

A close-up photograph of a platypus snout, showing the dark, leathery skin and the two small, circular eyes. The snout is the central focus of the image, with the fur of the head visible at the top.

Assessing the impacts of the 2019/20 bushfires on **platypus populations in south eastern Australia**



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EnviroDNA



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CONSERVATION
FOUNDATION

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We acknowledge the Traditional Owners of this country and their continuing connection to land, waters and community. We pay respect to elders both past and present.

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13/11/20	2.0	Final Report	JG, EMG	

Abbreviations

Abbreviations	Description
eDNA	environmental DNA
qPCR	quantitative polymerase chain reaction
GLMM	generalised linear mixed models
BACI	before-after-control-impact
ACF	Australian Conservation Foundation

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Executive Summary

Devastating bushfires impacted southeastern Australia during the 2019/20 summer with over 11 million hectares burnt and an estimated 3 billion animals killed or affected. The area was previously considered a stronghold for the iconic platypus – a species under ongoing threats in other parts of its range from drought, altered flow regimes, and habitat destruction. However, little empirical data exists on the impacts of bushfires on platypuses with previous studies hampered by a lack of rigorous pre-fire data.

Leveraging extensive environmental DNA surveys of platypus populations throughout southeastern Australia, we were able to re-survey sites in both fire-affected and control sites from March to September 2020 using a robust Before-After-Control-Impact design. Pre- and post-fire data of platypus occupancy was obtained from 142 sites – 48 sites within the impacted area and 94 in surrounding control areas. Prior to the 2019/20 bushfires, platypuses were detected at 40 of the 48 sites (83%) within the fire extent. After the fires, this decreased to 33 out of the 48 sites (69%), a decline of 14%. In comparison, of the 94 control sites, platypuses were detected at 68 sites prior to the bushfires (70%), and 70 after (75%). The estimated decline was slightly higher (18%) when data was restricted to native vegetation classes only.

Using generalised linear mixed models, we estimated a significant negative impact of bushfires on platypus presence, when the dataset was restricted to native vegetation classes only, reflecting the majority of area impacted by the fires. Other modelling approaches resulted in similar trends although relatively low sample sizes, particularly in impact areas, have limited the statistical power of these analyses. Further surveys scheduled for early 2021 will enable more rigorous analysis and assessment of longer term impacts.

Nature of work

Platypuses occur in a variety of aquatic ecosystems distributed throughout eastern Australia (Grant 1992; Grant and Temple-Smith 1998). Despite challenges in studying platypuses in the wild and assessing population trends (Grant and Temple-Smith 2003; Lunney *et al.* 2008), there is mounting evidence of population declines and localised 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 *et al.* 2019; Serena and Williams 2011; Serena *et al.* 2002; Griffiths *et al.* 2020; Serena *et al.* 2014; Williams 2010). Based on this evidence, the conservation status of platypuses was recently upgraded to Near Threatened by the International Union for Conservation of Nature (Woinarski and Burbidge 2016) with populations in Victoria considered under the most stress. The major threats to platypuses are considered land use changes in the surrounding catchment, clearing riparian vegetation, drought, water diversion and impoundment altering flow regimes, and poor water quality with major threats expected to increase due to climate change and growing human population (Grant and Temple-Smith 2003; Bino *et al.* 2019, 2020). However, bushfires have not previously been considered a major threat to platypus populations.

Southeastern Australia experienced devastating bushfires during late 2019 and early 2020. Over 11 million hectares were burnt with an estimated 3 billion animals killed or affected (WWF Australia 2020). While much of the media attention was focused on terrestrial species such as the koala, aquatic species like the platypus may also have been severely impacted. Although aquatic species may be somewhat buffered from the immediate impacts, bushfires can have significant short- and long-term impacts of aquatic ecosystems. Smaller waterways may completely vaporise during the fires, while subsequent runoff of sediment and ash from the surrounding catchment that has been stripped of vegetation, is expected to degrade waterways for an extended period. However, little empirical data exists on the impacts of bushfires on platypuses populations and the consequences of the recent bushfires on their conservation status (Bino *et al.* 2020).

