# Large-scale grid-based detection in occupancy surveys of a threatened small mammal: A comparison of two non-invasive methods

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## ARTICLE INFO

## ABSTRACT

Monitoring the status and trends of wildlife is key to understand how species respond to natural and human-derived threats, and to evaluate and improve conservation planning and management. Large-scale, grid-based assessment of species distribution, abundance, and population trends over time is an important component of biodiversity monitoring. However, such assessments still present important challenges related, for instance, to how the choice of the sampling method may affect species detectability and thus, overall data accuracy. Here, we address this issue, focusing on the Cabrera vole (Microtus cabrerae), a threatened small mammal, listed in the Habitats Directive (Annexes II and IV), hence requiring regular evaluation of its population status and trends. We used occupancy modelling to estimate method-specific detection probability of the species over large-scale, grid-based (10 × 10 km²) surveys relying on two non-invasive sampling techniques: sign surveys and owl pellet analysis. Results provided evidence for a greater cost-effectiveness of sign surveys compared to owl pellet analysis for detecting the species in occupancy surveys, suggesting that large-scale population monitoring of Cabrera voles (or other species also producing easily identifiable signs of their presence) may fairly rely on sign-surveys. Overall, our study supported the view that while owl pellet analysis provides a valuable option when the aim is to assess small mammal assemblages (i.e. multiple species) in a region, other complementary methods may be required to increase the detection probability of certain species that because of their secretive behaviour or rarity remain less predated by owls. We thus argue that the choice of the sampling method should be context-dependent and evaluated based on the study aims, the surveyed area (i.e. local factors), the target species (i.e. life history traits) and the available resources. Based on our results we recommend that researchers and managers explore survey-design trade-offs to ensure the proposed designs have sufficient power to detect real population trends.

## 1. Introduction

Conservationists and ecologists are increasingly concerned about global biodiversity declines resulting from habitat destruction and fragmentation, climate change, and introduction of exotic species (Sala et al., 2000). Despite the plethora of international conservation conventions and agreements (Hill et al., 2018), biodiversity continues to decline at unprecedented rates (Powers & Jetz, 2019). To counteract

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ongoing biodiversity loss, it is pivotal to implement regional to global-scale biodiversity assessments, based on survey methods that provide an accurate picture of species distribution changes over time (Gaston & Blackburn, 2003).

Large-scale biodiversity sampling and monitoring are of major significance for understanding macroecological patterns and their mechanistic drivers, including species distribution, abundance, richness, rarity, turnover, assemblage composition, and the design of protected areas (Araújo et al., 2005). Notably, large-scale data on species occurrence provide the baseline information for producing atlases representing species presence in near-equal-area grid cells on maps (Araújo et al., 2005). Such grid-based data also provide an important source of information for updating species red lists according to the IUCN criteria, particularly regarding the distribution parameters assessed under criteria B1 (extent of occurrence) and B2 (area of occupancy), as well as several other subcriteria (e.g., under criteria A and D) (IUCN Standards and Petitions Committee, 2022). Large-scale, grid-based biodiversity monitoring is also a major requirement for those species included in environmental regulations, such as the EU Habitats Directive (Directive 92/43/EEC), which requires that every 6 years, member states should draw up a report on the conservation status of species listed in Annexes II and IV. These species are often referred to act as effective umbrella species for other coexisting species in the community, being therefore of highest interest for biodiversity conservation (Lisón et al., 2015).

Despite their relevance to macroecology and conservation science, large-scale grid-based sampling and monitoring of species entail important challenges that often raise concerns related to data accuracy (Marta et al., 2019). Similarly to other ecological surveys, grid-based data are sensitive to the sampling method. Best practice suggests that an adequate effort should be allocated to each cell, which may be difficult to apply when too many grid cells are required to be surveyed in limited amount of time (Joseph & Possingham, 2008). Because most monitoring and conservation programs are under-resourced, this places constraints on the choice of the target species, the sampling methods, and the sampling effort used to detect target species and to infer their population trends (Zamora-Marín et al., 2021). Such limitations in large-scale grid-based survey designs may well contribute to large biases in species detection probabilities, leading to the misinterpretation of abundance and distribution patterns and their variations across time (Lahoz-Monfort et al., 2014). This may be particularly true for small elusive or rare species of conservation concern that are patchily distributed. For these species, confirming a presence or an absence may require labour-intensive and time-consuming field methods often involving invasive sampling of animals (e.g., trapping) potentially affecting individuals and populations (Jeliazkov et al., 2022). Therefore, there is a need for cost-effective, grid-based sampling methods reliant on non-invasive techniques that maximise the detectability of such species at large spatial scales, while also minimising any potential animal welfare conflicts (Zemanova, 2020).

