Project number 2126CR2
November 23, 2022

Assessing the conservation status of platypuses of the Orara Valley and potential impacts of the 2019/20 bushfires.

Prepared for:
Justin Couper

Jaliigirr Biodiversity Network

Prepared by:
Josh Griffiths, Emma Walker, Dr Rachael Impey, and Sarah Licul

EnviroDNA
95 Albert St
Brunswick, VIC 3056
Australia

Disclaimer

Project team

Title	Name
Project Manager	Josh Griffiths
Project Consultant	Emma Walker
Project Consultant	Sarah Licul
Project Consultant	Dr. Rachael Impey

Version control

Date	Version	Description	Author	Reviewed By
12/08/2022	1.0	Draft Report	EW	JG
3/11/2022	1.1	Draft Report	EW, JG	JC
22/11/2022	2.0	Final Report		

Abbreviations

Abbreviations	Description
eDNA	environmental DNA
qPCR	quantitative Polymerase Chain Reaction
JBA	Jaliigirr Biodiversity Network
ALA	Atlas of Living Australia
IUCN	International Union for Conservation of Nature
NPWS	NSW Parks & Wildlife Service

Introduction

The platypus is an endemic Australian mammal that inhabits a variety of freshwater systems throughout eastern Australia (Grant 1992; Grant and Temple-Smith 1998). Due to their reliance on aquatic ecosystems, platypuses are potentially vulnerable to a range of anthropogenic threats that can degrade or reduce their habitat. These threats include altered flow regimes (generally a reduction in available surface water) due to drought and water diversion and extraction, land use changes in the surrounding catchment, clearing riparian vegetation, and poor water quality with major threats expected to intensify due to climate change and an increasing human population (Grant and Temple-Smith 2003; Bino *et al.* 2019, 2020). The extent and relative impacts of these threats are poorly understood due to challenges in studying platypuses in the wild and lack of quantitative historical data. However, the species was recently listed as Near Threatened by the International Union for Conservation of Nature (Woinarski and Burbidge 2016) in response to mounting evidence of localised population declines and extinctions across their range, particularly in urban and agricultural landscapes (Grant 1992, 1993, 1998; Lintermans 1998; Lunney *et al.* 1998, 2004; Rohweder and Baverstock 1999; Serena and Williams 2004; Griffiths and Weeks 2018; Griffiths, Maino, *et al.* 2019; Serena and Williams 2011; Serena *et al.* 2002; Griffiths, Maino, *et al.* 2020; Serena *et al.* 2014; Williams 2010). Populations in Victoria were considered under the most stress and platypuses have now been listed as Vulnerable in the state.

Like many regions throughout their geographic range, there is no current information on the status of platypus populations in the Orara Valley near Coffs Harbour, NSW. There have been no systematic surveys undertaken and anecdotal records on wildlife databases (i.e. Atlas of Living Australia, BioNet, platypusSPOT) are scarce. The catchment area has been significantly modified by widespread land clearing for agriculture, presence of invasive species (e.g. erosion, and urbanisation with unknown impacts on water quality and river flows. Like much of southeastern Australia, the area was also impacted by the Black Summer bushfires during 2019/20 – an event that burnt over 11 million hectares included an estimated 13.56% of all platypus habitat in Australia (WWF Australia 2020). It is unclear how platypus populations respond to bushfires with consequences likely to vary depending on fire extent and severity, post-fire conditions, and resilience of the population (Serena and Williams 2004; Griffiths and Weeks 2014; Griffiths, McColl-Gausden, *et al.* 2020). While platypuses are expected to be buffered from the direct impacts of bushfires, subsequent runoff of sediment and ash from the surrounding catchment that has been stripped of vegetation, may degrade waterways for an extended period (Wallbrink *et al.* 2004) that may reduce their macroinvertebrate food resources (Vieira *et al.* 2004; Verkaik *et al.* 2014, 2015). Fire-impacted catchment areas are highly susceptible to erosion and heavy rainfall events following fires can result in large quantities of ash, sediment, and nutrients entering waterways and adversely affecting water quality and ecosystems for large distances downstream.

This project aimed to address the lack of contemporary knowledge on platypus populations in the Orara Valley region by assessing their occurrence and distribution using environmental DNA techniques. Environmental DNA (eDNA) is a non-invasive sampling technique that detects genetic material from a target species secreted into its surrounding environment (i.e. 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), enabling effective detection at low densities. Environmental DNA can be a highly sensitive and cost-effective method of determining platypus presence over large spatial scales by detecting traces of genetic material in the water (Lugg *et al.* 2018; Weeks *et al.* 2015). Importantly, simple water sampling methodology allows local organisations and citizen scientists to actively participate in rigorously designed projects with limited training and experience. The data collected will be critical for understanding the current status of platypus populations, identify possible impacts from various threats, provide a systematic baseline for future monitoring, and inform whether management actions are required.

Methods

Water sampling

The current occurrence of platypuses throughout the Orara Valley region was spatially mapped using eDNA techniques. Survey sites were selected to provide broad spatial coverage across the landscape where platypuses have previously been recorded (i.e. historical records from wildlife databases) and incorporate a range of different waterways and land use types. The sampling program was interrupted by several large rainfall events that caused safety and access issues to waterways as well as unsuitable conditions for water sampling (i.e. high turbidity).

During March and June 2022, water samples were collected from 32 sites (Figure 1) by Coffs Harbour City Council and Jaliigirr Biodiversity Alliance staff as well as some local citizen scientists following training in correct water sampling techniques through online workshops. At each site, two replicate samples were collected by passing 300-700 ml water (avg. 490 ml) through a 1.2 µm syringe filter. 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 between sites including new sampling equipment at each site, not entering water, and taking care not to transfer soil, water or vegetation between sites. A preservative was added to the filters after filtering to minimise DNA degradation. Filters were stored out of sunlight and at ambient temperature before being transported to the laboratory for processing.

eDNA analysis

DNA was extracted from the filters using a commercially available DNA extraction kit (Qiagen Power Soil Pro Kit) that minimizes compounds that can inhibit PCR reactions. A species-specific probe targeting a 57 base- pair sequence of the mitochondrial cytochrome b (CytB) gene was used to screen all samples for the presence of platypus DNA (Lugg *et al.* 2018). Real-time quantitative Polymerase Chain Reaction (qPCR) TaqMan® assays were used to amplify and quantify the target DNA. Assays were performed in triplicate on each sample. Positive and negative controls were included for all assays. To minimise false positives, a threshold of at least 2 positive assays out of 6 undertaken for each site was required to assign the site as positive for platypus. 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, facilitated movement of DNA between waterbodies (i.e. water birds, recreational anglers, water transfers, predator scats), or dispersal from further upstream.

