Tracking platypus populations through genetic fingerprints.

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Key Points

- Platypuses have recently been upgraded to 'Near Threatened' by the IUCN due to mounting evidence of population declines and localized extinctions.
- Traditional methods of surveying platypuses are time and labour intensive and can have low sensitivity resulting in limited information on population trends across their range.
- Environmental DNA is an effective and cost-effective method to ascertain the distribution of platypuses over a large spatial scale.
- These data provide a baseline to assess the impacts of future threats and effectiveness of management actions.

Abstract

The platypus was recently upgraded to 'Near Threatened' by the IUCN based on mounting evidence of population declines, local extinctions and fragmentation. Platypus populations in Victorian were identified as under the greatest stress. However, it is recognised that there is a scarcity of data on distribution or population trajectories at a local and catchment scale to fully understand their conservation status and threats.

Traditional monitoring methods based on live-trapping using mesh or fyke nets is time and labour intensive and restricted to certain habitats by water depth and flow, which has inherently limited the acquisition of data over a broad spatial scale. Over the past few years, EnviroDNA, in collaboration with University of Melbourne and Melbourne Water, has developed and tested environmental DNA (eDNA) as a viable method to detect platypuses using a long-term monitoring dataset from the greater Melbourne region. Direct comparisons with traditional live-trapping methods has revealed eDNA to be much more sensitive and cost-effective to detect presence/absence of platypuses.

We present the results of the first large-scale assessment of platypus distribution using eDNA across five major river catchments and highlight localised declines throughout greater Melbourne. We also demonstrate how site-occupancy data can provide reasonable estimates of abundance for the species. Environmental DNA now provides the opportunity to investigate the contemporary distribution of platypuses at a large spatial scale, understand the species' threatening processes and help predict future impacts.

Keywords

Environmental DNA, platypus, survey methods, presence/absence, distribution

Introduction

Knowledge of the distribution of a species is fundamental to conservation planning and developing management strategies at the appropriate scale. It is also important to understand the natural and anthropogenic factors that may limit a species distribution. For a semi-aquatic species like the platypus, these

may include water availability, flow regimes, water quality, extent of riparian vegetation, abundance of food resources, and suitable banks for burrow construction (Ellem *et al.* 1998; Grant 2004; Milione and Harding 2009; Serena 1994; Serena *et al.* 1998; Serena *et al.* 2001).

Platypuses inhabit a variety of aquatic systems throughout eastern Australia (Grant 1992; Grant and Temple-Smith 1998). Until recently, the platypus was considered a species of "Least Concern" by the International Union for the Conservation of Nature (IUCN) due to its relatively wide distribution and the lack of empirical evidence for significant declines in abundance or distribution (Lunney et al. 2008). Following a comprehensive analysis of the conservation status of Australian mammals (Woinarski *et al.* 2014), the IUCN now lists the species as "Near Threatened" (Woinarski and Burbidge 2016) due to mounting evidence population declines and localised extinctions within the species' broader geographic distribution (Grant 1992; Grant 1993; Grant 1998; Griffiths and Weeks 2011; Griffiths and Weeks 2013; Griffiths and Weeks 2014; Lintermans 1998; Lunney *et al.* 2004; Lunney *et al.* 1998; Rohweder and Baverstock 1999; Serena and Williams 2004; Serena and Williams 2011; Serena *et al.* 2014). However, it is recognised that a lack of population studies and difficulties in effectively monitoring the species results in insufficient data to reliably predict population trends at a local or catchment scale (Grant and Temple-Smith 2003; Gust and Griffiths 2009; Lunney *et al.* 2008). Populations in Victoria are predicted to be under the greatest stress (Woinarski *et al.* 2014).

