Baseline monitoring of platypus and blackfish using eDNA to assess efficacy of future riparian restoration of Birch's Creek.

cesar

sustainability through science and innovation

biodiversity conservation

Baseline monitoring of platypus and blackfish using eDNA to assess efficacy of future riparian restoration of Birch's Creek.

Confidential Report

June 30, 2016

Prepared for:

Greg Barber

North Central Catchment Management Authority

PO Box 18

Huntly, VIC 3551

Australia

Prepared by:

Josh Griffiths, Anthony van Rooyen

and Dr Andrew Weeks

cesar

293 Royal Parade

Parkville, VIC 3052

Australia

www.cesaraustralia.com

+61 3 9349 4723

ABN 26 123 867 587

Project number 1419CR1

Disclaimer

The professional analysis and advice in this report has been prepared for the exclusive use of the party or parties to whom it is addressed (the addressee) and for the purposes specified in it. This report is supplied in good faith and reflects the knowledge, expertise and experience of the consultants involved. The report must not be published, quoted or disseminated to any other party without prior written consent from **cesar** pty ltd.

cesar pty ltd accepts no responsibility whatsoever for any loss occasioned by any person acting or refraining from action as a result of reliance on the report. In conducting the analysis in this report **cesar** pty ltd has endeavoured to use what it considers is the best information available at the date of publication, including information supplied by the addressee. Unless stated otherwise, **cesar** pty ltd does not warrant the accuracy of any forecast or prediction in this report.

www.cesaraustralia.com
+61 3 9349 4723

Project team

Title	Name
Project Manager	Josh Griffiths
Project Consultant	Anthony van Rooyen
Project Supervisor	Dr. Andrew Weeks

Version control

Date	Version	Description	Author	Reviewed By
14/06/16	1.0	Final Report_Draft	JG	AW
29/06/16	1.1	Final Report_Draft	JG, AW	

Abbreviations

Abbreviations	Description
eDNA	environmental DNA
NCCMA	North Central Catchment Management Authority
NCWS	North Central Waterway Strategy
qPCR	quantitative polymerase chain reaction
LWD	Large woody debris

Contents

www.cesaraustralia.com
+61 3 9349 4723

Executive Summary

The North Central Catchment Management Authority (NCCMA) considers Birch's Creek in the upper Loddon catchment a priority waterway due to its provision of irrigation water and the environmental values it supports, particularly platypuses (*Ornithorhynchus anatinus*) and a regionally significant population of river blackfish (*Gadopsis marmoratus*). However, the creek is significantly degraded due to altered flow regimes and widespread clearing of the surrounding catchment for agriculture. The riparian zone in the upper reaches is dominated by extensive willow infestation. (*Salix* spp).

The NCCMA have committed to a long-term rehabilitation program of the riparian zone of Birch's Creek to improve conditions for platypuses and blackfish (and other aquatic species) and restore ecosystem function. This includes removal of invasive willows and other exotic weeds, revegetation with native species, and fencing to exclude stock access. **cesar** was engaged to provide baseline data on the distribution of platypuses and blackfish in Birch's Creek for future comparison to understand the impacts of the riparian restoration works. The distribution of platypuses and blackfish was determined using eDNA techniques which detect the DNA of the target species that has been shed into the water.

Water samples were collected at 28 sites along the length of Birch's Creek and the adjoining Creswick and Tullaroop Creeks in December 2015 and May 2016. Results indicate platypuses are distributed throughout Birch's Creek but are likely to be in relatively low density. Platypus DNA was detected at 21 of 26 sites sampled in December 2015 and 25 of 27 sites in May 2016. The distribution of blackfish was more patchy and largely clustered in the lower and upper reaches. Blackfish DNA was detected at 11 of 26 sites in December 2015 and 10 of 27 sites in May 2016. The presence of blackfish in the upper reaches eases concerns the species had disappeared from this area.

The possible fragmentation of populations from several in-stream structures along Birch's Creek could not be confirmed from the results presented herein. However, inspection of the structures suggests they are likely to present a barrier to dispersal, especially for blackfish. The removal or modification of these structures will facilitate movement of aquatic organisms along the creek and improve long-term viability of populations.

This project has provided detailed data on the distribution of platypuses and blackfish in Birch's Creek and a good baseline for future investigations to gauge the impacts of habitat improvement works.

www.cesaraustralia.com
+61 3 9349 4723

Nature of work

Extensive land clearing for agriculture and urbanisation has contributed to significant degradation of aquatic ecosystems across regional Victoria. Riparian vegetation provides a number of benefits to aquatic ecosystems and the resident biota such as reducing bank erosion and sedimentation, shading the water to reduce temperature fluctuations, cover while foraging, in-stream habitat in the form of woody debris, and organic material as a food resource. Further degradation has been caused by changes to natural flow regimes due to water extraction, altered run-off, and construction of dams and weirs.

