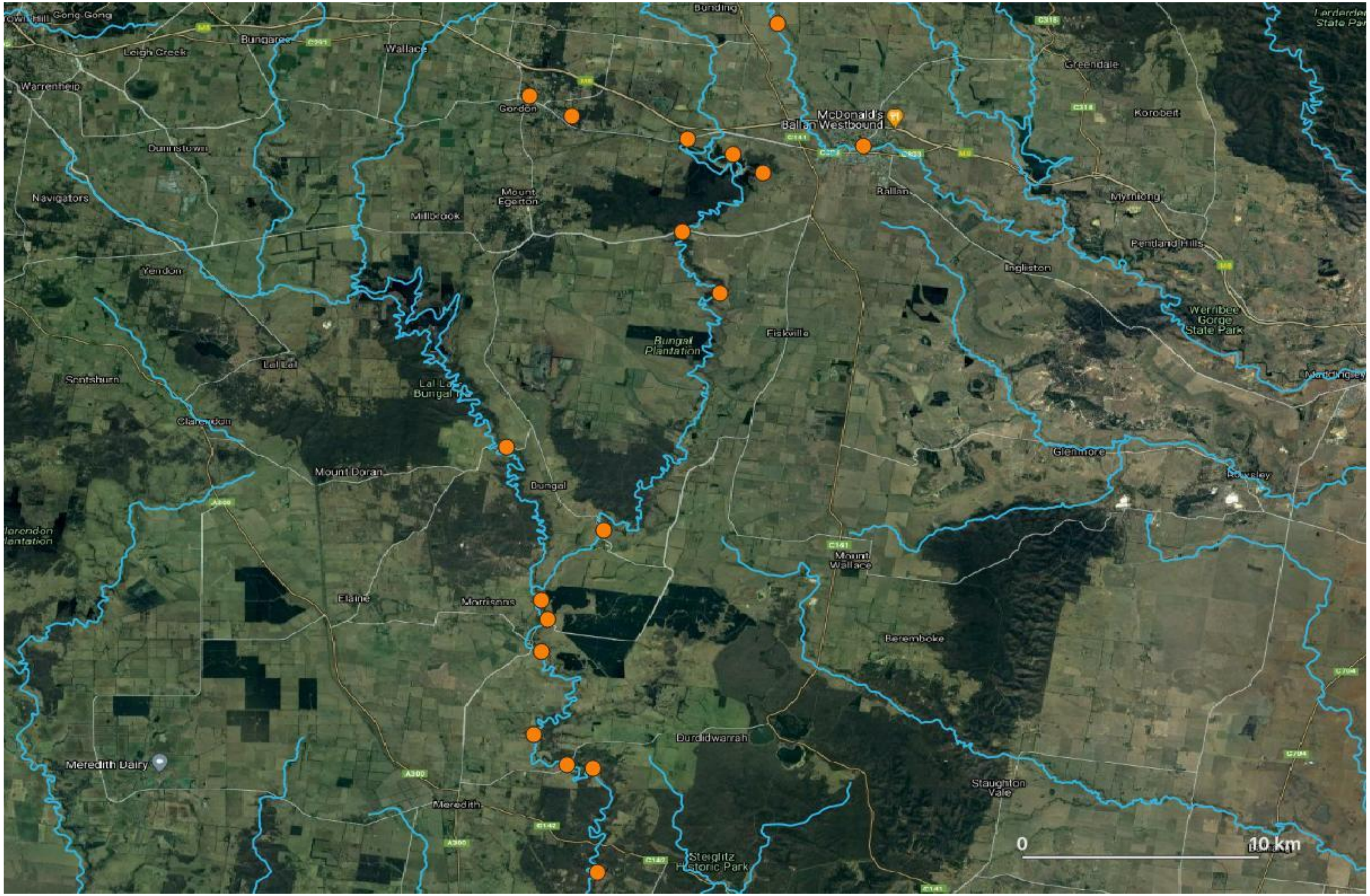

Figure 1. Location of sampling sites for eDNA analysis.

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Assessing condition of waterways.