Impacts of 2019/20 bushfires

To investigate the possible impacts of the devastating “Black Summer” fires of 2019/20, we pooled data from the current project, which had limited fire-impacted sites, with data from a recent project in the adjacent Dorrigo Plateau area (Griffiths and Licul 2022) to increase overall sample size and incorporate more fire-impacted sites. Survey sites were separated into control and impact sites using the National Indicative Aggregated Fire Extent Dataset (NIAFED) with a 5 km buffer to account for potential effects beyond the fire extent. Sites which were located within this fire extent were classified as “burnt” with sites outside of the extent were considered “unburnt”. Due to a lack of rigorous pre-fire data, a before-after-control-impact design was not possible. Rather, platypus occupancy was compared between impact and control sites only. Using site occupancy detection modelling, both the Dorrigo Plateau and Orara Valley datasets were used to further understand platypus detection probability in burnt and unburnt landscapes. The majority of burnt sites were located in forested, upper catchment areas while unburnt sites are primarily in lower catchment, agricultural or urbanized landscapes. To help explain likely confounding factors between burnt and unburnt areas, a broad land use type was assigned to each site based on the area they were located within using the NSW government land use dataset. Three main land use types were identified – forest, agriculture and residential.

Data analysis – Site occupancy detection modelling

To investigate patterns of platypus occurrence across the landscape, we used a site occupancy detection framework. Our model was defined by a sequence of Bernoulli trials:

$$z_i \sim \text{Bernoulli}\ (\psi_i)$$
$$\text{logit}(\psi_i) = \text{logit}(\gamma) + \beta_1 * \text{burnt}_i + \beta_2 * \text{landuse}$$
$$a_{ij} | z_i \sim \text{Bernoulli}\ (z_i \theta)$$
$$y_{ijk} | a_{ij} \sim \text{Bernoulli}\ (a_{ij} \omega)$$

where z_i describes the latent presence (z_i =1) or absence (z_i =0) of platypus eDNA at site i given the probability of occupancy ψ_i. The occupancy parameter ψ_i was a function of an intercept (γ) and two covariates: (i) whether a site was burnt or not and (ii) land-use (forested, agricultural, or residential). Parameter a_{ij} denotes the presence (a_{ij} = 1) or absence ($a_{ij} = 0$) of platypus eDNA in water sample j from site i, as a function of the occurrence of eDNA, z_i, and the availability probability θ. The observed detection data y_{ijk} are a function of the occurrence of eDNA in water sample j from site i, and the probability of detecting eDNA in quantitative PCR (qPCR) replicate k, ω.

Models were fit in R v4.2.1 (R Development Core Team 2015) using JAGS v4.3.1 (Plummer 2003) and the R2jags package (Su and Yajima 2015). Three Markov chains were each run for 5,000 iterations, discarding the first 1,000 iterations of each chain. Prior distributions for ψ, θ and ω, and for the two regression coefficients β_1 and β_2, were specified as normally distributed with mean = 0 and precision = Y on the logit scale. All R hat values were ~1.0, indicating successful convergence.

EnviroDNA Pty Ltd
+61 3 9028 8753
envirodna.com

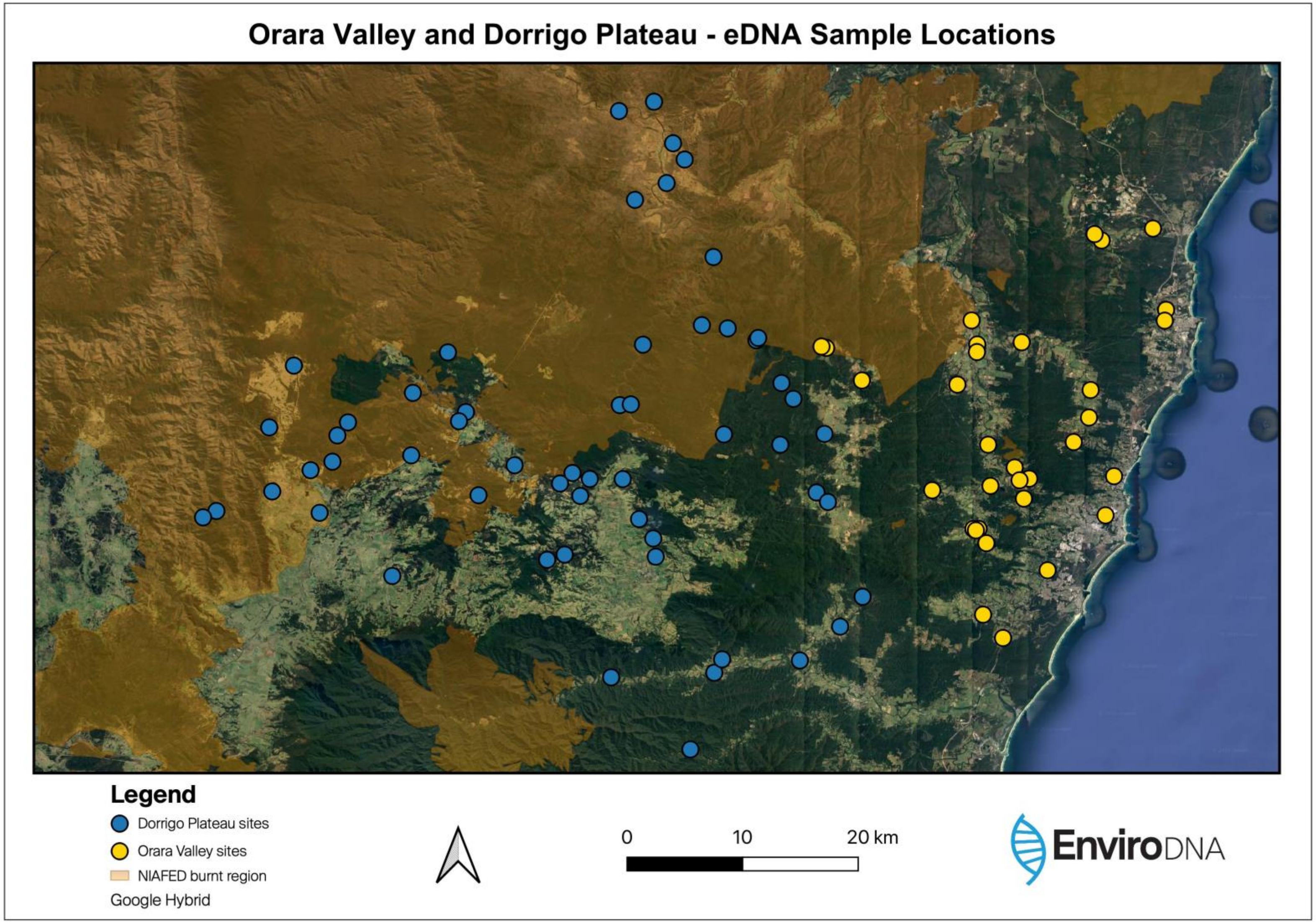


Figure 1. Location of sampling sites for eDNA analysis in the Orara Valley region (yellow) and Dorrigo Plateau (blue; from Griffiths and Licul 2022). Orange shaded areas indicate extent of the 2019/20 fires.

