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Comparing the effectiveness of short-focal camera trapping, live trapping, and soil eDNA for surveying small mammals: A case study on Eurasian water shrew (*Neomys fodiens*)

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Abstract

Small mammals are potential bio-indicators of various ecosystems and their populations are often studied. However, many small mammal species are difficult to detect due to their small size and elusive behaviour. Camera trapping and live trapping are commonly employed survey techniques, but they both have their limitations. Recently developed techniques such as adjusted short-focal camera trapping and environmental DNA (eDNA) are promising new approaches, but their relative performance remains poorly quantified. We compared the effectiveness of three survey protocols for detecting a semi-aquatic and elusive small mammal, the Eurasian water shrew (*Neomys fodiens*), by (1) short-focal camera trapping, (2) live trapping, and (3) soil eDNA. During September and October 2022, we surveyed 20 transects of each 100 m in length alongside the Kleine Dommel, a lowland brook in the Netherlands. The effectiveness of the three survey protocols was compared based on detection probabilities. Short-focal camera trapping yielded a significantly higher detection probability than the eDNA protocol. Detection probabilities between short-focal camera trapping and live trapping and, between the eDNA protocol and live trapping, were not significantly different. Short-focal camera trapping is an effective technique to survey Eurasian water shrews. Furthermore, this method detected additional species compared to live trapping and is non-invasive and less labour-intensive. Short-focal camera trapping showed a promising method for small mammal surveys in general and we recommend further evaluation of its applicability for other small mammal species.

Keywords Bio-indicators · Biomonitoring · Conservation · Detection probability · Environmental DNA · Wildlife

Introduction

Monitoring of bio-indicator species is useful for assessing ecosystem integrity and effective wildlife conservation (Carnignan and Villard 2002; Holt and Miller 2010). Small mammals such as mice, shrews, and voles are potential

bio-indicators (Pierce and Venier 2005), as they are key components of food webs and tend to respond rapidly to environmental changes (Leis et al. 2007). However, the small size and elusive behaviour of some species make them hard to detect, particularly in habitats that are difficult to survey. Detection of a species is imperative to any conservation strategy or monitoring program (Thomas et al. 2020), but relies on effective monitoring techniques (Bovendorp et al. 2017). A variety of techniques to monitor small mammals can be employed, each with their own limitations and constraints (Hoffman et al. 2010). Selecting the most effective and cost-efficient technique could greatly improve monitoring schemes, but the relative performance of the various techniques remains poorly studied.

One conventional technique to study small mammals is live trapping (Hoffman et al. 2010; Powell and Proulx 2003), which involves the deployment of large numbers of baited traps in which animals are captured and subsequently

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released. Live trapping can yield high capture rates and has been deployed to study species abundance, composition, and richness in diverse habitats (e.g. Bergers and La Haye 2000). An advantage of trapping and handling live animals is that species identification is usually unambiguous, while biometric data such as weight, sex, and reproductive status can be collected. However, live trapping also has several drawbacks. Being trapped interferes with an individual's natural behaviour, causes stress, and can even lead to the mortality of target species as well as the bycatch. A methodological bias of this technique is trap saturation (Distiller and Borchers 2015), especially when small mammal densities are high. Furthermore, live trapping requires multiple inspections per day and is, therefore, labour-intensive and costly.

Alternatively, camera trapping is a non-invasive, cost-efficient survey method that has become increasingly popular in conservation and management (De Bondi et al. 2010). This technique includes a wide range of equipment, but most often involves the use of remote cameras with passive infrared (PIR) motion sensors that are triggered by passing animals, producing time-stamped digital recordings. Camera trap surveys are now widely deployed in various habitats to study the distribution, abundance, and behaviour of a single species (e.g. Bischof et al. 2014) or broader wildlife communities (e.g. Welbourne et al. 2015). However, this technique also has several limitations. Detection probabilities are affected by camera trap characteristics, such as the sensitivity of the PIR sensor, the size and shape of the detection zone, and trigger speed, which all vary greatly among manufacturers and models (Meek et al. 2015). This is particularly relevant when monitoring small mammals, which may fail to trigger camera traps due to their small size and movement speed (Rowcliffe et al. 2011). Another drawback is that species identification is not always unambiguous, especially that of small mammals (Burns et al. 2018; Potter et al. 2018). However, novel camera trapping methods have recently been developed that are optimized for detecting small mammals (Gracanin et al. 2018; Littlewood et al. 2021; Smaal and van Manen 2022). These methods all involve enclosing a camera trap in a baited tunnel and placing a close-focus lens in front of the camera lens. When positioned at ground level, these adjusted camera traps effectively detect small mammals at short-focal distances (Smaal and van Manen 2022).

Another novel method to survey small mammals is the application of environmental DNA (eDNA; e.g. Deiner et al. 2017). This technique involves the extraction of DNA from environmental samples, such as surface water or topsoil, in which animals have shed faeces, hair, saliva, or skin (Leempoel et al. 2020). Samples can be screened for eDNA from a single target species by qPCR-sequencing (Lugg et al. 2018) or for broader taxa by metabarcoding (Harper et al. 2019). Sampling for eDNA is non-invasive and less labour-intensive than live trapping or camera trapping. However,

since the size, local densities, and behaviour of species most likely affects DNA shedding in the environment (Adams et al. 2019), it is unclear whether all species are detectable in the environment using eDNA methods (Leempoel et al. 2020). In particular, how well eDNA performs in detecting small mammals that occur in low abundances remains poorly quantified.

In this study, we compared the effectiveness of short-focal camera trapping, live trapping, and soil eDNA to detect a semi-aquatic small mammal species: the Eurasian water shrew *Neomys fodiens* (hereafter 'EWS'). This species is broadly distributed in the Palearctic and is a possible bio-indicator of riparian and wetland habitats with a high degree of naturalness (French et al. 2001; Greenwood et al. 2002). Throughout its range, EWS occurs solitary and in low densities (Churchfield 1990; Sheftel 2018).

Previous studies found that various small mammal species were detected by eDNA but not detected by camera traps (Harper et al. 2019; Leempoel et al. 2020; Lyet et al. 2021). Furthermore, it was demonstrated that detection probabilities were higher in a single eDNA water sample than during a single live trapping visit (Lugg et al. 2018). Therefore, we hypothesized the effectiveness of eDNA to be higher than camera trapping or live trapping, with effectiveness defined as the probability of successfully detecting EWS if the species is present. Furthermore, the effectiveness of short-focal camera traps was hypothesized to be higher than live trapping (De Bondi et al. 2010; Thomas et al. 2020; Welbourne et al. 2015), since the capture probability of the target species is not affected by the densities of other small mammals. For instance, other small mammals may quickly occupy live traps, thereby reducing the capture probability of less abundant target species such as EWS. We tested our hypotheses in a field study by surveying EWS presence using short-focal camera traps, live traps, and soil eDNA alongside a lowland brook in the Netherlands.

