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Platypus Results Report

The Great Australian Platypus Search Victoria 2021

Prepared by:
Josh Griffiths, Dr Rachael Impey, Sam Marwood, Hilary van Leeuwen, and Dr Andrew Weeks

EnviroDNA
Level 1, 95 Albert St
Brunswick VIC 3056

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Project team - Victoria

Title	Name	
Project Manager	Josh Griffiths	EnviroDNA
Laboratory Manager	Dr Rachael Impey	EnviroDNA
Project Coordinator	Sam Marwood	Odonata
Project Communications	Hilary van Leeuwen	Odonata
Project Supervisor	Dr. Andrew Weeks	EnviroDNA

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Abbreviations

Abbreviations	Description
GAPS	Great Australian Platypus Search
eDNA	environmental DNA
ALA	Atlas of Living Australia
VBA	Victorian Biodiversity Atlas

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Contents

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Summary

- The Great Australian Platypus Search is the largest systematic investigation of platypuses ever undertaken with platypus occupancy assessed at over 1800 sites throughout all Victorian River Basins in 2021.
- Results broadly agree with expected platypus occurrence from available previous data at a landscape scale.
- Results indicate a general pattern of lower occupancy and restricted distribution in the drier western and central basins and higher occupancy and more extensive distribution in eastern basins in Victoria.
- A comparison of results from the audit subprogram indicates that samples collected by citizen scientists for eDNA analysis provide reliable data although slightly higher detections may be achieved by trained ecologists.
- The Generalised Random Tesellation Stratified sampling design purposefully selected many sites in smaller waterways where no previous data existed and filled many gaps in our knowledge of platypus occurrence within basins. Platypus DNA was not detected in many of these streams leading to relatively low occupancy estimates at the basin scale.
- The results include several interesting detections, or possible detections, where no recent records of platypuses exist and were thought to have disappeared such as Eumeralla River in Portland Coast Basin, upper Wimmera River near Crowlands, and the furthest downstream detection in the Mackenzie River for decades.
- Results also included several anomalous detections where platypuses are not expected to occur such as Tidal River on Wilsons Promontory and Forge Creek near Paynesville.
- The remaining sites funded for the program (187) will now be used to investigate some of these results above, such as areas where higher detections were expected (e.g. Glenelg River, Hopkins River), as well as areas to fill some gaps in the sampling program (e.g. Broken River).

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Background

Project background

Freshwater systems possess substantial economic, cultural, and scientific value, but are among the most threatened in the world (Dudgeon *et al.* 2006; Geist 2011). In Australia, aquatic ecosystems and their dependent fauna, such as the platypus, are facing significant threats from anthropogenic pressures which are likely to intensify in the future, plus in some instances be compounded by climate change, particularly in southern Australia (Lake and Bond 2007). Effective and systematic monitoring programs underpin biodiversity conservation efforts and are critical to understand and mitigate these threats. Yet accurate knowledge on the distribution, abundance and ecology of freshwater species is hindered by the fact that current monitoring methods are often expensive, inaccurate and can be highly invasive (Goldberg *et al.* 2011). These limitations are amplified when considering species that occupy large spatial extents and are difficult to survey.

Odonata and EnviroDNA have developed a national aquatic monitoring program utilizing environmental DNA and citizen scientists to generate landscape scale data across a range of aquatic ecosystems. The platypus was chosen as a flagship species in recognition of growing concerns of its conservation status, lack of systematic data, and capacity to engage the broader community. However, the sampling undertaken will support investigations into the landscape scale distribution of a range of other aquatic vertebrates in addition to platypus. Stage 1 of this program was rolled out in Victoria in late 2021. This report focuses on the platypus results with the broader aquatic biodiversity data to be reported later.

Platypus background

The platypus (*Ornithorhynchus anatinus*) is a semi-aquatic mammal that inhabits a variety of freshwater habitats along the east coast of Australia (Grant 1992; Grant and Temple-Smith 1998). Unlike many Australian mammals, the broad geographic distribution of platypuses does not appear to have changed significantly since European settlement leading to little concern about their conservation status until recently. However, as a semi-aquatic species, the platypus is potentially vulnerable to a range of natural and anthropogenic threats that degrade aquatic ecosystems including drought, altered flow regimes from water diversion and impoundment, changes to surrounding catchment area due to agriculture or urbanisation, removal of riparian vegetation, habitat fragmentation, poor water quality, and predation from invasive predators (Grant and Temple-Smith 1998, 2003; Bino *et al.* 2020). Challenges in assessing platypus abundance and occurrence, as well as the lack of long-term studies and systematic historical data, have hampered attempts to quantitatively assess the impacts of

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various threatening processes at a local or landscape scale or to rigorously assess their conservation status (Hawke *et al.* 2019; Bino *et al.* 2019).

Despite difficulties 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 (Serena and Williams 2004; Grant 1992, 1998; Lintermans 1998; Lunney *et al.* 1998, 2004; Rohweder and Baverstock 1999; Grant 1993; Griffiths and Weeks 2018; Griffiths, Maino, *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 (IUCN) (Woinarski and Burbidge 2016). Platypus populations in Victoria are considered under the greatest stress with significant declines revealed through long term studies in the greater Melbourne region (Griffiths and Weeks 2011; Griffiths *et al.* 2017, 2018) and Wimmera Catchment (Mitrovski 2008; Griffiths and Weeks 2018; Josh Griffiths *et al.* 2016). Localised declines have also been reported elsewhere in Victoria (Serena and Williams 2008, 2010; Serena *et al.* 2002) including in the Coliban River (Williams 2010). These data as well as concerns for future impacts of climate change (Klamt *et al.* 2011) and increasing human population growth led the Victorian Government to list the platypus as Vulnerable under the FFG Act in 2021.

Landscape scale data on populations is required to properly understand the status of a species like the platypus that is widely and relatively sparsely distributed across a vast area. Until recently, such data was impossible to accumulate due to limitations of traditional monitoring techniques. Live-trapping surveys require specialist training and equipment, can be limited by environmental conditions, are time and labour intensive, logistically difficult and cost-prohibitive. Observational surveys can be difficult to implement in remote areas or where visibility is limited, by necessity need to occur during daylight when platypuses are much less active, are typically biased towards population centres and have moderate risk of false positives due to misidentification. Both techniques can have poor sensitivity to detect platypuses at low abundance, and are difficult to systematically implement over large spatial scales. More recently, non-invasive sampling techniques have been developed that detect species-specific DNA from environmental samples such as water or soil. Quantitative comparisons with traditional sampling methods indicate that environmental DNA (eDNA) methods are superior in terms of sensitivity and cost efficiency, particularly for scarce, elusive or cryptic species (Biggs *et al.* 2015; Smart *et al.* 2015), including platypuses (Lugg *et al.* 2018; Weeks *et al.* 2015), enabling effective detection at low densities.

In response to the platypus being listed as Vulnerable in Victoria, Odonata formed a partnership with EnviroDNA (along with other funding and technical partners) to develop the Great Australian Platypus Search (GAPS) in recognition of the need for systematic, landscape scale data on platypuses across their range to improve understanding of the species' true conservation status, help identify major threats and inform management interventions. The

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initial phase of the project focussed on platypuses; however, the overall aim is to document aquatic vertebrate biodiversity more generally using eDNA detection methods across Australia.