Results

Results from the eDNA targeted species analysis are summarised in Table 1 below for the Orara Valley region. During 2022, eDNA surveys investigated platypus occurrence at a total of 32 sites (Figure 2). Overall, platypus DNA was positively detected at 9 sites (28%) with a further 3 sites yielding Equivocal results and the remaining 20 sites (63%) having no platypus DNA detected. Most detections were clustered in the Orara River and adjacent tributaries.

Across both the Dorrigo Plateau and Orara Valley sites a total of 65 out of 88 sites had detections of platypus DNA (positive and equivocal) (Appendix 1, Table A1). Survey sites were distributed across four subcatchment areas (HydroBASINS Level 8). Historical records (1990-2020) from online wildlife databases indicate platypuses were widespread throughout all subcatchments surveyed, although recent (<10 years) were sparse. The current results indicate platypuses remain widespread throughout 2 subcatchments (Nymboida and Bellinger), possibly slightly restricted in one subcatchment (Orara), and sparse or absent in one subcatchment (coastal). Waterways that have historical platypus records but weren't detected during the current surveys include upper Bucca Bucca Creek, Corindi River, Poundyard Creek, Woolgoolga Creek, Pine Brush Creek, Bonville Creek, and Pine Creek although survey effort was quite limited in several waterways.

Thirty-three sites were classified as "burnt" within the fire extent of the National Indicative Aggregated Fire Extent Dataset (NIAFED) and 55 as unburnt control sites that were more than 5 km from the fire impacted areas. Of the unburnt sites, 60% had detections of platypus DNA, while the burnt sites had platypus DNA detections in 78% of sites. Although platypus occupancy was higher in burnt than unburnt sites, with land use included as a model covariate, there was no significant effect of fires evident. With the inclusion of the land use data across the combined dataset (n=88), sites within forest (76%) and agricultural (66%) areas have greater site occupancy than residential sites (0%), with forest sites having the highest probability of occupancy of all land use types (Figure 3). This indicates that while bushfires did not have a significant impact on platypus populations in the area, land use changes and development in the area may be a greater factor in changes to platypus occurrence. The site occupancy detection model concluded that across all sites, irrespective of burnt status or land use type, the mean probability of platypus detection at any given site was approximately 52%.

EnviroDNA Pty Ltd
+61 3 9028 8753
envirodna.com

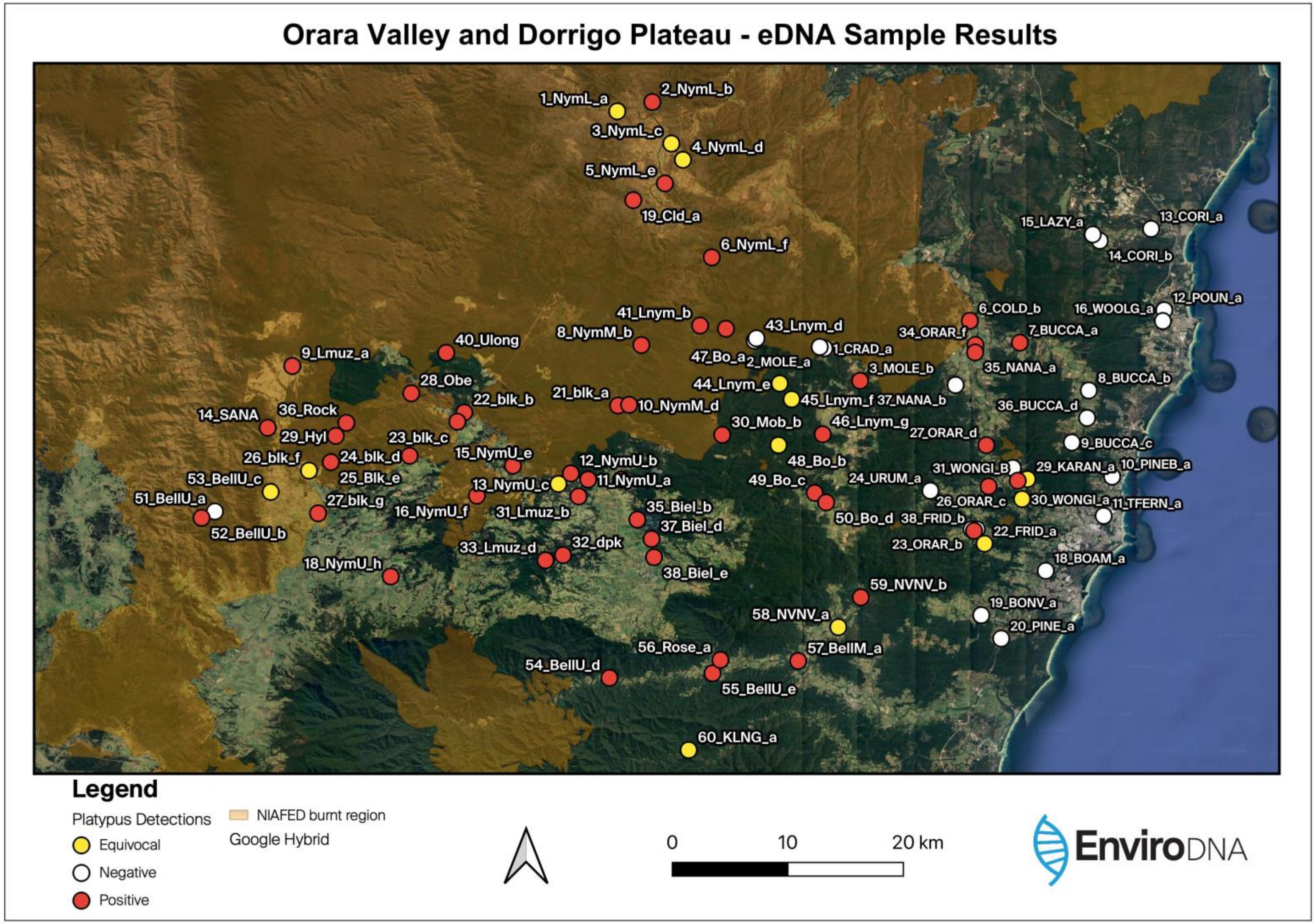
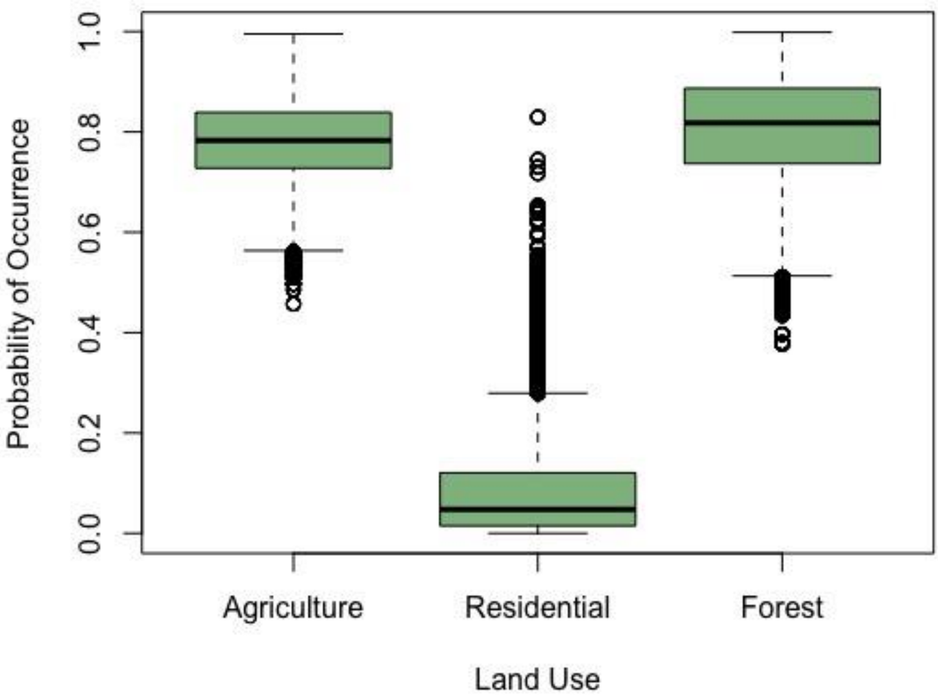