Burnt areas are highly susceptible to erosion and heavy rainfall events following fires can result in large 'slugs' of ash, sediment, and nutrients entering waterways and adversely affecting water quality and ecosystems for large distances downstream (Wallbrink *et al.* 2004). Previous research has demonstrated that bushfires can potentially have significant impacts on aquatic fauna. Mass fish die-offs and local extirpation of fish species have been recorded following bushfires, attributed due to large sediment slugs and resultant low dissolved oxygen levels (Lyon and O'Connor 2008; Rinne 2003). Recovery of fish populations following fires in 2003 was not recorded for 24 months (Lyon and O'Connor 2008). Aquatic macroinvertebrates are also known to be significantly impacted by bushfires with an overall reduction in abundance and diversity (Vieira *et al.* 2004; Verkaik *et al.* 2014, 2015). Such impacts on macroinvertebrate communities can last for years (Malison and Baxter 2010) with some populations taking 15 years to fully recover (Minshall 2003). Aquatic macroinvertebrates are the primary food source for platypuses (McLachlan-Troup *et al.* 2010; Marchant and

Grant 2015) and therefore any reduction in macroinvertebrates communities are likely to have a significant impact on platypuses.

To date, limited research on the impacts of bushfires on platypuses has indicated variable outcomes. Following the “Black Saturday” fires in 2009, some populations were able to persist or rapidly recolonise fire affected areas while other populations disappeared and have still not returned. Bushfires were identified as a major factor behind the local extirpation of an isolated platypus population near Melbourne (Serena and Williams 2004). Conversely, an intense localised fire in western Victoria appeared to have limited impact on a vulnerable local population although consistent flows in the river facilitated by releases from an upstream impoundment may have ameliorated any impacts (Griffiths and Weeks 2014). The response to bushfires is likely to depend extent and severity of fires, as well as other variables such as isolation, size of waterway, and conditions following the fires. However, determining impacts of bushfires on platypuses has been hampered by availability of systematic pre-fire data at a local or landscape scale. Demonstrating that platypuses persist in fire affected areas does not preclude reductions in abundance, survival or juvenile recruitment. The extent and severity of the 2019/20 bushfires as well as the significance of the area affected for platypuses in southeastern Australia is unique and unprecedented and it's critical to understand the impacts on platypus populations and potential consequences for their conservation status.

During 2018-19, **cesar** (and our collaborators San Diego Zoo Global and University of Melbourne) were undertaking a large-scale investigation of the conservation status of platypuses and identifying their major threats. This included a number of sites within the fire-affected area as well as the surrounding landscape. Here, we leverage this extensive pre-fire baseline data, to provide a comparison of platypus occupancy before and after the 2019/20 bushfires to quantify the impacts of bushfire on platypus populations at a landscape scale for the first time.

Methods

Platypus occurrence

The distribution of platypuses throughout the study area was spatially mapped using environmental DNA (eDNA). 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). Sampling sites for the original project used for pre-fire data were originally selected through a hierarchical stratified random design to evaluate the impacts of major threatening processes such as land use, changes to flow regimes, and riparian vegetation. To assess the impacts of the 2019/20 bushfires, we re-surveyed these sites from March to October 2020 in both fire-affected (impact) and unburnt (control) areas where existing pre-fire eDNA data was available using a before-after-control-impact (BACI) design. BACI sampling designs are robust and statistically powerful to isolate impact effects from natural variability (Chevalier *et al.* 2019).

At each site, water samples were collected in duplicate by passing 140 – 500 ml (average 267 ml) through a 0.22 µm filter (Sterivex) on site using a sterile syringe. Filtering on site reduces DNA degradation that may occur during transport of water (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 refrigerated or on ice 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). A platypus specific probe targeting a 57 base-pair sequence of the mitochondrial cytochrome b (CytB) gene (Lugg *et al.* 2018) was used to screen all samples for the presence of platypus DNA. 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 as well as an Internal Positive Control (IPC) to detect inhibition (Goldberg *et al.* 2016). There was no evidence of sample contamination from field or laboratory procedures with all controls returning a negative result, indicating sampling and analysis protocols were robust.

Occupancy modelling

To explore the probability that platypuses occupy sites across the study area, we used site occupancy detection models. Occupancy models such as these are used to account for imperfect detection, an important consideration for whichever sampling method is used. The models are ideally suited to eDNA data where there are different levels of data collection (site, sample and PCR replicate) and the potential for false positives or false negative detections. To estimate the probabilities of occupancy and detection, we used a three-level occupancy model (Lugg *et al.* 2018). The three levels in this model enabled us to distinguish between occupancy process (where some sites are occupied or not), the sampling process (where some waters sampled contain eDNA and others do not) and the qPCR process (where, given eDNA is present in the

water sample, platypus DNA is detected or not). The observed data took the form of binary detection/non-detection observations of platypus in each qPCR, in each sample, at each site. To account for spatial variation in occupancy, we included a random effect for site on this parameter (see Appendix 2 for more detailed model description).