The Melbourne Water Urban Platypus Program (MWUPP) has been running since 1995 (Griffiths *et al.* 2015; Serena and Williams 2008) to understand population trends of Melbourne's platypuses and identify key factors influencing their distribution and abundance. It is the only program to investigate platypus populations in an urban area where threats are expected to be the greatest due to extremely modified catchments and high human population density. Although the program provides valuable data on the status of platypuses in Melbourne's catchments and identified widespread declines (Griffiths *et al.* 2016; Griffiths and Weeks 2011), the current survey program encompasses only a small portion of Melbourne's waterways. Live trapping surveys are time and labour intensive and limited by suitable environmental conditions to deploy nets. Platypus sightings from the community are valuable but platypuses are difficult to consistently observe in the wild, observations are biased towards areas where people are present, can yield false positives from misidentification (particularly water rats), and lack repeatability and sampling rigour. As a consequence, there are many waterways where we have little or no information on platypus populations. This presents a real risk that this iconic species may disappear from some Melbourne waterways without our knowledge or understanding of the causes behind extirpation.

Environmental DNA (eDNA) is a rapidly emerging field and can be used to detect the presence of target species from a water sample (Thomsen and Willerslev 2015). We recently developed and verified the use of eDNA as a reliable method to assess platypus presence with a much higher sensitivity than traditional fyke netting surveys (Lugg *et al.* 2018). Here, we use eDNA to undertake widespread surveys throughout Melbourne Water's management areas to determine the presence of platypuses and address gaps in our knowledge of platypus distribution. We compare current presence with historical information, where available, to ascertain potential localised declines in platypus populations.

Methods

Survey sites

Survey sites were selected to provide broad information on the presence of platypuses throughout Melbourne's waterways and fill gaps in our knowledge of platypus distribution (i.e. where no recent live-trapping surveys have been undertaken). Within each catchment, sites were selected to provide good spatial coverage throughout the catchment and sample most of the major perennial waterways. Larger waterways were subdivided into reaches (upper, middle, lower). Where possible, at least two independent sites (>2 km apart) were chosen in each waterway/reach to provide greater confidence of detecting platypus presence. A

number of on-stream and off-stream waterbodies (i.e. wetlands, lakes, stormwater basins) were also sampled and were considered part of the waterway/reach they were associated with. A total of 326 sites were sampled across all five catchments (Figure 1).

