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Comparison of camera trapping, visual stakeouts and eDNA surveys for detection of platypuses (*Ornithorhynchus anatinus*) in north-eastern New South Wales

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ABSTRACT

The platypus (*Ornithorhynchus anatinus*) is a unique monotreme mammal from eastern Australia which can be difficult to study due to its semiaquatic lifestyle and nocturnal nature. Understanding which methods are most effective for studying this elusive species is key to optimising conservation actions. We compared the effectiveness of detecting platypuses using eDNA surveys with qPCR and metabarcoding analysis, camera trapping and visual stakeouts in waterways across the New England Tablelands and quantified activity patterns to optimise survey design. Out of ten survey sites, eDNA surveys with qPCR analysis, camera trapping and visual stakeouts were all effective, detecting platypuses at eight, seven and six sites, respectively. The least effective method was eDNA surveys using metabarcoding, which detected platypuses at only three sites. Camera traps showed platypuses were most active immediately after sunrise and just before sunset. For occupancy surveys, eDNA sampling using qPCR analysis is the most effective method; however, camera trapping and visual stakeouts were also useful, particularly in providing additional data on platypus behaviour.

Keywords: camera trapping, conservation, eDNA, environmental DNA, New England tablelands, New South Wales, platypus, platypus activity, platypus distribution, survey techniques, visual survey.

Introduction

The development and implementation of effective survey techniques to monitor animals is key to the conservation of species. A range of different methods exists, each with various uses; furthermore, new methods are continuously being developed as new technologies emerge. Classical methods, such as visual observation or live-trapping are still relevant and useful today, with the latter allowing for the collection of biometric data (González-Esteban *et al.* 2004; De Bondi *et al.* 2010). However, such methods may require considerable time investment from researchers, or, in the case of live trapping, place animals at risk of harm. More recently developed methods, such as camera trapping, provide an indirect method of detection that can gather data passively without continued intervention by researchers (Ridout and Linkie 2009). Environmental DNA (eDNA) surveys are among the most recently developed methods for surveying animals *in situ* and involve detecting genetic material in the environment to determine whether taxa are present (Rees *et al.* 2014). Understanding the benefits and limitations of newer methods is crucial in assisting ecologists to study a target species efficiently, particularly for cryptic or rare animals.

The platypus (*Ornithorhynchus anatinus*) is an Australian monotreme that can be difficult to study due to its nocturnal behaviour and elusive nature (Brunt *et al.* 2021). Platypuses are one of Australia's only native semiaquatic mammals (Grant and Temple-Smith 2003) and have several features which fascinate both scientists and the public at large, including their oviparity, electroreceptive bills and the venomous spurs possessed by males (Carrick *et al.* 2023). Despite being protected across Australia (Grant and Temple-Smith 2003), platypus populations are currently decreasing and have been listed as Near Threatened by the IUCN (Woinarski and Burbidge 2016). Platypuses face numerous threats including modification of rivers and streams by dams and weirs (Kolomyjec 2010; Mijangos *et al.* 2022), urbanisation and pollution (Serena and Pettigrove 2005; Martin *et al.* 2014;

Serena and Williams 2021) and adverse effects from other organisms, such as disease-causing pathogens (Gust and Griffiths 2011) and introduced predators (Grant 2004). Additionally, anthropogenic removal of riparian vegetative cover exposes platypuses to predators (Scott and Grant 1997), and leads to increased erosion of riverbanks (Abernethy and Rutherfurd 1998; Docker and Hubble 2008) in which platypuses excavate burrows for shelter (Grant and Temple-Smith 1998; Serena *et al.* 1998). Erosion also increases sedimentation within waterways, creating an unfavourable environment for platypus prey (Scott and Grant 1997).

