The use of eDNA to understand spatial and temporal patterns of platypus occupancy in Kosciuszko National Park, New South Wales

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Key Points

- **Platypus were detected using eDNA methods in waterways at high altitudes and low-stream orders where they had not previously been recorded.**
- **Platypus occupation remained relatively localised at sites with lower altitude, high stream-order and closer to dams, during both sampling seasons.**
- **There were seasonal and stream order effects on platypus detection frequency.**
- **Environmental factors such as seasonality and stream order should be considered when using eDNA to detect platypus occupancy.**

Abstract

The platypus is a semi-aquatic mammal endemic to waterways of eastern Australia. Understanding of spatial and temporal patterns of platypus occupancy within alpine and sub-alpine waterways in NSW is limited, particularly for low-order, high altitude streams or streams typically snowed over in winter. We sampled for platypus occupation using eDNA technology at 46 sites across Kosciuszko National Park (KNP). Eighteen sites were sampled using eDNA methods over a two-year period (2021-23) with seasonal sampling periods in November-December and April-May, to access the influence of abiotic conditions on platypus detection such as seasonality, altitude, and stream order. Stream orders varied from 2 to 8, and altitude from 300 m to 1750 m. Platypus were detected in waterways at high altitudes and low-stream orders where they had not previously been recorded. During the autumn sampling period, platypus detection at higher altitudes and low stream-order waterways increased compared to summer sampling months, such that overall, stream order and seasonality had the strongest significant effect on platypus detection. Overall, positive detection of platypus was more likely in autumn. Furthermore, platypus detection remained relatively likely at sites with lower altitude, and significantly likely at higher stream-orders, during both sampling seasons. This seasonal use of alpine streams that are subject to large variation in abiotic conditions, may suggest a relationship to platypus metabolic demands and foraging efficiency trade-offs. Increases in spatial-temporal occupation during spring may be attributed to breeding and territorial behavior of males, or dispersing juveniles. Understanding platypus spatial and temporal occupation across alpine catchments is pertinent for their conservation, as water resource development and habitat modification from feral pest activity and hydrologic alterations may reduce connectivity between populations across their distribution.

Keywords

Platypus, eDNA, survey methods, environmental conservation, alpine streams

Introduction

The platypus is a semi-aquatic mammal, endemic to freshwater environments of eastern Australia. A broad-brush distribution of platypus places them from Cook Town in Far North Queensland, down the east coast of Queensland, NSW and most of Victoria and Tasmania (Grant 2007). Platypuses are now deemed extinct in South Australia except for a translocated population on Kangaroo Island (Grant 2007). Platypuses depend on permanent water bodies whether it be lakes, large rivers, or the moving waters of riffles and connected pools. This aquatic environment is key to providing habitat for their prey species, (Faragher et al. 1979; McLachlan-Troup et al. 2010; Marchant and Grant 2015; Hawke et al. 2022) and healthy bank structure for protection in burrows (Serena et al. 1998; Thomas et al. 2019). Although, their distribution when visualised on a map can seem broad, their habitat is mostly confined to the linear nature of waterways and the irregular mosaic pattern of catchments across the landscape (Grant 2007). Therefore, their distribution is discontinuous and possibly uneven based on preference for fragments of more suitable and productive habitat (Grant 2007, Hawk et al 2019, Bino et al 2020). It has been demonstrated that this distribution has been further fragmented by human influences on changed flow regimes from both irrigation and river regulation, loss, and degradation of habitat from pollutants, sedimentation, stream and bank erosion and riparian loss (Grant 2007, Hawk et al 2019, Bino et al 2020).