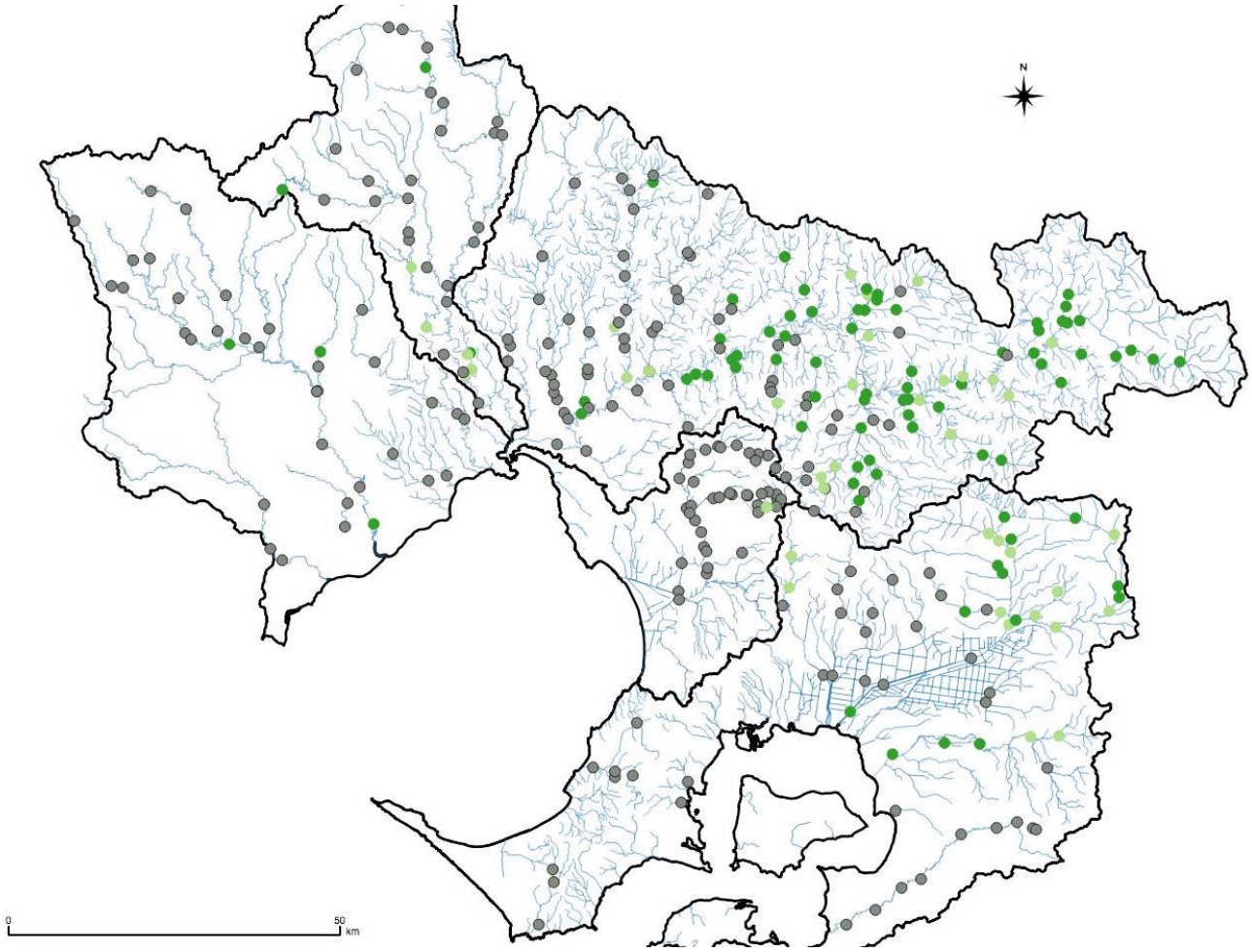

Figure 1. Results from the 2016 eDNA surveys indicating presence (green) or absence (grey) of platypus eDNA. Dark green markers represent sites with no recent information on platypus occurrence. .

Sampling methodology

Water samples were collected in triplicate at each site from June to August 2016 and filtered on-site by passing up to 500 mL of water through a 0.22µm filter using a sterile 60 ml syringe. Care was taken to avoid contamination by not entering the water to obtain samples, avoiding transfer of water or organic material between sites, and using clean (sterile) equipment at each site. Filters were stored on ice for a maximum of 48 hrs before transport to the laboratory for DNA extraction.

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 (Griffiths *et al.* 2014a) 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. For each sample, three replicate qPCR's were undertaken, for a total of nine at each site, and all assays included negative and positive controls. 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.

The high sensitivity of eDNA techniques reduces the incidence of false negatives (failing to detect a species that is present) over traditional techniques (Biggs et al. 2015; Smart et al. 2015; Thomsen et al. 2012). However, false positives (detecting a species when it is actually absent) may occur from non-specificity of probes used to detect the target species, field contamination, external contamination (e.g. transfer of genetic material by water birds, recreational anglers, water flow etc), or PCR artifacts at high replication cycles (Darling and Mahon 2011; Ficetola et al. 2015). To reduce the incidence of false positives, a site was only regarded as positive for platypus presence if at least two of the nine qPCR's detected the target DNA while sites with only one positive qPCR were considered negative.

Results

Platypus eDNA was detected in 118 water samples of the 326 sites surveyed (36%). The presence of platypuses was detected in all catchments although the proportion of positive sites varied significantly between catchments (Figure 2). The number of sites/waterways surveyed in each catchment were: Yarra (150 / 59), Maribyrnong (34 / 13), Westernport (66 / 28), Werribee (36 / 14) and Dandenong (40 / 12). The highest percentage of positive sites occurred in the Yarra Catchment (53%) followed by Westernport (41%), Maribyrnong (24%), Werribee (8%), and Dandenong (3%) had the lowest.