Visual observation surveys (Easton *et al.* 2008), camera trapping (Herrin 2009; Roberts and Serena 2024) and eDNA surveys (Lugg *et al.* 2018; Brunt *et al.* 2021) have all been used to study platypuses. Although each method is less intrusive than live trapping, their overall benefits and limitations vary. Camera traps increase the likelihood of observing natural behaviours (Caravaggi *et al.* 2017) and can be used to detect multiple species simultaneously (Wearn and Glover-Kapfer 2019). However, their usefulness can be limited by a target species' behaviour and morphology (Caravaggi *et al.* 2017). For example, as platypuses spend much time diving for prey, cameras will only detect platypuses when at the water surface. Visual observation surveys are simple to conduct and do not require specialised equipment, so observations by landowners and the public can be incorporated into studies which use this method (Rohweder and Baverstock 1999). However, time for personnel and travel is expensive, and public sightings might suffer from misidentification of the Australian water rat, or rakali (*Hydromys chrysogaster*) which are similar to platypuses in habit and habitat, so visual surveys may not always be optimal (Rohweder and Baverstock 1999). Furthermore, species may be over- or undercounted in visual observation surveys due to non-detection or repeat identifications of an individual (Jachmann 2002; Davis *et al.* 2022), thereby making estimating accurate population sizes difficult.

The most recently developed method for studying platypuses is eDNA surveys. eDNA surveys are non-invasive and can reliably detect species up to 2 km from the precise survey location (Van Driessche *et al.* 2023). Furthermore, eDNA surveys can be more sensitive and cost-effective than classical survey methods, such as live trapping (Pilliod *et al.* 2013; Smart *et al.* 2015). However, eDNA surveys can provide no, or very little, information on a species' abundance or demography in a given location (Beng and Corlett 2020). Additionally, DNA can be introduced into water bodies without an organism itself being present, via predators and cross-contamination by humans (Goldberg *et al.* 2016; Beng and Corlett 2020).

As the use of methods such as eDNA surveys and camera trapping to study platypuses is relatively new, little research has assessed the relative effectiveness of these methods for answering different questions. Such studies are important to provide researchers and managers with the knowledge to optimise methodology for research and conservation. Therefore, in this study we aimed to determine (1) the broad distribution of platypus in the New England Tablelands in north-eastern New South Wales, (2) the comparative effectiveness of visual stakeouts, camera traps, qPCR analysis and DNA metabarcoding analysis for detecting platypuses, including the benefits and disadvantages of each method, and (3) the activity cycles of platypuses so as to better inform survey design.

Methods

Study sites

eDNA sampling was completed between June and October 2023 to assess the broad distribution of platypuses in the New England Tablelands at 38 sites including lentic and lotic, and permanent and non-permanent waterbodies (Fig. 1). Following this, 10 sites at which platypuses were recently recorded were selected to allow us to compare platypus sampling methods (eDNA surveys, camera trapping and visual stakeouts). Sites were selected based on expected platypus habitat preferences and evidence of multiple platypus records during the last five years in the Atlas of Living Australia, or that had been communicated by property owners. Visual stakeouts and camera trapping was conducted from September to October 2023.

Camera trapping and visual stakeouts

Swift Enduro 4G cameras were deployed from 1 September to 14 October 2023. Two cameras were placed at each site, at least 50 m apart. The cameras were attached to a tree and positioned overlooking a section of river or, when a suitable tree was unavailable, strapped to a metal stake placed at the top of the riverbank. Each camera was set on 'timelapse' and programmed to take one photograph per minute. In two cases, cameras could not be placed at the same site as eDNA surveys. At site Macdonald River 1, cameras were placed approximately 1.7 km upstream of eDNA collection and visual stakeouts in a location requested by the property owners. At Uralla Creek, it was deemed too risky for theft to place camera traps on public property within the town so they were placed on private property 0.8 km downstream of eDNA collection and visual stakeout sites. Cameras were collected after 10 days.

Visual stakeouts were undertaken between 11 September 2023 and 19 October 2023. Four visual stakeouts were conducted at each site; two in the morning between 0630 and 1000 hours and two in the afternoon–evening between 1600 and 1930 hours. Stakeouts involved one to three observers positioned covertly by the riverbank and using binoculars to constantly scan the water surface for platypuses. The time of the first sighting of a platypus was recorded on each visual stakeout, at which point the survey was ended. If no platypuses were detected, surveys were concluded after 60 min.

