CONFIDENTIAL REPORT

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Assessing the impacts of riparian restoration along Birchs Creek on platypus and blackfish populations.

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14/12/2021	1.0	Final Report_Draft	JG	NCCMA
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Abbreviations

Abbreviations	Description
eDNA	environmental DNA
NCCMA	North Central Catchment Management Authority
qPCR	quantitative Polymerase Chain Reaction
ISC	Index of Stream Condition 2010
IUCN	International Union for the Conservation of Nature

Contents

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Background

Waterways and catchments of the North Central CMA (NCCMA) region are highly modified by land clearing, invasive species (i.e. *Sallix* sp.), stock access, reduced water quality, and altered flow regimes (e.g. water diversion and extraction, reduced run-off). Most recently, aquatic ecosystems were severely impacted by the Millennium Drought. Birchs Creek in the upper Loddon catchment originates on the northern slopes of the Great Dividing Range and flows northwest through agricultural lands before joining Creswick Creek near Clunes to form Tullaroop Creek. Natural flows in the creek have been significantly altered by the construction of Newlyn Reservoir and Hepburn Lagoon, numerous farm dams in the surrounding catchment, and extraction of water for irrigation (North Central CMA 2015). Birchs Creek is considered a priority waterway by NCCMA due to its role in irrigation for surrounding landowners as well as the natural values it supports, including platypus and river blackfish.

The platypus (*Ornithorhynchus anatinus*) was recently listed as "Vulnerable" in Victoria and "Near Threatened" by the IUCN (Woinarski *et al.* 2014; Woinarski and Burbidge 2016) in recognition of mounting evidence of population declines and localised extinctions throughout its range (Bino *et al.* 2020). As a semi-aquatic species, the platypus is potentially vulnerable to a range of natural and anthropogenic threats that degrade aquatic ecosystems 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). However, difficulties in assessing platypus populations and lack of systematic historical data have hampered attempts to quantify the impacts of various threats as well as the effectiveness of management actions.

The river blackfish (*Gadopsis marmoratus*) is considered widespread throughout southeastern Australia and is not currently listed on Victorian or national threatened species lists. However, extensive declines are suspected due to overfishing, in stream sedimentation, altered flow regimes, and predation and competition from introduced species, particularly trout (Allen *et al.* 2002; Bray 2017). There were serious concerns over the status of river blackfish in Birchs Creek after extended drought conditions followed by severe flooding with the species not detected during traditional surveys (McGuckin 2015).

In 2015, the NCCMA committed to a long-term restoration project to improve conditions in Birchs Creek. The waterway was considered to be in poor condition by the last Index of Stream Condition assessment in 2010 (Department of Environment and Primary Industries 2010). Restoration actions included woody weed removal (primarily willows), native revegetation, and exclusion of stock. At the start of the works, surveys for platypuses and river blackfish were undertaken using environmental DNA techniques to provide a baseline for assessing the effectiveness of the riparian restoration program (Griffiths *et al.* 2016). 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. Comprehensive surveys in

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December 2015 and May 2016 revealed platypuses were widespread throughout the system. River blackfish were also confirmed to still persist in Birchs Creek although their distribution was more restricted and patchy.

This project aimed to re-assess populations of platypuses and river blackfish, five years after the baseline surveys to see if the riparian restoration works had resulted in increases in distribution and/or site occupancy for each species.

Methods

Survey sites attempted to remain the same as the 28 sites surveyed in 2015/16 (Griffiths *et al.* 2016) to maintain consistency and provide comprehensive coverage along Birchs Creek downstream of Newland Reservoir. However, several sites could not be accessed during the current surveys due to safety concerns or an inability to gain landowner's permission. In total, 27 sites were sampled including 25 from previous surveys and two exploratory sites upstream of Newland Reservoir (Figures 1 & 2, Appendix 1). For comparison with previous surveys, only results from sites downstream of Newland Reservoir were considered.

Water sampling was undertaken on 16-17th September 2021 by EnviroDNA as well as NCCMA and Dja Dja Wurrung Clans Aboriginal Corporation staff following a workshop to demonstrate correct water sampling techniques. At each site, water samples were collected in duplicate by passing up to 600 ml water (average 321 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 out of sunlight and 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 of the target species. Assays were performed in triplicate on each sample. Positive and negative controls were included for all assays as well as an Internal Positive Control (IPC) to detect inhibition (Goldberg *et al.* 2016). At least two positive PCR's (out of six assays undertaken for each site) were required to classify the site as positive for the presence of the target species. To minimise false positives, sites were considered equivocal if only one of six assays returned a positive result, indicating very low levels of target DNA. While trace amounts of DNA may indicate the target species is actually present in low abundance, it may also arise from sample contamination through the sampling or laboratory screening process (minimised through strict protocols and negative controls), facilitated movement of DNA between waterbodies (i.e. water birds, recreational anglers, water transfers, predator scats), or dispersal from further upstream. If greater confidence is required, further sampling is recommended at equivocal sites to confirm the presence or absence of the target species. Repeat sampling is also recommended to help determine the tenure of the species at a site (i.e. resident or transient).