Figure 2. Results of eDNA surveys during 2021, approximately 21 months after the 2019/20 bushfires. Red = platypus DNA detected (positive), yellow = equivocal result, white = platypus DNA not detected (negative).

Table 1. Results of eDNA surveys throughout the Orara Valley region, including site location, date of collection and volumes. Also see Figure 2.

Site Code	Waterway	Latitude	Longitude	Date sampled	Volume 1 (mL)	Volume 2 (mL)	Positive assays	Test Result
18_BOAM_a	Boambee Ck	-30.336425	153.071239	23/03/2022	400	400	0/6	Negative
19_BONV_a	Bonville Ck	-30.376414	153.013052	23/03/2022	400	400	0/6	Negative
7_BUCCA_a	Bucca Bucca Ck	-30.13095201	153.0478615	22/06/2022	550	550	4/6	**Positive**
8_BUCCA_b	Bucca Bucca Ck	-30.173991	153.1100512	22/06/2022	600	600	0/6	Negative
9_BUCCA_c	Bucca Bucca Ck	-30.220886	153.094808	23/03/2022	450	450	0/6	Negative
36_BUCCA_d	Bucca Bucca Ck	-30.19876617	153.108572	22/06/2022	650	650	0/6	Negative
6_COLD_b	Coldwater Ck	-30.111296	153.002695	19/06/2022	650	650	2/6	**Positive**
13_CORI_a	Corindi River	-30.02847451	153.1662624	23/03/2022	300	300	0/6	Negative
14_CORI_b	Corindi River	-30.0391873	153.1199633	23/03/2022	350	350	0/6	Negative
1_CRAD_a	Cradle Ck	-30.136025	152.871521	23/03/2022	400	400	0/6	Negative
22_FRID_a	Friday Ck	-30.312	153.016	24/06/2022	700	700	1/6	Equivocal
38_FRID_b	Friday Ck	-30.300424	153.0065728	26/06/2022	650	650	3/6	Positive
29_KARAN_a	Karangi Ck	-30.253862	153.0546305	23/06/2022	600	600	1/6	Equivocal
15_LAZY_a	Lazyman Ck	-30.033489	153.1136063	23/03/2022	400	400	0/6	Negative
2_MOLE_a	Mole Ck	-30.134807	152.867334	23/03/2022	400	400	0/6	Negative
3_MOLE_b	Mole Ck	-30.165337	152.90376	23/03/2022	400	400	2/6	**Positive**
35_NANA_a	Nana Ck	-30.139758	153.007510	23/03/2022	500	500	3/6	**Positive**
37_NANA_b	Nana Ck	-30.16911	152.98988	23/06/2022	700	700	0/6	Negative
22_ORAR_a	Orara River	-30.29913808	153.0090202	25/06/2022	700	700	0/6	Negative
23_ORAR_b	Orara River	-30.299138	153.0042901	26/06/2022	550	550	0/6	Negative

Site Code	Waterway	Latitude	Longitude	Date sampled	Volume 1 (mL)	Volume 2 (mL)	Positive assays	Test Result
26_ORAR_c	Orara River	-30.2603898	153.0196924	23/03/2022	500	500	3/6	**Positive**
27_ORAR_d	Orara River	-30.223281	153.017624	23/03/2022	450	450	3/6	**Positive**
34_ORAR_f	Orara River	-30.132903	153.007798	23/03/2022	450	450	3/6	**Positive**
10_PINEB_a	Pine Brush Ck	-30.251628	153.131144	23/03/2022	400	400	0/6	Negative
20_PINE_a	Pine Ck	-30.397471	153.031048	23/03/2022	300	300	0/6	Negative
28_POPER_a	Poperaperin Ck	-30.2438072	153.0415524	22/06/2022	600	600	0/6	Negative
12_POUN_a	Poundyard Ck	-30.101711	153.178092	23/03/2022	350	350	0/6	Negative
11_TFERN_a	Tree Fern Ck	-30.286912	153.123477	23/03/2022	350	350	0/6	Negative
24_URUM_a	Urumbilum River	-30.264324	152.967154	23/03/2022	500	N/A	0/6	Negative
30_WONGI_a	Wongiwomble Ck	-30.271856	153.0498657	25/06/2022	600	N/A	1/6	Equivocal
31_WONGI_B	Wongiwomble Ck	-30.25531165	153.0459488	27/06/2022	550	550	2/6	**Positive**
16_WOOLG_a	Woolgoolga Ck	-30.111471	153.176874	23/03/2022	350	350	0/6	Negative



agricultural, residential and forested land use types across the Orara Valley and Dorrigo plateau regions.

