

Camera traps and genetic identification of faecal samples for detection and monitoring of an endangered ungulate

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Abstract. Almost all Indochinese ungulates are classified as globally threatened but efforts to assess and monitor population status have been hampered by their rarity, cryptic nature, and uncertainty in accurate identification from sightings. An improved approach is urgently needed to gather information about threatened ungulate species in order to effectively conserve them as a lack of reliable monitoring methods means that basic information such as population sizes, distribution and habitat associations is currently unknown. Here, we used a combination of camera trapping and genetic detection of the endangered Eld's deer, *Rucervus eldii*, to investigate the utility of these methods to infer intensity of site use within a protected Cambodian dry forest. We asked: 1) Are Eld's deer present in our study area?; 2) How is site use influenced by local habitat?; and 3) Do camera traps or genetic detection perform better in terms of detection and monitoring? Camera traps were deployed and faecal samples collected from Chhaeb Wildlife Sanctuary in northern Cambodia during the 2017 dry season. Faecal samples were identified as Eld's deer using newly developed species-specific mitochondrial DNA primers. Camera traps recorded 20 Eld's deer observations across 3,905 trap-nights and 44 out of 71 collected faecal samples, identified by fieldworkers as likely to belong to Eld's deer, were positively identified to be so. Camera trap surveys and genetic detection demonstrated that Eld's deer were present in Chhaeb Wildlife Sanctuary, although the number of detections relative to sampling effort was low in both methods (detected at 29% and 1% of sample sites, respectively). Occupancy models showed that water level and tree diameter both had positive relationships, whilst human and domestic or feral pig activity had a negative relationship, with the relative intensity of Eld's deer site use. Overall, our data suggest that both of our methods can prove effective for monitoring Eld's deer but that repeated sampling is necessary to account for their low detectability in this area. We suggest that faecal samples are collected during future camera trap monitoring visits to maximise efficiency, increase detectability, and provide the most information to support conservation.

Key words. occupancy, genetic detection, camera trap, Eld's deer, Cambodia

INTRODUCTION

Tropical and subtropical dry forests comprise many of the top 200 ecoregions worldwide in need of conservation based on their irreplaceability, with the dry dipterocarp forests (DDF) of Indochina particularly recognised for their diverse large vertebrate faunas (Olson & Dinerstein, 1998). Central Indochina has one of the last remaining expanses of tropical dry forest worldwide, but deforestation has resulted in the loss of much of this forest in only two decades, with 2% of the global area of tropical dry forests lost from Southeast

Asia alone (Miles et al., 2006). What remains of the DDF in Central Indochina is highly fragmented and forest loss is projected to continue (Trisurat & Bhumpakphan, 2018).

Ungulates are key members of Indochinese DDF systems as they disperse seeds, maintain habitat structure and support larger carnivores (Du Toit & Cumming, 1999). However, almost all Indochinese large ungulates are now classified as globally threatened (O'Kelly et al., 2012; IUCN, 2022). Eld's deer (*Rucervus eldii*) is an endangered species of ungulate, consisting of three recognised subspecies (Gray et al., 2015a; Ghazi et al., 2021). The Siamese Eld's deer subspecies, *R. e. siamensis*, is highly restricted in range, and found only in fragments of DDF in Cambodia, Thailand, Laos, and Hainan Island, China (Angom & Hussain, 2013; Gray et al., 2018; Trisurat & Bhumpakphan, 2018). The majority of the remaining Siamese Eld's deer are found in Cambodia. They are highly threatened by hunting and habitat destruction due to mining and agriculture, and this population is thought to have declined by 90% between 1998–2008 (Gray et al., 2015a; Ghazi et al., 2021). Despite the urgency of management actions needed to support the conservation of this endangered species, little is known about the presence of Eld's deer, current geographical distribution, population

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density, and population viability within Cambodia, which may have experienced rapid declines in a number of locations (Gray et al., 2012; Trisurat & Bhumpakphan, 2018).

Efforts to assess and monitor the status of threatened ungulate species are often hampered by their rarity, cryptic nature, and complexity of species identification (Gray et al., 2012; Woodruff et al., 2015). A comprehensive line transect survey conducted in eastern Cambodia had such low encounter rates with Eld's deer through sightings on line transects that they were unable to reliably estimate their density (Gray et al., 2012). Furthermore, the logistical difficulties of working in tropical environments can lead to the use of sub-optimal sampling strategies, which can limit further analyses (O'Dea et al., 2004; Muir & Muir, 2011). Trisurat & Bhumpakphan (2018) were able to compile occurrence data for Eld's deer in northern Cambodia from camera traps, visual sightings (including footprints), and interviews, highlighting the utility of combining data gathering approaches to assess species status. However, due to the inherent limitations of non-standardised sampling strategies, they were only able to estimate distribution and not population size.