Previous studies on platypus ecology and movement using traditional methods such as mark-recapture, radio tracking, and more recently acoustic implants, have mostly been limited to sections of river or catchment that will allow ease of access and provide the minimum number of platypus individuals for a viable study on an ecological aspect of the species (Grant 2007). Such studies have relatively low recapture rates in mark-recapture regardless of repeated efforts in the same location (Grant 2004, Grant 2007, Serena and Williams 2012). Tracking studies have shown over lapping home ranges between individuals, shared home ranges and individuals that seem to be more transient to a study area, with juveniles being rarely recaptured or leaving the bounds of the study area (Serena 1994, Gust and Handasyde 1995, Grant 2004, Bethge et al 2009, Bino et al 2018). Some of these findings can be attributed to limitations of the methods. For example acoustic and microchip tracking are confined to the boundary of the study area's placement of receivers and readers, mark-recapture can have issues of net avoidance and radio tracking can be very labour intensive which often makes the study area size limited by the willing effort and land access by the researcher (Grant 2004, Serena and Williams 2012, Griffith et al 2013, Bino et al 2018, Roberts and Serena 2024). As a result, our spatial and temporal knowledge of platypus occupation and movement across their entire distribution has gaps (Grant 2007, Griffith et al 2018, Bino et al 2020). With that said, there have been significant efforts and contributions in selected study areas with long-term survey data such as those in the Shoalhaven River NSW (Grant 2004), Badgers Creek Healesville (Serena 1994 and Thomas 2018), and Yarra River (Serena and Williams 2008, Griffith et al. 2014b, 2015 and 2016). Such studies have provided invaluable long-term data and insights into platypus behaviour in movement and habitat selection, feeding, reproduction, general biology, and ecology.

Where traditional methods are limited, opportunity exists for investigations via other methods. Environmental DNA (eDNA), a relatively new monitoring technique, has in some cases been able to partially answers questions regarding broader spatial distribution and occupation of platypus. In some areas of wildlife monitoring, it has enhanced our understanding of aquatic biodiversity and helped inform resource management and conservation decision-making (Yang et al 2021). eDNA sampling has allowed simple and cost-effective assessment of platypus occupation across almost all regions of platypus distribution (Lugg et al 2018, Griffith et al 2018, McColl-Gausden et al 2023). In Brisbane, Sydney and Melbourne, longer-term eDNA studies have been used to identify platypus occupancy and infer platypus preferred habitat (Griffith et al. 2014b, 2015 and 2016, Webb et al 2021, Brunt et al 2021). More recently, extensive pre and post fire eDNA sampling was employed to estimate platypus occupancy across fire affected and non-fire affected regions from the 2019-20 Australian bushfires (McColl-Gausden et al 2023). However, like all methods before it, eDNA has limitations, for example eDNA surveys will only truly provide presence-absence data. Furthermore, the probability of detection is affected by many environmental factors, including exposure to heat, water chemistry and UV light, suspended solids and filter clogging, stream dilution from discharge volumes and rainfall, and the unknown temporal rate at which individual animals shed DNA (Pilliod et al. 2014; Strickler et al. 2015; Barnes and Turner 2016; Stoeckle et al. 2017; Harrison et al. 2019).

Regardless of the limitations of eDNA, with the right pre-emptive strategies, appropriate questions, and understanding of survey design, there exists great opportunity for eDNA gathering of baseline data, and as an occupancy tool in planning and development assessment and reserve management for resource managers where vast and remote coverage is a challenge. Kosciuszko National Park offers such a landscape because it covers 6900km^2. Although the park waterways are much changed by river regulation from the snowy hydro scheme and past land use, much of the park is still vast and remote wilderness with pristine alpine waters that feed into large river systems such as the Murrumbidgee, Murray, and Snowy River. Many of the very upper reaches of these river systems reside in KNP at low streams orders, deep into the park wilderness areas, that experience large transitions in environmental gradients across seasons, temperature, altitude, and stream order. Large areas of this park have little to no data on platypus occupancy, with previous concentrated studies around major river sections in the Thredbo, Eucumbene, Murray, and Snowy River (Grant et al 1992, Grigg et al 1992, Hawke et al. 2020, 2021a and 2021b). It is also unknown how these environmental factors (stream order, altitude, and seasonality) influence platypus detection using eDNA. Therefore, the main purpose of this study was to determine whether these factors influence the occupation of platypus as inferred by eDNA detection in a selection of sites across the Kosciuszko National Park.