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Findings & Discussion

Current distribution of platypuses.

Platypus eDNA was positively detected at 23 of the 25 sites sampled downstream of Newlands Reservoir (92%; Figure 1, Appendix 1). Trace amounts of DNA was detected at one other site but was not above the defined threshold level (at least two positive PCR's) to be considered positive (indicated as equivocal in Appendix 1). Platypus eDNA was also detected in one of the exploratory sites upstream of the Reservoir. The results confirm that platypuses are currently distributed throughout Birchs Creek.

Current distribution of river blackfish.

Fourteen of the 25 sites sampled downstream of Newlands Reservoir returned positive results for river blackfish DNA (56%, Figure 2, Appendix 1). Trace amounts of DNA were detected at five other sites but was not above the defined threshold level (at least two positive PCR's) to be considered positive (indicated as equivocal in Appendix 1). No river blackfish DNA was detected in sites upstream of the Reservoir.

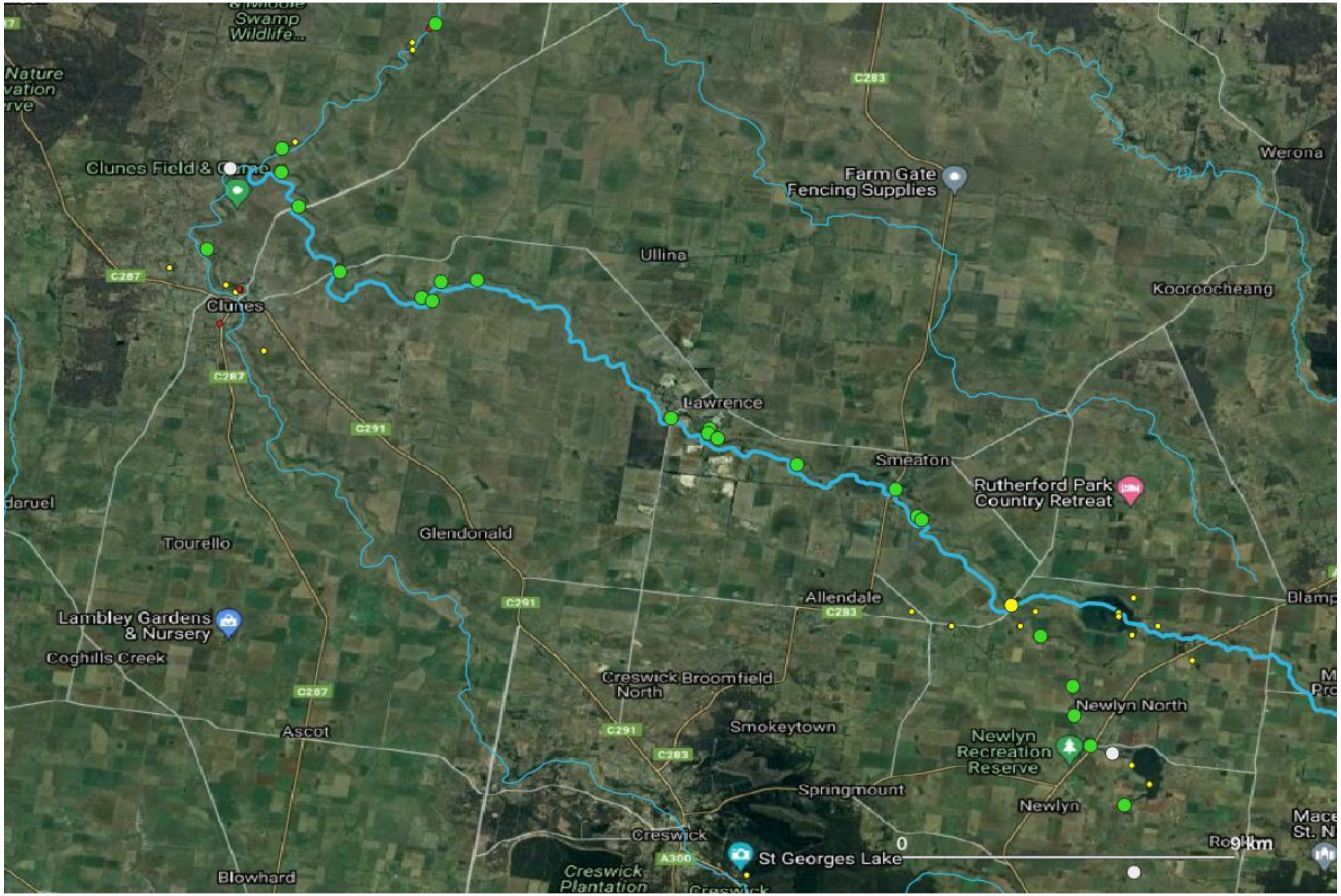

Figure 1. Results from the eDNA sampling indicating positive detection (green), equivocal (yellow) or non-detection (grey) of platypus DNA and historical platypus records from online databases (red <10yrs, yellow >10yrs).

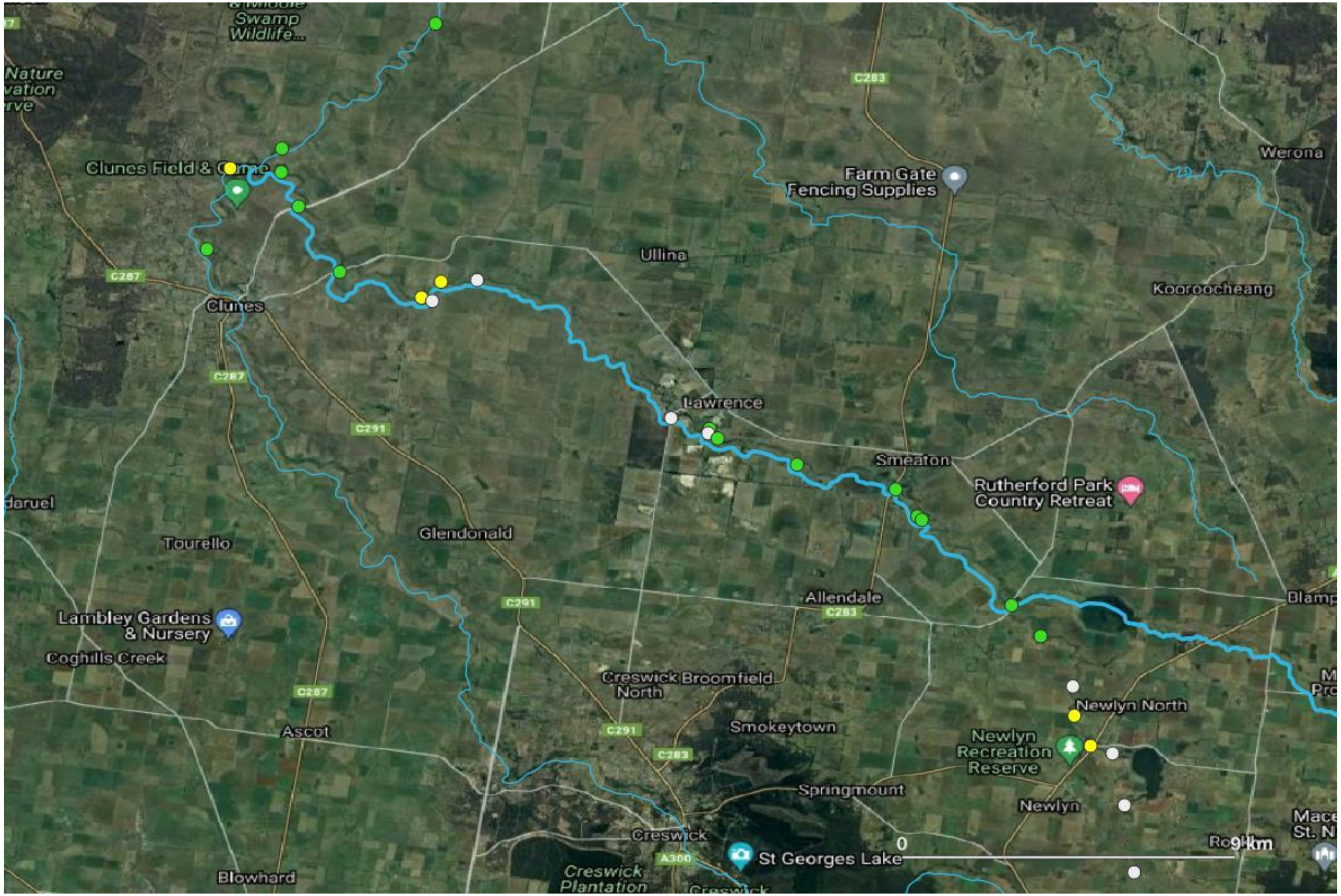

Figure 2. Results from the eDNA sampling indicating positive detection (green), equivocal (yellow) or non-detection (grey) of river blackfish DNA.