Discussion

EnviroDNA Pty Ltd
+61 3 9028 8753
envirodna.com

Results from this study indicate the platypus populations across the Orara Valley and Dorrigo Plateau regions are currently widespread, although there are some concerns in more modified landscapes. While it is impossible to determine the trajectory of the platypus populations in the region with such sparse and anecdotal historical data, the current site occupancy (percentage of positive sites) indicates populations are relatively healthy in the forested areas of the upper catchments (76%) but occupancy was lower in the agricultural areas (66%) and populations were not detected in residential areas (0%). This pattern is similar to that found across other projects where comparable landscape-scale projects have been undertaken although occupancy within agricultural areas is somewhat higher, potentially due to the close proximity to forested areas here. Site occupancy of platypuses can vary widely depending on habitat quality (and other factors) from approximately 25-50% in agricultural regions of western and northern Victoria (Griffiths, Song, *et al.* 2019; Griffiths, Licul, *et al.* 2021; Griffiths and Licul 2020), 10-25% in heavily urbanized catchments (Griffiths *et al.* 2018; Brunt *et al.* 2021), and over 80% in comparable forested catchments throughout southeastern Australia (Griffiths, McColl-Gausden, *et al.* 2020; Griffiths, Impey, *et al.* 2021). Of concern is an apparent decline in platypus distribution in several waterways within the agricultural and residential landscapes, particularly in the coastal areas.

Favourable habitat characteristics for platypuses include reliable surface water with suitable flow regimes, large riparian trees, overhanging vegetation, coarse benthic substrates (i.e. cobbles, rocks, pebbles) and large woody debris pools 1-3 m deep, and near vertical or undercut banks at least 0.5 m above the water (Bethge et al. 2003; Ellem et al. 1998; Grant 2004b; Serena et al. 1998a; Serena et al. 1998b; Serena et al. 2001; Worley and Serena 2000). Many of these variables are important for supporting abundant benthic macroinvertebrate food resources. Modified landscapes, either for agriculture or urbanisation, can impact a number of these variables to varying degrees through land clearing, bank erosion, in-stream sedimentation, and altered flow regimes. Therefore, management actions should be directed to these areas and include removal of invasive species, stock exclusion, and native vegetation of riparian zones with large trees and woody understorey.

Quantifying the impacts of the bushfires is difficult without systematic and quantitative pre-fire data on platypus occurrence. In these combined datasets, platypus occupancy was very high in burnt areas, in fact higher than unburnt areas. However, the analyses shows that this higher occupancy is due to the underlying forested habitat rather than any effect of the fires. The most significant impacts from bushfires are expected to be indirect effects including degradation of instream habitat from high volumes of ash and sediment entering the waterway resulting in a reduction in food resources (Vieira *et al.* 2004; Verkaik *et al.* 2014, 2015). Platypuses may be better equipped to cope with altered food resources than some species due to their generalist diet (McLachlan-Troup *et al.* 2010; Marchant and Grant 2015; Klamt *et al.* 2016), allowing them to exploit a variety of prey depending on availability. Nevertheless, the high occupancy at burnt sites approximately 2.5 years after the devastating 2019/20 bushfires, indicates there hasn't been major impacts on the platypus population in this area. Of course, the occupancy data from eDNA surveys does not preclude more subtle impacts on abundance, survival, or juvenile recruitment due to a possible reduction in food resources.

For the first time, this study (with Griffiths and Licul 2022) has provided a systematic assessment of the platypus populations throughout the Orara Valley and Dorrigo Plateau regions. It is important to note that results from the current study were obtained from a single sampling event and represent a snapshot of platypus 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 survey site at the time of sampling. 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; 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 *et al.* 2014). Therefore, some temporal variation in localised occurrence of platypuses 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 and under different land use practices. Importantly, there is now baseline data against which to assess future changes in platypus populations in response to environmental changes such as natural disturbances, human activities, or management actions.

Key recommendations:

Platypus populations throughout the area appear to be widespread and relatively healthy. However, it will be important to maintain habitat quality and connectivity to prevent future declines and ensure long-term viability of populations in the ongoing threats posed by climate change and altered land-use. Maintaining habitat connectivity and preventing fragmentation is critical as small, isolated populations have significantly higher risk of extinction due to stochastic events and loss of genetic diversity (Frankham *et al.* 2010; Soule 1980). Therefore, protecting habitat quality and connectivity is critical to maintain carrying capacity, population size, gene flow, and population viability.

Although habitat assessments were not undertaken as part of this project, general recommendations are outlined below:

- Protect/maintain existing platypus populations in the upper forested catchment areas to prevent any contraction of suitable habitat area.
- Suitable flow regimes are critical for healthy platypus populations (Griffiths, Maino, *et al.* 2019). Key elements to be aware of are protecting baseflows and preventing cease to flow events due to water extraction/diversion or climate change, and minimising high flow variability in urbanized areas along the coast.
- Ensure healthy riparian corridors through agricultural areas by protecting remnant vegetation patches and restoring degraded areas (exclude stock, remove invasive weeds, revegetate with native species). Where possible, aim for a 30 m buffer zone around waterways. With no obvious gaps in the current distribution of platypuses, prioritise reaches adjacent to or connecting existing patches of native vegetation.
- Establish an ongoing platypus monitoring program to build on the baseline provided here to assess the future status of populations, effectiveness of management actions, or impacts of disturbance events. This would include repeat sampling of the sites established in this study and depending on objectives may be undertaken every 2-5 years. This monitoring program could also be effectively undertaken by citizen scientists with simple instructions and training.

Acknowledgments

Funding for the Orara Valley project was provided by Coffs Harbour City Council Environmental Levy program and the Dorrigo Plateau project was funded by Australian Governments Bushfire Recovery for Wildlife and Habitat Community Grants Program awarded to Jaliigirr Biodiversity Alliance.