Collection of occurrence and non-detection data and subsequent occupancy modelling can provide a reliable method of estimating population distribution (Rovero & Marshall, 2009; Sollmann et al., 2013). Occupancy modelling is particularly useful where difficulties in individual recognition limit capture–mark–recapture analysis (Rowcliffe et al., 2008); a common limitation in ungulate studies (Rovero & Marshall, 2009; Parsons et al., 2017). Data for occupancy modelling can be obtained through camera trapping, which provides a non-invasive method of monitoring cryptic species in difficult forest environments and determining habitat use (Tobler et al., 2009; Li et al., 2014). However, detection rates from camera traps can be very low for some species and careful evaluation of the most effective camera placement is necessary (Matthews et al., 2023). In the current Southeast Asian context (excepting a very few areas well secured against hunting), camera trap encounter rates of threatened ungulates are only high when placed close to seasonal pools, locally called trapeangs, which introduces potential bias into population estimates (Pin et al., 2020). Both faecal sampling and camera trapping offer the opportunity to maximise detectability of individuals in a landscape by avoiding the requirement to observe highly cautious animals, but faecal sampling additionally allows the use of genetic tools to confirm species identification (Woodruff et al., 2015). This method has proven effective in a range of species, including large ungulates (Hajji et al., 2007; Ramón-Laca et al., 2015; Woodruff et al., 2015). By extracting and amplifying DNA from shed epithelial cells in faecal samples, conservation genetic techniques can be used to confirm species presence (at least at time of defecation) at specific geographic locations within a landscape (Waits & Paetkau, 2005).

Here, we used a combination of camera trapping and genetic detection of Eld's deer, *R. e. siamensis*, to investigate the

utility of both methods to infer species presence within a legally protected Cambodian dry forest. Specifically, we asked the following questions: 1) Are Eld's deer present in Chhaeb Wildlife Sanctuary?; 2) Do camera traps or genetic identification of faecal samples perform better in terms of detection and monitoring and 3) How is intensity of site use influenced by local habitat? Information on detection, habitat preferences and the effectiveness of these two monitoring methods will help to optimise field methods in Northern Cambodia by informing practitioners about how and where to monitor this difficult to survey species.

MATERIAL AND METHODS

Study Area. Chhaeb Wildlife Sanctuary (also known as Chhep, formerly Preah Vihear Protected Forest) in Northern Cambodia is an IUCN Management Category IV wildlife sanctuary, managed by the Ministry of Environment. Chhaeb Wildlife Sanctuary has one of the largest continuous tracts of dry dipterocarp forest (DDF) in the region (Suzuki et al., 2017), and is home to Eld's deer and other threatened ungulates (Trisurat & Bhumpakphan, 2018). The forest within this area is 67% DDF, with some small patches of semi-evergreen dense forest and open grassland (Suzuki et al., 2017). Trapeangs, forest pools are common throughout the forest (Pin et al., 2020).

Camera Traps. Bushnell Trophy Cam HD 119437 and Bushnell Trophy Cam HD MAX 119476 camera traps were deployed (Fig. 1) between January and June 2017 for a total of 3,905 trap-nights. A total of 22 cameras were set up in late January, with a further 20 in late February and early March. Cameras took three consecutive images when triggered and then could not be triggered again for ten seconds following this. Images of the same species were considered notionally independent when taken at least one hour apart (Bowkett et al., 2008). No baits or lures were used and cameras were set to operate for 24 hours each day.

Camera traps were deployed by Wildlife Conservation Society (WCS) staff with many years' experience of camera trapping in Chhaeb Wildlife Sanctuary. In order to maximise the probability of recording Eld's deer, all camera traps were located at trapeangs. WCS staff selected trapeang sites known to be frequented by Eld's deer in previous years, using historical camera trapping records and local knowledge (Rovero & Zimmermann, 2016). Trapeangs were selected mostly within DDF, with some close to semi-evergreen forest or grasslands. Locations were selected by WCS staff based on local knowledge and cameras were orientated so that cameras were not directly facing the sun in areas where vegetation was unlikely to obscure the camera. Only one camera was placed at each selected trapeang and the minimum distance between cameras was 583 m. The median distance between each camera and its nearest neighbour was 1,243 m. Cameras were strapped to trees adjacent to animal trails at an average of 4.25 m (S.E. = 0.14) from the trail. Cameras were at an average of 45 cm (S.E. = 1) from the ground and 14.78 m