In this study, we address this issue focusing on the Cabrera vole (*Microtus cabrerae*), a small mammal endemic to the Iberian Peninsula, which is patchily distributed and typically occurs at low numbers (Pita et al., 2014; Sabino-Marques et al., 2018). The Cabrera vole is considered 'Near-threatened' at the global scale (Fernandes et al., 2019), and as 'Vulnerable' in the Spanish and the Portuguese national red lists (Cabral et al., 2005; Palomo et al., 2007). In addition, it is included in the Annexe II and IV of the Habitats Directive, meaning that its conservation may possibly support the management and protection of other similar species, and that regular assessment of its population status is legally required to accomplish international commitments. Specifically, we use multi-method occupancy models (MacKenzie et al., 2002; Zamora-Marín et al., 2021) to assess occupancy detection probability of Cabrera voles at large spatial scales ($10 \times 10$ km² grid cells) provided by two non-invasive sampling methods: sign surveys and owl pellet analysis. Barn owl (*Tyto alba*) pellets are referred to offer a powerful, cost-effective mean for sampling small mammals across broad scales, as

barn owls are often considered generalist predators (Avenant, 2005; Heisler et al., 2015; van Strien et al., 2015), potentially allowing the detection and monitoring of rare small mammal species (e.g., Kiamos et al., 2019; McDonald et al., 2013). Likewise, sign surveys have been also shown to be useful for surveying elusive small mammals that produce identifiable signs of their presence (e.g., Seidlitz et al., 2021), as is the case of the Cabrera vole (e.g., Peralta et al., 2023; Pita et al., 2007; Valerio et al., 2020). Our specific aims were (1) to compare the detection effectiveness of large-scale grid-based surveys of the Cabrera vole from owl pellet analysis versus sign surveys; and, based on that, (2) to provide recommendations for the design of monitoring programmes, allowing resource optimisation and maximising species detectability, thereby improving information about species conservation status at large scales.

## 2. Material and methods

### 2.1. Study area and survey grids

The study was carried out in central and southern Portugal (provinces of Alentejo, Algarve, and part of Estremadura, Ribatejo and southern Beira interior), covering about 43,000 km² (Fig. 1a). The region is included in the meso-Mediterranean and thermo-Mediterranean bioclimatic belts, characterised by a hot, dry Mediterranean climate (Rivas-Martínez et al., 2002). The landscape is dominated by plains or low hills with elevation ranging from the sea level to ca. 1000 m altitude. The main habitats are agricultural fields (cereal steppes, olive groves, and vineyards) and Mediterranean scrublands, as well as holm oak (*Quercus rotundifolia*) and cork oak (*Quercus suber*) pastoral woodlands (*montados*) (e.g., Pinto-Correia & Vos, 2004). During the past decades there has been an increasing shift from traditional farming to intensive agriculture, which may have negative impacts on overall biodiversity (Simonson et al., 2018). Within the study region, 56 grid cells of $10 \times 10$ km² were considered for the study (Fig. 1a). These grid cells were part of a larger set included in a nationwide research program designed to survey small mammal species (Rodentia and Eulipotyphla) and to aid the assessment of their extinction risk and conservation status (project POSEUR-03–225-FC-000097). For this study, each selected grid cell was sampled for Cabrera voles between November 2019 and July 2020, as described below.