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Potential impacts of riparian restoration works.

When compared to the baseline surveys undertaken at the start of the restoration program (Griffiths *et al.* 2016), both species have increased in overall occupancy. Platypuses were found to be relatively widespread along Birchs Creek in 2015/16 but now occupy almost all sites surveyed, including sites in the adjacent Creswick and Tullaroop Creeks where they were not previously detected. Total site occupancy of platypuses has increased from 69% in December 2015 and 59% in May 2016 to 92% in the current surveys. Similarly, river blackfish were found to have a restricted and patchy distribution in 2015/16, with positive sites largely constrained to the lower and upper reaches. Total site occupancy of river blackfish has increased from 42% in December 2015 and 37% in May 2016 to 56% in the current surveys. Most notably, river blackfish have expanded into the middle reaches of Birchs Creek which is a critical step in reconnecting previously isolated subpopulations in the lower and upper reaches. Although eDNA surveys can't directly reveal whether recruitment is occurring, the expansion in distribution and overall site occupancy indicates the population is expanding, either due to local recruitment or immigration from the surrounding areas.

When comparing site occupancy between survey periods, it's important to note there is likely to be some variation due to seasons and environmental conditions on results. Unlike the previous surveys, the current surveys were undertaken during the platypus breeding season where we expect increased movements as males search for females and defend territories which is likely to result in higher detection rates. Similar increases in sightings and captures have been recorded for observational and live-trapping surveys respectively (Griffiths *et al.* 2020; Easton *et al.* 2008). However, blackfish are expected to spawn in late spring or early summer as water temperatures rise (Bray 2017). Therefore, summer may be the optimal eDNA detection time for the species and may explain the slightly higher site occupancy recorded in December 2015 compared to May 2016 (Griffiths *et al.* 2016). Conversely, the current surveys were undertaken during very high flows following significant rainfall. Increased water levels and volumes are likely to reduce overall detectability as available DNA is diluted and dispersed.

Riparian vegetation is an important habitat feature for both species. Riparian vegetation stabilises banks to facilitate platypus burrow construction, provides critical habitat for macroinvertebrate prey for both species, and large woody debris is important substrate for river blackfish spawning. With small samples sizes and likely confounding factors, it is difficult to accurately quantify the impacts of the riparian restoration works along Birchs Creek on the local platypus and river blackfish populations. However, an overall increase in distribution and site occupancy for both species shows the population trajectory of both species is increasing.

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Appendix 1. Sampling site details and eDNA results.

Table A1.1 – Results for current platypus eDNA surveys as well as previous results from Griffiths *et al*. 2016.

Site ID	Waterway	Latitude	Longitude	Dec2015 result	May2016 result	Sept2021 result
CRE2	Creswick Ck	-37.28224	143.77921	Negative	Negative	**Positive**
CRE1	Creswick Ck	-37.26263	143.78486	Equivocal		Negative
TUL2	Tullaroop Ck	-37.25782	143.79741		Equivocal	**Positive**
TUL1	Tullaroop Ck	-37.2276	143.83462	Negative	Equivocal	**Positive**
BIR1	Birchs Ck	-37.26356	143.79725	**Positive**	**Positive**	**Positive**
BIR2	Birchs Ck	-37.27187	143.80143	**Positive**	**Positive**	**Positive**
BIR3	Birchs Ck	-37.28067	143.80554	**Positive**	**Positive**	
BIR4	Birchs Ck	-37.28771	143.81142	**Positive**	**Positive**	**Positive**
BIR6	Birchs Ck	-37.29393	143.83122	Negative	**Positive**	**Positive**
BIR7	Birchs Ck	-37.29477	143.83383	Equivocal	**Positive**	**Positive**
BIR8	Birchs Ck	-37.29009	143.83595	**Positive**	**Positive**	**Positive**
BIR9	Birchs Ck	-37.28969	143.84469	**Positive**	**Positive**	**Positive**
BIR10	Birchs Ck	-37.29609	143.86796	Equivocal	Equivocal	
BIR11	Birchs Ck	-37.30773	143.87781	**Positive**	**Positive**	
BIR11A	Birchs Ck	-37.32312	143.8917		Equivocal	**Positive**
BIR12	Birchs Ck	-37.32572	143.901	Negative	Equivocal	**Positive**
BIR13	Birchs Ck	-37.3268	143.90067	**Positive**	Equivocal	**Positive**
BIR14	Birchs Ck	-37.32804	143.90298	**Positive**	**Positive**	**Positive**
BIR15	Birchs Ck	-37.33439	143.92221	Negative	**Positive**	**Positive**
BIR16	Birchs Ck	-37.34037	143.9461	**Positive**	**Positive**	**Positive**
BIR17	Birchs Ck	-37.34693	143.95136	**Positive**	**Positive**	**Positive**
BIR17A	Birchs Ck	-37.34776	143.95244	**Positive**	Equivocal	**Positive**
BIR18	Birchs Ck	-37.36845	143.97415	**Positive**	**Positive**	Equivocal
BIR19	Birchs Ck	-37.37594	143.98124	**Positive**	**Positive**	**Positive**
BIR20	Birchs Ck	-37.38809	143.98904	**Positive**	Equivocal	**Positive**
BIR21	Birchs Ck	-37.39519	143.9894	**Positive**	**Positive**	**Positive**
BIR22	Birchs Ck	-37.40249	143.99333	**Positive**	Negative	**Positive**
BIR23	Birchs Ck	-37.40433	143.99865	**Positive**	Equivocal	Negative
BIR24	Birchs Ck	-37.41687	144.00154			**Positive**
BIR25	Birchs Ck	-37.43311	144.00388			Negative