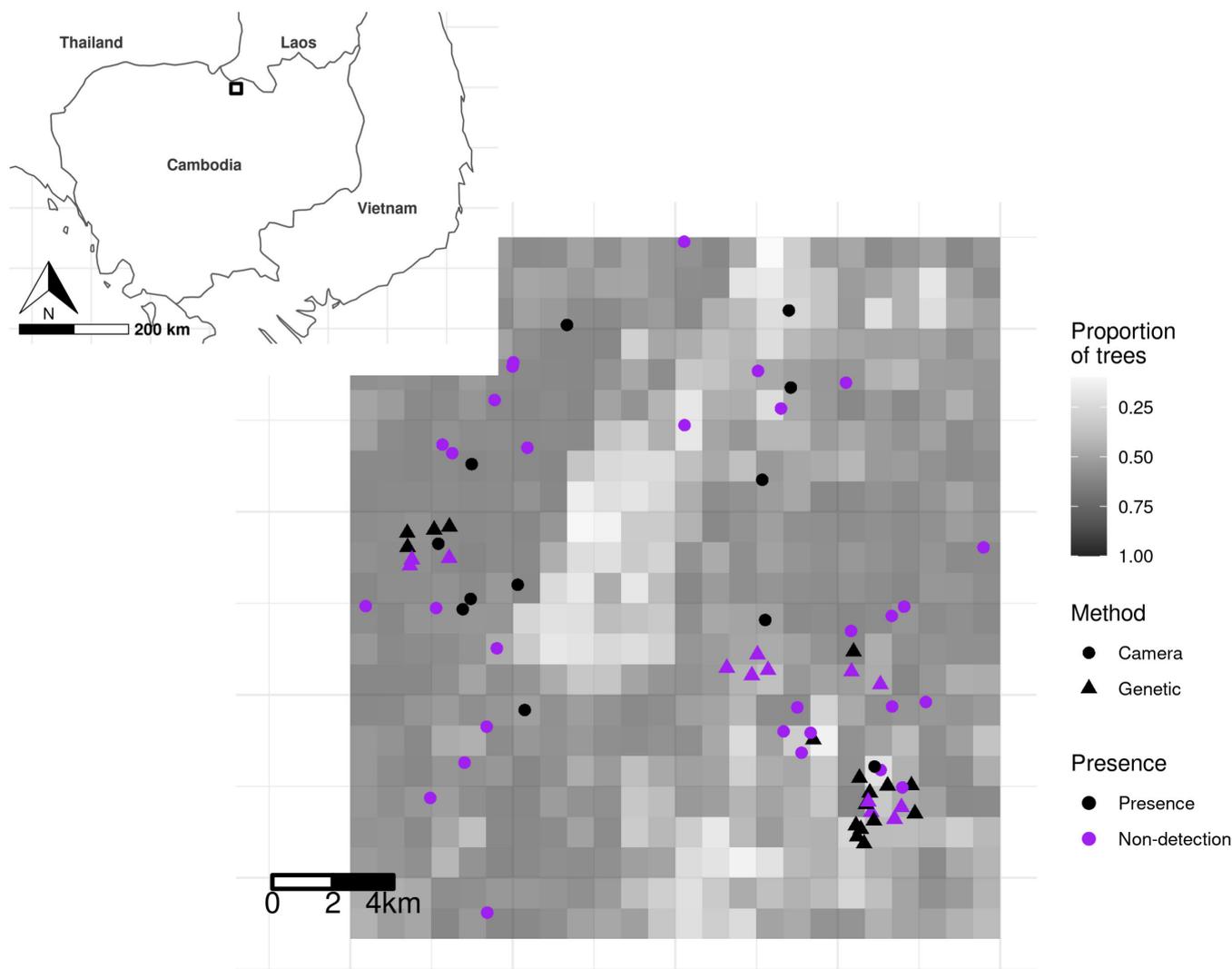


Fig. 1. Distribution of deployed camera traps showing presence (black circles) and non-detection (purple circles) and genetic sampling locations showing presence (black triangles) and non-detection (purple triangles) of Eld’s deer. Inset map shows the location of Chhaeb Wildlife Sanctuary in Cambodia (black rectangle). Background shows proportion of tree cover from WorldCover land cover map (© ESA WorldCover project 2020 / Contains modified Copernicus Sentinel data (2020) processed by ESA WorldCover consortium).

(S.E. = 2.1) from the edge of the trapeang, however, four were set up within the natural boundaries of the trapeang as these were fully dried up.

Environmental data. Habitat surveys were conducted for each trapeang where a camera had been placed. Trapeang habitat surveys were completed in February 2017 at the peak of the dry season. Trapeang composition was estimated in percentages in a method similar to that of Wright et al. (2010). The percentage of open water, exposed vegetation cover (including grass, sedge and *Sesbania*), dry grass, submerged grass, sedge (dry and submerged), *Sesbania* (dry and submerged), and bare ground were all estimated visually by the same observer at each site. The relative area of each trapeang was estimated by multiplying the maximum length and breadth of the pool measured using a handheld GPS (Garmin GPSMAP® 64s). Pig activity, which can affect vegetation structure (Gray et al., 2020), was assessed categorically with a score from zero (no pig activity) to five (entire perimeter of the trapeang disturbed). The effect of Asian elephant (*Elephas maximus*) and cattle on trapeangs

was not assessed as elephants were not present in the study area and it was not possible to consistently identify the impact of cattle on trapeangs. Percentage canopy cover was estimated visually, and the distance between the tree used for the camera trap and the next closest two trees was also measured to represent forest density. Larger-scale covariates such as distance to forest and human infrastructure are also likely to play a role in determining Eld’s deer distribution, however, as we did not have access to such data, we were unable to include it in our analyses.

Eld’s deer site use. These distances between our camera traps fall well within home range estimations for Eld’s deer (Pan et al., 2014). Therefore, we have focused on intensity of use at each site rather than site occupancy in our analyses. Relative intensity of site use by Eld’s deer at each trapeang was modelled using a state occupancy model in the unmarked package (Fiske & Chandler, 2011) in R (R Core Team, 2021) based on the presence data recorded from camera traps. As our detections were expected to be low, we used a month as our trapping period, giving at least two

(maximum five) samples at each camera trap station which we treated as repeats in our statistical analyses. Habitat features were used as explanatory variables in order to investigate preferred trapeang habitat for Eld's deer. Distance of the nearest tree to the camera, distance of the second closest tree to the camera, trapeang size, percentage of the trapeang affected by pig activity, percentage of submerged grass and percentage canopy cover were used as potential predictors of site use intensity. Models were compared and ranked using AIC scores with the 'best' model having the lowest score. Models with $\Delta AIC < 2$ were considered to have equal support and the most parsimonious model was preferred (Burnham & Anderson, 1998). Model selection was first applied to detection covariates (distance to the trapeang, camera height, aspect and distance to the next closest tree) and, once selected, these were held constant in models where occupancy covariates were selected.