Birch's Creek is approximately 39 km long, located in the upper catchment of the Loddon River. Birch's Creek originates on the northern slopes of the Great Dividing Range near Rocklyn and flows northwest through the townships of Newlyn and Smeaton until joining Creswick Creek near Clunes to form Tullaroop Creek, a tributary of the Loddon River. The surrounding catchment is largely cleared for agriculture (predominantly grazing, cropping, and potatoes) with only a narrow, discontinuous corridor of riparian vegetation remaining. While some native vegetation remains in the lower reaches, upstream of Smeaton, the riparian vegetation is dominated by willows (*Salix* spp). Natural flows in the creek have been severely 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).

Birch's Creek has been identified as a priority waterway in the North Central Waterway Strategy (NCWS) due to its provision of irrigation water and the environmental values it supports, particularly platypuses (*Ornithorhynchus anatinus*) and a regionally significant population of river blackfish (*Gadopsis marmoratus*)(North Central CMA 2014). The creek is currently considered to be in poor condition (Index of Stream Condition 2010). There was concern regarding the status of platypuses and blackfish following the Millennium Drought (1996 – 2010) and subsequent severe flooding in early 2011. In addition, the presence of several structures along the creek (weirs, road crossings) pose potential barriers to recolonisation of the upper reaches, particularly for blackfish (SKM 2005).

The NCCMA have committed to restoring the riparian zone of Birch's Creek over the next 4-5 years through removal of invasive willows and other exotic weeds, revegetation with native species, and fencing to exclude stock access. This work is expected to improve the ecological condition of Birch's Creek and improve habitat for platypuses, blackfish, and other aquatic fauna. The NCCMA engaged **cesar** to provide baseline data on the distribution of platypuses and blackfish in Birch's Creek for future comparison to understand the impacts of the riparian restoration works. Additional aims were to determine whether blackfish still inhabit the upper reaches of Birch's Creek and assess whether the in-stream structures along the creek are presenting barriers to dispersal.

Methods

Data on the distribution of platypuses and blackfish was obtained using environmental DNA (eDNA) techniques (Thomsen and Willerslev 2015). eDNA is a relatively new technique that detects genetic material of the target organism(s) that has been shed into the water. The water sampling required is non-invasive and quantitative comparisons indicate that eDNA methods are more sensitive than traditional sampling methods in detecting species, particularly at low densities (Biggs *et al.* 2015; Smart *et al.* 2015; Weeks *et al.* 2015).

Water samples were collected at 28 sites throughout Birch's Creek downstream of Newlyn Reservoir and the adjoining reaches of Creswick and Tullaroop Creeks (Figure 1). Sites were selected to provide comprehensive information on the distribution of platypuses and blackfish throughout the system. Most sites were >2 km apart to generate independent data. However, more intensive sampling was undertaken around potential barriers to investigate fragmentation of populations. Access to the creek is generally through private property, and thus permission from relevant landowners was obtained. This provided an opportunity to engage with landowners about the current project as well as the larger restoration of Birch's Creek. Water samples were collected over consecutive days in December 2015 and May 2016.

Two water samples were collected at each site by passing 300-500 ml of water through a 0.22 µm filter (Sterivex). Care was taken to minimise contamination between sites by using sterile equipment at each site and avoiding transfer of water, soil, or organic material between sites. Filters were stored at 4°C for a maximum of 48 hrs before processing. DNA was then extracted from the filters using a commercially available extraction kit (Qiagen DNeasy Blood and Tissue Kit). A species-specific blackfish quantitative polymerase chain reaction (qPCR) assay (TaqMan®, Life Technologies) was developed targeting the mitochondrial *cytochrome b* (CytB) gene. The qPCR assay was then tested using local blackfish tissue samples provided by the NCCMA. **cesar** has previously developed a platypus-specific qPCR assay also targeting the mitochondrial *cytochrome b* gene (Griffiths *et al.* 2014; Weeks *et al.* 2015). All samples were analysed by qPCR in triplicate. Previous research has shown two water samples with two qPCR's performed on each sample yields >95% confidence in detecting platypuses (Weeks *et al.* 2015). All assays included negative and positive controls.

PCR assays can produce three possible outcomes. A positive result confirms the presence of the target DNA at that site. Where target DNA is not detected, a negative result is assigned. However, a result was regarded as equivocal when only one or two (out of six in this study; two samples per site with three qPCR's on each sample) qPCR's detect the target DNA at high amplification cycles. This could indicate only trace amounts of the target species DNA is present in the sample due to very low abundance in the creek, but can also be a false positive due to dispersal of DNA from upstream, sample contamination, or background noise at high PCR amplification cycles. The careful field and laboratory protocols employed (i.e. hygienic water sampling, specificity of genetic markers, assay controls) reduce the probability of false positives

(Smart *et al.* 2015) and results from adjacent sites provide good confidence of the reliability of equivocal results in this study.