Materials and methods

Study area and design

The study was conducted near the lowland brook Kleine Dommel in the Netherlands (Fig. 1). This area concerns one of the few localities where EWS occur in the province of North Brabant (NDFP 2022). The Kleine Dommel is a 21 km long, low-energy, predominantly meandering tributary of the larger lowland brook the Dommel (Fig. 1). The mean flow rate in summer is 0.15 m s^{-1} (Water Authority De Dommel 2022). Fieldwork was conducted along a 3.5 km long section of the Kleine Dommel that has been rehabilitated into a natural state since 2021. Local vegetation

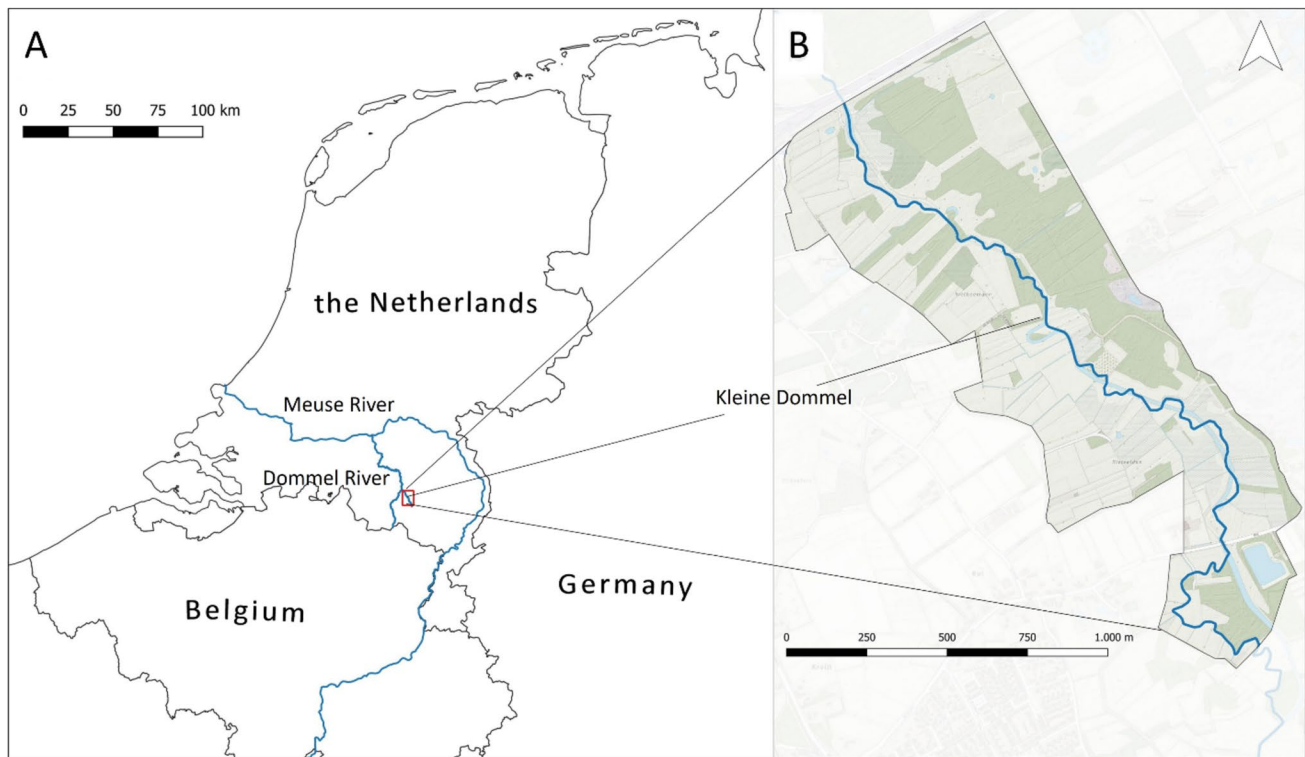


Fig. 1 **A** Map displaying the location of the study area in the Netherlands. **B** The study site alongside the lowland brook Kleine Dommel

is characterized by Alder *Alnus glutinosa* forests with an understory of predominantly sedges *Carex* spp., enclosed meanders, swampy meadows, and riparian habitats. We simultaneously conducted live trapping, short-focal camera trapping, and eDNA sampling between 31 August and 31 October 2022. This period marks the end of the reproduction season, which is when EWS densities and hence detection probabilities are highest (Bekker 2016). In total, 20 transects of 100×5 m alongside the Kleine Dommel were surveyed (see the Supplementary Material for a map displaying the exact location of these transects). Transects were selected based on the known occurrence of EWS (NDFP 2022) or the presence of suitable habitat based on expert judgement. Due to labour intensity and equipment constraints, surveys were carried out during two consecutive periods, each involving ten transects. The first survey (transects 1–10) was conducted between 31 August and 27 September 2022. The second (transects 11–20) between 26 September and 31 October 2022.

Live trapping

Live trapping was conducted between 9–16 September 2022 (transects 1–10) and 30 September – 7 October 2022

(transects 11–20). In each transect, 20 Heslinga live traps were deployed in pairs every 10 m (Fig. 2) following Bergers' (1997) standard method for sampling small mammals. This method has a high success rate and is recommended when target species occur in low abundances (e.g. Bergers and La Haye 2000), as is the case for EWS (Sheftel 2018). Traps were placed in dense vegetation on the ground and baited with carrot, granola, and live mealworm larvae. The traps were filled with hay to provide insulation. They were inactive for a pre-baiting period of 3 days to increase capture probabilities (Chitty and Kempson 1949), after which they were activated and checked with a 12 h interval for 1.5 to 3 consecutive days (i.e. 3–6 checking moments, Table 1). In eight transects, traps were recovered in less than 3 days to prevent recapturing individuals that were radio-tagged for another study. Therefore, the number of trap days varied among transects between 30 and 60 (Table 1). The live trapping surveys resulted in 1060 trap days in total. All individuals were identified to species and EWS were marked by fur clipping, after which they were released at the capture location. All procedures requiring a permit under the Dutch nature conservation legislation were allowed under a permit of the Netwerk Groene Bureaus (permit ODH-2020-00001393).

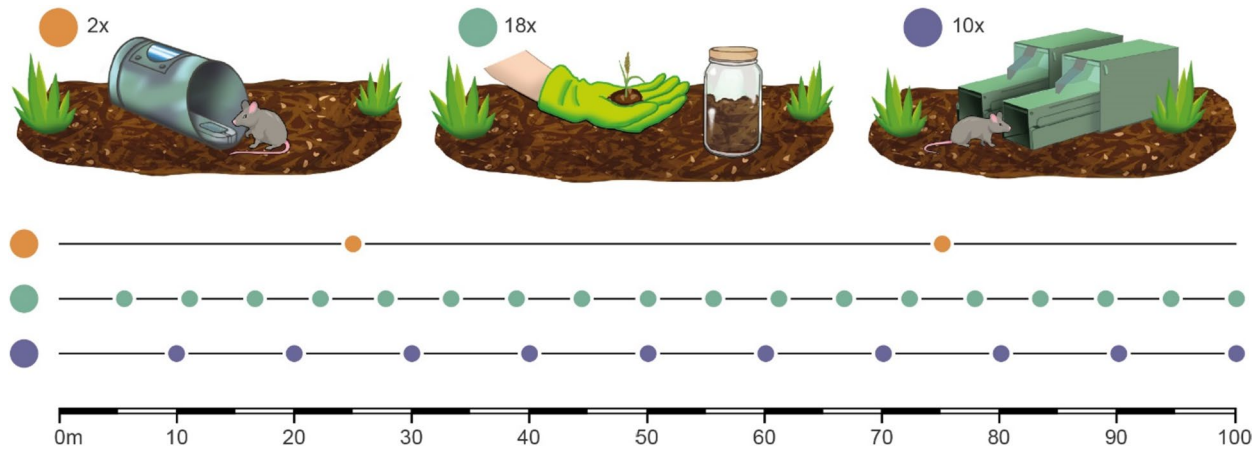


Fig. 2 Study design of the three deployed protocols in each transect to survey Eurasian water shrews: (1) short-focal camera trapping at 25 and 75 m (orange dots), (2) soil eDNA sampling every 5.5 m (green dots), and (3) live trapping by placing paired traps every 10 m (purple dots)