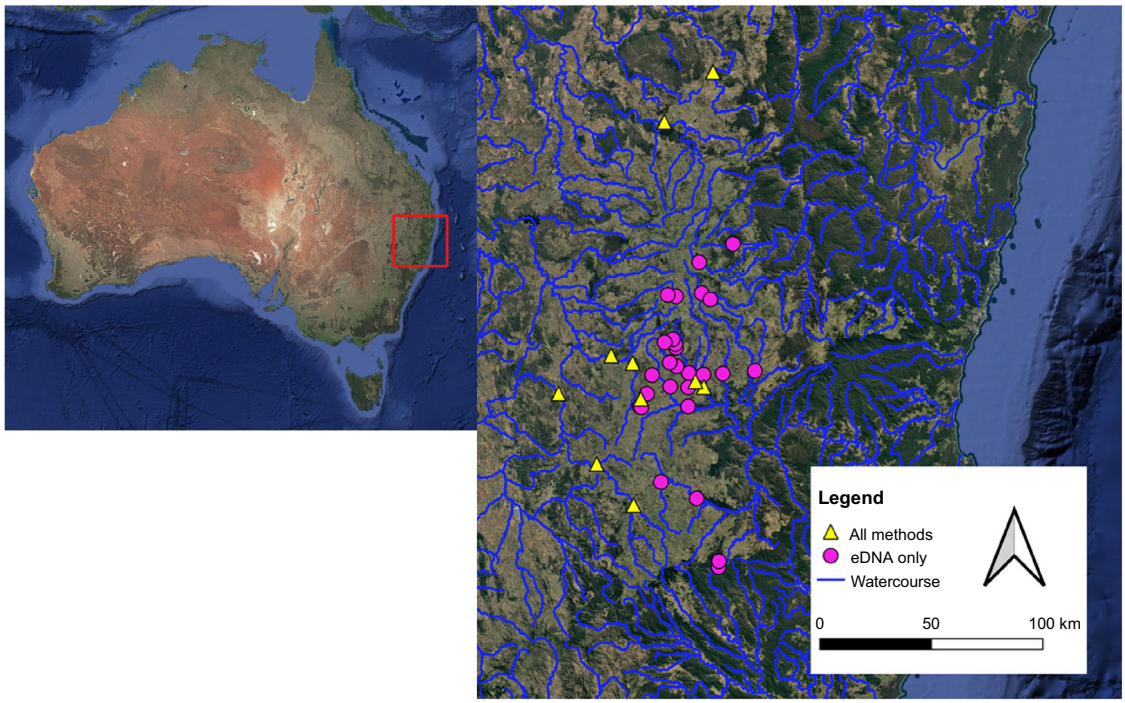

Fig. 1. Map showing sites on the New England Tablelands, NSW, Australia, where eDNA surveys, camera trapping and visual stakeouts were conducted from June to October 2023. Surveys at each site comprised all three methods (yellow triangles) or eDNA surveys only (pink circles).

eDNA methodology

Water samples were collected from each waterbody using sampling protocols developed by EnviroDNA, Melbourne. Platypuses are highly detectable (>95% probability) with two replicate eDNA samples (Lugg *et al.* 2018). Thus, two replicate samples were collected at each site. For each sample, water was passed through a 1.2 μm syringe disc filter until filtration became physically impossible, or when 1000 mL of water had been filtered. Filtration occurred on-site to reduce DNA degradation during the transport of water samples (Yamanaka *et al.* 2016). Clean sampling protocols were employed to minimise contamination between sites. Equipment that contacted water was cleaned with 10% bleach solution and then rinsed with water from the next site at which it was to be used. A preservative was added to the filter once filtration was complete to minimise DNA degradation. The filters were stored out of sunlight, at ambient temperature, before being sent to EnviroDNA for processing. In running waters, water was collected from a single location to be filtered. In standing waters, approximately 1 L of water was collected from five different areas in the waterbody and mixed into a bucket before being filtered. Each litre of water was collected from a different microhabitat (e.g. open water, reedbeds, shallow water) or at least 50 m from the other collection areas.

All DNA analyses were performed by EnviroDNA, Melbourne, using Quantitative Polymerase Chain Reaction (qPCR) and metabarcoding analyses. For qPCR analyses, a commercially available DNA extraction kit (Qiagen PowerSoil Kit) was used to extract DNA from filters. This kit minimises compounds that can inhibit PCR reactions in environmental samples. Real-time qPCR analyses were used to amplify platypus DNA, using a species-specific probe that targeted a small region of the platypus's mitochondrial DNA (Lugg *et al.* 2018). Analyses were performed three times on each sample and positive and negative controls were included for all analyses. Two or more positive qPCRs strongly suggested the presence of platypuses and considered a positive detection. Samples that yielded one positive qPCR indicated lower levels of target DNA were collected and considered equivocal.