River health was assessed at each site by volunteers based on an established methodology previously used by Victorian CMA's and WaterWatch that incorporates in-stream and riparian variables (River Detectives www.riverdetectives.net.au). An additional platypus-specific variable was added to rate availability of burrowing habitat. The river health assessment was explained and demonstrated to the volunteer group during the training session and habitat variables related to platypus requirements to provide context. The six habitat variables are evaluated and assigned to five categories from Very Poor to Excellent. These are then tallied to provide an overall river health rating for the site.

A measure of river health at the reach level was also derived from 2010 Index of Stream Condition (ISC) scores (Department of Environment and Primary Industries 2010) to support volunteer assessments for the waterway reaches where ISC assessments had taken place. ISC provides an overall measure of river health (very poor, poor, moderate, good, excellent) based on five key metrics: hydrology, streamside zone, physical form, water quality, and aquatic life.

Findings

Current distribution of platypuses.

Six of the 18 sites sampled returned positive results for platypus DNA (33%; Figure 2, Appendix 1). Trace amounts of DNA was detected at four other sites but was not above the defined threshold level (at least 2 positive PCR's) to be considered positive (indicated as equivocal in Appendix 1). While equivocal results may indicate the species presence at very low abundance, it can also arise from field contamination during sampling or dispersal of DNA from further upstream. Repeat sampling is recommended to confirm presence or absence at these sites. Detections of platypus DNA was clustered in sites around and downstream of the junction of the east and west branches of the Moorabool River although their distribution may also extend up the east branch in low abundance (Figure 2).

Current distribution of shortfin eels.

Four of the 18 sites sampled returned positive results for shortfin eel DNA (22%, Figure 3, Appendix 1). Trace amounts of DNA was detected at four other sites but was not above the defined threshold level (at least 2 positive PCR's) to be considered positive (indicated as equivocal in Appendix 1). Positive detections of shortfin eel DNA were recorded in the upper reaches of both east and west branches of the Moorabool River with equivocal results indicating their distribution may extend further downstream at low abundance (Figure 3).

Condition of waterways

River health assessments were completed at 15 sites. The sites were assessed as Degraded (1), Poor (2), Fair (3), Good (7), or Excellent (2) (Appendix 2). Fifteen sampling sites were located in reaches that also had corresponding ISC 2010 scores that were rated as poor (3) or fair (12)(Appendix 1). Detections of platypus eDNA tended to occur at sites with better health ratings by both volunteers or ISC although sample sizes were too small for analysis.