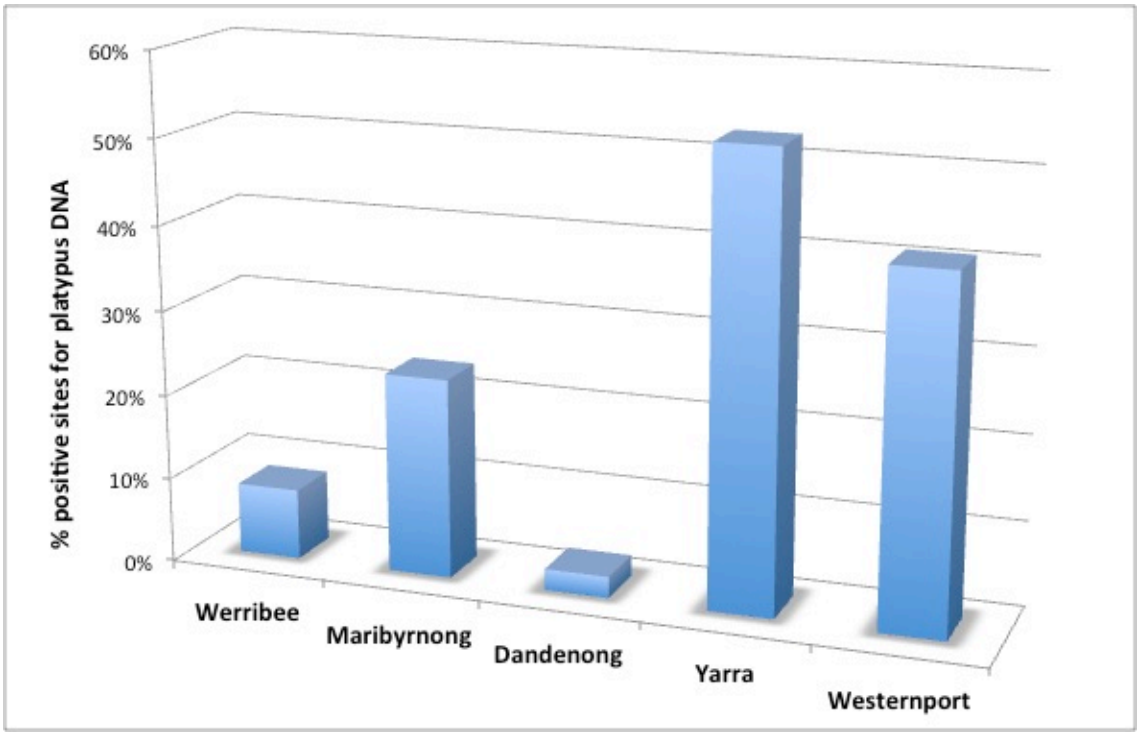

Figure 2. Percentage of total surveyed sites in each catchment that returned a positive result for platypus DNA.

Of the 126 waterways examined, platypus DNA was detected in 63 waterways (50%). Based on recent live-trapping and reliable sighting data, platypuses are known to occur in a further four waterways despite negative eDNA results during the current study. Conversely, positive eDNA results were recorded in several waterways where recent exploratory live-trapping surveys failed to capture any platypuses (e.g. Stringybark Creek, Don River). Interestingly, low amounts of platypus DNA were detected at one site in Jacks Creek where

they were thought to be locally extinct. Limited sampling was conducted in this area and further investigation is required to verify these results.