During spring 2021, the first stage of GAPS was implemented throughout Victoria. Importantly, the relatively simple water sampling methods that can be used for eDNA detection presented an opportunity for the project to actively engage with citizen scientists with limited training or experience (e.g. Biggs *et al.* 2015; Griffiths, Song, *et al.* 2019). The platypus is an ideal flagship species for aquatic ecosystems with broad habitat requirements, extensive distribution, and widespread public appeal as a charismatic and iconic Australian species. This project aimed to address the lack of contemporary data on platypus populations by systematically investigating their occurrence in a range of waterways and habitat types across Victoria. By actively involving citizen scientists, project costs were minimised while raising awareness of broader river health and conservation issues in the community. This model is likely to be used as the program moves to other states.

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Methods

The contemporary distribution of platypuses was investigated using environmental DNA at up to 2000 sites throughout Victoria (Figure 1). Sampling sites for the core program (1500 sites) for platypuses and other aquatic vertebrates were generated using a Generalised Random Tessellation Stratified (GRTS) design by experts at the Centre for Freshwater Ecosystems, LaTrobe University (Shackleton *et al.* 2021). Using a GRTS design ensured that sampling was spatially balanced across river basins, and clumping among sites that can arise through the use of other random sampling methods was avoided. There were some limitations placed on the pool of prospective sites that were considered, with unnamed streams likely to be highly ephemeral excluded, and only sites on public land where a stream intersects with a road or track for ease of access included. Sites were then reviewed by staff from relevant Catchment Management Authorities and Waterwatch to check likely accessibility and water availability. Where required, replacement sites were selected within the same system (see Shackleton *et al.* 2021 for further details of the sampling design). A further 300 sites were allocated to project collaborators (WWF, Ross Trust, Environment Education Victoria, Waterwatch, Traditional Owner groups, Parks Victoria, La Trobe University) to complement the main survey design. Approximately two hundred sites from the original sampling design were also randomly sampled by the GAPS team to verify sample quality and results from citizen scientists.

Water sampling for eDNA was undertaken from September 2021 to January 2022. Sampling was originally intended to be completed during September and October to ensure adequate surface water availability in many waterways and target the breeding season for platypuses in Victoria when they are most active and therefore likely to be more detectable (Bethge 2002; Grant 2007; Griffiths *et al.* 2014). Unfortunately, surveys were delayed due to Covid movement restrictions (Melbourne was in lockdown during September and October), sourcing consumables and delivery delays (also affected by Covid), and weather conditions; thus many of the sites were actually surveyed in November and December 2021.

Water sampling was primarily undertaken by citizen scientists following detailed instructions and demonstration of correct sampling techniques by EnviroDNA (water sampling procedure can be viewed here - https://www.youtube.com/watch?v=30G16kOFN7U&t=1s). Staff from the Victorian Department of Environment, Land, Water and Planning (DELWP) and Parks Victoria also assisted with sampling in more remote areas. Citizen scientists registered their interest and selected sites through the project website (www.thegreataustralianplatypussearch.org). Sampling kits were mailed out to participants for each of their assigned sites along with safety and sampling instructions and a reply-paid mailing satchel to return samples. A smartphone app (The Great Aus Platypus Search) was also developed for participants to record sample details, undertake a river health assessment (Appendix 1 – primarily for education and engagement rather than data for analysis) and automatically record location coordinates. If citizen scientists were unable to collect samples from their designated sites for any reason (e.g. road closures, no safe access, inadequate water) they were instructed to initially search

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the immediate area (i.e. up to 500 m up- and down-stream) for a suitable site. If the area is still not suitable for sampling, they could then select another site within 5 km and along the same waterway if possible.

At each site, water samples were collected in duplicate by passing water through two 1.2 μM syringe disc filters. Sample volumes varied widely from 7 to 3430 mL (average 347 mL). Filtration was undertaken on site to reduce DNA degradation during transport of whole water samples (Yamanaka et al. 2016). Clean sampling protocols were employed to minimise contamination including new sampling equipment at each site, not entering water, and taking care not to transfer soil, water or vegetation between sites. A preservative (approx. 0.5 ml 10xTris-EDTA) was added to the filters after filtering to minimise DNA degradation. Filters were stored out of sunlight and at ambient temperature before being sent back to the laboratory for processing. Due to concerns about sample quality, samples were excluded from analysis if sample volume was less than 50 mL, samples were not received within two weeks of sample date (typically due to postage delays), or if adequate metadata was not supplied.

DNA was extracted from the filters using a commercially available DNA extraction kit (Qiagen Power Soil Pro Kit) that minimises compounds that can inhibit the PCR reaction in environmental samples. Real-time quantitative Polymerase Chain Reaction (qPCR) assays were used to amplify the target DNA, using species-specific markers targeting a small region of the mitochondrial DNA, previously developed and assessed for specificity and sensitivity (e.g. Weeks *et al.* 2015; Lugg *et al.* 2018). Assays were performed in triplicate on each DNA extraction for a total of six assays per site. Negative controls were included for both the DNA extraction and qPCR steps. At least two positive qPCR assays (out of six assays undertaken for the site) were required to classify the site as positive for the presence of platypus DNA. This approach is conservative but minimizes false positives. If only a single qPCR out of six was positive, we highlight these as 'possible detections' that should be investigated further. While trace amounts of DNA may indicate the target species is present in low abundance, it may also arise from sample contamination, non-specific amplification through the sampling or laboratory screening process, inadvertent movement of the target species DNA by other fauna (or humans) or dispersal from further upstream.