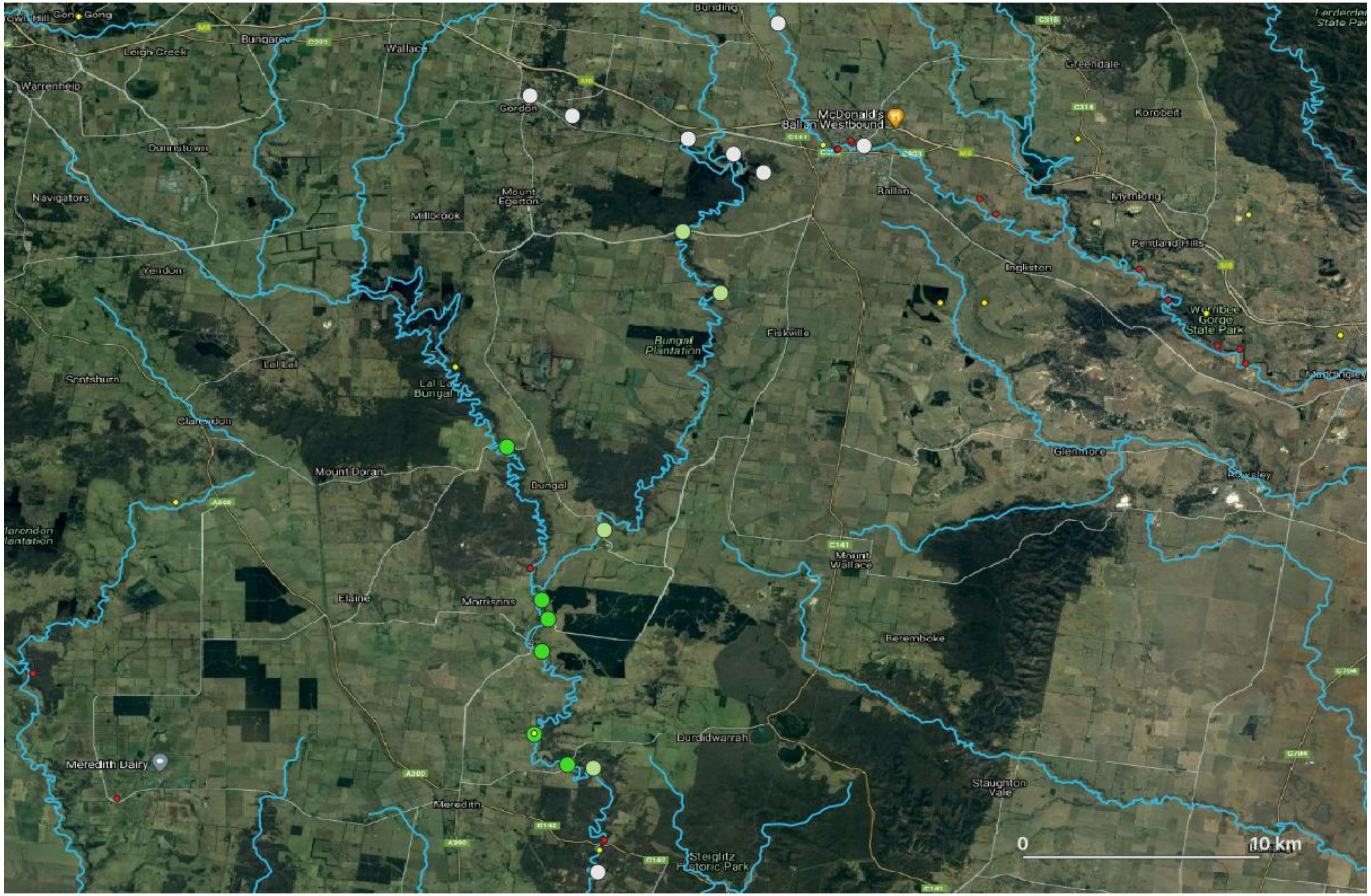

Figure 2. Results from the eDNA sampling indicating positive detection (green), equivocal (pale green) or non-detection (grey) of platypus DNA and historical platypus records from online databases (red <10yrs, yellow >10yrs).