### 2.2. Vole sign surveys

In each selected grid cell, up to three transects were sampled for Cabrera vole signs, with a mean of 1.70 ($\pm$0.61 SD) transects per grid. Transects length ranged from 484 to 926 m (mean 627.48 $\pm$ 81.84 SD) and were established in habitat patches suitable for Cabrera voles, which were previously identified based on Google Earth imagery and subsequent ground validation. These include wet habitats dominated by herbs, sedges and rushes, often found along small streams, ponds, road verges and field margins (Luque-Larena & López, 2007; Pita et al., 2007; Santos et al., 2006, see Fig. S1A in Supplementary material). In one of the selected grid cells no transects were defined due to the difficulty of identifying suitable areas for Cabrera voles. Therefore, a total of 55 grid cells were considered for vole sign sampling (see Fig. 1a, Table S1 in Supplementary material). Along each transect (e.g., Fig. 1b), we carefully searched for the distinctive presence signs of the Cabrera vole, which include its droppings, runways made on grasses and heaps of grass clippings (see e.g., Garrido-García & Soriguer, 2015; Luque-Larena & López, 2007; Peralta et al., 2023; Pita et al., 2007; Santos et al., 2006; Proença-Ferreira et al., 2019; see Fig. S1B and C in Supplementary material). In our study area there is no possible misidentification of these signs with those of other similar species, as signs made by the sympatric southern water vole (*Arvicola sapidus*) are much larger, while those of other voles (e.g., *Microtus duodecimcostatus*) are much smaller (e.g., Garrido-García & Soriguer, 2015). On the other hand, the Portuguese field vole (*Microtus rozianus*), which may produce similar signs to
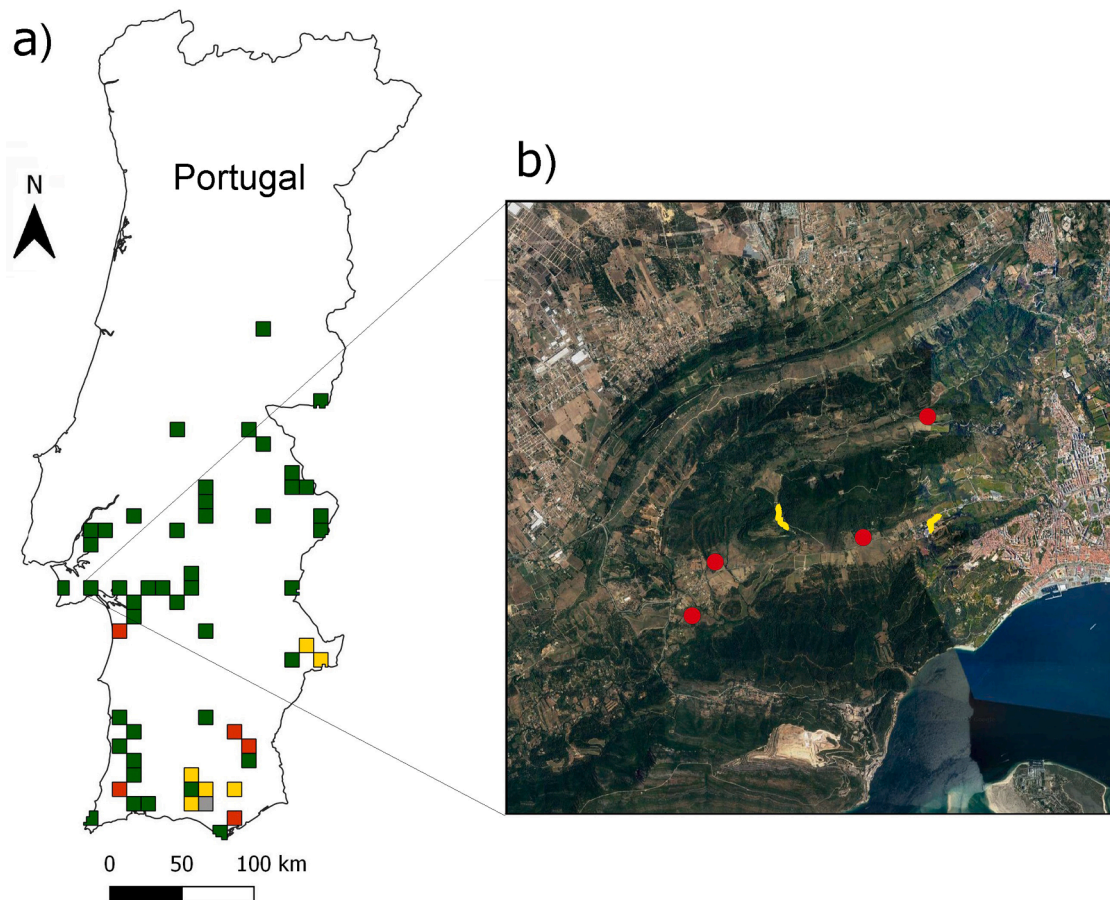
**Fig. 1.** (a) Study area and location of the 56 grid cells of 10 × 10 km² in southern Portugal, selected to sample Cabrera voles based on sign surveys and owl pellet analyses. Each colour indicates the sampling method(s) employed (see text for details): Green – Sign surveys and owl pellet data totalling ≥100 small mammal prey items; Red – Sign surveys and owl pellets with <100 prey items; Yellow – Sign surveys only; Grey – not sampled with either method. (b) Example of a 10 × 10 km² grid cell showing the location of two transects for surveying vole signs (yellow lines) and 4 barn owl nests where pellets were collected (red circles). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

those of Cabrera voles, is only found in the north of the country (Paupério et al., 2012). All transects were thoroughly walked by the same trained observers (2 observers/transect), and each detection of Cabrera voles based on presence signs was recorded.