EnviroDNA would like to thank Justin Couper (Jaliigirr Biodiversity Alliance) and Samantha Hessey (Coffs Harbour City Council), for facilitating the project and comments on this report, as well as all the citizen scientists who participated in water sample collection.

Jaliigirr Biodiversity Alliance acknowledge the traditional custodians of country throughout Australia and their continuing connection to land, sea and community. We respect the Elders past, present and emerging on Gumbaynggirr country

References

Biggs J., Ewald N., Valentini A. *et al.* (2015) Using eDNA to develop a national citizen science-based monitoring programme for the great crested newt (*Triturus cristatus*). *Biol. Conserv.* doi: 10.1016/j.biocon.2014.11.029. [online]. Available from: http://dx.doi.org/10.1016/j.biocon.2014.11.029.

Bino G., Kingsford R. T., Archer M. *et al.* (2019) The platypus: evolutionary history, biology, and an uncertain future. *J. Mammal.* **100** , 308–327.

Bino G., Kingsford R. T. & Wintle B. A. (2020) A stitch in time – Synergistic impacts to platypus metapopulation extinction risk. *Biol. Conserv.* **242** , 108399. [online]. Available from: https://doi.org/10.1016/j.biocon.2019.108399.

Brunt T., Cecil M., Griffiths J., Adams-Hosking C. & Murray P. (2021) Where are the platypuses (*Ornithorhynchus anatinus*) now? A snapshot in time of their distribution in the Greater Brisbane region. *Aust. Mammal.* **43** , 368–372. [online]. Available from: https://doi.org/10.1071/AM20052.

Frankham R., Ballou J. D. & Briscoe D. A. (2010) *Introduction to conservation genetics*. 2nd edn. Cambridge University Press, Cambridge, UK.

Gardner J. L. & Serena M. (1995) Spatial-Organization and movement patterns of adult male platypus, ornithorhynchus-anatinus (monotremata: Ornithorhynchidae). *Aust. J. Zool.* **43** , 91–103.

Grant T. R. (1992) Historical and current distribution of the platypus, *Ornithorhynchus anatinus*, in Australia. In: *Platypus and Echidnas* (ed M. L. Augee) pp. 232–254 Royal Zoological Society of New South Wales.

Grant T. R. (1993) The past and present freshwater fishery in New South Wales and the distribution and status of the platypus Ornithorhynchus anatinus. *Aust. Zool.* **29** , 105–113.

Grant T. R. (1998) Current and historical occurrence of platypuses, Ornithorhynchus anatinus, around Sydney. *Aust. Mammal.*

Grant T. R., Grigg G. C., Beard L. A. & Augee M. L. (1992) Movements and burrow use by platypuses, Ornithorhynchus anatinus, in the Thredbo River, New South Wales. In: *Platypus and Echidnas* (ed M. L. Augee) pp. 263–267 Royal Zoological Society of New South Wales. [online]. Available from: file:///M:/RMC_Res_Mgmt_Cons/BCO_Biodiv_Conserv/WMC_WildlifeAndMarine/WMC_SHARED/fauna/Platypus/DATA/Pdfs/Grant et al 1992.pdf.

Grant T. R. & Temple-Smith P. D. (1998) Field biology of the platypus (*Ornithorhynchus anatinus*): historical and current perspectives. *Philos. Trans. R. Soc. London Ser. B-Biological Sci.* **353** , 1081–1091.

Grant T. R. & Temple-Smith P. D. (2003) Conservation of the platypus, *Ornithorhynchus anatinus*: Threats and challenges. *Aquat. Ecosyst. Heal. Manag.* **6** , 5–18.

Griffiths J., Impey R. & Licul S. (2021) *Assessing landscape-scale impacts of bushfires on platypuses using environmental DNA and citizen scientists*. East Gippsland Landcare Network.

Griffiths J., Kelly T. & Weeks A. (2014) *Impacts of high flows on platypus movements and habitat use in an urban stream. (Report to Melbourne Water)*. Parkville, VIC.

Griffiths J. & Licul S. (2020) *Mapping the distribution of platypuses and blackfish in the upper Campaspe region using citizen scientists and environmental DNA. Prepared for: Upper Campaspe Landcare Network*. EnviroDNA, Parkville, VIC.

Griffiths J. & Licul S. (2022) *Assessing the conservation status and potential impacts of the 2019 / 20 bushfires on platypuses of the Dorrigo Plateau. (Report for Jaliigirr Biodiversity Alliance)*. Brunswick, VIC.

Griffiths J., Licul S. & Weeks A. (2021) *Determining the distribution of platypuses & short-finned eels in the Moorabool River subcatchment. Report for Moorabool Catchment Landcare Group.* EnviroDNA, Parkville, VIC.

Griffiths J., Maino J., Tingley R. & Weeks A. (2020) *Distribution and relative abundance of platypuses in the greater Melbourne area: survey results 2018-20 (Report for Melbourne Water)*. Parkville, VIC.

Griffiths J., Maino J. & Weeks A. (2019) *Identifying key flow variables and quantifying their impact on platypus populations (Report to Melbourne Water)*. **cesar**, Parkville, VIC.

Griffiths J., McColl-Gausden E. F., Connelly F. & Weeks A. (2020) *Assessing the impacts of the 2019/20 bushfires on platypus populations in south eastern Australia*. Cesar Australia, Parkville, VIC.

Griffiths J., van Rooyen A., Song S. & Weeks A. (2018) *Distribution and relative abundance of platypuses in the greater Melbourne area : MWUPP 2013-18*. **cesar**, Parkville, VIC.

Griffiths J., Song S. & Weeks A. (2019) *Determining the distribution of platypuses in the upper Barwon region using environmental DNA. (Prepared for Upper Barwon Landcare Network)*. EnviroDNA, Parkville, VIC.

Griffiths J. & Weeks A. (2014) *Platypus distribution and abundance in the MacKenzie River: impact of fire, April 2014 (Report to Wimmera Catchment Management Authority)*. cesar, Parkville.

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

Griffiths J. & Weeks A. (2018) *Investigating the current distribution of platypuses, rakali, and blackfish in the upper Wimmera region (Report to Project Platypus)*. EnviroDNA, Parkville, VIC.

Gust N. & Handasyde K. (1995) Seasonal Variation in the Ranging Behaviour of the Platypus (Ornithorhynchus anatinus) on the Goulburn River, Victoria. *Aust. J. Zool.* **43** , 193–208.