Table A1.2 – Results for current river blackfish eDNA surveys as well as previous results from Griffiths *et a*l. 2016.

Site ID	Waterway	Latitude	Longitude	Dec2015 result	May2016 result	Sept2021 result
CRE2	Creswick Ck	-37.28224	143.77921	Negative	Negative	**Positive**
CRE1	Creswick Ck	-37.26263	143.78486	**Positive**		Equivocal
TUL2	Tullaroop Ck	-37.25782	143.79741		**Positive**	**Positive**
TUL1	Tullaroop Ck	-37.2276	143.83462	Negative	Negative	**Positive**
BIR1	Birchs Ck	-37.26356	143.79725	**Positive**	**Positive**	**Positive**
BIR2	Birchs Ck	-37.27187	143.80143	**Positive**	**Positive**	**Positive**
BIR3	Birchs Ck	-37.28067	143.80554	Negative	Negative	
BIR4	Birchs Ck	-37.28771	143.81142	**Positive**	**Positive**	**Positive**
BIR6	Birchs Ck	-37.29393	143.83122	Negative	Negative	Equivocal
BIR7	Birchs Ck	-37.29477	143.83383	Negative	Negative	Negative
BIR8	Birchs Ck	-37.29009	143.83595	Negative	Negative	Equivocal
BIR9	Birchs Ck	-37.28969	143.84469	**Positive**	Negative	Negative
BIR10	Birchs Ck	-37.29609	143.86796	Negative	Negative	
BIR11	Birchs Ck	-37.30773	143.87781	Negative	**Positive**	
BIR11A	Birchs Ck	-37.32312	143.8917		Negative	Negative
BIR12	Birchs Ck	-37.32572	143.901	Negative	**Positive**	**Positive**
BIR13	Birchs Ck	-37.3268	143.90067	Negative	**Positive**	Negative
BIR14	Birchs Ck	-37.32804	143.90298	Negative	Negative	**Positive**
BIR15	Birchs Ck	-37.33439	143.92221	Negative	Negative	**Positive**
BIR16	Birchs Ck	-37.34037	143.9461	Negative	Negative	**Positive**
BIR17	Birchs Ck	-37.34693	143.95136	**Positive**	Negative	**Positive**
BIR17A	Birchs Ck	-37.34776	143.95244	Negative	Negative	**Positive**
BIR18	Birchs Ck	-37.36845	143.97415	Negative	Negative	**Positive**
BIR19	Birchs Ck	-37.37594	143.98124	**Positive**	**Positive**	**Positive**
BIR20	Birchs Ck	-37.38809	143.98904	**Positive**	**Positive**	Negative
BIR21	Birchs Ck	-37.39519	143.9894	**Positive**	Negative	Equivocal
BIR22	Birchs Ck	-37.40249	143.99333	**Positive**	Negative	Equivocal
BIR23	Birchs Ck	-37.40433	143.99865	**Positive**	**Positive**	Negative
BIR24	Birchs Ck	-37.41687	144.00154			Negative
BIR25	Birchs Ck	-37.43311	144.00388			Negative

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