Short-focal camera trapping

Camera traps (Browning Strike Force HD Pro X) were deployed from 1 to 27 September 2022 (transects 1–10) and from 27 September to 31 October 2022 (transects 11–20), resulting in a total number of 1200 trapping days. Halfway through each survey, batteries and SD cards (SanDisk 32 GB) were replaced to secure collected data and ensure trapping continuity. Camera traps were mounted in a Struikrover, a PVC tube of 40 cm in length with a closed back end (Smaal and van Manen 2022). A prescription eyeglass (+3 dioptres) placed in front of the camera lens reduced the focal distance to approximately 35 cm. In each transect, two Struikrovers were installed at 25 and 75 m (Fig. 2). They were positioned horizontally at ground level in dense vegetation. At the tube opening, a pierced can of oiled sardines was used as bait (Smaal and van Manen 2022). Camera traps were programmed to take three photographs (i.e. one image sequence) when triggered with a 30 s capture delay to avoid excessive capture events (De Bondi et al. 2010) and to minimize battery use. We chose a conservative approach and only considered a detection as an independent event if 60 min passed since the last EWS detection (Welbourne et al. 2015).

Table 1 Survey effort per method for each transect

Transect no.	Survey	Subsamples soil eDNA	Camera trap days	Checking moments live trapping	Live trap days
1–6	1	18	52	6	60
7–10	1	18	52	5	50
11–14	2	18	68	3	30
15–20	2	18	68	6	60

Photographs were manually examined and all animals were identified up to species level when possible.

Soil eDNA sampling and laboratory analysis

We exclusively sampled for eDNA from soil and not from water, since the lowland brook was the only surface water in our study area. Relating the location of EWS based on a one-time sampling event from this running water would be uncertain. Soil samples were collected on 31 August 2022 (transects 1–10) and 26 September 2022 (transects 11–20). To prevent cross-contamination, each transect was first surveyed for eDNA prior to camera trapping or live trapping. Per transect, in total 18 subsamples were taken every 5.5 m in a uniform direction by scraping off the first centimetre of soil and organic litter of a surface area of approximately 10 cm² using sterile gloves (Fig. 2). Subsamples were pooled in a sterile 1 L jar prefilled with 250 mL MilliQ water and shaken for 60 s for homogenization. Using new sterile gloves, 25 mL of the pooled sample was divided into three 50 mL Falcon tubes, each prefilled with 25 mL denatured ethanol (Datura 2022). Samples were stored at 7 °C until further analysis.

To exclude potential false detection of eDNA, three additional samples (i.e. fictional transects) were added as controls. Two of these were collected near a pond in a fenced garden (WGS84 coordinates 51.695, 5.215) where EWS do not occur, following the same sampling procedure as described above. We also included a positive control by placing a dead and frozen EWS in a 1 L jar for 5 h to ensure sufficient release of DNA. This individual was found dead on 24 July 2021 (WGS84 coordinates 51.415, 5.915) and was directly stored in a freezer at –20 °C.

All samples were analysed by Datura Molecular Solutions BV (Wageningen, the Netherlands) in October 2022. At first,

eDNA in the soil samples was extracted using the 'DNeasy Blood & Tissue kit' (Qiagen, Hilden, Germany). To check the inhibition of eDNA detection, 30,000 molecules of an artificial DNA fragment were added to a PCR reaction. If inhibition took place, the sample was purified. It was then checked again whether the inhibitory substances were sufficiently removed following the same procedure.

Detection of eDNA was conducted by real-time quantitative PCR (qPCR) using species-specific primers developed by Datura. In addition, Datura works with species-specific probes (a type of primer) that bind exclusively to eDNA of EWS using a qPCR platform (CFX96 Touch™, Bio-Rad, Berkeley, USA). The qPCR detection was performed with 12 replicates (Ficetola et al. 2015; Nichols et al. 2018) using the TaqMan® Environmental Mastermix 2.0 (Life Technologies, Carlsbad, USA).

Data analysis

We modelled the detection probabilities of each survey protocol in a conservative way by first converting the detection success to binary data (i.e. 1 if EWS was detected and 0 if EWS was not detected) at the transect level. For live trapping, detection success was 1 for transects where EWS were trapped at least once. For camera trapping, detection success was 1 if EWS were photographed at least once, while for the eDNA survey, detection success was 1 if at least 1 out of 12 replicates was positive. Subsequently, detection success was included as a response variable in a Logistic Mixed Effect Regression Model with survey protocol included as a fixed factor and transect as a random term. We used a Tukey HSD test for pairwise comparisons of the survey protocols.

We further modelled the effectiveness of short-focal camera trapping and live trapping using a time-to-event analysis (viz. survival analysis) (Bischof et al. 2014). To estimate detection curves, we used the first detection of EWS as an event and transect as the individual from which the event occurred. We included all transects, including those where neither survey protocol detected an EWS, to allow for right-censoring. For live trapping, the full survey duration was maximally 3 days; for camera trapping, we used 21 days since longer deployment did not result in additional EWS detections (Fig. 5). Hence, the time of the first detection event was converted to a percentage where the full survey duration represented 100%, to be able to compare detection probabilities between these two methods.

To analyse the effect of deploying a single Struikrover or two Struikrovers per transect, we included each as a separate method (i.e. StruikroverA, StruikroverB, and StruikroverAB) in detection curves. The first detection event by either StruikroverA or StruikroverB was used as the first detection event for the method StruikroverAB. Empirical detection curves were estimated by

constructing Kaplan-Meier curves. Differences in detection probabilities among survey protocols were analysed with a log-rank test with a Bonferroni correction. All analyses were conducted in R v4.2.1 (R Core Team 2020) using the packages 'lme4' (Bates et al. 2015), 'emmeans' (Lenth 2020), 'survminer' (Kassambara et al. 2021), and 'survival' (Therneau 2012).