The current eDNA surveys failed to detect platypuses in nine waterways where they were previously known to occur within the last 20 years (i.e. over the duration of the MWUPP). Waterways where platypuses are now thought to be locally extinct include Running Creek, Arthurs Creek, Toorourrong Reservoir and tributaries (although see Jacks Creek above), upper Dandenong and Dobsons Creeks, lower reaches of Monbulk Creek, Ferny Creek, and Deep and Boyd Creeks near Darrweit Guim. These results are supported by recent live-trapping surveys in most waterways except Ferny Creek (Griffiths et al. 2015; Griffiths et al. 2016; Kelly et al. 2013).

Discussion

This project demonstrates the value of eDNA for detecting the presence/absence of platypuses to determine the species' distribution over large spatial scales. For the first time platypuses were detected in 20 waterways where no reliable previous information existed. Platypus presence was confirmed in a further 14 waterways with no recent data, as well as expanding the known range of platypuses in several other waterways. The results filled large gaps in our knowledge of platypus distribution in the greater Melbourne region with almost half (62 of 126) of the waterways examined having either no previous information or only unconfirmed sightings.

Extensive sampling did not detect platypus DNA in several areas where unverified sightings have been reported (e.g. Little River, the Mornington Peninsula, Bass and Little Bass Rivers) suggesting the sightings are likely to be misidentifications (i.e. possible water rats Hydromys chrysogaster) or true sightings of transient individuals. The ability to quickly confirm or disprove sightings is a valuable application of eDNA. Importantly, platypuses were identified from several waterways where recent live-trapping surveys failed to detect them. This highlights the sensitivity of eDNA and its value as a monitoring technique where platypus abundance is likely to be low (Lugg et al. 2018).

The results from the eDNA sampling confirm platypuses are relatively widespread throughout Melbourne's waterways although the extent of distribution varies between catchments (Figure 1). Combined data from the eDNA sampling, recent trapping, and reliable sightings indicate platypuses currently occur in 50% of Melbourne's waterways. However, a reduction in the distribution of platypuses throughout the greater Melbourne area is evident over the last 20 years. Detailed information on platypus distribution at a local scale is scarce but a little over a decade ago, the species was reported in 73% of 45 river reaches investigated in the greater Melbourne region (Serena and Pettigrove 2005).

Not surprisingly, platypuses have disappeared from the lower reaches of the Yarra and Maribyrnong Catchments where they were regularly sighted up to the 1950's (Faithfull 1987). This is the most heavily urbanised region of Melbourne (CBD district and adjacent inner suburbs) where waterways are subjected to significant impacts of urban stormwater run-off, identified as a key factor limiting platypus distribution (Martin et al. 2013; Serena and Pettigrove 2005). A subsequent result of this range contraction is the disconnection between platypus populations in the Yarra and Maribyrnong Catchments, which is supported by genetic evidence (Weeks and van Rooyen 2014). Platypuses are also assumed to have been historically more widespread in the Werribee Catchment where they are now restricted to a couple of small, isolated reaches of the Werribee River. More recently (<20 years), platypuses appear to have disappeared from Running and Arthurs Creeks, Toorourrong Reservoir and tributaries, upper Dandenong and Dobsons Creeks, the lower reaches of Monbulk Creek and adjoining Ferny Creek, and most of upper Deep Creek. Coinciding with these localised extinctions, widespread declines in abundance have also been recorded at key

populations (Griffiths and Weeks 2011) although some recovery of populations has been evident in recent years (Griffiths et al. 2016).