Using the DNA extracted samples, a library was constructed using two rounds of PCR. The first round used gene-specific vertebrate primers (Riaz *et al.* 2011) to amplify the target region and the second round included sequencing adapters and unique barcodes for each sample–amplicon combination included in the library. Negative controls were included in the construction of the library. These consisted of the extraction negative as well as PCR negatives, in which nuclease-free water was used in place of DNA during both rounds of PCR. Positive controls consisted of mock communities which included a variety of vertebrate species with known but variable DNA concentrations. Sequencing was conducted on an Illumina sequencing platform. After quality control filtering removed primer sequences, truncated reads, and low frequency reads, DNA sequences were denoised

(UNOISE3 algorithm implemented in VSEARCH) to a set of unique amplicon sequence variants (ASVs). Default filters applied removed detections with <30 reads per ASV and sample, or <0.02% of reads per assay and sample. The remaining DNA sequences were clustered into Operational Taxonomic Units (OTUs) based on the similarity of sequences. Taxonomic assignment was performed with VSEARCH software (Rognes *et al.* 2016), whereby each OTU cluster was assigned a species identity using a threshold of 95% by comparing against a reference sequence database. If a species could not be assigned (i.e. reference database was deficient and/or taxa were poorly characterised), taxonomic assignments were manually vetted. This was done by obtaining a list of possible species through BLASTN searches against the public repository Genbank (www.ncbi.nlm.nih.gov), followed by elimination of species based on their geographic distributions, using information from the Atlas of Living Australia. When an OTU could not be adequately identified to a single species (e.g. due to shared haplotypes), the OTU was assigned to the lowest taxonomic rank without further classification.

Survey effort

Two eDNA samples were collected at 10 sites on the New England Tablelands. In total, 12,062 mL of water was filtered ($\bar{x} = 603.10$ mL/sample). A total of 207,818 photographs was taken from 20 cameras deployed across 10 sites. This included 248 photographs of vertebrates that yielded 172 independent events. One camera trap, at Macdonald River 2, failed after two days and ceased taking photographs beyond this time. Forty hours of visual stakeouts were conducted across 10 sites.

Data analyses

Camera trap data were prepared using the programs DataOrganize and DataAnalyse in the CameraSweet software package (Sanderson and Harris 2013) and statistical analyses were performed using R 4.1.2 (R Core Team 2021). A map was created using QGIS and the watercourses were mapped based on the NSW named watercourses dataset from the NSW Spatial Collaboration Portal (NSW Government 2025). Photographs of the same number of individuals of the same species within 30 min by the same camera were considered as one independent event (O'Brien *et al.* 2003; Ridout and Linkie 2009). Logistic regression modelling was conducted to compare the effectiveness of each method in detecting platypuses, using platypus presence as the reference factor. qPCR and metabarcoding methods were considered separate methods for the analysis. As the data analysis relied on the presence/absence data (i.e. positive or negative results), equivocal results from qPCR analyses were considered positive as platypus presence for these sites was confirmed by other methods. The dependent variable was the presence of platypuses at a site as detected by each method, whereas the independent variable was the method used to detect platypuses. The Akaike Information Criterion (AIC) values for the logistic regression model we created were compared to a null model to determine how well the model fitted the data. Additionally, a density plot was constructed to visually represent platypus activity throughout the day using the package 'overlap' (Meredith and Ridout 2014).

Results

Broad distribution

Platypuses were detected in eight of the 38 sites using eDNA surveys. Platypuses were only detected in connected, flowing water bodies and not in upland lagoons. Occupied sites were Commissioners Waters, Deepwater River, Gwydir River, Macdonald River (at three sites), Severn River and Uralla Creek. For six sites, platypus DNA was detected with certainty while at two sites results were equivocal. However, platypus presence at these two sites was confirmed by camera traps and visual stakeouts.