Data was split into 4 categories: before/control, before/impact, after/control and after/impact. Two separate analyses were conducted using different variations of the collected data. The first analysis separated control and impact sites using the National Indicative Aggregated Fire Extent Dataset (NIAFED) with a 5 km buffer to take into account the potential effects beyond the fire extent. Sites which were located within this fire extent were classified as “impact”. Sites outside of the extent were “control”. To control for variation due to differences in habitat types, the second analysis restricted both control and impact sites to the VAST (Vegetation Assets, States and Transitions) native vegetation classes — classifications 0 to 3 — as most of the fire impacted sites were within these classes.

BACI GLMM

To investigate how occupancy of platypuses has changed as a result of the 2019/20 bushfires in the short term, we used a before-after, control-impact analysis. We used generalised linear mixed models (GLMM) to quantify the influence of before/after, control/impact and the interaction between these factors, on the detection of platypuses. The response data took the form of binary detection/non-detection (0/1) where a detection of platypus DNA in any of the qPCRs in a sample, at a site, would class that site as positive for that visit. We fitted binomial GLMM's using the package “glmer”. The model had 3 fixed effects: Period, SiteClass and Period:SiteClass (the interaction)(Pardini *et al.* 2018). Site was included as a random effect. Model residuals were analysed using the “DHARMA” package. Analysis of the simulated model residuals indicated good model fit. We extracted model coefficients for the predictor variables and explored the relationship between detection and a predictor variable by considering the size and uncertainty (95% confidence intervals) of model coefficients. This analysis was conducted using the two variations of the dataset described above. We also investigated the relationship between before/after and control/impact using the GLMM to predict the probability of a presence at a site.