Methods

Study Site

Kosciuszko National Park (KNP) is the largest National Park in New South Wales, with an area of 6900 km^2. KNP is part of the greater Australian Alpine region in the Great Dividing Range with Australia's highest mountain peak, Mount Kosciuszko at 2228 m ASL. The alpine region of the park includes a unique vegetation community particularly above the tree line, where there is a fragile patchwork of alpine heaths, herbfields, feldmarks, bogs, and fens. This region experiences large environmental variation across temperature, altitude, seasonality, stream levels, and vegetation types. In winter, the mountainous regions >1300 m ASL are covered in snow, bringing recreational snow sports to the region. In the spring/summer months the snow melt drains into the catchments of the Snowy River, Murray River, and Murrumbidgee River. These catchments are water sources of large, regulated rivers and dams in the Snowy Hydro Scheme. The region has also been much changed by historical land use such as gold mining and shale oil in Kiandra and the legacy of high-country grazing and stockmen. Feral horses remain and continue to influence the KNP landscape particularly in the very northern and southern region of the park (Robertson et al 2019).

Study Design

The surveys were conducted on four separate seasonal sampling occasions from December 2021 to May 2023 (Tab.1) with 46 sites and 106 samples collected across the KNP. Most sites were pre-selected because of existing NSW National Parks and Wildlife Service monitoring program locations and 6 additional sites were selected to increase geospatial coverage, sampling stratification across the independent environmental variables (altitude and stream order) and to increase records across areas of the park with no previous records (Fig. 1).