Results

General

Live trapping resulted in 776 capture events, representing nine mammal species (Supplementary Materials Table S1). Of these, 29 capture events were of EWS (3.74%), involving 17 individuals. In total 14 specimens of three species were found dead in live traps (Supplementary Materials Table S1). Camera trapping yielded 19,161 image sequences, of which in 352 (1.8%) were EWS (Fig. 3), representing 338 unique detection events. All species detected using live traps were also detected using camera traps. Additionally, short-focal camera traps detected ten mammal species that could not be live trapped due to their size, as well as various avian species (Supplementary Materials Table S1).

The eDNA survey resulted in the detection of EWS DNA in eight out of 20 transects (Fig. 4 and Supplementary Materials Table S1). The number of positive replicates varied among transects between 1/12 and 12/12, thus ranging from 8.3 to 100.0% positive replicates, indicative of eDNA concentrations (Supplementary Materials Table S2). Both negative controls tested negative, while the positive control resulted in 12/12 positive replicates (Supplementary Materials Table S2), indicating reliable qPCR analysis. The eDNA survey detected EWS in one transect where it was not detected with camera traps or live traps (Fig. 4). We found a positive correlation between the eDNA concentration and the number of caught EWS in live traps (Pearson's $r(18)=0.492$, $p=0.027$), but the number of detection events obtained via short-focal camera trapping did not correlate with eDNA concentrations (Pearson's $r(18)=0.178$, $p=0.452$).

Detection probabilities

The total number of transects with EWS detections based on live trapping, short-focal camera trapping, and soil eDNA was 10, 14, and 8, respectively (Fig. 4 and Supplementary Materials Table S1). The Logistic Mixed Effect Regression Model showed that camera trapping had the highest effectiveness in terms of detection probability (0.798), followed by live trapping (0.494) and eDNA (0.332). Pairwise comparisons between survey protocols indicated

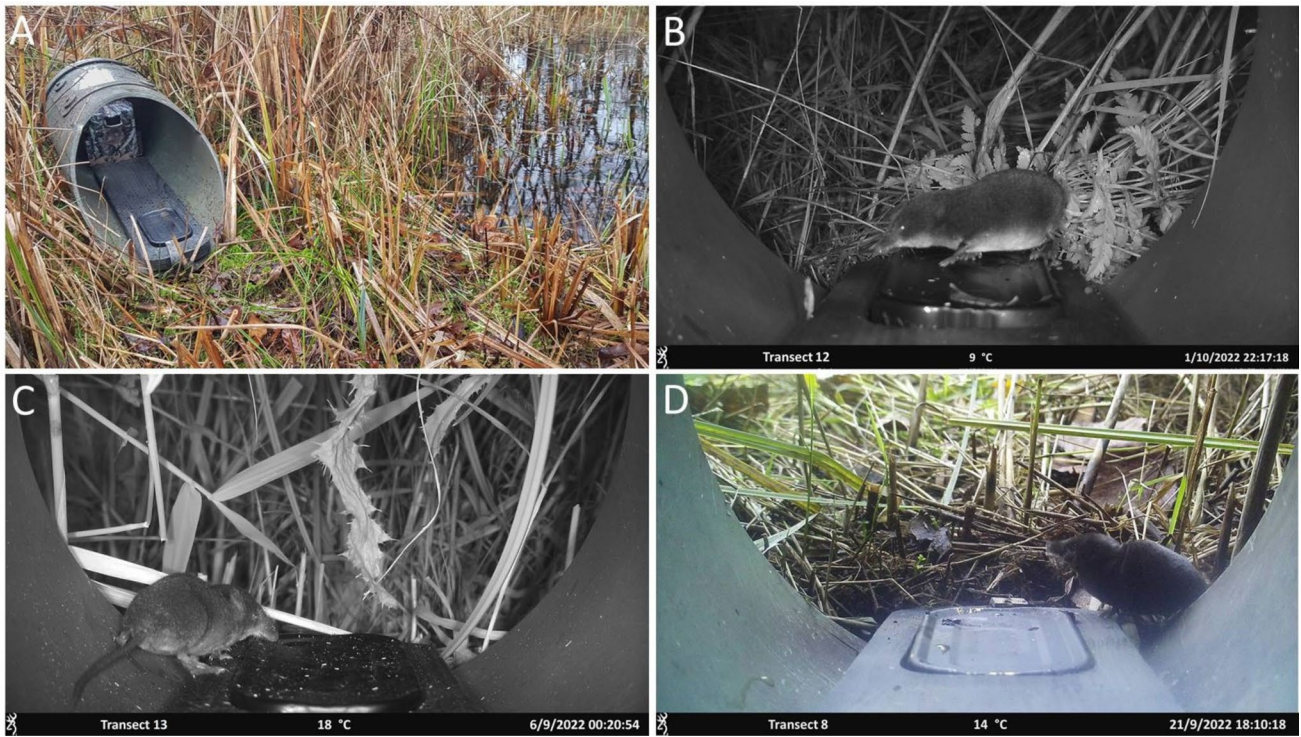


Fig. 3 A Short-focal camera trap in situ and **B–D** images of Eurasian water shrews that were recorded with a short-focal camera trap

no significant difference between live trapping and eDNA (Tukey HSD, $Z = -0.821$, $p = 0.690$), nor between live trapping and short-focal camera trapping (Tukey HSD, $Z = -1.621$, $p = 0.237$). However, the detection probability by eDNA was significantly lower than by short-focal camera trapping (Tukey HSD, $Z = -2.486$, $p = 0.034$; Fig. 4).

Effectiveness of camera trapping versus live trapping

Short-focal camera trapping and live trapping resulted in 14 and 10 transects with EWS detections, respectively. Camera trapping reached this number after 21 survey days, while live trapping reached this number already after 2.5 survey

Fig. 4 A Eurasian water shrew detections per survey protocol. Each circle represents a transect. B Estimated marginal means of the detection probability of Eurasian water shrews per survey protocol. Bars represent \pm SE and the asterisk (*) indicates a significant difference ($p < 0.05$) based on a Tukey HSD test