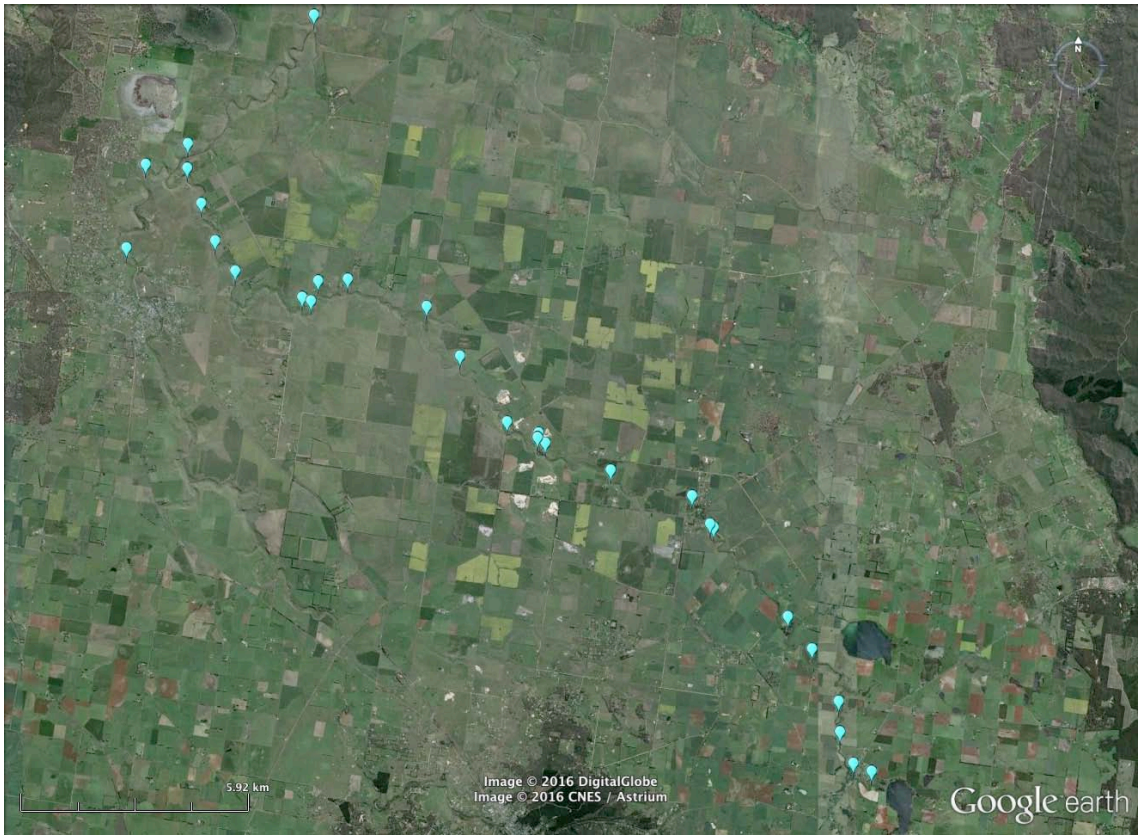

Figure 1. Sampling sites for eDNA analysis to detect platypuses and blackfish along Birch's Creek and the adjoining Creswick Creek and Tullaroop Creek. See Appendix for site details.

www.cesaraustralia.com
+61 3 9349 4723

Findings

Platypus

Platypus DNA was detected throughout Birch's Creek during both sampling periods. In December 2015, 21 of the 26 sites sampled were positive for platypuses (Figure 2a). Several negative results scattered through the middle reaches may have been caused by a lack of flow in the creek at this time. As a result, there was poor connectivity along the creek with some sampling in isolated pools. This would have limited movement of platypuses (Griffiths and Weeks 2015), restricted the dispersal of genetic material and reduced detectability through eDNA techniques. Similar results were found in May 2016 with 25 of 27 sites sampled positive for platypus DNA (Figure 2b). Slightly better flows in May would have facilitated platypus movement and dispersal of DNA along the creek.

Sites sampled in Creswick and Tullaroop Creeks, however, were largely negative or equivocal for platypus DNA on both sampling dates, suggesting that platypuses are not using these creeks. However, few samples were taken in these creeks and therefore the results must be interpreted with some caution.

Blackfish

The eDNA results indicate blackfish have a patchy distribution in Birch's Creek. Eleven of 26 sites sampled during December 2015 were positive for blackfish DNA with blackfish distribution clustered in the lower and upper reaches (Figure 3a). Results were similar in May 2016 (10 of 27 sites positive) with a slight change in distribution (Figure 3b). Several sites in the upper section that were positive in December recorded negative results in May (e.g. BIR 21, BIR22) and several sites in the middle section that were negative became positive (e.g. BIR 12, BIR13).