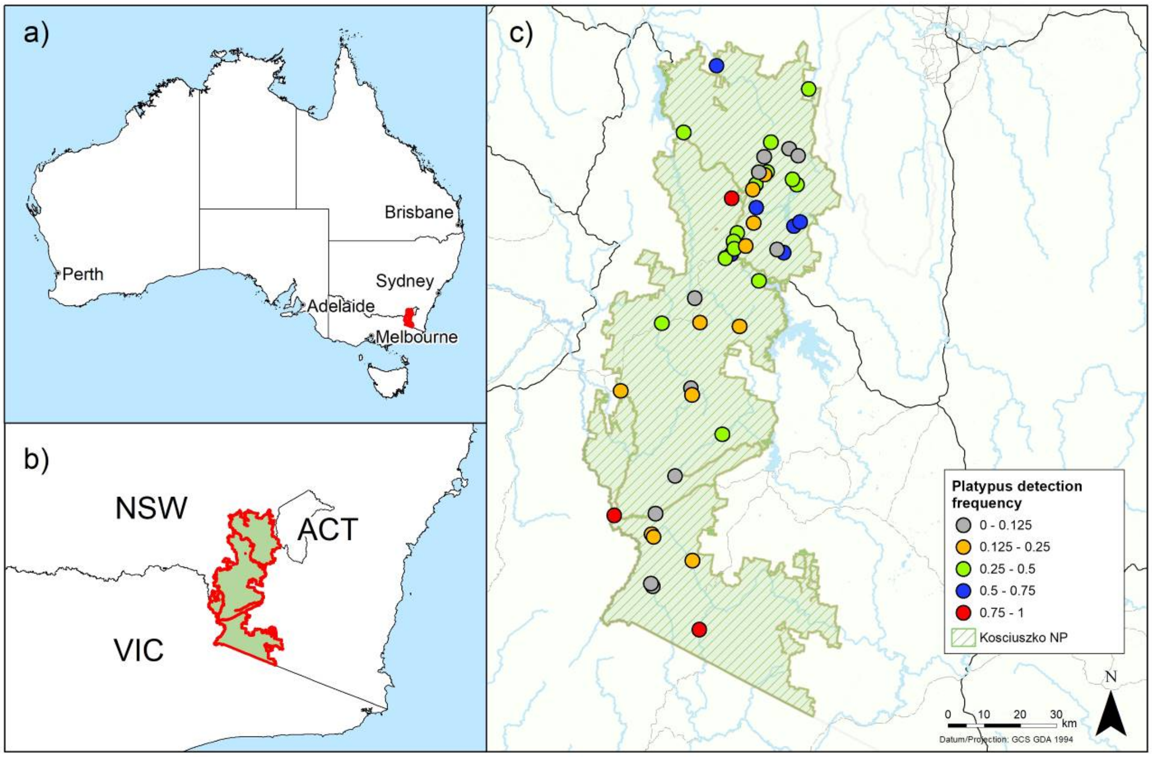

Figure 1. a) Overview map of study location in Australia; b) Location reference of study site, Kosciuszko National Park, in South-east Australia; c) Platypus detection frequency in Kosciuszko National Park.

eDNA sampling

Water samples were collected in duplicate. Water was filtered on-site by pumping 500-2000mL of sample water from the site through a 5µm filter using the Smith-root eDNA sampler. Smith-root self-preserving filters were used for each sample and stored in a dark cool box for up to three weeks before being transported to EnviroDNA (Parkville, Victoria) for DNA extraction and processing by real-time quantitative Polymerase Chain Reaction (qPCR) (Positive > 2 qPCRs +ve; equivocal ≤ 2 qPCR +ve and negative = 0 qPCR +ve of 6-9 assays) as per methods outlined in Griffith et al 2018.

Environmental variables and statistical analysis

Environmental variables altitude and Strahler stream order for each site were collected from geolocation coordinates in QGIS 3.32 with basic terrain layers and Hydro Line layers available from NSW Government SEED data sets. Sites were categorised and grouped by their environmental variables as per table. 1. Platypus positive detection frequency was calculated for each site and averaged for each category. As the data was non-parametric a chi-square goodness of fit test was used to test whether the observed mean positive platypus detection frequencies for each category (altitude, season, or stream order) were different to what was expected (i.e., equal frequencies).

Results

Platypus were detected in waterways at high altitudes and low stream orders where they had not been previously recorded (Fig.1). The highest positive detection site was Valentine creek, a very upper tributary of the Geehi River at 1670m ASL and stream order 3 (Tab.1). The lowest stream order locations where platypus were detected was three at eight different sites. During the autumn sampling period, platypus detection at higher altitudes and low stream-order waterways increased compared to summer sampling months, such that overall, stream order and seasonality had the strongest effect on platypus detection; Chi-sq. (13.7, 4), p = 0.008 and Chi-sq. (24.23, 6), p<0.001, respectively (Fig. 3 (a-b)). Overall, positive detection of platypus was more likely in autumn (Fig. 3). However, platypus detection remained relatively likely at sites with lower altitude, and significantly more likely at higher stream-orders during both sampling seasons. Sites >1500m ASL only reported a positive platypus detection in autumn.