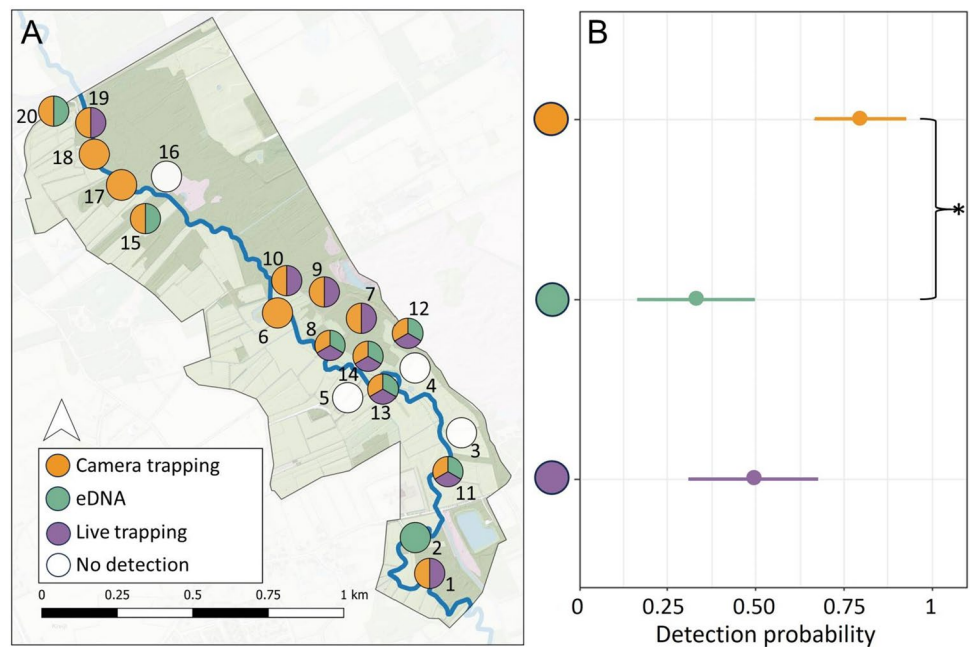
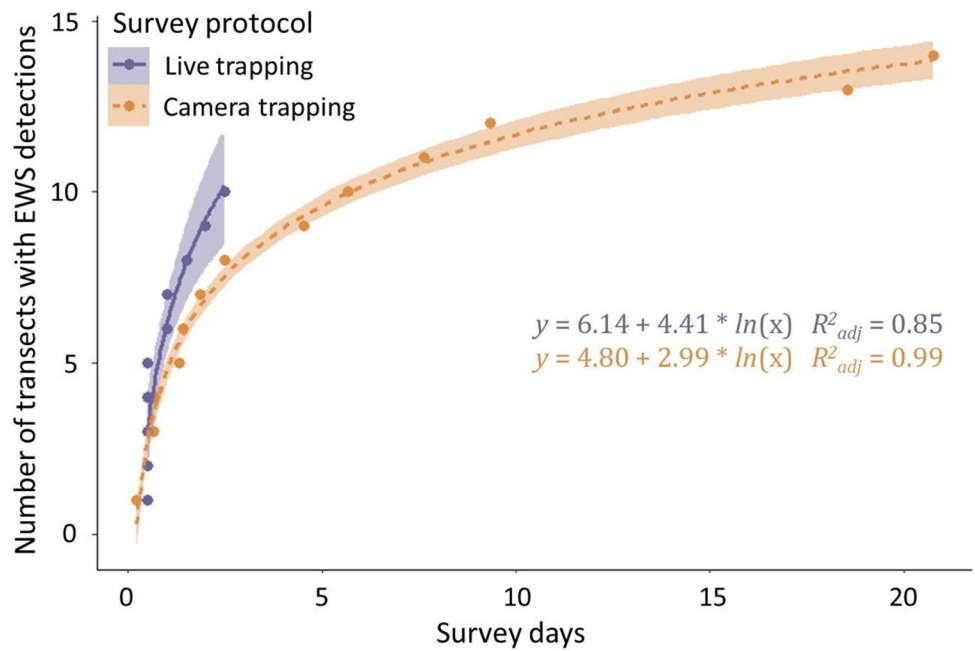


Fig. 5 The cumulative number of transects with Eurasian water shrew detections by live trapping (solid purple line) and short-focal camera trapping (dashed orange line). Shaded areas display 95% confidence intervals

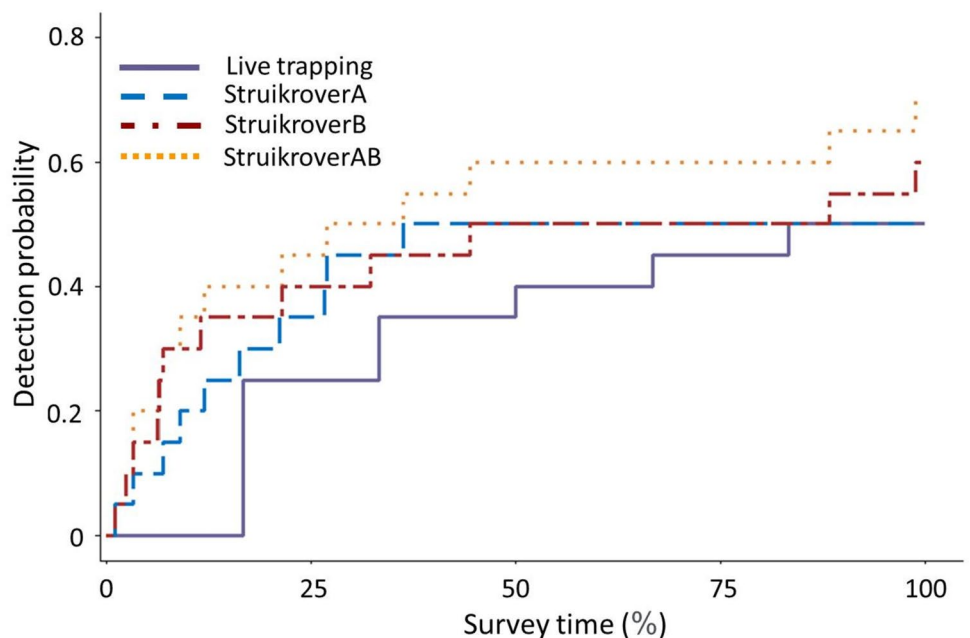


days (Fig. 5). However, live trapping involved a high trap intensity with 20 traps per transect, whereas only two camera traps were deployed. The effectiveness of camera trapping exceeded that of live trapping after 6 consecutive days but did not increase any further after 21 days (Fig. 5).

Empirical estimates of the detection curves showed that short-focal camera trapping resulted in the highest effectiveness in terms of overall detection probability (0.700) when two Struikrovers were deployed per transect (Fig. 5). With only one Struikrover, the mean overall detection probability was 0.550. This is still higher than the overall detection

probability for the live trapping survey protocol (0.500). The median time to first detection of an EWS occurred at 31.6% (i.e. 6.6 days) of the total camera trapping survey duration with two Struikrovers, while this was reached at 46.1% (i.e. 10.8 days) with a single Struikrover. The median time to the first detection of an EWS with live trapping occurred at 83.3% (i.e. 2.5 days) of the total survey duration (Fig. 6). The EWS detection probability was not significantly different between the camera trapping and live trapping survey protocols (log-rank test, $p=0.420$), regardless of whether a single or two Struikrovers were deployed per transect.

Fig. 6 Kaplan-Meier curves showing empirical estimates of the detection probabilities of the first detection event of a Eurasian water shrew at a given time per survey protocol, with the total survey duration set to 100% (i.e. 21 days for short-focal camera trapping and 3 days for live trapping). Short-focal camera trapping is divided into three groups consisting of a single Struikrover (A or B; dashed blue and dash-dotted brown lines) and two Struikrovers (AB; dotted orange line) per transect, while the solid purple line represents the live trapping protocol. The detection probability among groups did not significantly differ



Discussion

We compared the effectiveness of live trapping, short-focal camera trapping and soil eDNA for detecting Eurasian water shrews (EWS), an elusive semi-aquatic small mammal. Short-focal camera trapping had the highest detection probability, followed by live trapping and eDNA. Although differences were only significant between short-focal camera trapping and eDNA, we argue that short-focal camera trapping is also preferable over live trapping as it is non-invasive and less labour-intensive. In practice, however, the choice for a specific survey method also depends on available time and budget. If both are limited, eDNA is a suitable method to detect EWS at large spatial scales, for instance, at the landscape level. At local scales, however, ecologists should be cautious interpreting no detection by eDNA as true absence, especially during environmental impact assessments, which often require unambiguous conclusions.