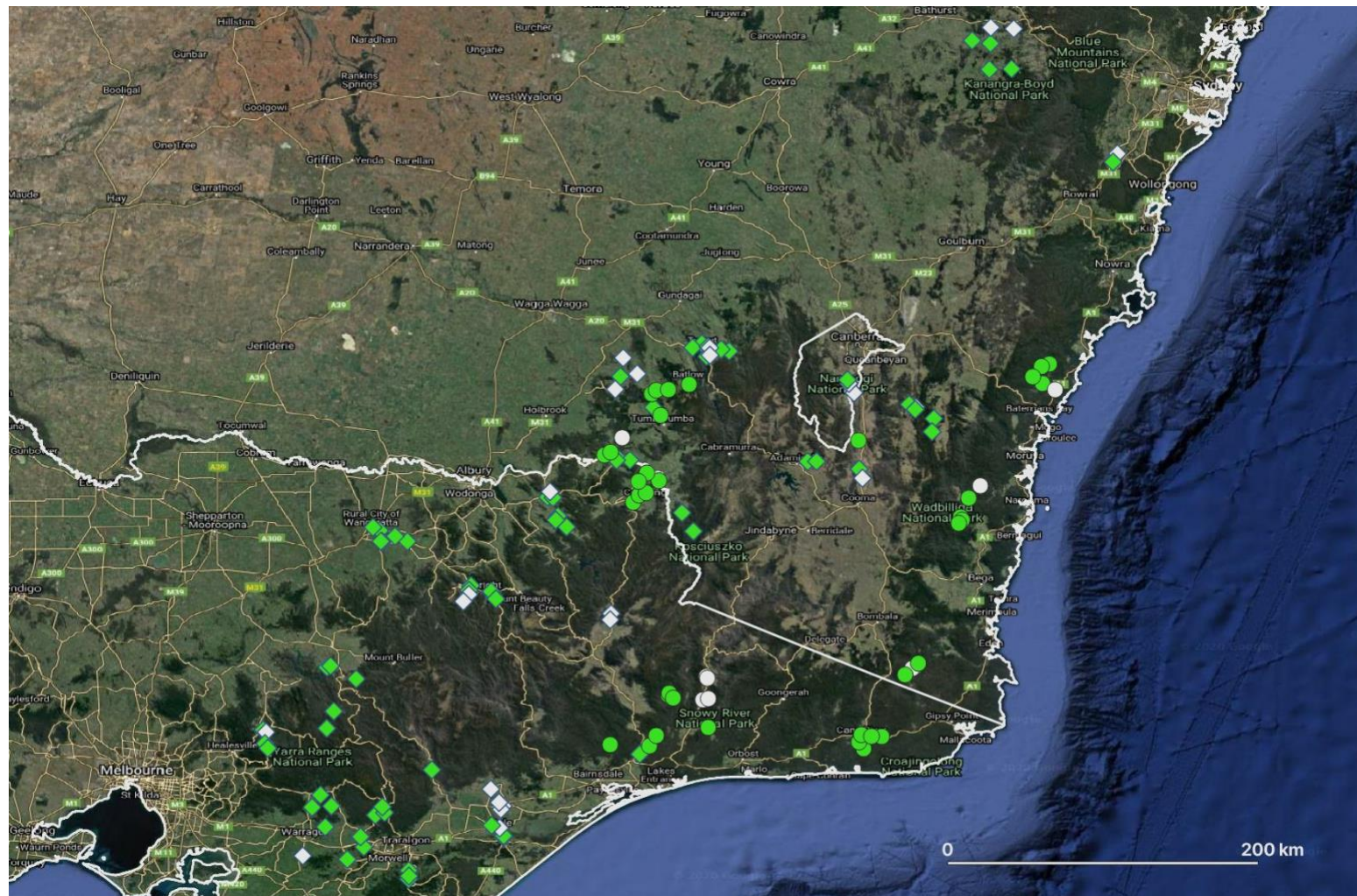


Figure 1. Occurrence of platypuses at control (diamonds) and impact sites (circles) before the 2019/20 fires. Green = detected; Grey = not detected.