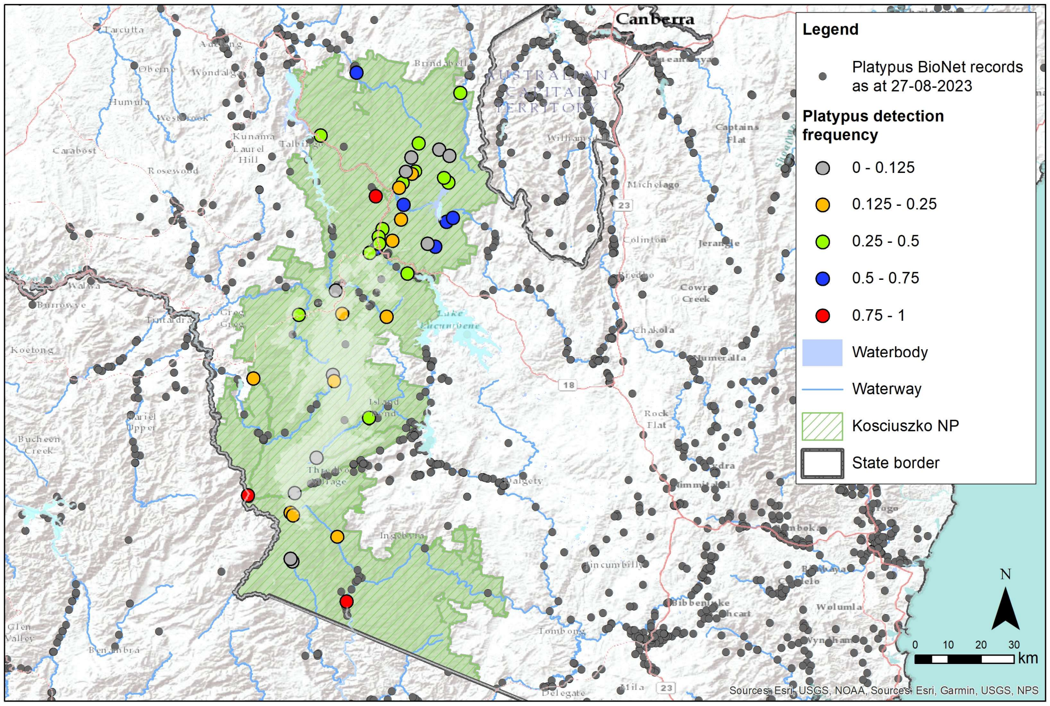

Figure 2. Study location with previous platypus detection records (BioNet as at 23/08/2023) and eDNA detection frequency at sites over the sampling regime from 2021-2023.

Table 1. Number sites for each category classification; a) Altitude, b) stream order, and c) season

a)

Altitude (m ASL)	*No. Sites*
>1500	5
1250-1500	28
1000-1250	6
<1000	7

b)

Strahler Stream Order	*No. sites*
2-3	14
4-5	24
6-8	8

c)

Season	*No. sites*
Summer 21/22	16
Autumn 22	33
Summer 22/23	23
Autumn 23	40

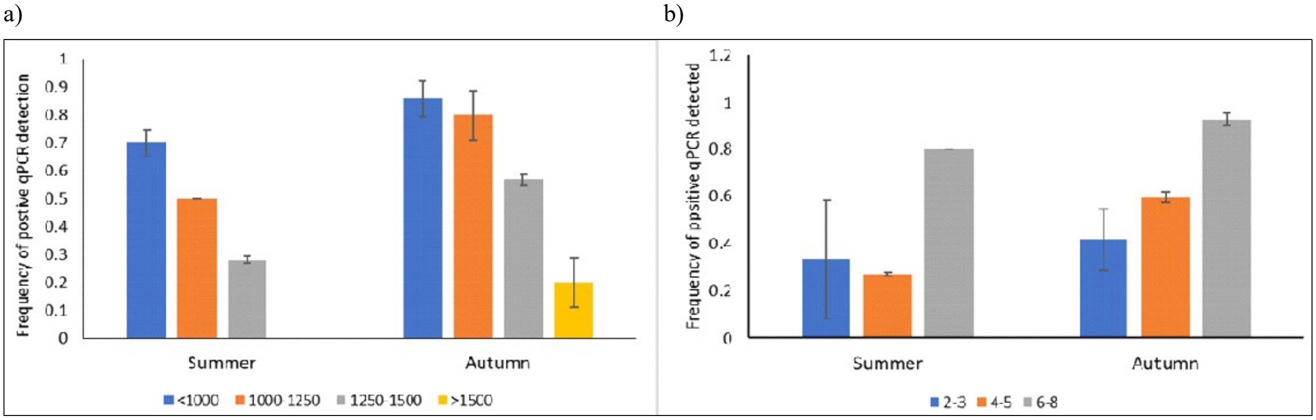

Figure 3. Frequency distribution of positive eDNA detection by qPCR by season for each category; a) altitude (m ASL) and b) Strahler stream order; inclusion of standard error bars