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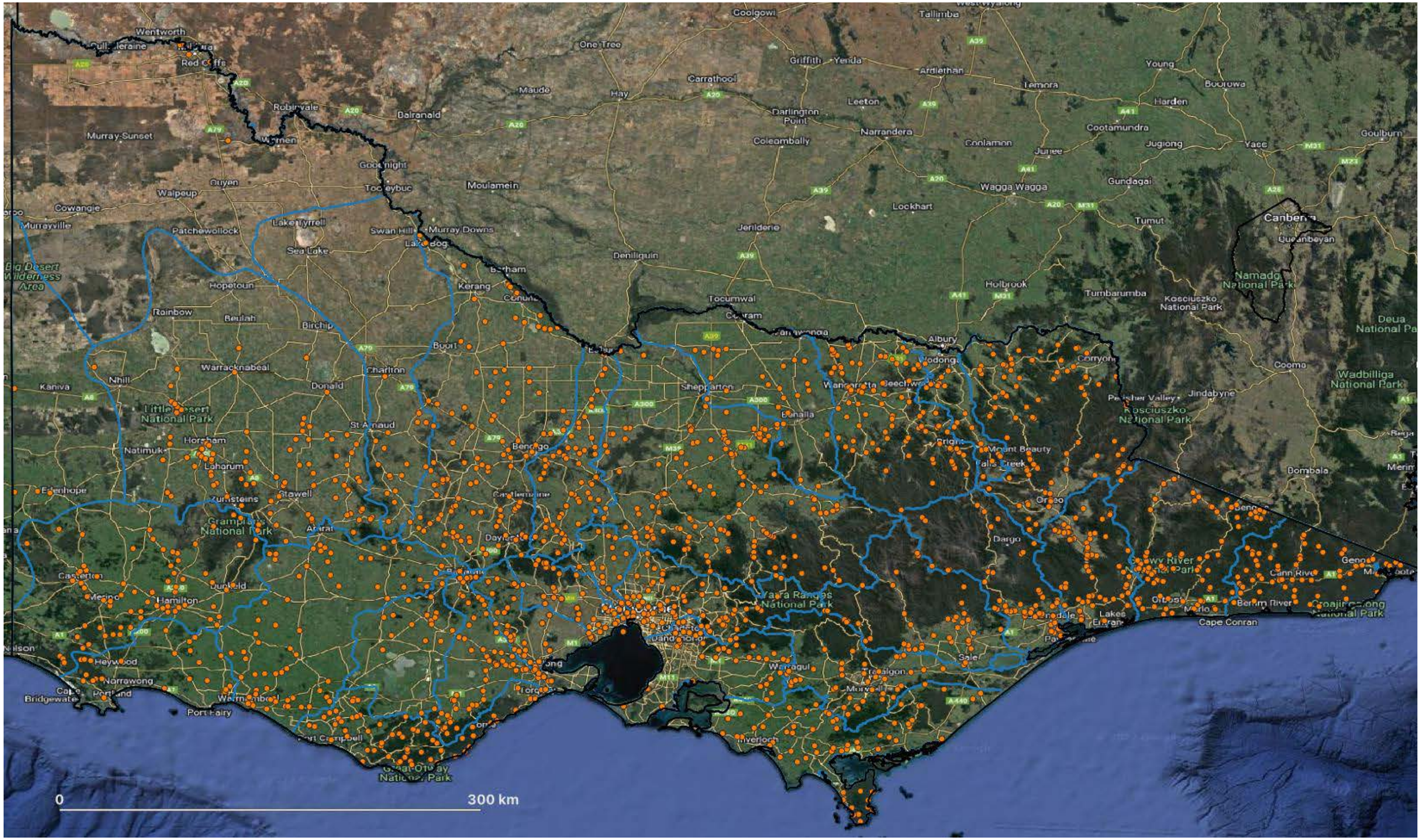

Figure 1. Location of sites surveyed (orange dots) across Victoria for the Great Australian Platypus Search Sept-Dec 2021.

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Quality control

Audit Sites

The inclusion of citizen scientists in projects such as these is critical for reducing overall project costs and engaging the community on river health and conservation issues. However, inexperienced and untrained volunteers also bring some risk in terms of quality of samples or data collected. To test this, we selected 200 sites that were re-sampled by the GAPS team and compared results to those obtained from volunteers. Due to some uncertainty of when sites were being sampled by citizen scientists, temporal variation in sampling is likely to contribute error around these validation sites due to the high mobility of platypuses and relatively dispersed distribution. We therefore looked for general concordance in detection rates between samples taken by citizen scientists and trained GAPS ecologists.

Expected/known platypus distribution

State-wide data on platypuses in Victoria is largely restricted to anecdotal sighting records on online databases with some localised systematic surveys using live-trapping or eDNA. Data was restricted to the last 30 years prior to this project (1st June 1991 to 1st June 2021) as this is approximately the time period used by the IUCN to determine conservation status (three generations). Due to the scarcity of data and longevity of platypuses, we considered the previous 10 years as recent or evidence of contemporary occurrence. Many records on online databases are anecdotal unverified sightings from the public with known errors in location accuracy and misidentification (typically with rakali). Even unrepeated captures or eDNA detections may represent transient individuals. To minimize false positives of platypus occurrence in areas where they are not local residents, we set a threshold of at least five database records in any time period to have confidence of platypus presence.

Expected platypus occurrence in each river basin (29) was qualitatively assessed using recent records (<10 years) from online databases (Atlas of Living Australia, Victorian Biodiversity Atlas, platypusSPOT) and previous eDNA surveys by EnviroDNA and collaborators. This assessment does not indicate abundance or population trajectories and recognizes that available data is typically sparse in most basins with large areas of no data. The following statuses were defined for basins:

1. Widespread – records in multiple waterways throughout the basin.
2. Restricted – multiple records but limited to less than 50% coverage of total available stream length of the basin.
3. Absent – No recent data available that indicates platypus occurrence.

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Findings

We obtained water samples for eDNA analysis from 1649 sites and 164 validation sites (total 1813) across all major river basins in Victoria (Figure 1). A number of proposed sites (*n* = 187) were not sampled from the original goal of 2000 sites due to access issues, lack of water, or non-participation by citizen scientists or collaborators. Twenty-two sites were excluded as they did not meet our quality control standards due to very low sample volumes, replicate samples not taken, extended postage delays, or inadequate metadata provided. No platypus DNA was detected in any of these samples but we could not be confident of the sample quality and therefore the accuracy of the result. We also excluded sites (*n* = 12) in areas where platypuses are not expected to occur (Millicent Coast, Mallee Basins) and some off-stream billabongs and lakes that are not connected to waterways and therefore inaccessible for platypuses. These sites were not part of the original sampling program but selected by program partners from their sampling allocation. While these sites are not relevant for platypuses, they will provide data on other aquatic vertebrates as part of the broader biodiversity assessments being undertaken.

The remaining survey sites (187) from the original 2000 will still be used for assessing 'possible detections' and validating some negative locations that were expected to be positive. These surveys will be undertaken in July and August 2022.

Audit program

As part of the quality control process, 164 sites were repeat sampled by the GAPS team. As expected, there was some differences in results between sampling undertaken by citizen scientists and the GAPS team due to differences in sampling dates (up to 119 days, av. 30 days). Sample volumes tended to be higher in samples taken by the GAPS team (av. 885 mL compared to av. 315 mL from citizen scientists, Figure 2), which included a number of sites (*n* = 33) sampled using a Smith-Root eDNA backpack sampler and 5 μM self-preserving filters (Thomas *et al.* 2019, 2018). However, these differences did not result in a significant increase in detections. With a threshold level of two positive qPCR assays to consider a site as positive for platypus eDNA, we found 82% concurrence between audit sites (121 not detected, 13 detected). Where results did not correspond, 12 sites (7%) had detections by citizen scientists but not GAPS, and 18 sites (11%) had the reverse results. Average differences in sample volumes were actually lower at sites where results did not correspond. The results therefore indicate that in most cases, sampling by citizen sciences is likely to be as reliable as sampling by the GAPS team.