Klamt M., Davis J. A., Thompson R. M., Marchant R. & Grant T. R. (2016) Trophic relationships of the platypus: Insights from stable isotope and cheek pouch dietary analyses. *Mar. Freshw. Res.* **67** , 1196–1204.

Lintermans M. (1998) The status and distribution of the platypus (Ornithorhynchus anatinus) in the Australian Capital Territory with notes on some localised declines. *Aust. Mammal.*

Lugg W. H., Griffiths J., van Rooyen A. R., Weeks A. R. & Tingley R. (2018) Optimal survey designs for environmental DNA sampling. *Methods Ecol. Evol.* **9** , 1049–1059.

Lunney D., Grant T. & Matthews A. (2004) Distribution of the platypus in the Bellinger catchment from community knowledge and field survey and its relationship to river disturbance. *Proc. Linn. Soc. New South Wales*. [online]. Available from: file:///M:/RMC_Res_Mgmt_Cons/BCO_Biodiv_Conserv/WMC_WildlifeAndMarine/WMC_SHARED/fauna/Platypus/DATA/Pdfs/Lunney, Grant & Matthews 2004.TIF.

Lunney D., Grant T., Matthews A., Esson C., Moon C. & Ellis M. (1998) Determining the distribution of the platypus (Ornithorhynchus anatinus) in the Eden region of south-eastern New South Wales through community-based surveys. *Aust. Mammal.* [online]. Available from: file:///M:/RMC_Res_Mgmt_Cons/BCO_Biodiv_Conserv/WMC_WildlifeAndMarine/WMC_SHARED/fauna/Platypus/DATA/Pdfs/Lunney et al 1998 community survey.pdf.

Marchant R. & Grant T. R. (2015) The productivity of the macroinvertebrate prey of the platypus in the upper Shoalhaven River, New South Wales. *Mar. Freshw. Res.* **66** , 1128–1137.

McLachlan-Troup T. A., Dickman C. R. & Grant T. R. (2010) Diet and dietary selectivity of the platypus in relation to season, sex and macroinvertebrate assemblages. *J. Zool.* **280** , 237–246.

Pilliod D. S., Goldberg C. S., Arkle R. S. & Waits L. P. (2014) Factors influencing detection of eDNA from a stream-dwelling amphibian. *Mol. Ecol. Resour.*

Rohweder D. A. & Baverstock P. R. (1999) Distribution of platypus Ornithorhynchus anatinus in the Richmond river catchment, northern New South Wales. *Aust. Zool.* **31** , 30–37.

Serena M., Thomas J. L., Williams G. A. & Officer R. C. E. (1998) Use of stream and river habitats by the platypus, *Ornithorhynchus anatinus*, in an urban fringe environment. *Aust. J. Zool.* **46** , 267–282.

Serena M. & Williams G. A. (2004) PROGRESS REPORT : AN EXPERIMENTAL REINTRODUCTION OF PLATYPUS (ORNITHORHYNCHUS ANATINUS) TO CARDINIA CREEK APRIL-JUNE 2004).

Serena M. & Williams G. A. (2011) *Distribution and status of platypus in the Gunbower Creek system. (Report to North Central Catchment Authority)*. Australian Platypus Conservancy, Wiseleigh.

Serena M. & Williams G. A. (2012) Movements and cumulative range size of the platypus (Ornithorhynchus anatinus) inferred from mark-recapture studies. *Aust. J. Zool.* **60** , 352–359.

Serena M., Williams G. A. & Johnston L. D. (2002) *Status of platypus in the Curdies River catchment: live-trapping and local sightings, summer 2002. (Report to Corangamite Catchment Management Authority)*. Australian Platypus Conservancy, Whittlesea.

Serena M., Williams G. A., Weeks A. R. & Griffiths J. (2014) Variation in platypus (Ornithorhynchus anatinus) life-history attributes and population trajectories in urban streams. *Aust. J. Zool.* **62** , 223–234.

Smart A. S., Tingley R., Weeks A. R., Van Rooyen A. R. & McCarthy M. A. (2015) Environmental DNA sampling is more sensitive than a traditional survey technique for detecting an aquatic invader. *Ecol. Appl.* **25** , 1944–1952.

Soule M. E. (1980) Thresholds for survival: maintaining fitnessand evolutionary potential. In: *Conservation Biology: An Evolutionary-Ecological Perspective* (eds M. E. Soule & B. A. Wilcox) pp. 151–169 Sinauer, Sunderland, MA.

Thomsen P. F., Kielgast J., Iversen L. L. *et al.* (2012) Monitoring endangered freshwater biodiversity using environmental DNA. *Mol. Ecol.* **21** , 2565–2573.

Verkaik I., Prat N., Rieradevall M. & Reich P. (2014) Effects of bushfire on macroinvertebrate communities in south-east Australian streams affected by a megadrought. *Mar. Freshw. Res.* doi: 10.1071/MF13039.

Verkaik I., Vila-Escale M., Rieradevall M. *et al.* (2015) Stream macroinvertebrate community responses to fire: Are they the same in different fire-prone biogeographic regions? *Freshw. Sci.* doi: 10.1086/683370.

Vieira N. K. M., Clements W. H., Guevara L. S. & Jacobs B. F. (2004) Resistance and resilience of stream insect communities to repeated hydrologic disturbances after a wildfire. *Freshw. Biol.* **49** , 1243–1259. [online]. Available from: https://doi.org/10.1111/j.1365-2427.2004.01261.x.

Wallbrink P., English P., Chafer C. *et al.* (2004) *Impacts On Water Quality By Sediments and Nutrients Released During Extreme Bushfires: Report 1: A Review of the Literature Pertaining to The Effect of Fire On Erosion And Erosion Rates, With Emphasis on The Nattai Catchment, NSW, Following the 2001 Bushf*. Canberra, ACT.

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

Williams G. A. (2010) *Results of a platypus live-trapping survey along Coliban River, Malmsbury, October 2010. (Report to North Central Catchment Management Authority)*. Australian Platypus Conservancy, Wiseleigh.

Woinarski J. & Burbidge A. A. (2016) *Ornithorhynchus anatinus*. The IUCN Red List of Threatened Species.

WWF Australia (2020) *Australia's 2019-2020 Bushfires: The Wildlife Toll - Interim Report*.

Yamanaka H., Motozawa H., Tsuji S., Miyazawa R. C., Takahara T. & Minamoto T. (2016) On-site filtration of water samples for environmental DNA analysis to avoid DNA degradation during transportation. *Ecol. Res.* **31** , 963–967.