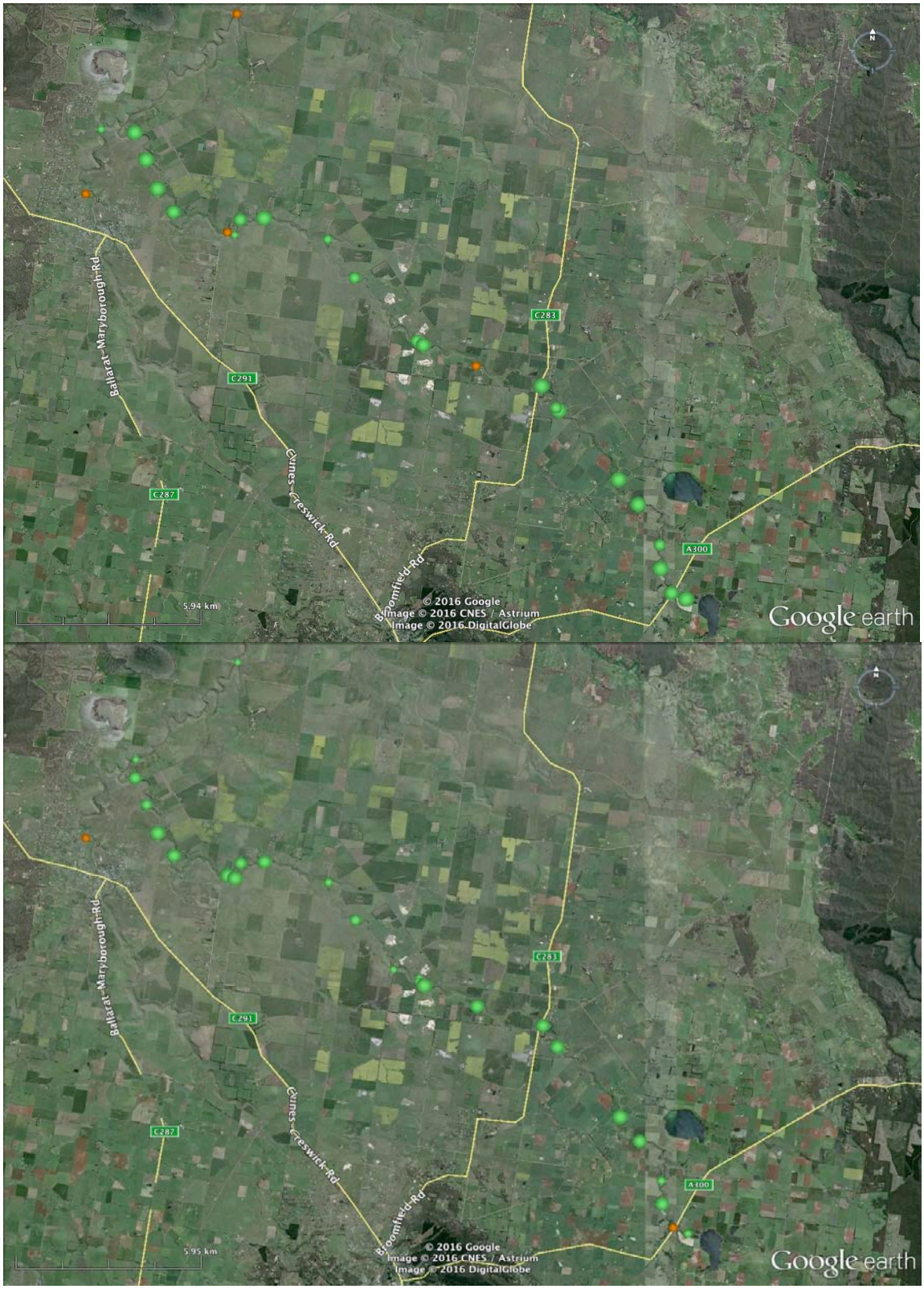

Figure 2. Detection of platypus DNA in water samples collected during a) December 2015 and b) May 2016. Green symbols are positive for platypus, orange are negative. The size of positive symbols indicates the signal strength (number of positive qPCR's, Appendix 1).