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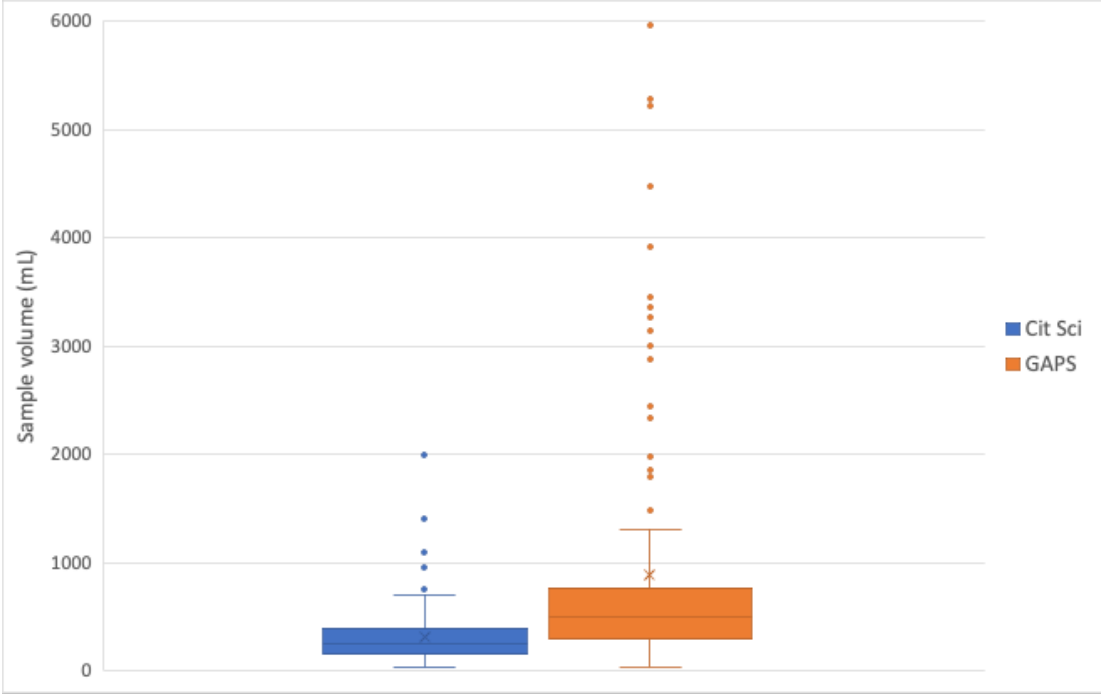

Figure 2. Comparison of sample volumes between citizen scientists and trained GAPS ecologists.

Contemporary platypus distribution

Prior to this project, less than 1000 platypus records were available throughout Victoria on our major wildlife databases (ALA, VBA) over 10 years between 2011 and 2021 (Figure 2). There is evidence for contemporary platypus occurrence (i.e. at least three records in the last 10 years) in all major river basins except Mallee, Millicent Coast, Avoca River, Portland Coast, and Lake Corangamite (Figure 3). When comparing records from the last 30 years, no recent records (<10 years) exist suggesting the status of platypuses in the Avoca River and Portland Coast Basins is currently unknown, while distributions may have contracted in the Wimmera-Avon Rivers and possibly Glenelg River and South Gippsland Basins. However, these data need to be interpreted cautiously due to the scarcity of overall data, bias towards areas of human activity, and lack of systematic surveys.

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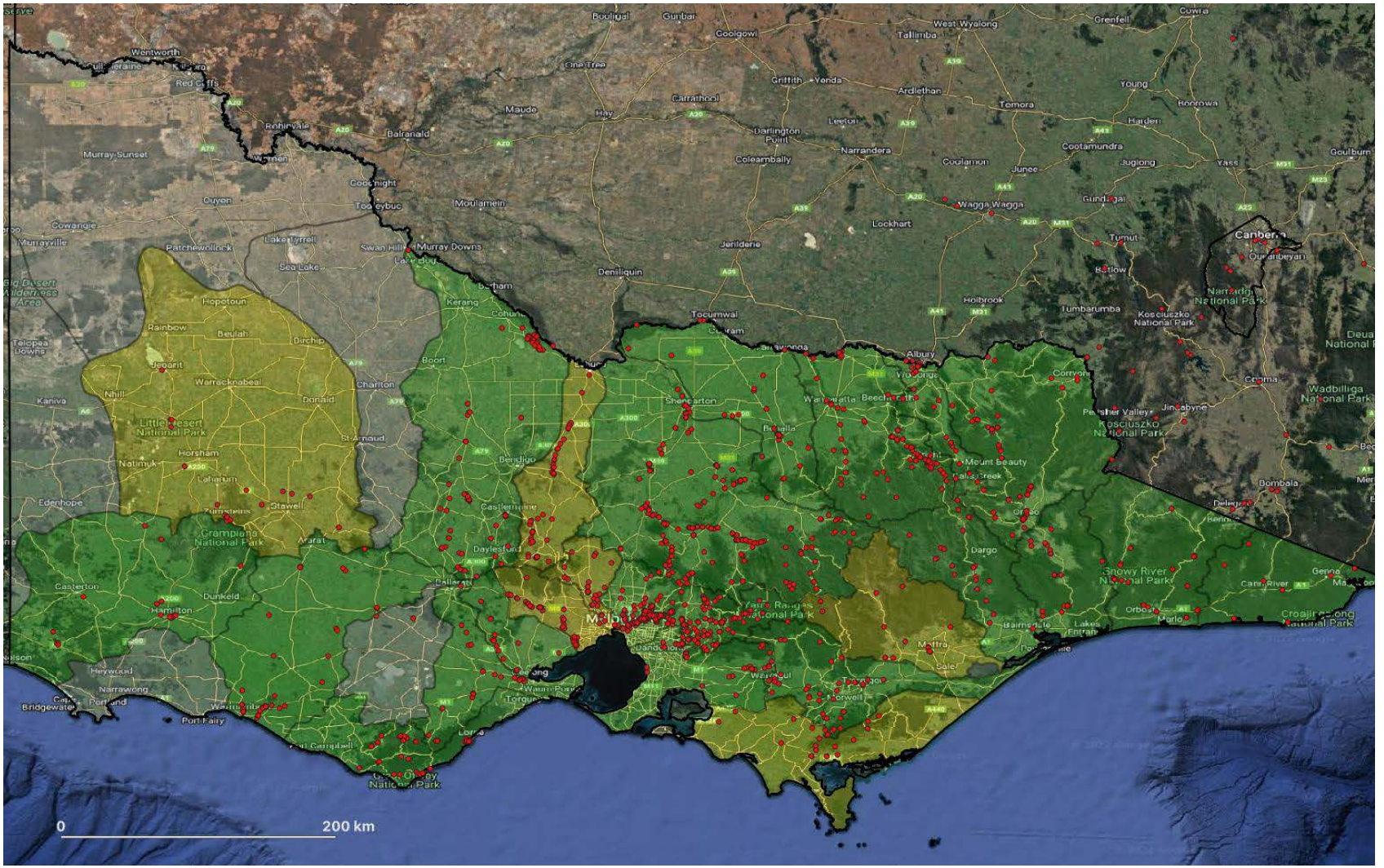

Figure 3. Predicted status of platypus occurrence in river basins across Victoria from available data. Green = widespread, Yellow = restricted, grey = absent/unknown. Markers represent recent data (<10 yrs) from online databases (ALA, VBA, platypusSPOT).