Genetic sample collection. Faecal samples were collected from Chhaeb Wildlife Sanctuary during February and March 2017 (Fig. 1), which are Cambodia's driest and hottest months, when temperatures average between 24°C to 35°C (Thoeun, 2015). During the dry season, species are unable to obtain sufficient moisture from surface water sources that are frequently available during the wet season, and thus visit trapeangs, more persistent water sources, more frequently (Valeix, 2011). Therefore, active trapeangs (where water was present) were targeted for faecal sampling to maximise discovery. At each trapeang location, three observers walked, in turn, a loop around the trapeang scanning a transect width of approximately 2 metres. Putative Eld's deer samples were identified by the shape (oblong) and size (1.5–3 cm) of the pellets, based on comparison with pellets from captive Eld's deer in Cambodia. The only other species within the study area that produces a faecal pellet similar in shape and size to that of the Eld's deer is the northern red muntjac (*Muntiacus vaginalis*) which, although smaller, has dung that is visually congruous and may have been collected as an assumed Eld's deer pellet (Gray et al., 2012). All collected samples were assigned a confidence of Eld's deer origin score: 3 = highly likely, 2 = likely, and 1 = possible Eld's deer dung. Pellets were identified as being from a single individual by their close proximity to one another within the dung pile, and similar visual appearance.

Faecal samples were swabbed using an easy-snap cotton swab (Fisherbrand, Loughborough) that had previously been dipped in ASL buffer (Qiagen Inc., Crawley), as per Ramón-Laca et al. (2015). The swab tip was then placed in 500 µl of ASL buffer and stored at –20°C upon arrival at base camp until shipment to the UK, under the DEFRA import permit ITIMP17.0352. No export permit was required for these samples.

DNA extraction. DNA extractions were carried out using the QIAGEN DNeasy Blood and Tissue kit (Qiagen, Manchester, UK), as per the manufacturer's protocol, with the following alterations to maximise yield (Peters et al., 2020). The incubation step was increased to 48 hours in order to achieve complete sample lysis and swab tips were

then placed into a QIAshredder spin column to remove all of the lysate retained within the cotton. Samples were eluted using 70 µl of elution buffer, which had been heated to 70°C, with a five-minute incubation. This was then repeated for a final elution volume of 140 µl.

Primerless PCR. Primerless PCR has been shown to increase DNA quality and improve amplification success in subsequent primed PCR reactions (Peters et al., 2020). DNA extracted from each sample was exposed to a primerless PCR cycle using illustra™ PuReTaq Ready-To-Go™ PCR Beads with a final volume of 25 µl including 5 µl of DNA template. Samples were subjected to a PCR cycle with the following cycling parameters: initial denaturation at 95°C for 5 minutes, 10 cycles of 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 60 seconds, 35 cycles of 95°C for 30 seconds, 50°C for 30 seconds, 72°C for 60 seconds, and a final extension at 72°C for 5 minutes.

Species identification. Species-specific primers were designed, in MEGA7 (Kumar et al., 2016), within the cytochrome b region of the mitochondrial DNA (mtDNA) (GenBank accession no.: NC_014701) (unpublished data). The target length of the species-specific sequence was 69 bp using the following primers: SS5F: 5'–CCATACATCGGCACAAATC–3' and SS5R: 5'–GTTGGGTTATTGGATCCT–3'. The species-specific primers were tested against the other most likely species to have been collected during the faecal sampling, the northern red muntjac. In the known species trials, Eld's deer samples had positive amplification whereas the red muntjac had no positive amplification.

PCR was conducted using illustra™ PuReTaq Ready-To-Go™ PCR Beads with a final volume of 25 µl including 19.5 µl of sterile H₂O, 0.5 µl of primers and 5 µl of primerless treated DNA sample. Samples were subjected to a PCR cycle with the following cycling parameters: initial denaturation at 95°C for 10 minutes, 35 cycles of 95°C for 45 seconds, 53°C for 60 seconds and 72°C for 120 seconds, and a final extension at 72°C for 10 minutes. The presence of a band when PCR product was visualised using gel electrophoresis was recorded as a positive result for the presence of Eld's deer DNA. All samples identified as belonging to Eld's deer were used in further analyses.

RESULTS

Eld's deer presence. Camera traps made 20 Eld's deer records across 3,905 trap-nights giving a trapping rate of 0.005 records per trap-night and a naive occupancy of 0.29. Swabs from 71 faecal samples were collected in the field and 45 were positively identified as Eld's deer following DNA extraction and amplification with species-specific primers. There were 26 remaining samples which did not positively amplify as Eld's deer. Positive identification to species level of all faecal samples collected in the field was thus 62%. Confidence scoring of physical dung identification determined that 60 of the 71 samples were Highly Likely to be Eld's

Table 1. Confusion matrix for potential Eld's deer faecal samples indicating the confidence category to which each sample was assigned and the number of samples for which identification was confirmed genetically.

	Confidence Category		
	Possible (1)	Likely (2)	Highly Likely (3)
Genetic identification confirmed	2 (33%)	0 (0%)	43 (72%)

deer. Of these samples, genetic identification confirmed 72% (43 samples) of this category were in fact Eld's deer (Table 1). Within the 26 samples that did not positively amplify as Eld's deer, nine had a confidence score of 1 or 2, meaning it was felt only Possible or Likely that the faecal pellets were deposited by an Eld's deer. The remaining 17 samples did have a confidence rating of 3, Highly Likely to be Eld's deer, however, notes made at the time of sampling recorded that 14 samples were in a degraded state. In the areas which were both sampled for faeces and had camera traps deployed, we detected Eld's deer at a considerably larger number of sites using cameras (29%) than faecal sampling (1%).