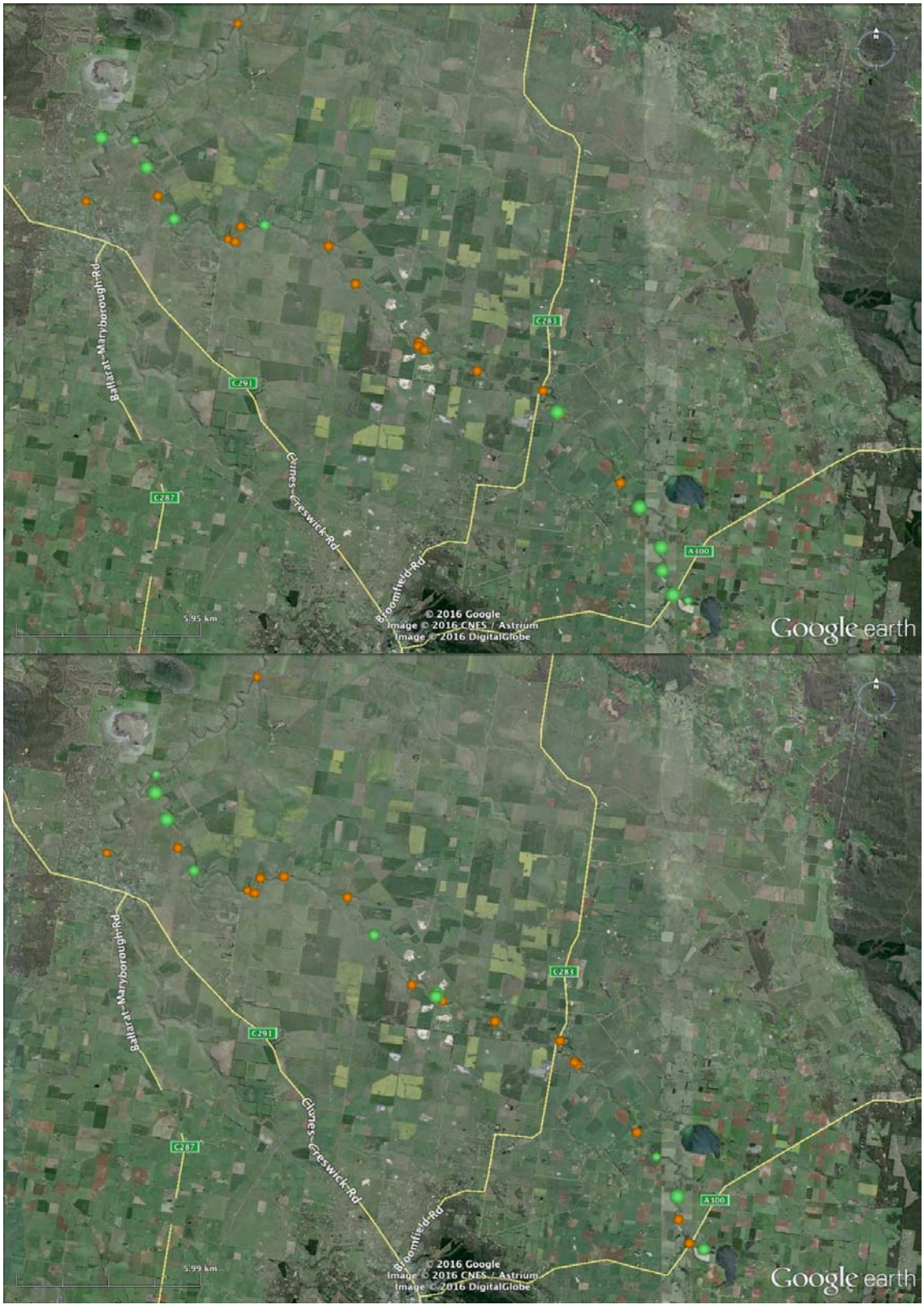

Figure 3. Detection of blackfish DNA in water samples collected during a) December 2015 and b) May 2016. Green symbols are positive for blackfish, orange are negative. The size of positive symbols indicates the signal strength (number of positive qPCR's; Appendix 2).

www.cesaraustralia.com
+61 3 9349 4723

Conclusions

The results indicate that platypuses currently have a fairly broad distribution in Birch's Creek but blackfish are more patchily distributed. Platypus DNA was detected at most sites sampled along the creek although results suggest lower abundance/activity in the middle reaches (BIR10-BIR15). There were no obvious differences in the distribution of platypuses between the two sampling periods. There is a lack of empirical data on the distribution or abundance of platypuses in Birch's Creek although seven platypuses were recently captured in the lower reaches of the creek during fish surveys (McGuckin 2015). However, the current results support anecdotal information obtained in discussions with landowners around platypus sightings as well as records from online databases (Atlas of Living Australia, Victorian Biodiversity Atlas, platypusSPOT). Several negative and/or equivocal sites for platypus DNA suggest that the density of platypuses is quite low.

Significant declines in the distribution and abundance of platypus populations during the Millennium Drought have been recorded elsewhere in Victoria (Griffiths and Weeks 2011; Griffiths and Weeks 2012; Griffiths and Weeks 2013) and it is highly probable that platypuses in Birch's Creek were also impacted. This is supported by observations from several landowners (e.g. R. Cosgrove; North Central CMA 2015) who reported seeing fewer platypuses during this period. When streamflow ceased and portions of the creek dried completely during 2008-09, platypuses would have been forced to migrate further downstream or restricted to remnant pools. Therefore, it is pleasing to note that platypuses have now recolonised most of Birch's Creek, at least as far as Newlyn Reservoir. There is no evidence to suggest the in-stream structures found along Birch's Creek pose barriers to platypus dispersal. Platypuses are known to leave the water to travel around such barriers but they are vulnerable to predation while out of the water. Therefore, the removal or modification of these structures would facilitate safer movement of platypuses.

Historical data from fish surveys (Department of Natural Resources and Environment 2002; Koster 2003; Pittman and Saddlier 2006), online databases (Atlas of Living Australia, Victorian Biodiversity Atlas), and anecdotal evidence from local landowners indicates blackfish were once distributed along the entire length of Birch's Creek prior to the Millennium Drought. During the extended periods of no flow at the height of the drought (2008-09), locals reported substantial fish death of multiple species, including blackfish, along the dried channel sections. A more recent post-drought assessment of the fish fauna in Birch's Creek found a significant reduction in the distribution and abundance of blackfish (McGuckin 2015). The species was only recorded at sites downstream of Tourella Estate (BIR6, BIR7) where a road crossing forms a potential barrier to upstream dispersal (Figure 4).