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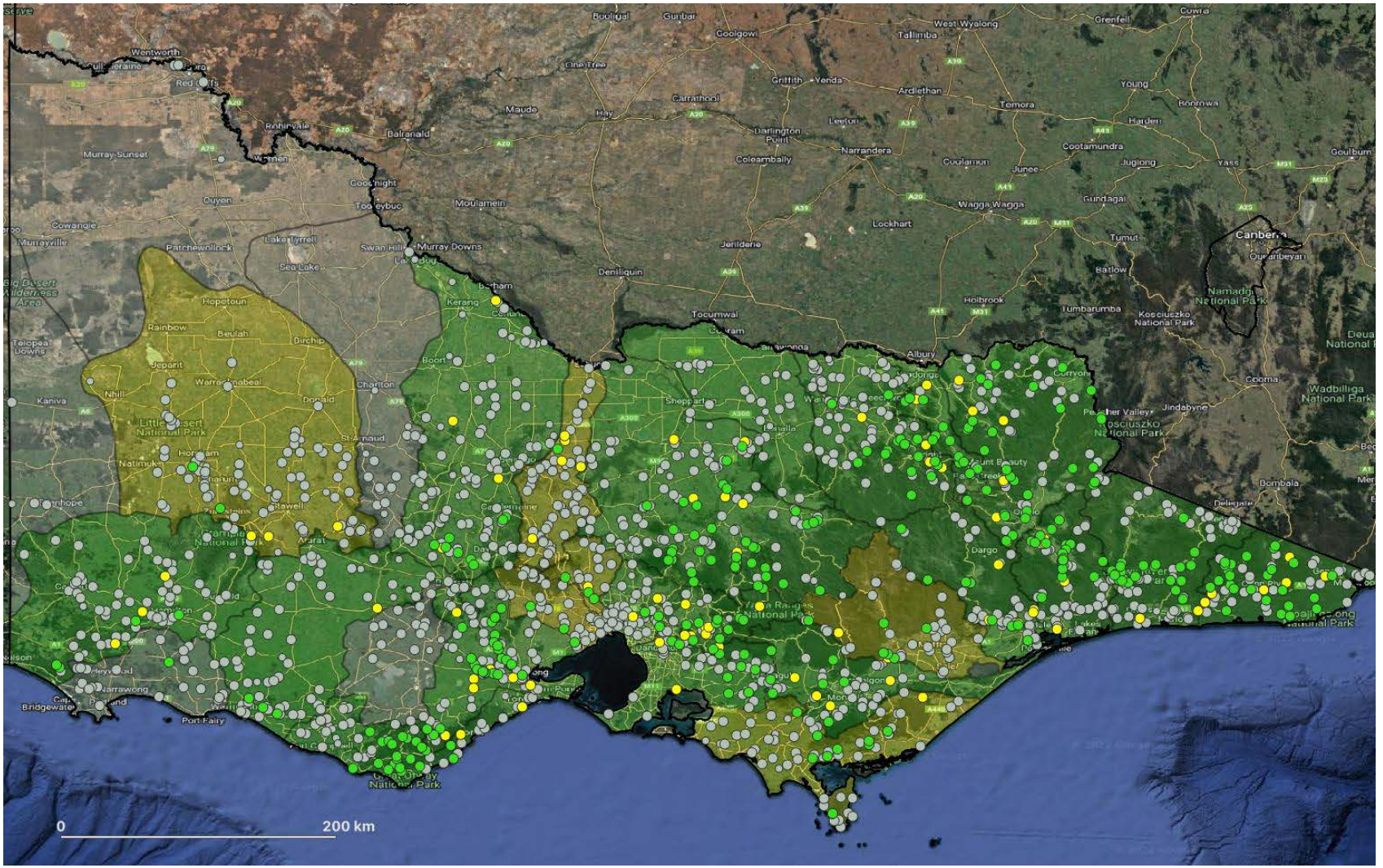

Figure 4. Results of GAPS eDNA surveys for platypus from 1611 sites across Victoria. Green = detection, Yellow = possible detection, grey = not detected. River basin status from Figure 2.

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GAPS results

Excluding audit sites, repeated sites and sites that failed our quality control standards (e.g. very low sample volume, lacking metadata), we assessed platypus occurrence at 1611 sites with positive detections of platypus eDNA at 312 sites with 'possible detections' at another 89 sites (Figure 4). Overall, the site occupancy estimate of platypuses across the state was 19-25%, depending on whether possible detections are included. Detections were generally higher and more widespread in eastern Victoria, with lower and more localised detections in the drier northern and western districts. Positive detections were recorded in all major river basins where platypuses were expected to currently occur (i.e. multiple records in the previous 10 years), except for Broken River Basin although some basins have quite low occupancy estimates or restricted distributions. For the basins with no recent records of platypus occurrence, platypuses were not detected in the Avoca River (n = 16) or Lake Corangamite Basins (n = 17) but a single detection at one site was recorded in the Portland Coast Basin (n = 23, see below). An overview of results for each river basin is provided below and in Table 1.

Glenelg River – n = 70, 4 positive, 3 possible. Occupancy estimate 6-10%.

Positive detections recorded in the lower Glenelg River, Grange Burn Creek and Malakite Creek, possible detections in other tributaries. Given the widespread historical records, surprisingly low detections, particularly in the Glenelg River although many sites were located in smaller tributaries rather than the main river channel. Previous eDNA surveys in 2018/19 recorded platypuses throughout the Glenelg River with low occupancy in the upper reaches and moderate occupancy in the lower reaches (GHCMA, EnviroDNA unpublished data). This area warrants further investigation but suggests platypus occurrence may be quite low outside of the main Glenelg River.

Portland Coast – n = 23, 1 positive. Occupancy estimate 4%.

A single detection was recorded in the Eumeralla River at Macarthur. While there are no recent records of platypuses in the Portland Coast Basin, there is a sighting from 2005 on ALA at this site as well as several historic records (<10 years) from elsewhere in the basin. No previous systematic surveys have been undertaken here and these data fill a valuable gap in our knowledge. More detailed investigations are required to understand the current extent of distribution.

Hopkins River – n = 64, 3 positive, 1 possible. Occupancy estimate 5-6%.

Positive detections recorded in the lower Hopkins River, Merri River and Brucknell Creek and a possible detection in Mt Emu Creek at Skipton (given previous knowledge this is likely to represent a true detection). Overall detections were surprisingly low with previous research showing extensive platypus distribution throughout the Hopkins River (EnviroDNA unpublished data), lower Merri River and Mt Emu Creek (Griffiths, Song, *et al.* 2019b). The current survey

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program had relatively few sites in these waterways and warrants further investigation, but results demonstrate that platypuses are scarce outside of these main river systems.

Wimmera-Avon Rivers – *n* = 81, 2 positive, 3 possible. Occupancy estimate 2-6%.