Eld's deer site use intensity. The 'best' model for predicting site use intensity for Eld's deer in Chhaeb Wildlife Sanctuary contained no covariates affecting either detectability or site use intensity. The model predicted Eld's deer occupancy in the park to be 0.5 (S.E. = 0.171) and detectability to be 0.21 (S.E. = 0.076). This model was the simplest from a candidate set in which six models had $\Delta AICc < 2$. Model averaging of this subset of models suggests that the diameter of nearby trees ($\beta = 6.3$) and the percentage of water visible in the trapeang ($\beta = 1.7$) both positively influence Eld's deer site use intensity (Ψ) whereas signs of human ($\beta = -60.1$) and pig activity ($\beta = -31.3$) both negatively influence Eld's deer site use intensity. Medium ($\beta = 37.3$) and, particularly, small trapeangs ($\beta = 106.7$) were associated with a higher intensity of site use than large trapeangs.

DISCUSSION

Both our camera trap surveys and genetic analyses demonstrate that Eld's deer are present in Chhaeb Wildlife Sanctuary, although the number of detections relative to sampling effort was low in both cases. Eld's deer are highly restricted in range in Cambodia and occur at very low densities within fragments of remaining habitat due to hunting and habitat loss (Gray et al., 2012). Previous surveys have had very low detection rates. McShea et al. (2005) recorded Eld's deer in only 0.03% of the 1 km² blocks surveyed using camera trapping and transect surveys, Suzuki et al., (2017) found a naive occupancy of only 0.02 using camera traps, and Gray et al. (2012) had only two Eld's deer detections using line transect surveys across 143 transects. However, despite their low detectability, our camera trap and faecal records demonstrate widespread use of Chhaeb Wildlife Sanctuary over several months by Eld's deer. Surveys for Eld's deer in this area are likely to be costly and have low success and so, optimising methods for long-term monitoring of Eld's deer that meet both economic and scientific objectives is an

important step for this species. Therefore, survey methods need to assume detectability will be low and concentrate on longitudinal approaches (i.e., surveys repeated over time rather than 'point' surveys)—both of the methods we trialled do this to some extent but our faecal sampling could have been repeated during camera maintenance to increase this aspect.

Our models suggest that the trapeangs sampled have a 50% chance of being occupied by Eld's deer during the time of survey and that the intensity of site use was not strongly related to habitat features. However, all of our cameras were in forest previously shown to contain Eld's deer (Trisurat & Bhumpakphan, 2018) and trapeangs are important water sources, so all areas likely represent potentially good habitat for the species (Pin et al., 2020). Therefore, our models may have lacked the necessary power to detect small differences in habitat quality. We were also unable to investigate the impact of landscape scale drivers of Eld's deer distribution such as anthropogenic infrastructure and habitat configuration due to a lack of data but these factors are likely to have an influence on resultant habitat use. Nevertheless, indications from model averaging suggest some aspects of trapeang structure may also be important. Water levels were positively related to Eld's deer site use which may indicate permanence and provide a more stable water source for deer throughout the year. Trapeang depth and area have previously been shown to be related to use of trapeangs by Eld's deer (Pin et al., 2020). Very few trapeangs retain water all year around (Koehncke, 2010). Gray et al. (2015b) demonstrated that deepening trapeangs can improve their water retention, with Eld's deer recorded at these modified trapeangs. A positive relationship with tree diameter suggests more mature forest and tree cover as has previously been shown to be related to Eld's deer presence (McShea et al., 2005). Further surveys across a wider range of habitats and with more specific measurements of trapeang structure and the surrounding habitat could offer further insight into potentially beneficial habitat management within the forest and Eld's deer distribution throughout the annual cycle.

Model averaging showed a negative relationship with both human and pig activity (wild or domestic/feral). Pigs can act as ecosystem engineers and pig activity could change water chemistry as well as the structure of both trapeangs and surrounding habitat (Mihailou & Massaro, 2021). Harvesting of Eld's deer is a problem across their range, and hunting of both Eld's deer and other species remains a problem in Cambodia (Loucks et al., 2009; Harrison et al., 2016; Gray et al., 2018). Previous work has demonstrated that Eld's deer presence is negatively related to proximity to villages,

which suggests, at the very least, a negative impact from some aspect(s) of human presence (Gray et al., 2018; Pin et al., 2020).