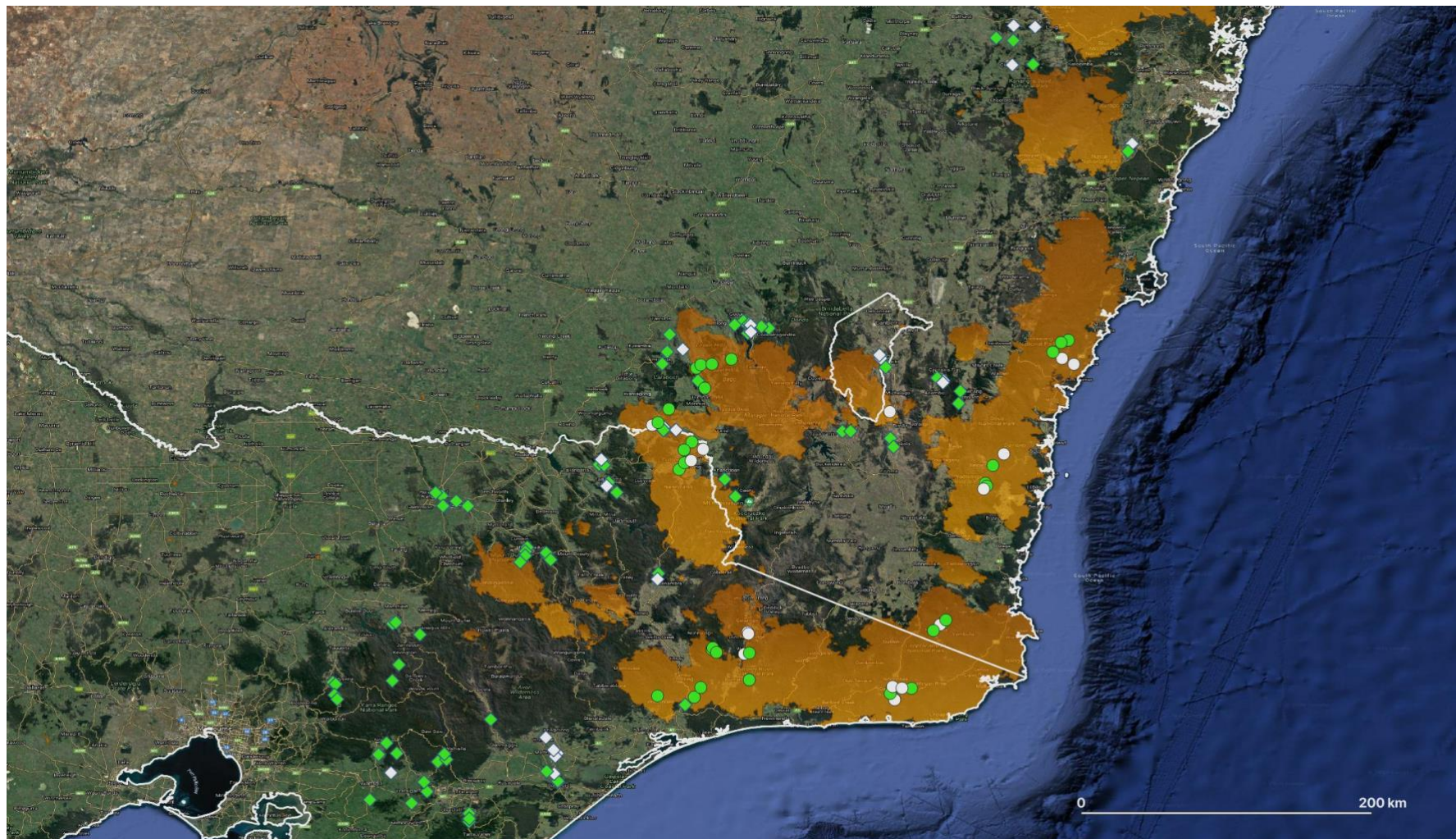


Figure 2. Occurrence of platypuses control (diamonds) and impact sites (circles) after the 2019/20 fires. Green = detected; Grey = not detected.

Findings

A total of 142 sites were sampled in both the pre-fire (2018/19) and post-fire period (2020). Forty-eight of these sites were within the fire extent of the National Indicative Aggregated Fire Extent Dataset (NIAFED). Prior to the 2019/20 bushfires, platypuses were detected at 40 of the 48 sites (83%) within the fire extent. After the fires, this decreased to 33 out of the 48 sites (69%), a decline of 14%. In comparison, of the 94 control sites, platypuses were detected at 68 sites prior to the bushfires (70%), and 70 after (75%).

Occupancy modelling

For the site occupancy models, we included a 5 km buffer around the fire extent to expand the potential affected area, to incorporate adjacent and downstream impacts, acknowledging that downstream impacts may be evident over a much larger extent. The mean posterior estimate for the occupancy probability was high for all categories (Before/Control, Before/Impact, After/Control and After/Impact). Before the fires, the impact sites had a slightly higher occupancy probability compared to the control sites. This relationship was reversed after the fires where the control sites then had a higher probability of occupancy (Figure 3). The estimate for After/Impact (post-fire sampling in fire-affected areas) was lower than the other three categories, suggested a potential decline in occupancy, but with larger uncertainty. The 95% credible intervals overlapped for all four groups.

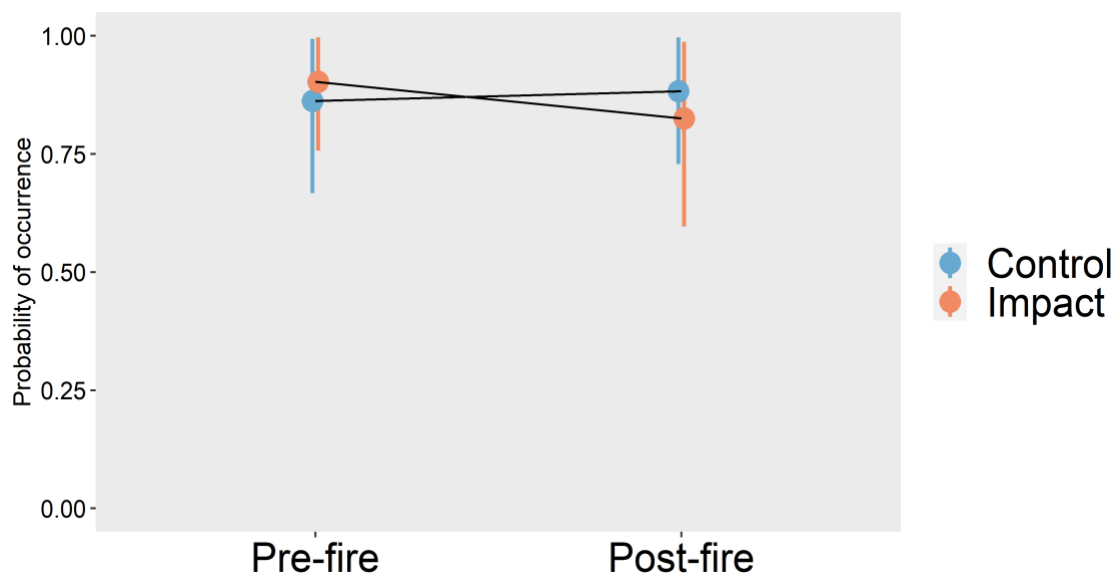


Figure 3. Mean estimates of occupancy of platypuses for the 4 categories. Dots represent means and vertical line represent 95% credible intervals. 5 km buffer of fire extent used.