Positive detections recorded in the Mackenzie River and possible detections in Fyans Creek, Mt William Creek, and upper Wimmera River. A positive detection in lower Mackenzie River represents the furthest downstream platypuses have been recorded. Mackenzie River supports the last known resident population of platypuses in the Wimmera catchment and has been slowly expanding since the end of the Millennium Drought in response to an environmental water program provided by Wimmera CMA. This result raises hope that platypuses may soon disperse and recolonise the lower Wimmera River. Elsewhere, the possible detections in Mt William Creek and upper Wimmera River near Crowlands are interesting as platypuses are thought to have disappeared from the upper Wimmera region although several unconfirmed sightings have reported. While very low numbers may still persist in the area, extensive repeated surveys have demonstrated a viable resident population does not occur (Griffiths and Weeks 2013; J Griffiths *et al.* 2016; Griffiths and Weeks 2018). There was no evidence of platypuses in the Avon River system.

Avoca River – *n* = 16, no detections. Occupancy estimate 0%.

Although there are several historical records in the Avoca Basin, these results provide further evidence that platypuses no longer occur in the area.

Loddon River – *n* = 105, 8 positive, 4 possible. Occupancy estimate 8-10%.

Low occupancy with restricted detections in the upper reaches of the system (Birchs Creek, Campbells Creek). No confirmed detections (although several possible detections) in the lower reaches of the basin, although many sites were located in smaller tributaries rather than the main Loddon River channel. Previous eDNA surveys have indicated low occupancy in the Loddon River (EnviroDNA, unpublished data). Current results indicate that platypuses are scarce outside upper reaches raising concerns for isolation of these populations.

Lake Corangamite – *n* = 16, no detections. Occupancy estimate 0%.

There is no evidence for platypus occurrence in this basin and there were no eDNA detections. No previous systematic surveys have been undertaken here and these data fill a valuable gap in our knowledge.

Otway Coast – *n* = 68, 22 positive, 3 possible. Occupancy estimate 32-37%.

Moderate overall occupancy with widespread detections throughout the central and eastern areas of the basin. Results concur with recent available data for the areas and confirm platypuses do not occur in the Curdies system.

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Barwon River – *n* = 67, 9 positive, 13 possible. Occupancy estimate 13-33%.

Moderate occupancy and widespread detections throughout the basin, particularly the Leigh River. Detections were lower in the Barwon River indicating a sparser and possibly fragmented population. Many of the possible detections are likely to represent actual occurrence. Results support previous eDNA surveys undertaken by local citizen scientists in the area (Griffiths, Song, *et al.* 2019a; Griffiths and Impey 2020).

Moorabool River – *n* = 33, 12 positive. Occupancy estimate 36%.

Moderate occupancy and widespread detections throughout he Mooorabool River apart from the upper tributaries where permanent water may be less reliable. Platypuses do not appear to occur elsewhere in the basin. Results support previous eDNA surveys undertaken by local citizen scientists in the area (Griffiths *et al.* 2021)

Werribee River – *n* = 36, 3 positive. Occupancy estimate 8%.

Low occupancy with scattered detections along the Werribee River as far as Werribee Gorge. No evidence of platypuses in other waterways such as Skeleton Creek, Kororoit Creek or Lerederderg River. Results largely agree with extensive eDNA surveys undertaken in 2016 and 2017/18 for Melbourne Water although platypuses are known to occur as far upstream as Ballan (Griffiths *et al.* 2017, 2018).

Campaspe River – *n* = 60, 1 positive, 5 possible. Occupancy estimate 2-10%.

Low occupancy with scattered detections and possible detections through the Coliban River and lower Campaspe River. Platypuses are expected to be relatively widespread in these systems, but few sites were sampled in the Coliban River. Detections in the lower Campaspe are clustered where most recent records exist suggesting a hotspot between Axedale and Elmore. No detections were recorded in the upper Campaspe from extensive surveys, reinforcing recent surveys indicating platypuses are now at very low abundance and probably not supporting a viable population although a few individuals likely remain (Griffiths and Licul 2020).

Maribyrnong River – *n* = 22, 3 positive, 1 possible. Occupancy estimate 14-18%.

Low occupancy with several detections along Jacksons Creek only. No evidence of platypuses in other waterways such as Deep Creek and lower Maribyrnong River. Results largely agree with extensive eDNA surveys undertaken in 2016 and 2017/18 for Melbourne Water (Griffiths *et al.* 2017, 2018). Platypuses were previously widely distributed along Deep Creek but now appear to be in very low abundance, likely due to increasing cease-to-flow events.

Goulburn River – *n* = 161, 54 positive, 10 possible. Occupancy estimate 34-40%.

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Moderate occupancy from extensive surveys with widespread detections throughout the middle and upper basin waterways. Detections are sparser in the lower reaches.

Yarra River – *n* = 68, 15 positive, 9 possible. Occupancy estimate 22-35%.

Clear gradient of higher detections in the upper reaches and declining towards the lower reaches that are more urbanized. Overall low-moderate occupancy although sampling sites were somewhat biased towards the more urban areas. Results largely agree with extensive eDNA surveys undertaken in 2016 and 2017/18 for Melbourne Water (Griffiths *et al.* 2017, 2018).

Bunyip River – *n* = 41, 10 positive, 4 possible. Occupancy estimate 24-34%.

Low to moderate occupancy with detections in all the major waterways (Bunyip, Tarago, Lang Lang Rivers), as well as a small isolated population in Monbulk Creek. No detections were recorded on the Mornington Peninsula from limited sites. There are occasional unconfirmed reports of platypuses on Mornington Peninsula but have never been verified from multiple investigations. Results largely agree with extensive eDNA surveys undertaken in 2016 and 2017/18 for Melbourne Water (Griffiths *et al.* 2017, 2018).

South Gippsland – *n* = 64, 9 positive, 1 possible. Occupancy estimate 14-16%.

Low occupancy but relatively widespread detections across the basin. Results correspond to recent records from databases apart from an unusual detection in Tidal River where there is no evidence of current or historical platypus occurrence. In combination with the lack of recent sightings and negative results from live-trapping and previous eDNA surveys (Melbourne Water unpublished data), the data also provides further evidence the species no longer likely occurs in the Bass system.

Latrobe River – *n* = 41, 6 positive, 4 possible. Occupancy estimate 15-24%.

Low occupancy but relatively widespread detections throughout the basin including Latrobe River, Narracan Creek, Morwell River and Tyers River.

Broken River – *n* = 24, no detections. Occupancy estimate 0%.

Surprisingly, no detections in the basin although survey effort was relatively low compared to other areas. Platypuses are known to occur in the Broken River although little is known of their total distribution or abundance. Most sampling sites were located in the smaller tributaries with very few located in the main Broken River channel and this warrants further investigation to supplement the current results. However, results indicate platypuses have limited distribution outside of the main river system.

Ovens River – *n* = 91, 18 positive, 5 possible. Occupancy estimate 20-25%.

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Low to moderate occupancy with widespread detections but concentrated in the upper reaches of the basin. Results largely correspond with recent records from databases and eDNA surveys (EnviroDNA unpublished data) with maybe fewer than expected detections in the lower Ovens/King Rivers.

Kiewa River – *n* = 18, 11 positive, 2 possible. Occupancy estimate 61-72%.

High occupancy with widespread detections, although total number of survey sites is relatively low in this small basin. Results correspond with recent records and indicate platypuses are widely distributed throughout the Kiewa River and permanent tributaries.