Despite low estimated species detectability using faecal samples (1%), Eld's deer DNA amplification was successful in 62% of samples. Given the unknown period of time that faecal samples were in the field in tropical conditions and the impact that heat and time has on DNA quality, this amplification success rate is comparable to other herbivore faecal DNA studies (Maudet et al., 2004; Woodruff et al., 2015). We cannot be 100% confident in the physical identification of dung to be that of Eld's deer in this area due to the presence of red muntjac, whose dung has a similar physical appearance (Gray et al., 2012). However, 14 samples classified as Highly Likely to belong to Eld's deer based on their physical appearance were noted as degraded upon collection: old with white spots in appearance or collected after a heavy rainstorm. The condition of these faecal pellets prior to swabbing may have hindered DNA collection, resulting in a false negative for those samples. Failed DNA amplification could either be due to limited DNA extraction or the sample being from a non-target species. A DNA quantification step after extraction (Adams et al., 2019) could determine whether limited DNA extraction was the cause. Alternatively, faecal material could be searched for hair samples to provide samples with better chance of DNA amplification. Non-invasively collected genetic information has been widely applied for individual identification (Brook et al., 2012), to estimate population size (Hedges et al., 2013), and for creation of conservation management units (Hajji et al., 2007) among other analyses (for a review, see Waits & Paetkau, 2005). One advantage that remote methods such as camera trapping have over the faecal sampling protocol we used is that they allow the collection of data over long periods. We have evidence from camera trapping that in some locations, although no Eld's deer faeces was found on our surveys, Eld's deer later used the habitat in that location. One improvement to our faecal sampling protocol would be to repeat the sampling occasions over several months in the same way that the camera traps were deployed. In this way, we could account for imperfect detection in our genetic monitoring and increase the accuracy of any estimates of species distribution or abundance. This change would increase the costs of the fieldwork and laboratory analysis; however, it could still be argued that the cost of monitoring in this way would be comparable to the cost of purchasing and deploying a camera trap array.

One advantage of faecal sampling over camera traps is that faecal searches can be made over a wider area and are not subject to the same field of view restrictions of cameras (Apps & McNutt, 2018). Detection in the field of faecal samples could also be improved using trained dogs (Arandjelovic et al., 2015) or through increased search time and effort. Other than increasing the number of cameras and using differing grid designs, this increase in search efficiency is not possible with camera trapping. Additionally, genetic monitoring provides a range of information of value to population monitoring which cannot be collected through remote cameras such

as subspecies assignment, population structure, genetic diversity, and presence of inbreeding (Balakrishnan et al., 2003; Hajji et al., 2007; Edelhoff et al., 2020; Ghazi et al., 2021). However, camera trapping itself can provide additional information that genetic methods applied to degraded faecal DNA are unlikely to be able to produce, such as productivity and population age-structure (Macauley et al., 2020). Genetic methods also incur substantial additional costs per sample, whereas the increase in costs for additional image processing from camera traps is much smaller and camera traps have the potential to capture information about other important species with limited additional costs.

The gathering of information on the genetic status of Eld's deer would be highly beneficial for the conservation management of this species, as records indicate that populations in Cambodia are now mainly restricted to nine locations (Ladd et al., 2021); with no knowledge of population connectivity, gene flow, or inbreeding levels. The current captive populations of Eld's deer are not managed to promote genetic diversity (Gray et al., 2019). Without genetic monitoring of both captive and wild populations, reintroductions or translocations may have the potential to inadvertently further reduce the genetic health of Eld's deer (Ladd et al., 2021). Despite the additional costs associated with genetic monitoring, Eld's deer populations, such as this one in northern Cambodia, are at severe risk of extinction, and detailed information about their demographics, distribution and genetic health is sorely needed. As such, it is important that monitoring efforts are maximised wherever possible.

Overall, our data demonstrate that Eld's deer were still present in Chhaeb Wildlife Sanctuary in 2017 but our encounter rates were low. We suggest that camera traps are most suitable for maximising detectability in order to estimate occupancy or intensity of site use of Eld's deer, but recommend that faecal samples are collected during camera trap monitoring visits in order to maximise efficiency, increase detectability, and provide the most information to support conservation. Camera trapping surveys for this species are likely to require long periods of monitoring and covering large areas to monitor the species effectively. There are additional costs associated with the collection, storage, and processing of genetic samples, but the additional information which can be revealed using genetic approaches, alongside information from camera trapping, could be vital in understanding and mitigating the threats that this small and potentially isolated population continues to face.

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AUTHOR CONTRIBUTIONS

AM, MH and MG designed the experiment. MH, ZB, SW and MK carried out the fieldwork. SW, RB and KP carried out the laboratory work. MG carried out the statistical analyses. AM and MG wrote the manuscript. All authors read and approved the manuscript.