Appendix

Table A1. Results of eDNA surveys throughout the Dorrigo Plateau region, including site location, date of collection and volumes (from Griffiths and Licul 2022).

Site Code	Latitude	Longitude	Date sampled	Volume 1 (mL)	Volume 2 (mL)	Positive assays	Test Result
21_blk_a	-30.18807102	152.687643	30/9/2021	350	400	6/6	Positive
22_blk_b	-30.192727	152.547268	28/9/2021	350	400	6/6	Positive
23_blk_c	-30.20901698	152.529523	28/9/2021	400	400	6/6	Positive
24_blk_d	-30.22605697	152.485463	25/9/2021	320	350	6/6	Positive
26_blk_f	-30.25006299	152.385393	28/9/2021	400	400	1/6	Equivocal
27_blk_g	-30.28413802	152.383222	28/9/2021	400	400	6/6	Positive
51_BellU_a	-30.4718904	152.441114	29/9/2021	400	400	0/6	Negative
52_BellU_b	-30.481505	152.5165085	29/9/2021	400	400	5/6	Positive
19_Cld_a	-30.28525701	152.883079	28/9/2021	370	350	6/6	Positive
10_NymM_d	-30.19157197	152.694202	30/9/2021	300	300	5/6	Positive
53_BellU_c	-30.4461558	152.6192278	10/10/2021	230	800	1/6	Equivocal
8_NymM_b	-30.13310449	152.7063417	30/9/2021	400	400	3/6	Positive
15_NymU_e	-30.24217302	152.585359	28/9/2021	400	400	6/6	Positive
12_NymU_b	-30.25122698	152.638012	30/9/2021	400	400	6/6	Positive
16_NymU_f	-30.26726198	152.555967	28/9/2021	450	400	6/6	Positive
9_Lmuz_a	-30.25555556	152.6541667	8/10/2021	400	400	6/6	Positive

EnviroDNA Pty Ltd
+61 3 9028 8753
envirodna.com

Site Code	Latitude	Longitude	Date sampled	Volume 1 (mL)	Volume 2 (mL)	Positive assays	Test Result
31_Lmuz_b	-30.13160298	152.702814	8/10/2021	400	400	6/6	**Positive**
33_Lmuz_d	-30.32432204	152.621627	8/10/2021	400	400	5/6	**Positive**
18_NymU_h	-30.34987201	152.476903	8/10/2021	400	350	6/6	**Positive**
38_Biel_e	-30.32351503	152.717656	8/10/2021	400	400	3/6	**Positive**
11_NymU_a	-30.25222222	152.65	8/10/2021	400	400	5/6	**Positive**
32_dpk	-30.36694896	152.524387	8/10/2021	300	300	3/6	**Positive**
48_Bo_b	-30.22264704	152.830678	23/9/2021	400	400	2/6	**Positive**
49_Bo_c	-30.26632063	152.8628412	23/9/2021	275	230	4/6	**Positive**
50_Bo_d	-30.26611903	152.8607	23/9/2021	400	350	4/6	**Positive**
41_Lnym_b	-30.11522499	152.75288	22/9/2021	300	330	6/6	**Positive**
42_Lnym_c	-30.12296199	152.788591	22/9/2021	450	400	4/6	**Positive**
47_Bo_a	-30.16374003	152.797762	23/9/2021	350	350	0/6	Negative
43_Lnym_d	-30.12590001	152.808403	22/9/2021	400	400	0/6	Negative
44_Lnym_e	-30.16668501	152.831956	22/9/2021	450	400	2/6	**Positive**
45_Lnym_f	-30.17363504	152.837043	22/9/2021	350	350	1/6	Equivocal
46_Lnym_g	-30.21351828	152.8700337	22/9/2021	400	400	4/6	**Positive**
1_NymL_a	-29.92259	152.684677	21/11/2021	400	400	1/6	Equivocal
2_NymL_b	-29.922143	152.703581	21/9/2021	500	400	5/6	**Positive**
3_NymL_c	-29.95162801	152.7334561	21/9/2021	450	450	2/6	**Positive**

EnviroDNA Pty Ltd
+61 3 9028 8753
envirodna.com
—

Site Code	Latitude	Longitude	Date sampled	Volume 1 (mL)	Volume 2 (mL)	Positive assays	Test Result
4_NymL_d	-29.96628324	152.7439189	21/9/2021	400	350	1/6	Equivocal
5_NymL_e	-29.98749671	152.727644	21/9/2021	450	500	5/6	**Positive**
6_NymL_f	-30.05414627	152.7699075	21/9/2021	500	500	3/6	**Positive**
35_Biel_b	-30.28916499	152.703513	23/9/2021	350	400	3/6	**Positive**
37_Biel_d	-30.30623599	152.71489	23/9/2021	350	350	4/6	**Positive**
40_Ulong	-30.23343799	152.884016	22/9/2021	400	400	6/6	**Positive**
30_Mob_b	-30.213995	152.779341	23/9/2021	370	400	4/6	**Positive**
25_Blk_e	-30.23857165	152.4262829	19/10/2021	500	500	3/6	**Positive**
28_Obe	-30.1765657	152.4988272	18/10/2021	500	500	6/6	**Positive**
29_Hyl	-30.21493675	152.4307297	19/10/2021	500	500	6/6	**Positive**
54_BellU_d	-30.43287	152.6776	14/10/2021	725	825	6/6	**Positive**
55_BellU_e	-30.42877	152.77063	14/10/2021	850	750	6/6	**Positive**
56_Rose_a	-30.41669	152.77766	18/10/2021	510	610	6/6	**Positive**
57_BellM_a	-30.41767	152.84782	16/10/2021	160	635	6/6	**Positive**
58_NVNV_a	-30.38719	152.88414	18/10/2021	450	250	2/6	**Positive**
59_NVNV_b	-30.36033	152.90439	14/10/2021	150	150	3/6	**Positive**
60_KLNG_a	-30.49785	152.74911	17/10/2021	500	600	2/6	**Positive**
13_NymU_c	-30.39377	152.86703	18/11/2021	400	450	1/6	Equivocal
14_SANA	-30.3461774	152.6154874	18/11/2021	350	400	5/6	**Positive**

Site Code	Latitude	Longitude	Date sampled	Volume 1 (mL)	Volume 2 (mL)	Positive assays	Test Result
34_Biel_a	-30.3469836	152.6969219	18/11/2021	350	300	3/6	**Positive**
36_Rock	-30.3385844	152.7337334	18/11/2021	400	400	3/6	**Positive**