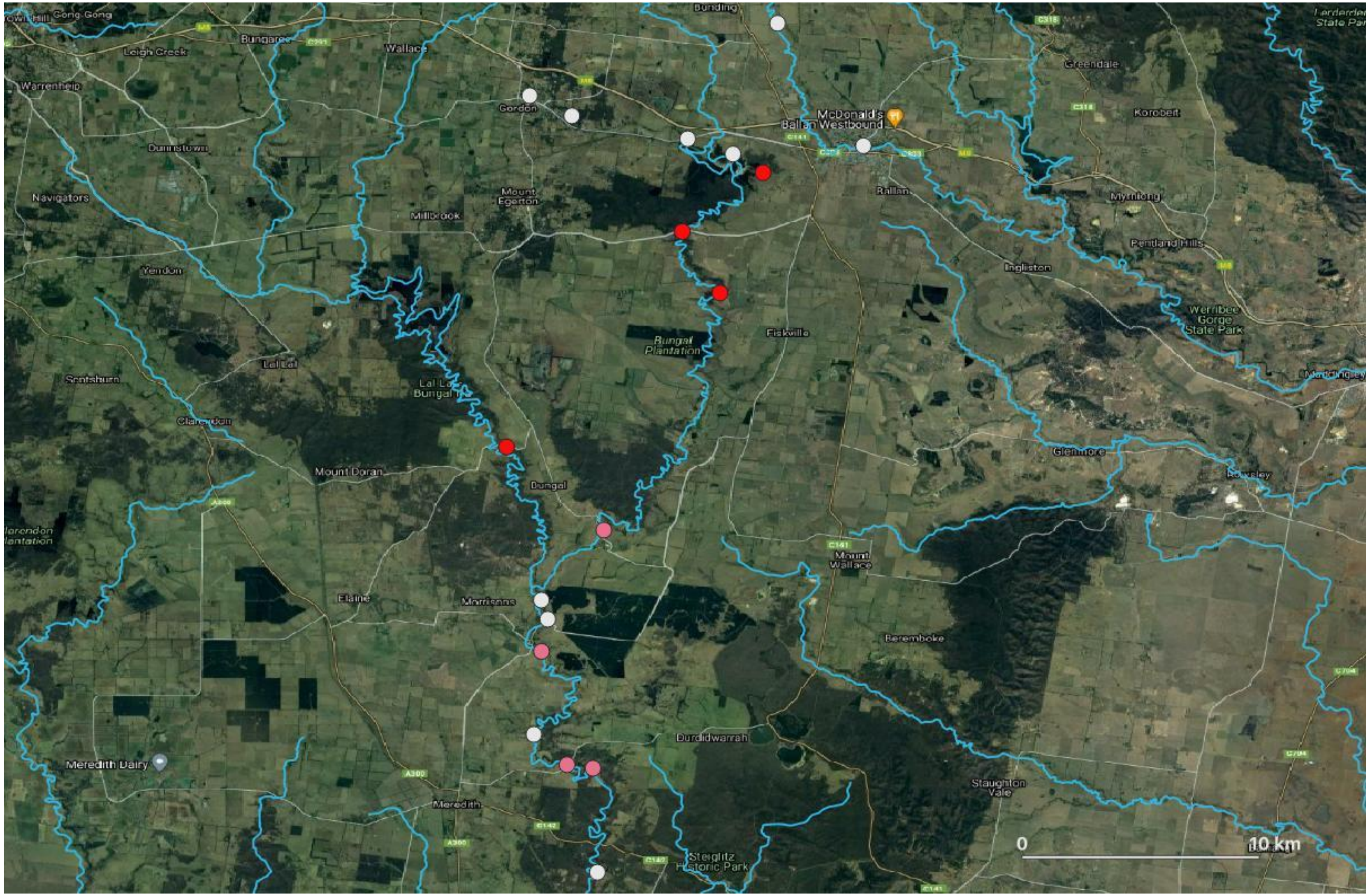

Figure 3. Results from the eDNA sampling indicating positive detection (red), equivocal (pink) or non-detection (grey) of shortfin eel DNA.

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Discussion and recommendations

There is very limited recent or historical data on platypuses in the Moorabool Catchment with just 16 records scattered throughout the study area between 1994 to 2019. Records from online wildlife databases (Atlas of Living Australia, Victorian Biodiversity Atlas, platypusSPOT) mostly comprise anecdotal sightings from residents or visitors and typically are concentrated near population centres or tourist locations. Despite these limitations, the data indicate platypuses were likely to have been widespread throughout the Moorabool River and its perennial tributaries as well as the upper Werribee River as far as reliable water is available. Results from the current study broadly correspond with these previous records apart from the upper Werribee River where no platypus eDNA was detected from two sampling sites. However, positive eDNA results have been previously recorded at Ballan (2019, EnviroDNA unpublished data) as well as recent sightings and low captures from live-trapping surveys (Griffiths *et al.* 2012; Griffiths, Kelly, van Rooyen, *et al.* 2014) all suggesting platypuses occur in low abundance in the upper Werribee River.

In the absence of any previous systematic data, it is impossible to infer a population trajectory. The current results indicate platypuses are widely distributed throughout the Moorabool River including the east and west branches although there was no evidence of platypuses upstream of Bostock Reservoir. Poor habitat quality in Paddock Creek and the barrier posed by the reservoir may prevent platypus dispersal into this area. Positive eDNA results have also been recorded downstream of the current study area (between Meredith and Bannockburn, EnviroDNA unpublished data) indicating platypuses likely occupy the lower Moorabool River downstream to the junction with the Barwon River near Geelong.