When the data was restricted to VAST native vegetation classes, 39 sites were within the impact area (plus 5 km buffer) with platypuses detected at 32 sites before (82%) and 25 sites after the fires (64%). In contrast, of the 46 control sites in native vegetation, platypuses were detected at 38 sites before (82%) and 40 sites after the fires (86%). The

site occupancy model produced a similar pattern as the previous model but suggested a larger decline in the After/Impact group compared to the other groups (Figure 4). However, confidence intervals were also larger due to smaller sample sizes and overlapped between all 4 groups.

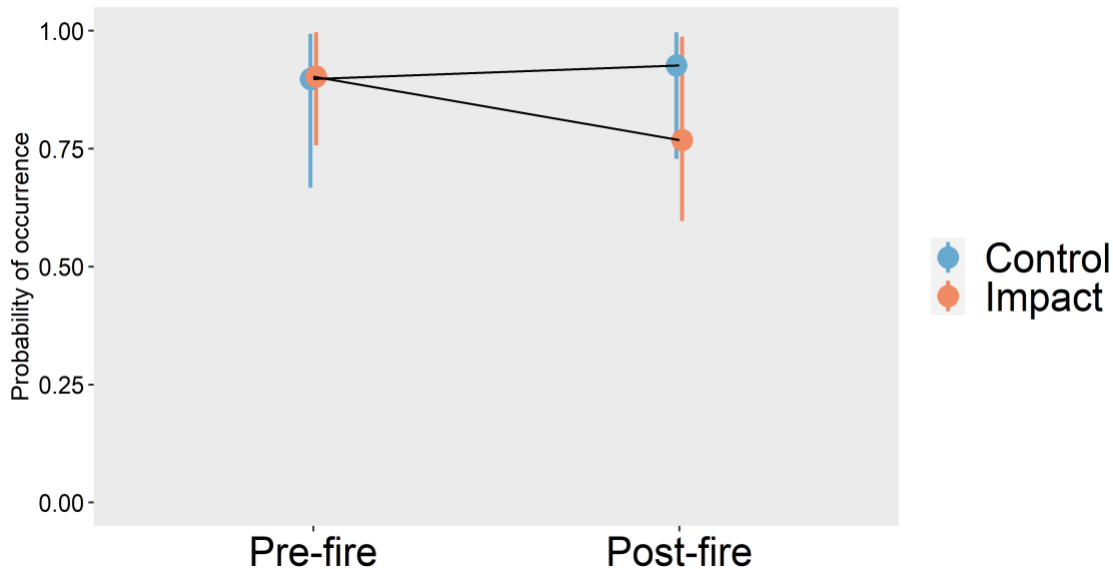


Figure 4. Mean estimates of occupancy of platypuses for the 4 categories. Dots represent means and vertical lines represent 95% credible intervals. Data restricted to native vegetation categories 0-3 of the VAST dataset. 5 km buffer of fire extent used.

BACI GLMM

The model coefficients from the GLMM's indicated a negative effect of "impact" compared to "control", with a significant interaction term of the time period (Before/After) and the site class (Control/Impact). This suggests there are significant differences between the groups (Control/Impact) over time (Before/After) corresponding with the observed trend of lower detections in the After/Impact group.

Using predictions from the GLMM, the mean predicted presence of platypus differed before and after fire, as well as between control and impact groups for the all data scenario (Figure 5). There is a downward trend in predicted presence for the impact sites but 95% credible intervals overlapped between all four groups. However, when the data was restricted to VAST native vegetation classes, a significant difference was detected between control and impact sites after fire (Figure 6).