Thomson River – *n* = 41, 6 positive, 2 possible. Occupancy estimate 15-20%.

Relatively low overall occupancy but detections are scattered across the basin suggesting platypuses are reasonably widespread. However, sampling sites were biased towards the lowland areas with relatively few sites in the more remote, less disturbed upper reaches where occupancy is expected to be higher. Recent data in the basin is scarce but, where available, results largely correspond apart from the lack of detections in the Mcallister River (*n* = 3).

Mitchell River – *n* = 54, 9 positive, 5 possible. Occupancy estimate 17-26%.

Low to moderate occupancy with scattered detections throughout the basin. A detection in Forge Creek near Paynesville is unusual and warrants further investigation.

Tambo River – *n* = 67, 19 positive, 4 possible. Occupancy estimate 28-34%.

Moderate occupancy with detections throughout the Tambo River and tributaries as well as the Nicholson and Timbarra Rivers. Detections were sparse in the lower reaches of the basin. Relatively few recent records exist in this basin so results have filled some knowledge gaps.

Upper Murray River – *n* = 82, 31 positive, 6 possible. Occupancy estimate 38-45%.

Relatively high occupancy and widespread distribution throughout the basin. Results largely confirm known occurrence with some knowledge gaps filled in more remote areas.

Snowy River – *n* = 80, 23 positive, 5 possible. Occupancy estimate 29-35%.

Moderate occupancy overall but higher in the middle reaches and lower in the northern and southern regions. Historical records (<30 years) exist in the northern areas but only 1 detection was recorded in the current surveys indicating distribution may have contracted.

East Gippsland – *n* = 68, 29 positive, 5 possible. Occupancy estimate 43-50%.

High occupancy and widespread distribution throughout basin. Relatively few records exist in this basin due to its remoteness.

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Basin	Positive detections	Possible detections	No detections	Occupancy estimate
Glenelg River	4	3	63	6-10%
Portland Coast	1	0	22	4%
Hopkins River	3	1	60	5-6%
Wimmera-Avon Rivers	2	3	76	2-6%
Avoca River	0	0	16	0%
Loddon River	8	4	93	8-10%
Lake Corangamite	0	0	16	0%
Otway Coast	22	3	43	32-37%
Barwon River	9	13	45	13-33%
Moorabool River	12	0	21	36%
Werribee River	3	0	33	8%
Campaspe River	1	5	54	2-10%
Maribyrnong River	3	1	18	14-18%
Goulburn River	54	10	97	34-40%
Yarra River	15	9	44	22-35%
Bunyip River	10	4	27	24-34%
South Gippsland	9	1	54	14-16%
Latrobe River	6	4	31	15-24%
Broken River	0	0	24	0%
Ovens River	18	5	68	20-25%
Kiewa River	11	2	5	61-72%
Thomson River	6	2	33	15-20%
Mitchell River	9	5	40	17-26%
Tambo River	19	4	44	28-34%
Upper Murray River	31	6	45	38-45%
Snowy River	23	5	52	29-35%
East Gippsland	29	5	34	43-50%

Table 1. Summary of GAPS results for each river basin

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Discussion

Using eDNA techniques and citizen scientists, we assessed platypus occurrence at 1612 sites in the largest systematic platypus survey ever undertaken. The results largely correspond with what was expected based on the contemporary distribution from online databases and localised monitoring programs. This indicates that using citizen scientists and eDNA techniques can be an effective and cost-efficient way to investigate platypus (and other species) occurrence at both local and landscape scales. Species detection from samples collected by citizen scientists largely corresponded to samples collected at the same sites by trained ecologists, helping to improve confidence in results. However, improvements in the sampling guidance and data recording process would help streamline the process, improve sample quality, and reduce confusion over sample metadata such as site codes and locations. This may include a combination of improvements to the app used to record data, clearer sampling instructions (including the importance of accurate sample metadata), and on-site workshops for citizen scientists which were initially planned but difficult to implement for this project due to Covid restrictions.

Platypuses remain widely distributed throughout Victoria, but populations are much more extensive in the eastern regions compared to the drier and more modified northern and western areas of the state, where populations appear much more restricted and fragmented (Figure 4). Critically, this data has filled valuable gaps in the current knowledge of the status of platypuses at a landscape scale, thereby helping to confirm their status in each of Victoria's major river basins, including many areas where no recent data existed. The results have indicated the species is widespread in the East Gippsland, Snowy River, Tambo River, Upper Murray River, Mitchell River, Ovens River, Thompson River, Latrobe River, Goulburn River, South Gippsland, Bunyip River, Yarra River, Moorabool River, Otway Coast, and Barwon River Basins as well as confirming the likely absence of platypuses in the Avoca and Lake Corangamite Basins. Platypuses appear to be quite scarce and/or have restricted distributions in the Broken River, Campaspe River, Loddon River, Maribyrnong River, Wimmera-Avon Rivers, Hopkins River and Glenelg River Basins. However, apparent low occupancy in several of these basins (Glenelg, Hopkins, Broken) may be due to a limitation of the sampling design, and also timing of the surveys (see below) and require further follow up surveys to establish their extent in these basins. The GRTS sampling design ensured spatially balanced sampling throughout a range of waterways within each basin but through this design, some major rivers were allocated comparatively few sites, which may have impacted results in some basins. Nevertheless, results do indicate that platypus distribution in these systems may be largely restricted to the major rivers which is of some concern. A positive detection at one site in the Portland Coast is also of significant interest as there is very limited records of platypuses in this system and none for more than 15 years. Several other notable detections recorded where platypuses are not expected to occur that warrant further investigation include Tidal River on Wilson's Promontory, Forge Creek near Paynesville, and Wimmera River near Crowlands.

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For a widespread and low density species such the platypus, occupancy estimates are useful indicators of overall population health at a river basin scale (extent of distribution) and for relative comparisons but should not be considered quantitatively. There are several methodological reasons that may have feasibly reduced overall detectability of platypuses during this project. Firstly, many surveys were undertaken later than desired and missed the breeding season for platypuses when they are likely to be more detectable due to increased activity. During the breeding season (late August to mid October), both adult males and females are more active as they endeavor to increase body condition, search for mates, and defend territories (Bethge 2002; Grant 2007; Griffiths *et al.* 2014). This increased activity leads to higher rates of captures during surveys as well as sightings (Griffiths *et al.* 2020; Easton *et al.* 2008) and declines once breeding activity has stopped. It is reasonable to assume this would also result in more DNA shed into the water and higher detection rates through eDNA, although this hasn't been empirically tested. Secondly, consistent high rainfall events across Victoria throughout spring created higher water levels and flows in many rivers. This contributed to delayed sampling for many sites, and high water levels in streams may also decrease species detectability through a dilution effect compounded by lower filtration volumes due to higher turbidity. Finally, although the audit program provided confidence that samples collected by citizen scientists were generally reliable, sample volumes and detection rates were slightly lower than those collected by experienced and trained ecologists. Sample volume is an important variable when considering eDNA data (similar to survey effort for traditional methods). While the effect of volume on detection rates is poorly understood, higher filtration volumes will increase detection rates, although the relationship is unlikely to be linear. Confounding variables such as species density, stream size and flow rates will also impact detection rates and effects of filtration volume. A small number of samples received did not pass our minimum quality control threshold of at least 50 mL and were therefore not included in the results. Platypus DNA was not detected in any of these samples. However, there is no defined threshold volume for excluding samples and a number of positive detections were recorded at low volumes (i.e. 50-100 mL). Any rigorous analysis of the data should include volume, as well as proportion of positive assays for each sample, as covariates to calculate probability of occurrence at a site, rather than binary detection/non-detection outcomes. The outcomes of reduced detectability are likely to be more evident in areas of relatively low species abundance. We recommend more intensive follow-up investigations in several sampling locations that had lower than expected detections rates, based on recent data from databases and eDNA surveys, or limited survey effort. These include the Glenelg River, Hopkins River, Mt Emu Creek, Loddon River, and Broken River.