Although widely distributed, the low site occupancy (33% although it could be as high as 56% if equivocal results are true positives) indicating platypuses are likely to be in relatively low abundance throughout the area. Similar results have been recorded in the adjacent upper Barwon region (23-31%; Griffiths *et al.* 2019). This likely reflects poor habitat quality with ISC ratings for the river reaches considered poor to fair. River health assessments at the sampling sites were generally higher than the ISC ratings (Appendix 2). Differences in timing, scale, and metrics between the two methods likely account for some of the discrepancies. The last ISC assessment was undertaken in 2010 at the end of an extensive period of drought and conditions may have changed since then and the current study in 2021. The ISC assessed condition at the reach scale which is usually several kilometres (or tens of kilometres) long while volunteers in this study assessed the visible area at each site (approximately 50 m). It is possible for small patches of relatively good habitat to exist along poor reaches (or vice versa). The ISC also incorporates several metrics such as hydrology and aquatic life (invertebrates) that are unable to be easily assessed by untrained volunteers in the field.

Results from the current study as well as previous eDNA testing indicate the middle reaches of the Moorabool River is the stronghold for platypuses in the region. Not surprisingly, this also corresponds with somewhat better habitat quality as rated by both ISC and our volunteers. Habitat variables known to be important for platypuses include large riparian trees, overhanging vegetation, pools 1-3 m deep, near vertical, undercut and stabilised banks at least 0.5 m above the water, large woody debris, and coarse benthic substrates (Bethge *et al.* 2003; Ellem *et al.* 1998; Grant 2004; M. Serena *et al.* 1998; Serena *et al.*

2001; Worley and Serena 2000; M Serena *et al.* 1998). Critically, platypuses require aquatic habitats that support adequate and reliable resources of macroinvertebrate prey and many of the habitat variables above provide shelter and food resources for aquatic macroinvertebrates. Waterways with reduced baseflows, and/or high flow variability, or poor water quality such as high turbidity (i.e. sedimentation arising from degraded riparian zones and subsequent erosion) or low dissolved oxygen can result in depauperate macroinvertebrate assemblages (Chessman 2009; Marchant and Grant 2015; Boulton and Brock 1999; Walsh and Webb 2013).

Like platypuses, shortfin eels also have fairly generic habitat requirements, although eels tend to prefer slower flowing or still waters. Critically for eels as a diadromous species, they require hydrologic connections to the marine environment for spawning migrations. The hydrologic score of the ISC in the lower Moorabool River was very poor. Poor flow regimes, including cease to flow events could limit dispersal of eels to the ocean as well as limit habitat suitability for platypuses and connection with the lower Barwon population. While flows may have improved in the last 10 years, ensuring adequate baseflows throughout the Moorabool system is critical to maintaining river health and habitat quality for a variety of aquatic species.

It is important to note that results from the current study were obtained from a single sampling event and represent a snapshot of platypus and shortfin eel distribution at the time of sampling only. Previous studies have demonstrated that eDNA has high sensitivity to detect platypuses, even at low densities (Lugg *et al.* 2018; Weeks *et al.* 2015), but negative site results may still arise in waterways where platypuses are known to occur if no platypuses have been active near the sampling site. In freshwater systems, eDNA generally degrades or disperses relatively quickly (i.e. within days) (Thomsen *et al.* 2012; Pilliod *et al.* 2014). In addition, platypuses are highly mobile with typical home ranges of several kilometers (Gardner and Serena 1995; Serena and Williams 2012; Grant *et al.* 1992; M. Serena *et al.* 1998). Species behaviour, movements, and habitat use can also change in response to seasons and environmental conditions (Gust and Handasyde 1995; Griffiths and Weeks 2015; Griffiths, Kelly and Weeks 2014). Therefore, some temporal variation in localised occurrence of platypuses and eels is expected although broad distribution should remain similar over short time periods. Therefore, these results provide a good indication of the target species' distribution throughout the area. Importantly, there is now baseline data against which to assess future changes in platypus or eel populations in response to natural disturbances or management actions.

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Appendix 1. Sampling site details, eDNA results, river health assessments and ISC scores.