Discussion

We detected platypus using eDNA sampling methods across the Kosciuszko National Park over 4 sampling periods from 2021-2023. We detected platypus in streams with no previous platypus records. Most notably platypuses were detected deep into the park at altitude >1600 m ASL, and those in the very upper reaches of the northern and southern catchments of the park. These records add valuable information on the platypus occupation in highly remote parts of the National Park where eDNA survey methods may be more efficient than traditional methods for ascertaining occupation. For that reason, among others, it may be that previous studies on the platypus in the region have been limited to sections of the major rivers that have high stream order and easier access (Hawke et al. 2020, 2021a and 2021b, Grant et al 1992, Grigg et al 1992). eDNA does not provide abundance data, rather only presence-absence (Roberts and Serena 2024). However, over the four sampling periods, stream order had a significant effect on the detection frequency such that platypus occupation remained relatively localized at sites with high stream-order and closer to dams, and very low frequency at low stream orders. Low detection in any detection method is often loosely attributed to low abundance (Griffith et al 2018). However, low detection for eDNA can also be influenced by many environmental factors, including exposure to heat, water chemistry and UV light, suspended solids and filter clogging, stream dilution from discharge volumes and rainfall, and the unknown temporal rate at which individual animals shed DNA (Pilliod et al. 2014; Strickler et al. 2015; Barnes and Turner 2016; Stoeckle et al. 2017; Harrison et al. 2019).

Considering the location and low detection of some of the low stream order sites there may have been a dilution affect from the seasonal snow melt. This seems likely given the significant difference in platypus detection frequency between the two sampling seasons. Platypus detection at all the sites was more likely during the autumn sampling period and provided the highest detection results for those sites at higher altitude and low stream order. During summer positive detections were more localised to low altitude, high stream order and larger bodies of water (i.e., dams and large pools). Although there may be an environmental effect on the sampling methods, platypus activity patterns and known habitat selectivity could also explain the seasonal and stream order effect on platypus detection frequency across the sampling sites. For instance, the spatial arrangement of male platypuses is temporal and dictated by the breeding season from September to November (Grant 2007). Gust and Handasyde (1995) found home range expansion of male platypuses and establishment of exclusive territories leading up to and during the breeding season. Autumn aligns with the emergence and dispersal of first year juveniles (Grant 2007). Juveniles and sub-adults have increased accumulative movements and range during the lead up to and during the breeding season (Bino et al 2018). Long-term mark recapture studies show low recapture rate of male juveniles (86%) within the original trapping location (Grant 2004). Territorial behavior by breeding males could cause male juveniles and sub-adults to disperse from the natal home range (Thomas 2018). This more mobile phase of both adult and juvenile males overlaps with our sampling period in Autumn. The known spatial and temporal patterns of male platypus at varying life stages could be a reason for the seasonal variance in our platypus detection frequency. Conversely or additively, female platypuses spend less time in the water during egg incubation (Grant 2007), which may reduce DNA detection rates of females at a site during that period (spring-early summer).

Lastly, those sites in our study at high altitude, and low stream order are often considered alpine drainage lines that are typically snowed over with little to no flowing water over the winter months, offering a harsh environment with very little food in the form of macroinvertebrates. During spring/summer, snow melt and increased rainfall results in high flow in these systems, which may prevent movement into these streams due to increased foraging energy expenditure (Griffith et al 2014a). However, we postulate that during autumn when stream levels subside these high altitude and small-low stream order systems become highly productive with reduced energetic demand, making it a lucrative habitat selection for dispersing or sub-adult males pushed out by more dominated breeding males.

Conclusion

We showed that environmental factors such as stream order, altitude, and seasonality may influence platypus eDNA detection frequency, but it is difficult to disentangle the effect due to sampling method limitations interacting with the environmental gradient providing false negatives, or the environmental gradient shaping the true occupation of the platypus across the study area. Thus, we recommend that eDNA studies on platypus occupation must understand this limitation when building the study design. Similarly, the research question does not stretch the bounds of what eDNA data can provide and infer about occupancy.

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