Accounting for imperfect detection in survey data is important for accurate estimates of site occupancy. Site occupancy detection models that estimate site occupancy and account for imperfect detection are ideal for eDNA data where replicate samples are collected at a set of sites (Guillera-Arroita *et al.* 2017; Lahoz-Monfort José *et al.* 2015; Lugg *et al.* 2018; Schmidt *et al.* 2013). Such models can help with interpretation of low-level detections (defined as 'possible detections' here). Possible detections are very low traces of target species DNA detected in samples and should not be considered definitive evidence of platypus occurrence

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at a site but should be interpreted based on results from surrounding sites as well as historical data. Low-level detections can indeed indicate presence of the target species at low abundance but can also arise from site-level or sample-level contamination (Darling *et al.* 2021). Site contamination can occur due to facilitated movement of DNA through the sampling process (minimsed through strict sampling protocols) or uncontrolled by water birds, recreational anglers, water transfers, predator scats, or natural dispersal of DNA from further upstream locations in lotic systems. Site-level contamination can also arise through the laboratory screening process. Previous research has indicated that false positives in eDNA surveys can be very low with appropriate control measures (Tingley *et al.* 2021). Some possible detections in the current survey are very likely to be true positive detections (i.e. lower Campaspe River, upper Yarra sites) while others are likely low probability of occurrence (i.e. Hope Creek near Inglewood, Fyans Creek near Halls Gap).

Additional eDNA surveys by the GAPS team at some locations with low or absent detection rates where platypuses were expected, as well as some 'possible detection' sites, will be undertaken as part of the program in July/August 2022. This will provide greater confidence around presence or absence of platypuses at these locations.

Conservation status in Victoria

These results provide support for the recent listing of platypuses as vulnerable under the Victorian FFG Act. While it remains difficult to quantitatively assess declines due to limited historical data, the data broadly support the decline of platypuses in the Wimmera-Avon Basin, Portland Coast Basin, upper Campaspe River, Bass River, and a number of waterways of the greater Melbourne region within the last 30 years. Longer term absences of platypuses from the lower Murray River and Curdies system is also supported by these eDNA results. Of further concern is the limited distribution and fragmentation of populations indicated in other river basins of central and western Victoria as well as low detections in the lowland reaches of many waterways of eastern Victoria that could lead to further fragmentation of populations in tributaries and upper reaches. Previous research has shown that fragmentation will exacerbate negative genetic effects, such as inbreeding, leading to further declines in populations.

The GAPS data, along with other recent eDNA survey data, now present an opportunity to assess the conservation status of platypuses more thoroughly in Victoria and identify areas of major concern.

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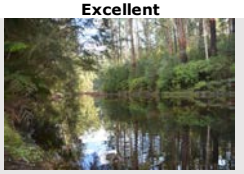
Appendix 1. River Health assessment undertaken by citizen scientists.

Site Code:		Date:			
Habitat area	**Very Poor**	**Poor**	**Fair**	**Good**	**Excellent**
Bank erosion					
	Extensive erosion. Little vegetation present to stablise banks	Evidence of recent erosion. Extensive areas of bare banks	Erosion occurring in specific areas. Moderate vegetation cover	Erosion only in very small spots. Good vegetation cover. Maybe gentle bank slopes.	No erosion evident. Lower banks covered with grass, reeds or shrubs.
SCORE	**1**	**2**	**3**	**4**	**5**
Bank vegetation (immediately adjacent to water, within 2m)					
	Mostly bare ground. Occasional tree. Concrete or paved channel.	Introduced ground cover. Little native woody vegetation.	Moderate cover of native or introduced vegetation. Variation between sides - one cleared, one vegetated.	Mainly native vegetation. Little disturbance to banks.	Mainly undisturbed native vegetation (i.e. forested).
SCORE	**2**	**4**	**6**	**8**	**10**
Burrowing habitat (earthern banks, relatively vertical profile at water surface, >1m high to allow burrow construction)					
	Low, eroded, gently sloping banks.	Limited potential burrowing sites.	Some potential burrowing sites in area. One side steep, other shallow sloping.	Extensive potential burrowing sites.	Steep, consolidated banks along both sides, >1m high
SCORE	**1**	**2**	**3**	**4**	**5**

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Instream complexity (supports abundant macroinvertebrate prey)	 No snags, boulders or vegetation over water. Silt or sandy substrate. Could be rock or concrete lined channel.	 Limited benthic complexity. Mostly silty/sandy substrate with occasional snag or rocks. Usually turbid.	 Some snags & rocks present. Some aquatic macrophytes & overhanging vegetation. Some gravel/cobbled substrate.	 >50% cobbled substrate with snags, logs, rocks. May have aquatic macrophytes and overhanging vegetation.	 Frequent snags, logs, rocks. Cobbled, rocky and gravel substrate throughout. Extensive aquatic & overhanging vegetation.
SCORE	**2**	**4**	**6**	**8**	**10**
Channel complexity (promotes habitat diversity to support diverse and abundant macroinverts	 Straightened stream. Uniform depth (i.e. all shallow). Could be irrigation channel etc.	 Limited variation in depth, not artificailly straightened.	 Occassional riffle or bend, some variation in depth.	 Pools and riffles present. Several bends.	 Riffles and pools of varying depth. Winding channel.
SCORE	**1**	**2**	**3**	**4**	**5**
Verge vegetation (riparian zone, within ~30m)	 Bare ground or pasture/grass cover next to water.	 Narrow band (<5m) of sparse native or introduced vegetation.	 Wide corridor (5-10m) native or introduced vegetation. One side cleared, other wide native vegetation.	 Mainly native but some introduced vegetation. Wide area (>10m).	 Mainly native vegetation on both sides (>30m wide).
SCORE	**2**	**4**	**6**	**8**	**10**
TOTAL SCORE	**(9-13) Degraded**	**(14-21) Poor**	**(22-30) Fair**	**(31-38) Good**	**(39-45) Excellent**
Surrounding land use: Forest_bushland / agriculture grazing / agriculture cropping / rural town / urban				**Stock access (Y / N)**	
Human activity (i.e. litter, fishing) (Y / N)					

EnviroDNA Pty Ltd
+61 3 9349 4723
envirodna.com