				Platypus		Shortfin eel			
Site	**Waterway**	**Latitude**	**Longitude**	**+ve assays**	**Site result**	**+ve assays**	**Site result**	**Site condition rating**	**ISC reach score**
1	Werribee River	-37.551041	144.198281	0	Negative	0	Negative	Fair	Poor
2	Werribee River	-37.597396	144.230777	0	Negative	0	Negative	Fair	Poor
3	Moorabool River East Branch	-37.629852	144.162233	1	Equivocal	4	**Positive**	Fair	Fair
4	Moorabool River East Branch	-37.65309	144.1765	1	Equivocal	2	**Positive**		Fair
5	Moorabool River East Branch	-37.742801	144.132492	1	Equivocal	1	Equivocal	Excellent	Poor
6	Moorabool River West Branch	-37.60757	144.19278	0	Negative	2	**Positive**		Fair
7	Moorabool River West Branch	-37.711349	144.09568	6	**Positive**	4	**Positive**	Good	Fair
8	Moorabool River	-37.769292	144.108861	4	**Positive**	0	Negative	Good	Fair
9	Moorabool River	-37.776527	144.111268	4	**Positive**	0	Negative	Good	Fair
10	Moorabool River	-37.78871	144.108906	4	**Positive**	1	Equivocal	Good	Fair
11	Moorabool River	-37.820109	144.106021	3	**Positive**	0	Negative	Good	Fair
12	Moorabool River	-37.831556	144.118596	3	**Positive**	1	Equivocal		Fair
13	Moorabool River	-37.832895	144.128392	1	Equivocal	1	Equivocal	Good	Fair
14	Moorabool River	-37.872274	144.130089	0	Negative	0	Negative	Excellent	Fair
15	Paddock Creek	-37.578436	144.104433	0	Negative	0	Negative	Degraded	
16	Paddock Creek	-37.58606	144.12042	0	Negative	0	Negative	Good	
17	Paddock Creek	-37.594752	144.164255	0	Negative	0	Negative	Poor	
18	Bostock Reservoir	-37.600503	144.181392	0	Negative	0	Negative	Poor	Fair

Appendix 2. Site habitat assessments by volunteers.

Site	Waterway	Latitude	Longitude	Bank erosion	Bank vegetation	Burrowing habitat	Instream complexity	Channel complexity	Verge vegetation	Site condition rating
1	Werribee River	-37.551041	144.198281	Fair	Fair	Good	Fair	Excellent	Fair	Fair
2	Werribee River	-37.597396	144.230777	Good	Good	Fair	Good	Fair	Poor	Fair
3	Moorabool River East Branch	-37.629852	144.162233	Good	Fair	Fair	Poor	Fair	Poor	Fair
4	Moorabool River East Branch	-37.65309	144.1765							
5	Moorabool River East Branch	-37.742801	144.132492	Good	Good	Good	Excellent	Excellent	Good	Excellent
6	Moorabool River West Branch	-37.60757	144.19278							
7	Moorabool River West Branch	-37.711349	144.09568	Good	Fair	Fair	Excellent	Excellent	Excellent	Good
8	Moorabool River	-37.769292	144.108861	Fair	Fair	Fair	Good	Excellent	Poor	Good
9	Moorabool River	-37.776527	144.111268	Excellent	Fair	Fair	Excellent	Good	Fair	Good
10	Moorabool River	-37.78871	144.10891	Good	Good	Good	Fair	Good	Fair	Good
11	Moorabool River	-37.820109	144.106021	Excellent	Fair	Excellent	Fair	Fair	Good	Good
12	Moorabool River	-37.831556	144.118596							
13	Moorabool River	-37.832895	144.12839	Good	Good	Fair	Excellent	Excellent	Fair	Good

			2							
14	Moorabool River	-37.872274	144.13008 9	Excellent	Good	Excellent	Excellent	Excellent	Fair	Excellent
15	Paddock Creek	-37.578436	144.10443 3	Degraded	Degraded	Poor	Degraded	Fair	Fair	Degraded
16	Paddock Creek	-37.58606	144.12042	Excellent	Good	Fair	Fair	Fair	Excellent	Good
17	Paddock Creek	-37.594752	144.16425 5	Degraded	Poor	Poor	Degraded	Poor	Poor	Poor
18	Bostock Reservoir	-37.60050	144.18139 2	Fair	Degraded	Poor	Poor	Poor	Fair	Poor

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