Figure 4. The road crossing at Tourella Estate (left) presents a barrier to dispersal along Birch's Creek whereas the bridge crossing (right) allows free movement of aquatic organisms.

The eDNA results from the current study reveal blackfish do still occur in the upper reaches of Birch's Creek. Due to the in-stream barriers present, it is more likely that a small number of blackfish persisted through the drought in remnant pools in the upper reaches rather than recolonisation from the lower creek. Sparse, patchy populations of blackfish are unlikely to be detected by electrofishing (McGuckin 2015), highlighting one of the advantages of eDNA techniques. It is currently unknown whether there is any connectivity and gene flow between blackfish in the upper and lower Birch's Creek. However, inspection of the barriers at Tourella Estate, Lawrence Weir, and the bluestone weir suggest they would inhibit movement of blackfish along the creek under most flow conditions. Removal or modification of these redundant barriers will facilitate connectivity and improve resilience of blackfish populations in Birch's Creek. Consideration should be given to the potential habitat and drought refuges created by the pools upstream of some of the barriers before removal.

Some changes in blackfish distribution were evident between the two sampling periods, particularly in the middle and upper reaches. Compared to the December 2015 results, the number of positive sites in the upper reaches declined slightly in May 2016, while the number of positive sites in the middle reaches increased marginally. One possible reason for this may be the minimal flows experience between the sampling periods due to very dry conditions over summer and autumn compounded by water releases from Newlyn Reservoir being stopped shortly before the second water sampling in May 2016 (G. Barber pers. comm.). Low water levels may have caused blackfish to contract to deeper pools (such as Lawrence weir pool BIR13). In addition, willow removal works just downstream of Newlyn Reservoir may have caused some temporary disturbance, although blackfish were still detected in the immediate area (BIR23) in May 2016.

Although habitat wasn't investigated during the current study, observations in the field suggest the lower reaches of Birch's Creek (downstream of Smeaton) provide better quality habitat than the upper reaches. The lower reaches retain some native riparian vegetation (red gums, blackwood, bottlebrush) beds of aquatic macrophytes and

reeds, with some deep pools present, undercut banks, large woody debris (LWD), and patches of cobbled or rocky substrate. This area has benefited from previous habitat improvement works (Department of Natural Resources and Environment 2002). Willows dominate the riparian zone of the upper reaches, fewer pools are present (>1 m deep), little LWD, and more sedimentation. Given the apparent differences in habitat quality, it was somewhat surprising to find similar results for both species in the upper and lower section. The middle section had the poorest results for both species (although platypuses were detected throughout the system), which may reflect less consistent flows through this area. Water for irrigation released from Newlyn Reservoir provides regular inputs to the upper section of Birch's Creek. However, this water often does not make it to the middle section. The lower reaches appear to receive some input from groundwater, which helps to maintain reliable surface water through this section. This pattern was evident during the December sampling period where low flows were observed in the upper and lower reaches but little flow was evident through the middle section and the creek in this area was reduced to a series of isolated pools (J. Griffiths pers. obs.).

Platypuses are highly adaptable and found in variety of aquatic habitats. However, favoured habitat characteristics include stable earthen banks with a near vertical or undercut profile for burrow construction and maintenance, overhanging riparian vegetation, reliable surface water, and in-stream structural complexity that supports abundant macro-invertebrates such as cobbled substrate and LWD (Ellem *et al.* 1998; Grant 2004; Milione and Harding 2009; Serena *et al.* 1998; Serena *et al.* 2001). Platypuses are also known to prefer native riparian vegetation over willows (Serena *et al.* 2001). Many of these habitat characteristics are also favourable to blackfish, particularly the presence of undercut banks, LWD, cobbled substrate (Allen *et al.* 2002).

It is expected that the restoration works along Birch's Creek, combined with an environmental flow program (North Central CMA 2015), will improve conditions for platypus, blackfish, and other aquatic species. The data presented herein provides a good baseline of the current status of platypuses and blackfish in Birch's Creek. It is recommended the sampling be repeated in future to determine the impact of restoration works on these two species with the potential to retrospectively analyse samples for additional species. As a minimum, monitoring should be repeated at the end of the restoration works but ideally annually to assess the temporal progression and allow adaptive management if necessary. Annual monitoring also provides a valuable opportunity to continue engagement with landowners and demonstrate environmental outcomes of the restoration program.

Acknowledgments

cesar would like to thank NCCMA for funding this project, particularly Greg Barber and Lang Dowdell for their support and advice during the project. **cesar** also thank the many landowners who granted access to their properties for obtaining water samples and provided anecdotal accounts of the historical state of the creek and its fauna.