Methods comparison

Methods differed in their effectiveness at detecting platypus. Metabarcoding detected platypuses at significantly fewer sites than the other methods ($\beta = -2.23$, s.e. = 1.05, $P = 0.03$, 95% CI [−4.29, −0.18]). Metabarcoding was the least effective method, detecting platypuses at three sites (Table 1). All other methods also successfully detected platypuses at these three sites. The most effective method of detecting platypuses was eDNA surveys using qPCR analysis, which detected platypuses at all eight sites (six positive, two equivocal). Camera traps were the next most effective, detecting platypuses in seven sites. Visual stakeouts detected platypuses at only six sites. Platypuses were detected by both camera traps and visual stakeouts at five sites. At Macdonald River 2, platypuses were detected by stakeouts and eDNA surveys but not by camera traps. At Commissioners Waters 2 and Uralla Creek, both camera traps and eDNA surveys detected platypuses where visual stakeouts failed to do so. The number of sites at which platypuses were detected by qPCR ($\beta = 0.00$, s.e. = 1.12, $P > 0.05$), camera traps ($\beta = -0.54$, s.e. = 1.05, $P > 0.05$) and visual stakeouts ($\beta = -0.98$, s.e. = 1.02, $P > 0.05$) was not significantly different from the number of sites that platypuses occupied.

Platypus activity

We recorded 172 independent events of platypuses using camera traps. Platypus activity increased in the pre-dawn hours and peaked just after sunrise (Fig. 2). A lull in activity occurred around midday, with another smaller peak in activity just prior to sunset (Fig. 2). Platypus activity declined after sunset and was again low in the few hours leading to midnight (Fig. 2).

Table 1. The success of various survey methods in detecting platypuses in winter and early spring in the New England Tablelands.

Site	Location (Latitude, Longitude)	qPCR	Camera traps	Visual stakeouts	Metabarcoding
Commissioners Waters 2	(30.57273°S, 151.75943°E)	Y	Y	N	N
Deepwater River	(29.29646°S, 151.84308°E)	Y	Y	Y	N
Gara River 1	(30.59542°S, 151.79914°E)	N	N	N	N
Gwydir River	(30.46671°S, 151.35867°E)	Y	Y	Y	N
Macdonald River 1	(30.62215°S, 151.10854°E)	E	Y	Y	Y
Macdonald River 2	(30.91065°S, 151.28995°E)	Y	N	Y	N
Macdonald River 3	(31.07811°S, 151.46531°E)	Y	Y	Y	Y
Rocky River	(30.49729°S, 151.46033°E)	N	N	N	N
Severn River	(29.50336°S, 151.61169°E)	Y	Y	Y	Y
Uralla Creek	(30.64340°S, 151.49966°E)	E	Y	N	N
Total		8	7	6	3

Y indicates platypuses were detected; N indicates platypuses were not detected; E indicates results were equivocal.