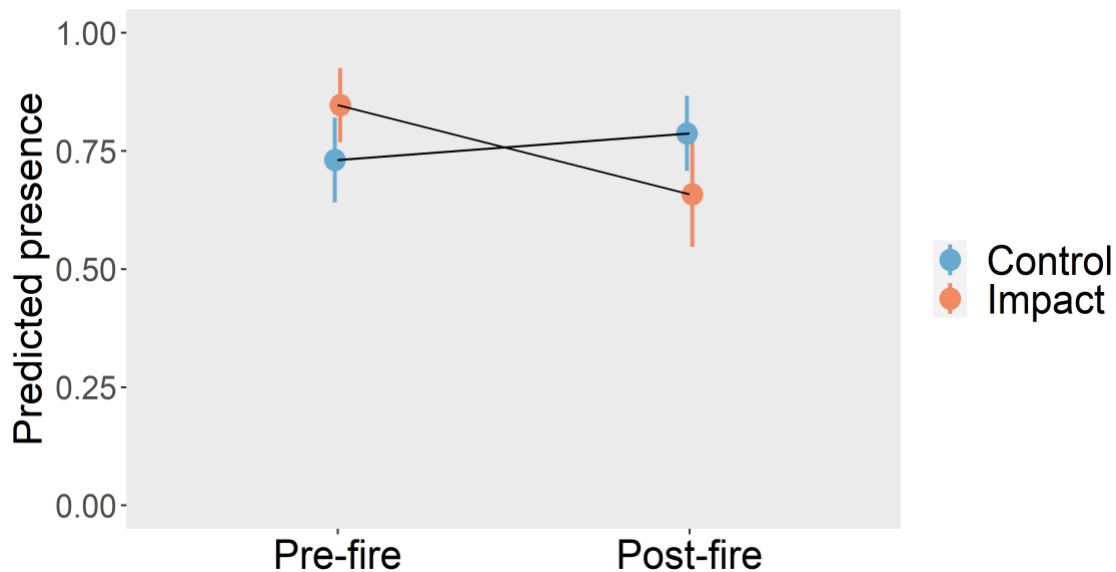


Figure 5. Predicted probability of platypus presence from the GLMM. Dots represent means and vertical lines represent 95% confidence intervals. 5 km buffer of fire extent used.

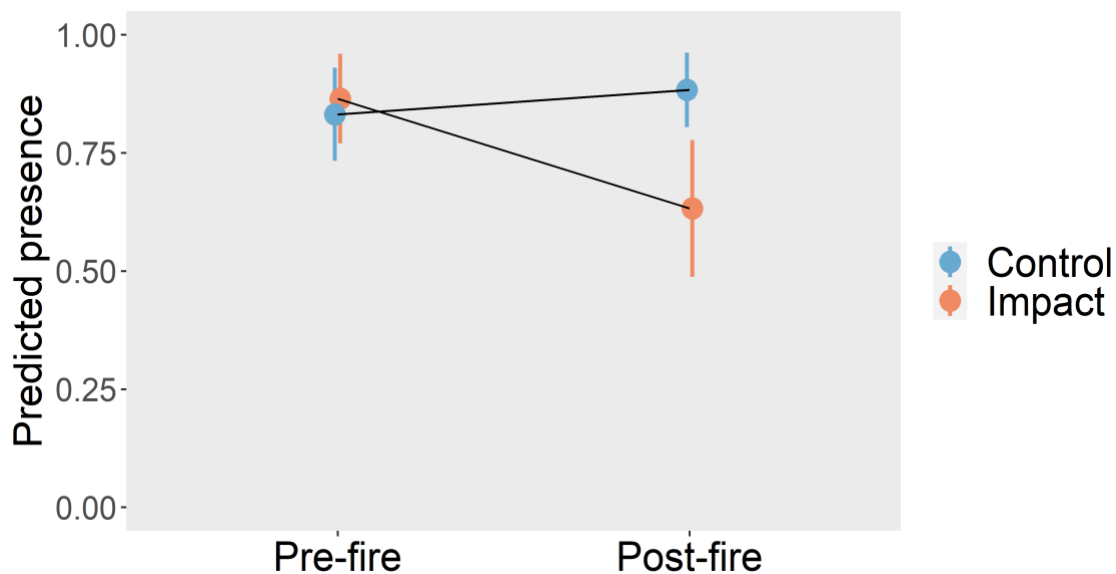


Figure 6. Predicted probability of platypus presence from the GLMM. Dots represent means and vertical lines represent 95% confidence intervals. Data restricted to native vegetation categories 0-3 of the VAST dataset. 5 km buffer of fire extent used.

Conclusions

The area impacted by the 2019/20 bushfires appeared to support relatively healthy platypus populations before the fires with over 80% estimated site occupancy based on recent surveys using environmental DNA. Raw data from pre- and post-fire eDNA surveys have indicated a decline of 14-18% in occupancy of platypuses in fire affected areas compared to unburnt control sites in the 9 months after the bushfires. Analysis of the data using a generalized linear mixed model using data restricted to VAST native vegetation classes only, predicted a significant decline in the probability of platypus presence at fire impacted sites compared to control sites. Similar trends were revealed with other modelling approaches although relatively low sample sizes, particularly in impact areas, have limited the statistical power of these analyses. Difficulties in site access due to road closures and then travel restrictions and border closures caused by Covid-19 severely limited field sampling during 2020. Further sampling during 2021 will enable more rigorous analysis and assessment of longer term impacts.