References

Allen, G.R., Midgley, S.H., and Allen, M. (2002) 'Field guide to the freshwater fishes of Australia.' (West Australian Museum: Perth)

Biggs, J., Ewald, N., Valentini, A., Gaboriaud, C., Dejean, T., Griffiths, R.A., Foster, J., Wilkinson, J.W., Arnell, A., Brotherton, P., Williams, P., and Dunn, F. (2015) Using eDNA top develop a national citizen science-based monitoring programme for the great crested newt (*Triturus cristatus*). *Biological Conservation* **183**, 19-28.

Department of Natural Resources and Environment (2002) Birch's Creek Habitat Rehabilitation for River Blackfish. Department of Natural Resources and Environment, Melbourne.

Ellem, B.A., Bryant, A., and O'Connor, A. (1998) Statistical modelling of platypus, *Ornithorhynchus anatinus*, habitat preferences using generalized linear models. *Australian Mammalogy* **20**, 281-285.

Grant, T.R. (2004) Depth and substrate selection by platypuses, *Ornithorhynchus anatinus*, in the lower Hastings River, New South Wales. *Proceedings of the Linnean Society of New South Wales* **125**, 235-241.

Griffiths, J., Kelly, T., van Rooyen, A., and Weeks, A. (2014) Distribution and relative abundance of platypuses in the greater Melbourne area: survey results 2013/14. (Report to Melbourne Water). **cesar**, Parkville.

Griffiths, J., and Weeks, A. (2011) Temporal patterns in abundance of platypus populations in Melbourne's catchments (Report to Melbourne Water). **cesar**, Parkville.

Griffiths, J., and Weeks, A. (2012) Distribution and relative abundance of platypuses in the MacKenzie River: survey results 2011/12 (Report to Wimmera Catchment Management Authority). cesar, Parkville.

Griffiths, J., and Weeks, A. (2013) Platypus distribution and abundance in the upper Wimmera region. (Report to Project Platypus). **cesar**, Parkville.

Griffiths, J., and Weeks, A. (2015) Impact of environmental flows on platypuses in a regulated river. Report to Melbourne Water. **cesar**, Parkville

Koster, W. (2003) Summary of Birches Creek Fish Survey, April 9th 2003. Department of Sustainability and Environment, Heidelberg, Victoria.

McGuckin, J. (2015) Post drought survey of Birch's Creek (Report for North Central CMA). Streamline Research Pty Ltd

Milione, M., and Harding, E. (2009) Habitat use by platypus (*Ornithorhynchus anatinus*) in a modified Australian wet tropics catchment, north-eastern Queensland. *Australian Mammalogy* **31**, 35-46.

North Central CMA (2014) Birch's Creek Seasonal Watering Strategy. North Central Catchment Management Authority, Huntly, Victoria.

North Central CMA (2015) Birch's Creek Environmental Water Management Plan. North Central Catchment Management Authority, Huntly.

Pittman, K.S., and Saddlier, S. (2006) Survey for River Blackfish following rehabilitation works in Birches Creek, North-Eastern Victoria. Arthur Rylah Institute for Environmental Research, Heidelberg, Victoria.

Serena, M., Thomas, J., Williams, G., and Officer, R. (1998) Use of stream and river habitats by the platypus, *Ornithorhynchus anatinus*, in an urban fringe environment. *Australian Journal of Zoology* **46**(3), 267-282.

Serena, M., Worley, M., Swinnerton, M., and Williams, G.A. (2001) Effect of food availability and habitat on the distribution of platypus (*Ornithorhynchus anatinus*) foraging activity. *Australian Journal of Zoology* **49**(3), 263-277.

SKM (2005) Environmental Flow Assessment for the Birch'ses Creek catchment: Issues Paper. Sinclair Knight Mertz, Melbourne.

Smart, A.S., Tingley, R., Weeks, A.R., van Rooyen, A.R., and McCarthy, M.A. (2015) Environmental DNA sampling is more sensitive than a traditional survey technique for detecting an aquatic invader. *Ecological Applications* **25**(7), 1944-1952.

Thomsen, P.F., and Willerslev, E. (2015) Environmental DNA – An emerging tool in conservation for monitoring past and present biodiversity. *Biological Conservation* **183**(0), 4-18.

Weeks, A., van Rooyen, A., Griffiths, J., and Tingley, R. (2015) Determining the effectiveness of eDNA for monitoring platypuses (Report to Melbourne Water). **cesar**, Parkville.