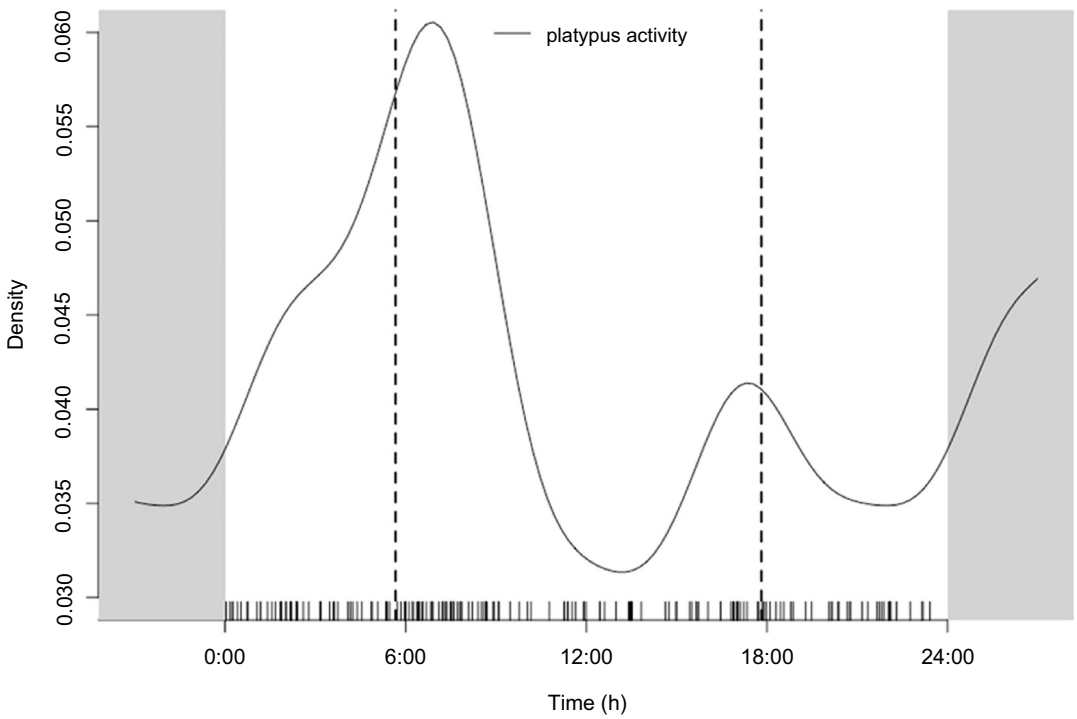

Fig. 2. Platypus activity detected by camera traps on the New England Tablelands in early spring. Dotted lines represent approximate sunrise and sunset times in Armidale during September and the 'rug' on the *x*-axis displays the raw platypus observations.

Platypus visual stakeouts

Platypuses were detected at 19 of the 40 visual stakeouts between six sites. Platypuses were detected in every stakeout at only one site. For the remaining five sites at which platypuses were detected, platypuses were detected at three stakeouts (75%). Of the 19 times platypuses were detected, they were observed 11 times within the first 10 min and in 16 visual stakeouts, platypuses were observed within the first 30 min. Only three times were platypuses not observed until after 30 min had passed.

Discussion

Platypus distribution

Platypuses were detected only in rivers; none were detected in upland lagoons. Although platypuses are capable of overland

migration, their adaptations to an aquatic lifestyle limit their capacities for terrestrial movement and aquatic dispersal is the most frequent method of migration (Ellem *et al.* 1998; Fish *et al.* 2001; Kolomyjec *et al.* 2009). As a result, platypuses are primarily found in connected river systems. Survey methods such as camera trapping and visual stakeouts are limited to detecting platypuses within the observers or cameras field of view. Therefore, understanding platypus habitat preferences allows ecologists to optimise survey effort by concentrating on areas where platypuses are most likely to be found. Our study supports findings that suggest ecologists aiming to find platypuses should focus their efforts on systems with connected, flowing water bodies.

Effectiveness of methods

Platypuses were detected by multiple methods at each site where they were present; however, qPCR analysis of eDNA was the method most successful in identifying platypus presence. This finding supports numerous studies showing qPCR analysis is a highly effective method of detecting platypuses (Lugg *et al.* 2018; Brunt *et al.* 2021). Unlike other methods, eDNA surveys can detect the presence of a target taxon, without the target taxon being present at the exact moment of sample collection or in the immediate vicinity as the DNA persists in the environment (Berger *et al.* 2020; Saito and Doi 2021; Villacorta-Rath *et al.* 2021; Van Driessche *et al.* 2023). Although the results were equivocal for two sites, alternate methods confirmed the presence of platypuses at these sites and thus qPCR analysis of eDNA could be considered successful in detecting platypuses. Therefore, eDNA surveys for platypuses can be conducted at any time of day, and in easy to access locations, regardless of whether platypuses are active during that time or likely to pass through that location.

Visual stakeouts and camera traps were also effective as platypus survey methods. Although visual stakeouts are a common method of studying platypus occupancy, camera traps have been rarely used for this species. All platypuses were detected by camera traps set on a timelapse and platypuses were most easily identified by camera traps facing down on the river from above. The success of camera traps in our study suggests that they are an underutilised method of studying platypuses which could be incorporated into future platypus research; this supports other recent findings exploring the use of camera traps to study platypuses (Roberts and Serena 2024).