Effectiveness and detection probabilities among survey protocols

Our study shows that short-focal camera trapping resulted in the highest EWS detection probabilities. This is in contrast to other studies in which eDNA surveys were at least as effective as camera trapping and/or live trapping surveys (Leempoel et al. 2020; Lugg et al. 2018; Lyet et al. 2021; Mena et al. 2021). Differences in methodology could explain this discrepancy. In previous comparative studies, camera traps were usually deployed conventionally by mounting them to trees or ground pegs (Harper et al. 2019; Leempoel et al. 2020; Ryan et al. 2022). Such a mounting design is effective for capturing medium to large-sized mammals (Wearn and Glover-Kapfer 2019) but often fails to detect fast-moving and/or small mammals (Rowcliffe et al. 2011). Conventional camera trapping, therefore, does not allow for a fair comparison with eDNA protocols when specifically targeting small mammals. Here, we used adjusted camera traps with a short-focal distance (i.e. Struikrovers; Smaal and van Manen 2022) that allowed for the effective detection of small mammals. Our comparative study shows that these short-focal camera traps have a significantly higher detection probability of small mammals such as EWS than soil eDNA.

Short-focal camera trapping as an alternative for live trapping

Our results show that short-focal camera trapping is more effective than live trapping in detecting EWS, in agreement with our prediction. These findings are also in accordance with several previous studies that compared (adjusted) camera trapping with live trapping surveys of small mammals (De Bondi et al. 2010; Thomas et al. 2020; Welbourne et al.

2015). However, we do note that the outcomes of such comparison depend on the study design, such as the number of cameras and live traps, distances between cameras/traps, camera trap mounting design, and deployment length of each method. In our study, we detected EWS in half of all surveyed sites after 2.5–3 days of live trapping, which is the total survey length according to the standard method of EWS surveys in the Netherlands (Bergers 1997). However, a pre-baiting period of 3 days preceded the live trapping survey to maximise detection probabilities. The camera trapping protocol yielded similar results as live trapping in approximately 6 survey days (equal to the live trapping survey including the pre-baiting period) when using two Struikrovers. Thus, the effectiveness of short-focal camera trapping was equal to that of live trapping during the first 6 days, but with a lower trapping intensity (2 versus 20 traps per transect, respectively). When short-focal camera traps were deployed for more than 6 days, the effectiveness exceeded that of live trapping, although it did not increase any further after 21 days. This is in agreement with a previous study that recommended a deployment duration of minimally 21 days for small mammal surveys using short-focal camera traps (Smaal and van Manen 2022). Therefore, we argue that short-focal camera trapping is superior to live trapping surveys in terms of effectiveness in detecting EWS when two Struikrovers are deployed per 100 m for at least 6 days, and in terms of being less labour-intensive (De Bondi et al. 2010). Moreover, camera trapping is less invasive than live trapping and is therefore also preferable from an animal welfare perspective. During live trapping, we observed mortality in three small mammal species: bank vole *Clethrionomys glareolus*, short-tailed field vole *Microtus agrestis*, and common/crowned shrew *Sorex araneus/coronatus* (Supplementary Material Table S1) totalling 1.8% of all captures.

Management implications and further research

The results of this study indicate clear differences in the effectiveness between EWS survey protocols. However, each survey protocol has its benefits and limitations. The choice of applying a specific survey protocol depends on the research objectives and management implications. Given the highest effectiveness of short-focal camera trapping and the high cost-efficiency of this method (e.g. Welbourne et al. 2015), we recommend its application for EWS surveys specifically. However, since all small mammal species that were caught in live traps were also detected using short-focal camera traps, and these species were detected at more sites, short-focal camera trapping is a promising and effective method to study the presence of small mammals in general. To further explore this potential, we encourage researchers to use short-focal camera traps to study other small mammal

species and evaluate these results compared to conventional techniques.

High detection probabilities were obtained during 6–21 days of short-focal camera trapping, which is generally longer than a standard live trapping survey or eDNA sampling, but this should not be problematic if studies are properly planned. We only recommend live trapping as a survey method if the research objective requires individuals to be handled or individually recognized, such as for mark-recapture studies. Otherwise, we consider short-focal camera trapping as a superior method for small mammal surveys due to its high effectiveness, while likely becoming more cost-efficient in the long-term and, more importantly, being non-invasive (De Bondi et al. 2010; Thomas et al. 2020; Welbourne et al. 2015).

When deploying short-focal camera traps, the relatively high one-time investment costs should be considered. However, during this study, live trapping was more than twice as labour-intensive as short-focal camera trapping. Therefore, during longer-lasting studies or repetitive use, short-focal camera trapping likely becomes economically beneficial over live trapping.

The detection success of eDNA varies between species (Rees et al. 2014), but due to the relatively low EWS detection probability using soil eDNA, we argue that the applicability is dependent on the scale of deployment. We currently advise against the mere use of this technique for presence/absence surveys on a local scale, such as banks of side ditches, since in our study, soil eDNA failed to detect EWS several times while its presence was confirmed by camera traps or live traps. However, if the objective is to establish the presence of EWS at a landscape level, for example, a river valley, soil eDNA can be used to detect EWS, particularly if multiple subsamples are pooled. The use of soil eDNA for sampling elusive species may also be preferable when survey sites are poorly accessible or cannot be visited frequently due to logistical constraints.

While we found that soil eDNA performed poorly in detecting EWS at a local scale, other studies have shown that EWS can readily be detected by eDNA from water sources (Broadhurst et al. 2021; Sales et al. 2020). A study on a related species, the Japanese water shrew *Chimarrogale platycephala*, also concluded that the use of water eDNA is a promising survey technique to detect this semi-aquatic species (Yonezawa et al. 2020). We did not sample eDNA from the lowland brook in our study area, because sampling of running water would have led to serious difficulties and high uncertainties in interpreting positive detections in relation to their location due to the downstream transportation of eDNA. In isolated water bodies, a positive eDNA detection could be better linked to the presence of EWS at that location. Sampling for eDNA from soil as well as surface waters, complemented by survey techniques such as short-focal

camera trapping, potentially increases the effectiveness and cost-efficiency of studies that are carried out at larger scales (Croose et al. 2023; Sales et al. 2020).

Conclusions

We simultaneously compared the effectiveness of three survey protocols at 20 transects alongside a natural lowland brook, targeting EWS as a case study for small mammal surveys. Our study demonstrated that short-focal camera trapping was most effective in detecting EWS, followed by live trapping, while the eDNA protocol was least effective. Short-focal camera trapping was the most effective when deploying two Struikrover camera traps per transect for 21 days, but longer deployment did not increase detection probabilities. The overall effectiveness of camera trapping exceeded that of live trapping after 6 survey days.