LITERATURE CITED

- Adams CIM, Knapp M, Gemmill NJ, Jeunen GJ, Bunce M, Lamare MD & Taylor HR (2019) Beyond biodiversity: Can environmental DNA (eDNA) cut it as a population genetics tool? *Genes*, 10(3): 192.
- Angom S & Hussain SA (2013) A review on genetic status of Eld's Deer *Rucervus eldii*: With notes on distribution, population status and future perspectives. *Octa Journal of Environmental Research*, 1(2): 65–76.
- Apps PJ & McNutt JW (2018) How camera traps work and how to work them. *African Journal of Ecology*, 56(4): 702–709.
- Arandjelovic M, Bergl RA, Ikfuingei R, Jameson C, Parker M & Vigilant L (2015) Detection dog efficacy for collecting faecal samples from the critically endangered Cross River gorilla (*Gorilla diehli*) for genetic censusing. *Royal Society Open Science*, 2(2): 140423.
- Balakrishnan CN, Monfort SL, Gaur A, Singh L & Sorenson MD (2003) Phylogeography and conservation genetics of Eld's deer (*Cervus eldi*). *Molecular Ecology*, 12(1): 1–10.
- Bowkett AE, Rovero F & Marshall AR (2008) The use of camera-trap data to model habitat use by antelope species in the Udzungwa Mountain forests, Tanzania. *African Journal of Ecology*, 46(4): 479–487.
- Brook SM, van Coeverden de Groot P, Scott C, Boag P, Long B, Ley RE, Reischer GH, Williams AC, Mahood SP, Hien TM, Polet G, Cox N & Hai BT (2012) Integrated and novel survey methods for rhinoceros populations confirm the extinction of *Rhinoceros sondaicus annamiticus* from Vietnam. *Biological Conservation*, 155: 59–67.
- Burnham KP & Anderson DR (1998) Model selection and inference: A practical information-theoretic approach. Springer New York, New York, xx + 355 pp.
- Clements T, Garrett L, John A, Keo O, Sreng K, Bunnat P, Vann R, Setha T, Sokha T & Rainey H (2010) Bird Nest Protection Program in the Northern Plains of Cambodia. TRANSLINKS. Case Study. Bird Nest Protection Program in the Northern Plains of Cambodia. Report Prepared for WCS TransLinks Program. <https://global.wcs.org/Resources/Publications/Publications-Search-II/ctl/view/mid/13340/pubid/DMX539600000.aspx>. (Accessed 01 July 2017).
- Du Toit JT & Cumming DH (1999) Functional significance of ungulate diversity in African savannas and the ecological implications of the spread of pastoralism. *Biodiversity & Conservation*, 8(12): 1643–1661.
- Edelhoff H, Zachos FE, Fickel J, Epps CW & Balkenhol N (2020) Genetic analysis of red deer (*Cervus elaphus*) administrative management units in a human-dominated landscape. *Conservation Genetics*, 21(2): 261–276.
- Fiske I & Chandler R (2011) unmarked: An R Package for Fitting Hierarchical Models of Wildlife Occurrence and Abundance. *Journal of Statistical Software*, 43(10): 1–23.
- Ghazi MG, Sharma SP, Tuboi C, Angom S, Gurumayum T, Nigam P & Hussain SA (2021) Population genetics and evolutionary history of the endangered Eld's deer (*Rucervus eldii*) with implications for planning species recovery. *Scientific Reports*, 11(1): 1–14.
- Gray SM, Roloff GJ, Kramer DB, Etter DR, Vercauteren KC & Montgomery RA (2020) Effects of wild pig disturbance on forest vegetation and soils. *Journal of Wildlife Management*, 84(4): 739–748.
- Gray TNE, Brook SM, McShea WJ, Mahood S, Ranjitsingh MK, Miyunt A, Hussain SA & Timmins R (2015a) *Rucervus eldii*: E.T4265A22166803. The IUCN Red List of Threatened Species 2015. (Accessed 03 October 2022).
- Gray TNE, Eames JC, Lyon JRA & Meyerhoff M (2019) Rewilding in Southeast Asia: an assessment of conservation opportunities in Western Siem Pang Wildlife Sanctuary, Cambodia. *Cambodian Journal of Natural History*, 2: 98–112.
- Gray TNE, Hughes AC, Laurance WF, Long B, Lynam AJ, O'Kelly H, Ripple WJ, Seng T, Scotson L & Wilkinson NM (2018) The wildlife snaring crisis: An insidious and pervasive threat to biodiversity in Southeast Asia. *Biodiversity and Conservation*, 27(4): 1031–1037.
- Gray TNE, McShea WJ, Koehncke A, Sovanna P & Wright M (2015b) Artificial deepening of seasonal waterholes in eastern Cambodia: Impact on water retention and use by large ungulates and waterbirds. *Journal of Threatened Taxa*, 7(6): 7189–7195.
- Gray TNE, Phan C, Pin C & Prum S (2012) Establishing a monitoring baseline for threatened large ungulates in eastern Cambodia. *Wildlife Biology*, 18(4): 406–413.
- Hajji GM, Zachos FE, Charfi-Cheikrouha F & Hartl GB (2007) Conservation genetics of the imperilled Barbary red deer in Tunisia. *Animal Conservation*, 10(2): 229–235.
- Harrison RD, Sreekar R, Brodie JF, Brook S, Luskin M, O'Kelly H, Rao M, Scheffers B & Velho N (2016). Impacts of hunting on tropical forests in Southeast Asia. *Conservation Biology*, 30(5): 972–981.
- Hedges S, Johnson A, Ahlering M, Tyson M & Eggert LS (2013) Accuracy, precision, and cost-effectiveness of conventional dung density and fecal DNA based survey methods to estimate Asian elephant (*Elephas maximus*) population size and structure. *Biological Conservation*, 159: 101–108.
- IUCN (2022) The IUCN Red List of Threatened Species. Version 2022-1. <https://www.iucnredlist.org> (Accessed 7 September 2022).
- Koehncke A (2010) Report on Monitoring Waterhole-Dynamics in Mondulhiri Protected Forest. (No. 32). WWF-Cambodia, Phnom Penh, Cambodia, 32 pp.
- Kumar S, Stecher G & Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution*, 33(7): 1870–1874.
- Ladd R, Crouthers R, Brook S & Eames J (2022). Reviewing the status and demise of the Endangered Eld's deer and identifying priority sites and conservation actions in Cambodia. *Mammalia*, 86(5): 407–421.
- Li X, Buzzard P & Jiang X (2014) Habitat associations of four ungulates in mountain forests of southwest China, based on camera trapping and dung counts data. *Population Ecology*, 56(1): 251–256.
- Loucks C, Mascia MB, Maxwell A, Huy K, Duong K, Chea N, Long B, Cox N & Seng T (2009) Wildlife decline in Cambodia, 1953–2005: Exploring the legacy of armed conflict. *Conservation Letters*, 2(2): 82–92.