www.cesaraustralia.com
+61 3 9349 4723

Appendix 1. Platypus eDNA results

				December 2015			May 2016		
Site	**Waterway**	**Easting**	**Northing**	**Site Result**	**No. positive PCR's (6)**	**DNA copies/Litre**	**Site Result**	**No. positive PCR's (6)**	**DNA copies/Litre**
CRE2	Creswick Creek	746392	5870196	Negative	0	0	Negative	0	0
CRE1	Creswick Creek	746957	5872357	Equivocal	1	465			
TUL2	Tullaroop Creek	748086	5872858				Equivocal	1	828
TUL1	Tullaroop Creek	751487	5876113	Negative	0	0	Equivocal	1	129
BIR1	Birch's Creek	748053	5872221	Positive	6	5505	Positive	4	1560
BIR2	Birch's Creek	748397	5871288	Positive	6	1803	Positive	3	813
BIR3	Birch's Creek	748732	5870301	Positive	6	781	Positive		
BIR4	Birch's Creek	749230	5869504	Positive	5	1652	Positive	5	1068
BIR6	Birch's Creek	750965	5868762	Negative	0	0	Positive	6	466
BIR7	Birch's Creek	751194	5868661	Equivocal	1	528	Positive	6	2757
BIR8	Birch's Creek	751397	5869174	Positive	4	1979	Positive	4	703
BIR9	Birch's Creek	752174	5869196	Positive	6	1325	Positive	5	1094
BIR10	Birch's Creek	754215	5868424	Equivocal	2	688	Equivocal	2	125
BIR11	Birch's Creek	755049	5867105	Positive	3	804	Positive	3	589
BIR11A	Birch's Creek	756228	5865360				Equivocal	1	186
BIR12	Birch's Creek	757044	5865046	Negative	0	0	Equivocal	1	122
BIR13	Birch's Creek	757011	5864927	Positive	4	1092	Equivocal	1	388
BIR14	Birch's Creek	757211	5864783	Positive	5	2879	Positive	6	721
BIR15	Birch's Creek	758894	5864026	Negative	0	0	Positive	5	791
BIR16	Birch's Creek	760990	5863296	Positive	6	2435	Positive	5	593
BIR17	Birch's Creek	761433	5862554	Positive	3	618	Positive	5	504
BIR17A	Birch's Creek	761526	5862458	Positive	6	1915	Equivocal	2	436
BIR18	Birch's Creek	763377	5860102	Positive	6	3191	Positive	6	2382
BIR19	Birch's Creek	763979	5859251	Positive	6	5069	Positive	5	830
BIR20	Birch's Creek	764627	5857880	Positive	3	498	Equivocal	2	407
BIR21	Birch's Creek	764634	5857092	Positive	6	3369	Positive	5	1049
BIR22	Birch's Creek	764956	5856271	Positive	5	1323	Negative	0	0
BIR23	Birch's Creek	765421	5856051	Positive	6	12481	Equivocal	2	886

Appendix 2. Blackfish eDNA results

				December 2015			May 2016		
Site	**Waterway**	**Easting**	**Northing**	**Site result**	**No. positive PCR's (6)**	**DNA copies /Litre**	**Site result**	**No. positive PCR's (6)**	**DNA copies /Litre**
CRE2	Creswick Creek	746392	5870196	Negative	0	0	Negative	0	0
CRE1	Creswick Creek	746957	5872357	Positive	5	1167			
TUL2	Tullaroop Creek	748086	5872858				Equivocal	2	33
TUL1	Tullaroop Creek	751487	5876113	Negative	0	0	Negative	0	0
BIR1	Birch's Creek	748053	5872221	Positive	2	866	Positive	6	49
BIR2	Birch's Creek	748397	5871288	Positive	5	642	Positive	6	878
BIR3	Birch's Creek	748732	5870301	Negative	0	0	Negative		
BIR4	Birch's Creek	749230	5869504	Positive	4	441	Positive	3	109
BIR6	Birch's Creek	750965	5868762	Negative	0	0	Negative	0	0
BIR7	Birch's Creek	751194	5868661	Negative	0	0	Negative	0	0
BIR8	Birch's Creek	751397	5869174	Negative	0	0	Negative	0	0
BIR9	Birch's Creek	752174	5869196	Positive	3	1088	Negative	0	0
BIR10	Birch's Creek	754215	5868424	Negative	0	0	Negative	0	0
BIR11	Birch's Creek	755049	5867105	Negative	0	0	Positive	3	57
BIR11A	Birch's Creek	756228	5865360				Negative	0	0
BIR12	Birch's Creek	757044	5865046	Negative	0	0	Positive	4	385
BIR13	Birch's Creek	757011	5864927	Negative	0	0	Positive	5	44
BIR14	Birch's Creek	757211	5864783	Negative	0	0	Negative	0	0
BIR15	Birch's Creek	758894	5864026	Negative	0	0	Negative	0	0
BIR16	Birch's Creek	760990	5863296	Negative	0	0	Negative	0	0
BIR17	Birch's Creek	761433	5862554	Positive	6	280	Negative	0	0
BIR17A	Birch's Creek	761526	5862458	Negative	0	0	Negative	0	0
BIR18	Birch's Creek	763377	5860102	Negative	0	0	Negative	0	0
BIR19	Birch's Creek	763979	5859251	Positive	6	1334	Equivocal	2	161
BIR20	Birch's Creek	764627	5857880	Positive	6	1410	Positive	6	24
BIR21	Birch's Creek	764634	5857092	Positive	5	1075	Negative	0	0
BIR22	Birch's Creek	764956	5856271	Positive	6	607	Negative	0	0
BIR23	Birch's Creek	765421	5856051	Positive	2	348	Positive	4	1005