We recommend short-focal camera trapping for EWS surveys due to its effectiveness and relatively low costs compared to eDNA and live trapping, with an additional advantage that this method is non-invasive and labour-extensive. Specifically for such surveys, we advise deploying two Struikrovers per 100 m for 21 days. Short-focal camera trapping showed a promising method as an alternative for live trapping regarding all detected small mammal species during our study. Therefore, we recommend further evaluation of its potential for small mammal surveys in general by studying other small mammal species and comparing these results to conventional techniques.

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Author contribution This study was conducted as the MSc thesis of J.V., who designed the study; J.V., P.L. and P.v.H. carried out the funding acquisition; J.V. and T.v.d.P. carried out the eDNA sampling; J.V., T.v.d.P., P.v.H. and D.H. conducted fieldwork for camera and live trapping.; J.V., P.v.H. and D.H. processed camera trap data; J.V. analysed data; J.V. wrote the manuscript; W.F.d.B. and H.E. supervised the study. All authors contributed to drafts and gave final approval for publication.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval All procedures requiring a permit under the Dutch nature conservation legislation were allowed under a permit of the Netwerk Groene Bureaus (permit ODH-2020-00001393).

Conflict of interest The authors declare no competing interests.

References

- Adams CIM, Hoekstra LA, Muell MR, Janzen FJ (2019) A brief review of non-avian reptile environmental DNA (eDNA) with a case study of painted turtle (*Chrysemys picta*) eDNA under field conditions. *Diversity* 11(4):1–22. <https://doi.org/10.3390/d11040050>
- Bates D, Maechler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. *J Stat Softw* 67(1):1–48. <https://doi.org/10.18637/jss.v067.i01>
- Bekker DL (2016) Waterspitsmuis – *Neomys fodiens*. In: Broekhuizen S, Spoelstra K, Thissen JBM, Canters KJ, Buys JC (eds) Atlas van de Nederlandse zoogdieren. Natuur van Nederland 12, Naturalis Biodiversity Center & EIS Kenniscentrum Insecten en andere ongewervelden, pp 169–171
- Bergers PJM (1997) Kleine zoogdieren inventariseren: het kan efficiënter. *Zoogdier* 8(3):3–7
- Bergers P, La Haye M (2000) Kleine zoogdieren betrouwbaarder en efficiënter inventariseren. *De Levende Natuur* 101(2):52–57
- Bischof R, Hameed S, Ali H, Kabir M, Younas M, Shah KA, Din JU, Nawaz AA (2014) Using time-to-event analysis to complement hierarchical methods when assessing determinants of photographic detectability during camera trapping. *Methods Ecol Evol* 5(1):44–53. <https://doi.org/10.1111/2041-210X.12115>
- Bovendorp RS, McCleery RA, Galetti M (2017) Optimising sampling methods for small mammal communities in Neotropical rainforests. *Mammal Rev* 47(2):148–158. <https://doi.org/10.1111/mam.12088>
- Broadhurst HA, Gregory LM, Bleakley EK, Perkins JC, Lavin JV, Bolton P, Browett SS, Howe CV, Singleton N, Tansley D, Sales NG, McDevitt AD (2021) Mapping differences in mammalian distributions and diversity using environmental DNA from rivers. *Sci Total Environ* 801:149724. <https://doi.org/10.1016/j.scitotenv.2021.149724>
- Burns PA, Parrott ML, Rowe KC, Phillips BL (2018) Identification of threatened rodent species using infrared and white-flash camera traps. *Aust Mammal* 40(2):188–197. <https://doi.org/10.1071/AM17016>
- Carnignan V, Villard MA (2002) Selecting indicator species to monitor ecological integrity: a review. *Environ Monit Assess* 78:45–61. <https://doi.org/10.1023/A:1016136723584>
- Chitty D, Kempson DA (1949) Prebaiting small mammals and a new design of live trap. *Ecology* 30(4):536–542. <https://doi.org/10.2307/1932457>
- Churchfield S (1990) The natural history of shrews. Christopher Helm Publishers Ltd
- Croose E, Hanniffy R, Harrington A, Pödra M, Gómez A, Bolton PL, Lavin JV, Browett SS, Pinedo J, Lacanal D, Galdos I, Ugarte J, Torre A, Wright P, MacPherson J, McDevitt AD, Carter SP, Harrington LA (2023) Mink on the brink: comparing survey methods for detecting a critically endangered carnivore, the European mink *Mustela lutreola*. *Eur J Wildl Res* 69:34. <https://doi.org/10.1007/s10344-023-01657-3>
- Datura (2022) eDNA Soil sampling protocol. <https://datura.nl/1556-2/protocol-en-sampleformulieren/>. Accessed 12 Jul 2022
- De Bondi N, White JG, Stevens M, Cooke R (2010) A comparison of the effectiveness of camera trapping and live trapping for sampling terrestrial small-mammal communities. *Wildl Res* 37(6):456–465. <https://doi.org/10.1071/WR10046>
- Deiner K, Bik HM, Mächler E, Seymour M, Lacoursière-Roussel A, Altermatt F, Creer S, Bista I, Lodge DM, de Vere N, Pfrender ME, Bernatchez L (2017) Environmental DNA metabarcoding: transforming how we survey animal and plant communities. *Mol Ecol* 26(21):5872–5895. <https://doi.org/10.1111/mec.14350>
- Distiller G, Borchers DL (2015) A spatially explicit capture–recapture estimator for single-catch traps. *Ecol Evol* 5(21):5075–5087. <https://doi.org/10.1002/ece3.1748>
- Ficetola GF, Pansu J, Bonin A, Coissac E, Giguët-Covex C, De Barba M, Gielly L, Lopes CM, Boyer F, Pompanon Gilles Rayé F, Taberlet P (2015) Replication levels, false presences and the estimation of the presence/absence from eDNA metabarcoding data. *Mol Ecol Resour* 15(3):543–556. <https://doi.org/10.1111/1755-0998.12338>
- French BI, Mezquita F, Griffiths H (2001) Habitat variables affecting the occurrence of *Neomys fodiens* (Mammalia, Insectivora) in Kent, UK. *Folia Zool* 50:99–105
- Gracanin A, Gracanin V, Mikac KM (2018) The selfie trap: a novel camera trap design for accurate small mammal identification. *Ecol Manage Restor* 20(2):156–158. <https://doi.org/10.1111/emr.12345>
- Greenwood A, Churchfield S, Hickey C (2002) Geographical distribution and habitat occurrence of the Water Shrew (*Neomys fodiens*) in the Weald of South-East England. *Mammal Rev* 32(1):40–50. <https://doi.org/10.1046/j.1365-2907.2002.00099.x>
- Harper LR, Lawson L, Angus H, Carpenter I, Ghazali M, Di Muri C, Macgregor CJ, Logan TW, Law A, Breithaupt T, Read DS, McDevitt AD, Hänfling B (2019) Environmental DNA (eDNA) metabarcoding of pond water as a tool to survey conservation and management priority mammals. *Biol Conserv* 238:1–11. <https://doi.org/10.1016/j.biocon.2019.108225>
- Hoffman A, Decher J, Rovero F, Schaer J, Voigt C, Wibbelt G (2010) Field methods and techniques for monitoring mammals. In: Eymann J, Degreef J, Häuser C, Monje JC, Samyn Y, VandenSpiegel D (eds) Manual on field recording techniques and protocols for all taxa biodiversity inventories, pp 482–529
- Holt EA, Miller SW (2010) Bioindicators: using organisms to measure environmental impacts. *Nat Educ Knowl* 3(10):8
- Kassambara A, Kosinski M, Biecek P (2021) *Survminer: Drawing survival curves using 'ggplot2'*. R package version 0.4.9. Available from <https://CRAN.R-project.org/package=survminer>
- Leempoel K, Hebert T, Hadly EA (2020) A comparison of eDNA to camera trapping for assessment of terrestrial mammal diversity. *Proc R Soc B* 287:20192353. <https://doi.org/10.1098/rspb.2019.2353>
- Leis SA, Leslie DM, Engle DM, Fehmi JS (2007) Small mammals as indicators of short-term and long-term disturbance in mixed prairie. *Environ Monit Assess* 137(1–3):75–84. <https://doi.org/10.1007/s10661-007-9730-2>
- Lenth RV (2020) Estimated marginal means, aka Least-Square Means. R package version 1.8.2. Available from <https://cran.rproject.org/web/packages/emmeans/index.html>
- Littlewood NA, Hancock MH, Newey S, Shackelford G, Toney R (2021) Use of a novel camera trapping approach to measure small mammal responses to peatland restoration. *Eur J Wildl Res* 67(12):1–10. <https://doi.org/10.1007/s10344-020-01449-z>
- Lyet A, Pellissier L, Valentini A, Dejean T, Hehmeyer A, Naidoo R (2021) eDNA sampled from stream networks correlates with camera trap detection rates of terrestrial mammals. *Sci Rep* 11:11362. <https://doi.org/10.1038/s41598-021-90598-5>