- Matthews N, Nixon S, von Hardenberg A, Isoke S & Geary M (2023) Targeting burrows improves detection in giant pangolin *Smutsia gigantea* camera trap surveys. *Oryx* (First View): 1–9.
- Maudet C, Luikart G, Dubray D, Hardenberg AV & Taberlet P (2004) Low genotyping error rates in wild ungulate faeces sampled in winter. *Molecular Ecology Notes*, 4(4): 772–775.
- McShea WJ, Koy K, Clements T, Johnson A, Vongkhamheng C & Aung M (2005) Finding a needle in the haystack: Regional analysis of suitable Eld's deer (*Cervus eldi*) forest in Southeast Asia. *Biological Conservation*, 125(1): 101–111.
- Mihailou H & Massaro M (2021) An overview of the impacts of feral cattle, water buffalo and pigs on the savannas, wetlands and biota of northern Australia. *Austral Ecology*, 46(5): 699–712.
- Miles L, Newton AC, DeFries RS, Ravilious C, May I, Blyth S, Kapos V & Gordon JE (2006) A global overview of the conservation status of tropical dry forests. *Journal of Biogeography*, 33(3): 491–505.
- Muir AP & Muir M (2011) A new rapid assessment technique for amphibians: Introduction of the species list technique from San José de Payamino, Ecuador. *Herpetological Review*, 42(2): 184–186.
- O'Dea N, Watson JE & Whittaker RJ (2004) Rapid assessment in conservation research: A critique of avifaunal assessment techniques illustrated by Ecuadorian and Madagascan case study data. *Diversity and Distributions*, 10(1): 55–63.
- O'Kelly HJ, Evans TD, Stokes EJ, Clements TJ, Dara A, Gately M, Menghor N, Pollard EHB, Soriyun M & Walston J (2012) Identifying conservation successes, failures and future opportunities; assessing recovery potential of wild ungulates and tigers in Eastern Cambodia. *PLoS One*, 7(10): e40482.
- Olson DM & Dinerstein E (1998) The Global 200: A representation approach to conserving the Earth's most biologically valuable ecoregions. *Conservation Biology*, 12(3): 502–515.
- Parsons AW, Forrester T, McShea WJ, Baker-Whatton MC, Millsbaugh JJ & Kays R (2017) Do occupancy or detection rates from camera traps reflect deer density? *Journal of Mammalogy*, 98(6): 1547–1557.
- Peters C, Nelson H, Rusk B & Muir A (2020) A novel method to optimise the utility of underused moulted plumulaceous feather samples for genetic analysis in bird conservation. *Conservation Genetics Resources*, 12(3): 457–467.
- Pan D, Song YL, Zeng ZG & Bravery BD (2014) Habitat selection by Eld's deer following relocation to a patchy landscape. *PLoS One*, 9(3): p.e91158.
- Pin C, Ngoprasert D, Gray TN, Savini T, Crouthers R & Gale GA (2020) Utilization of waterholes by globally threatened species in deciduous dipterocarp forest of the Eastern Plains Landscape of Cambodia. *Oryx*, 54(4): 572–582.
- R Core Team (2021) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. <https://www.R-project.org/> (Accessed 03 October 2022.)
- Ramón-Laca A, Soriano L, Gleeson D & Godoy JA (2015) A simple and effective method for obtaining mammal DNA from faeces. *Wildlife Biology*, 21(4): 195–203.
- Rovero F & Marshall AR (2009) Camera trapping photographic rate as an index of density in forest ungulates. *Journal of Applied Ecology*, 46(5): 1011–1017.
- Rovero, F & Zimmermann F (2016) Camera Trapping for Wildlife Research. Pelagic Publishing, Exeter, 320 pp.
- Rowcliffe JM, Field J, Turvey ST & Carbone C (2008) Estimating animal density using camera traps without the need for individual recognition. *Journal of Applied Ecology*, 45(4): 1228–1236.
- Sollmann R, Mohamed A, Samejima H & Wilting A (2013) Risky business or simple solution—Relative abundance indices from camera-trapping. *Biological Conservation*, 159: 405–412.
- Suzuki A, Thong S, Tan S & Iwata A (2017) Camera trapping of large mammals in Chhep Wildlife Sanctuary, northern Cambodia. *Journal of Natural History*, 2017(1): 63.
- Tobler MW, Carrillo-Percegué SE & Powell G (2009) Habitat use, activity patterns and use of mineral licks by five species of ungulate in south-eastern Peru. *Journal of Tropical Ecology*, 25(3): 261–270.
- Trisurat Y & Bhumpakphan N (2018) Effects of Land Use and Climate Change on Siamese Eld's Deer (*Rucervus eldii siamensis*) Distribution in the Transboundary Conservation Area in Thailand, Cambodia, and Lao PDR. *Frontiers in Environmental Science*, 6: 35.
- Waits LP & Paetkau D (2005) Noninvasive genetic sampling tools for wildlife biologists: A review of applications and recommendations for accurate data collection. *Journal of Wildlife Management*, 69(4): 1419–1433.
- Woodruff SP, Johnson TR & Waits LP (2015) Evaluating the interaction of faecal pellet deposition rates and DNA degradation rates to optimize sampling design for DNA based mark-recapture analysis of Sonoran pronghorn. *Molecular Ecology Resources*, 15(4): 843–854.
- Wright HL, Buckingham DL & Dolman PM (2010) Dry season habitat use by critically endangered white shouldered ibis in northern Cambodia. *Animal Conservation*, 13(1): 71–79.