Although both visual stakeouts and camera trapping detected platypuses at a similar number of sites, the sites in which platypuses were detected were not always consistent between methods which highlights potential limitations for each method. Although the automated nature of camera traps can be useful, technical failures that occur during the deployment period can impact survey data, as happened at the Macdonald River 2 site. Ideally, placing multiple cameras at one site can overcome technical failures; however, camera trap placement also needs to be considered as camera traps will not detect individuals that do not enter a camera's field of view. Conversely, visual stakeouts require the presence of an observer, and can be impacted by human presence and time constraints. For example, it is possible that platypuses were not detected visually at Uralla Creek due to high human activity. Due to the risk of camera traps being stolen at the Uralla Creek site, visual stakeouts and eDNA surveys were conducted in a public park whereas camera traps had to be placed on private property upstream. It is likely that cameras detected platypuses due to the more secluded nature of the area in which they were placed and that eDNA surveys detected the DNA of platypuses further upstream from where it was collected. Additionally, although 60 min was generally found to be long enough to detect platypuses, this period of time is too short for significant weather or temperature changes to occur. If conditions during the stakeout are suboptimal for platypuses to be active, they are less likely to be observed. Finally, platypuses may be cross-identified with other aquatic vertebrates, such as rakali (Rohweder and Baverstock 1999), and care must be taken to correctly discern between platypuses and other aquatic vertebrates when conducting visual stakeouts and camera trapping. The least successful method of detecting platypuses was eDNA surveys using metabarcoding analysis, which detected them significantly less than would be expected. This is likely due to primers for metabarcoding binding to common species or those that shed DNA at high rates (Kelly *et al.* 2014; Schneider *et al.* 2016) rather than binding to a target species as in qPCR analysis. Another reason for low detection may have been the application of detection thresholds, which reduce the likelihood of detecting false positives but can also omit rare or low abundance species (Deiner *et al.* 2017; McColl-Gausden *et al.* 2023). Although metabarcoding analysis is a useful tool when the aim is to survey many species in an area at once, for few or single species surveys other methods are likely to be more effective, particularly for less abundant species such as platypuses.

Platypus activity

In addition to detecting platypuses, camera traps provide additional information (Streeting *et al.* 2024) which we used to estimate when platypuses were most active. Although platypuses were active throughout the day, there was a distinct peak in activity soon after sunrise, and a smaller peak just before sunset. Studies in south-eastern New South Wales, north-eastern Victoria and southern Tasmania have supported our finding that platypuses are nocturnal with peak activity at dawn and dusk, and least active during the day (Grigg *et al.* 1992; Hawke *et al.* 2021; Roberts and Serena 2024). At our sites, platypus were least active at midnight, and exhibited low activity after midday in most sites. Our data, however, represents only a small seasonal window of platypus

activity throughout the year in the New England Tablelands and further study is required to provide an accurate representation of seasonal daily platypus activity. For example, platypuses have been reported as more diurnal during winter and early spring as movements increase in the lead up to breeding season (Menkhorst and Knight 2011; Hawke *et al.* 2021) and more active foragers as the cost of thermoregulation increases (Bethge *et al.* 2009; Hawke *et al.* 2021). Additionally, platypus activity at night may be underestimated due to limited light availability reducing the camera's field of view (Roberts and Serena 2024).

An accurate understanding of daily platypus activity throughout the year would aid ecologists in deciding upon the best time to survey for platypuses using visual stakeouts, which are the least specialised method of detecting platypuses. For many sites, platypuses could be easily detected during visual stakeouts within the first 20 min. Additionally, for all sites at which platypuses were observed during visual stakeouts, platypuses were detected within the first two stakeouts. Visual observations of platypuses can be prone to misidentification, require people to be searching for platypuses at the same time and place as they are active and are restricted by light availability and weather (Rohweder and Baverstock 1999; Easton *et al.* 2008; Grant 2012). However, our findings suggest that visual stakeouts can be an effective method of surveying for platypuses when resources may be too limited to use other methods.

Recommendations for platypus surveys

Various methods can be useful for studying platypuses; however, the method used should reflect the goal of the study and the available resources. For presence–absence surveys, eDNA surveys using qPCR analysis were found to be the most effective method for detecting platypuses. Occasionally however, too little eDNA may be collected during eDNA surveys to ensure conclusive results from qPCR analysis and methods which yield more certain results (e.g. camera traps, visual stakeouts) may be required to confirm platypus presence. eDNA surveys using metabarcoding analysis are a useful method when trying to survey for a large number of species in a limited number of samples. However, we found it to be an ineffective method of surveying for platypuses and should not be used when platypuses are the target species. If additional data are required, such as behaviour or activity, camera traps and visual stakeouts are a useful addition to a platypus survey. Furthermore, based on observations of platypus activity as determined by camera trapping, our results indicate that visual stakeouts should be conducted shortly after sunrise for approximately 30–60 min. Future research which aims to determine platypus activity throughout eastern Australia throughout the year would be beneficial in providing a broader understanding of platypus activity and the most effective times to survey for them. Understanding the various benefits of these different survey methods in detecting platypuses will assist ecologists in choosing the optimal method for their study, which will in turn improve our understanding of the behaviours of this iconic and unique Australian mammal.

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