- Lugg WH, Griffiths J, van Rooyen AR, Weeks AR, Tingley R (2018) Optimal survey designs for environmental DNA sampling. *Methods Ecol Evol* 9(4):1049–1059. <https://doi.org/10.1111/2041-210X.12951>
- Meek PD, Ballard G-A, Fleming PJS (2015) The pitfalls of wildlife camera trapping as a survey tool in Australia. *Aust Mammal* 37(1):13–22. <https://doi.org/10.1071/AM14023>
- Mena JL, Yagui H, Tejada V, Bonifaz E, Bellemain E, Valentini A, Tobler MW, Sanchez-Vendizú P, Lyet A (2021) Environmental DNA metabarcoding as a useful tool for evaluating terrestrial mammal diversity in tropical forests. *Ecol Appl* 31:e02335. <https://doi.org/10.1002/eap.2335>
- NDFD (2022) <https://ndff-ecogrid.nl/uitvoerportaal/secure/index.zul>. Accessed 24 Jun 2022
- Nichols RV, Vollmers C, Newsom LA, Wang Y, Heintzman PD, Leighton M, Green RE, Shapiro BE (2018) Minimizing polymerase biases in metabarcoding. *Mol Ecol Resour* 18(5):927–939. <https://doi.org/10.1111/1755-0998.12895>
- Pierce J, Venier L (2005) Small mammals as bioindicators of sustainable boreal forest management. *For Ecol Manag* 208(1–3):153–175. <https://doi.org/10.1016/j.foreco.2004.11.024>
- Potter LC, Brady CJ, Murphy BP (2018) Accuracy of identifications of mammal species from camera trap images: A northern Australian case study. *Austral Ecol* 44(3):473–483. <https://doi.org/10.1111/aec.12681>
- Powell RA, Proulx G (2003) Trapping and marking terrestrial mammals for research: integrating ethics, performance criteria, techniques, and common sense. *ILAR J* 44(4):259–276. <https://doi.org/10.1093/ilar.44.4.259>
- R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>
- Rees HC, Maddison BC, Middleditch DJ, Patmore JRM, Gough KC (2014) The detection of aquatic animal species using environmental DNA - a review of eDNA as a survey tool in ecology. *J Appl Ecol* 51(5):1450–1459. <https://doi.org/10.1111/1365-2664.12306>
- Rowcliffe JM, Carbone C, Jansen PA, Kays R, Kranstauber B (2011) Quantifying the sensitivity of camera traps: an adapted distance sampling approach. *Methods Ecol Evol* 2(5):464–476. <https://doi.org/10.1111/j.2041-210X.2011.00094.x>
- Ryan E, Bateman P, Fernandes K, van der Heyde M, Nevill P (2022) eDNA metabarcoding of log hollow sediments and soils highlights the importance of substrate type, frequency of sampling and animal size, for vertebrate species detection. *Environ DNA* 4(4):940–953. <https://doi.org/10.1002/edn3.306>
- Sales NG, McKenzie MB, Drake J, Harper LR, Browett SS, Coscia I, Wangensteen OS, Baillie C, Bryce E, Dawson DA, Ochu E, Hänfling B, Handley LL, Mariani S, Lambin X, Sutherland C, McDevitt AD (2020) Fishing for mammals: landscape-level monitoring of terrestrial and semi-aquatic communities using eDNA from riverine systems. *J Appl Ecol* 57(4):707–716. <https://doi.org/10.1111/1365-2664.13592>
- Sheffel BI (2018) Eurasian water shrew *Neomys fodiens*. In: Wilson DE, Mittermeier RA (eds) *Handbook of the mammals of the world – volume 8 insectivores, sloths and colugos*. Lynx Edicions, Barcelona, p 457
- Smaal M, van Manen W (2022) Detecting and monitoring small mammals with trail cameras. *Lutra* 65(2):247–257
- Therneau T (2012) A package for survival analysis in R. R package version 2.36–14. Available from <https://cran.r-project.org/package=survival>
- Thomas ML, Baker L, Beattie JR, Baker AM (2020) Determining the efficacy of camera traps, live capture traps, and detection dogs for locating cryptic small mammal species. *Ecol Evol* 10(2):1–15. <https://doi.org/10.1002/ece3.5972>
- Water Authority De Dommel (2022) HOW traject Kleine Dommel bovenstrooms A67
- Wearn OR, Glover-Kapfer P (2019) Snap happy: camera traps are an effective sampling tool when compared with alternative methods. *R Soc Open Sci* 6(3):181748. <https://doi.org/10.1098/rsos.181748>
- Welbourne DJ, MacGregor C, Paull D, Lindenmayer DB (2015) The effectiveness and cost of camera traps for surveying reptiles and critical weight range mammals: a comparison with labour-intensive complementary methods. *Wildl Res* 42(5):414–425. <https://doi.org/10.1071/WR15054>
- Yonezawa S, Ushio M, Yamanaka H, Miya M, Takayanagi A, Isagi Y (2020) Environmental DNA metabarcoding reveals the presence of a small, quick-moving, nocturnal water shrew in a forest stream. *Conserv Genet* 21:1079–1084. <https://doi.org/10.1007/s10592-020-01310-5>

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