As indicated by previous research, the potential impacts of bushfires on platypus populations are likely to be complex and varied. Direct impacts from heat and reduction in surface water may cause mortality or forced migration of platypuses and exacerbate predation pressure, particularly in smaller waterways. Mass dispersal from fire-affected areas may place additional stress on populations in surrounding areas that don't have the resources to support additional animals, exacerbating intra-species competition.

The largest and most widespread impacts 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. However, a decrease in overall food resources will lead to reduced carrying capacity of affected waterways and longer term mortality or migration from starvation. Such impacts may extend well beyond the burnt areas as ash and sediment is transported downstream. The timing and intensity of rainfall events following bushfires, as well as speed of vegetation regrowth, are likely to be significant factors governing the severity, extent, and duration of post-fire impacts on aquatic ecosystems.

Given that fire-affected aquatic systems may take years to recover (Lyon and O'Connor 2008; Minshall 2003; Malison and Baxter 2010) the impacts from these devastating bushfires may be felt by platypus populations for some time. In areas where platypuses still occur there may still be declines in abundance so the decline in occupancy identified here may not fully represent the true impacts of these bushfires. In addition, there may be longer term impacts of further declines or reduction in reproduction. Many mammals will halt or delay reproduction in times of nutritional stress and several long-term monitoring programs have revealed reproductive output was lower in platypus populations during the Millennium Drought (Griffiths *et al.* 2016; Griffiths and Weeks 2017). Conversely, some areas may see rapid recolonization if conditions

improve and platypuses persist in nearby, hydrologically connected refuge areas. Further monitoring planned for 2021 will help to assess these outcomes.

The 2019/20 bushfire affected a significant amount of the best remaining platypus habitat in southeastern Australia. Research undertaken immediately before the bushfires revealed the affected area to be a stronghold for platypuses with high occupancy compared to other areas of Victoria and southern NSW (**cesar** unpublished data). It has been estimated that 13.6% of available platypus habitat across their entire range was impacted during the 2019-20 bushfires (UNSW unpublished data). An estimated 15% decline in platypuses throughout fire affected areas would equate to a loss of 2% of Australia's entire platypus population in a single catastrophic event. This is not only significant for platypuses at a local and regional scale but would have consequences for their national conservation status.

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Appendix 1. Photos of fire-affected sites.



Appendix 2. Site occupancy model description

Occurrence:

$$z_i \sim \text{Bernoulli}(\psi_i)$$

Availability:

$$a_{ij} | z_i \sim \text{Bernoulli}(z_i \theta_{ij})$$

Detection:

$$y_{ijk} | a_{ij} \sim \text{Bernoulli}(a_{ij} p_{ijk})$$

Occupancy process:

$$\text{logit}(p_{ijk}) = \alpha +$$

Equation 1 describes the occupancy of the species at site i ($z_{ik} = 1$ where the species is present, and $z_{ik} = 0$ where the species is absent), given the mean probability of occurrence for that species across all sites (ψ_i).

Equation 2 describes the availability process ($a_{ijk} | z_i = 1$ where the species DNA is available to detect in a water sample, and $a_{ijk} | z_i = 0$ where the species DNA is not available to detect), in replicate j at site i . This is a function of the occurrence (z_i) of the species at site i as well as the availability probability θ .

Equation 3 describes the observed detection process ($y_{ijk} | a_{ij} = 1$ where the species is detected and $y_{ijk} | a_{ij} = 0$ where the species is not detected), at site i for replicate k and in PCR k . This is a function of the occurrence (z_i) of the species at site i as well as the probability of detecting an individual (p_{ijk}) at site i with replicate j in PCR k .

To account for spatial variation in platypus occupancy, we included site-level random effects, ϵ_s , for this parameter (on the logit scale).

Model fitting

Models were fit using the package rjags v.4-8 in R version 3.6.3 (R Development Core Team, 2018). Three model chains were run for 30,000 iterations each. The first 10,000 samples were discarded, and the remaining samples were thinned by a factor of 10, resulting in 2,000 samples per chain from the posterior distribution. Convergence was measured using traceplots as implemented in the jagsUI package v.1.5.0 and indicated that chains were well mixed. Gelman-Rubin statistic values \hat{R} were below 1.1, indicating successful convergence of chains.