Although the results provide a good indication of broad platypus distribution, the pattern of site occupancy of platypuses between catchments (Figure 2) is comparable to the pattern of relative abundance from live-trapping surveys (Griffiths et al. 2016; Kelly et al. 2013), indicating eDNA can also provide a broad indication of relative abundance at large scales. However, actual site occupancy is likely to be higher than reported here. The sampling regime for the current study was largely designed to fill gaps in our knowledge of platypus distribution. As such, relatively few sites were selected in areas of known platypus occurrence (i.e. waterways that currently have live-trapping surveys as part of the MWUPP). In addition, the distribution of platypuses during the sampling period may have been limited by extended dry conditions, particularly early in the sampling regime. Under these conditions, low water levels and lack of connectivity may have restricted the distribution of platypuses, particularly in the upper reaches of smaller waterways. The distribution of platypuses and number of positive sites may feasibly increase under wetter conditions. Supporting this hypothesis, platypus DNA was detected in the lower reaches of several small waterways but not in the upper reaches despite apparently good habitat (e.g. Watsons Creek, Diamond Creek). It is possible that platypuses will move into the upper reaches of such waterways when sufficient water is available. Indeed, several platypuses were recently captured in upper Diamond Creek (C. Bloink pers. comm.) following substantial winter rainfall, although platypus DNA was not detected in water samples obtained earlier in the year. Platypuses have previously been shown to be opportunistic in their habitat usage under different hydrologic conditions (Gust and Handasyde 1995). Identifying the habitat use and distribution of platypuses under different environmental conditions is important to understand the dynamics of populations and develop appropriate management strategies.

The results here largely correspond with known and predicted platypus distribution, providing further evidence of the validity of eDNA as an effective monitoring technique for platypuses. However, it is important to recognise that the results presented here provide a snapshot of platypus occurrence at the time of sampling. As described above, platypus distribution may change under different environmental conditions or during different life history stages (i.e. males travel extensively during breeding season (Griffiths et al. 2014b; Serena et al. 1998) or juvenile dispersal (Serena and Williams 2013)). As a result, some positive results may arise from transient individuals or occasional use in response to local conditions rather than a permanent population. For example, resident platypus populations are not known to occur in either Toolern Creek or Dixons Creek but platypus DNA was detected at a single site in each of these waterways. Both creeks typically have relatively low baseflows for extended periods (J. Griffiths pers. obs.) and these results may represent exploratory behavior by individuals from adjacent waterways in response to increased flows following rainfall. Although results such as this may be reflective of dispersing individuals and not represent a resident population, it is nonetheless valuable information as it demonstrates that platypuses can access these areas through natural dispersal and may establish a resident population if conditions improve or use them as a dispersal corridor for other populations. This information can be used to direct and prioritise areas for habitat improvement works to increase platypus distribution. Conversely, negative results may arise from no platypuses in that area at the time although they may regularly utilise the creek. As eDNA degrades relatively rapidly in aquatic environments (although variable depending on hydrological and environmental conditions: Barnes et al. 2014; Strickler et al. 2015), a negative result merely reveals that a platypus was unlikely to have been present at that site during the previous few days. For example, live-trapping surveys regularly capture platypuses in Mullum Mullum and Sassafras Creeks but both returned negative eDNA results in the current study from two and three sites respectively. Results such as these highlights the need for multiple sampling sites and sampling events to improve the confidence of detecting resident and transient platypuses in a waterway.

Conclusions

Determining the distribution of platypuses at a landscape scale is logistically challenging using traditional methods (live-trapping, observations). This study used eDNA to investigate platypus distribution across 126 waterways in Melbourne's catchments over a two-month period; the first study to do this over such a large spatial scale. The eDNA results provide a good baseline for the current distribution of platypuses throughout Melbourne's catchments. The main focus of the current eDNA surveys, however, were to investigate areas with little current information on platypus occurrence. This necessitated sampling in many waterways where platypuses were unlikely to occur. A future eDNA monitoring program should consider therefore consider the following: i) concentrate survey sites in known platypus populations and adjacent areas to more effectively detect changes in distribution; ii) reduce sampling effort in areas where platypuses are known to be absent; and iii) incorporate areas where habitat improvement works have been undertaken (are planned to be undertaken) to assess the impacts of these management actions. Here, we have demonstrated that eDNA can be used effectively as a way to monitor changes in platypus distribution and therefore should be used routinely to assess management actions.

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