### 2.3. Owl pellet sampling

Barn owl nests were searched within each grid cell by inspecting all favourable sites (e.g., old and abandoned buildings, windmills) (van Strien et al., 2015; see Fig. S1D in Supplementary material). These sites were firstly identified based on information previously provided by the rangers from the national authority for nature conservation (ICNF, Instituto da Conservação da Natureza e Florestas) and on Google Earth imagery, followed by ground confirmation. Because barn owls may be considerably elusive and nests are often placed in small crevices and false ceilings, searches included careful examination of these structures within identified sites. In 7 of the selected grid cells, we could not find any active barn owl nest/roost, so these grid cells were not surveyed with this method (see Fig. 1a and Table S1 in Supplementary material). Pellets detected in each site (see e.g. Fig. S1E in Supplementary material) were collected into plastic bags and frozen until later identification of prey content. Prey items were identified with the help of binocular lens amplifying bone remains up to 40x, and dedicated identification keys (e.g., Madureira, 1983; Alcántara, 1998; Blanco, 1998a, 1998b; Moreno & Balbotín, 1998; Turón, 2012; Román, 2019). In particular, Cabrera vole skull and mandible remains in owl pellets are relatively easy to identify based on their size, and the tooth form provides

unequivocal diagnostic feature to accurately discriminate the species from other *Microtus* species (see Cuenca-Bescós et al., 2014; see Fig. S1F in Supplementary material). We considered only those grid cells for which a minimum of 100 small mammal prey items were available for the analyses (Fig. 1a), which often implied the inclusion of data from different nests within each grid cell to increase the number of prey items (mean number of nests per grid cell = 1.63 ± 1.32 SD, range: 0–7) (e.g., Fig. 1b). Otherwise, the grid cell was not considered as being surveyed through this method, as small sample sizes may irremediably prevent the detection of Cabrera voles based on barn owl pellet analyses (Mira et al., 2008). Therefore, a total of 44 grid cells met these criteria and were considered in the analyses of pellet owl data (Fig. 1a and Table S1 in Supplementary material). Prey items identified in each grid cell were processed together as a batch (e.g., Love et al., 2000; van Strien et al., 2015).

### 2.4. Data analysis

In order to assess method-specific detection probability in occupancy surveys of Cabrera voles, we used multi-method, single-season detection-occupancy models (hereafter referred as occupancy models, MacKenzie et al., 2002; Nichols et al., 2008; Zamora-Marín et al., 2021), using the package 'unmarked' (Fiske & Chandler, 2011) for R program version 4.2.0 (R Core Team, 2022). Occupancy models are hierarchical models focused on estimating species detection and occupancy probabilities, assuming that no survey method provides perfect detectability of a species (MacKenzie et al., 2002). The approach is based on species

detection/non detection (1/0) histories, which are recorded from replicated surveys (either in time or in space) within study sites (in our case, each $10 \times 10$ km$^2$ grid cell). In our analysis we considered each transect as a spatial replicate scoring either 0 (if no Cabrera vole signs were detected) or 1 (if presence signs of the species were detected) (MacKenzie et al., 2006). We opted for spatial replicates in sign surveys due to logistic and time constrains in resampling the same locations through independent temporal replicates. Although the use of spatial replicates have been referred to induce potential biases in detection-occupancy estimates (Kendall & White, 2009), there is some controversy on the extent to which such biases may occur. In fact, both simulation (Guillera-Arroita, 2011) and empirical-based studies (e.g., Whittington et al., 2015; Srivathsa et al., 2018) suggested that spatial replication may perform equally well to temporal replication, as long as (i) the probability of species presence in one replicate is not influenced by species presence in other replicates within the same site (MacKenzie et al., 2002), and (ii) each replicate in a positive grid has non-zero probability of being occupied (Guillera-Arroita, 2011). Non-independence among replicated surveys typically occurs when considering successive segments along a single transect (e.g. Hines et al., 2010). However, in our case, each transect for detecting Cabrera voles within each grid was separated by a distance >1,5 km from other transects. Therefore, considering the range of movements of the species (mean home ranges of ca. 400 m$^2$, see Pita et al., 2010), we assumed that our spatial replicates within grids are fairly independent from each other. On the other hand, because the Cabrera vole has a metapopulation-like spatial dynamics (e.g. Pita et al., 2007; Mestre et al., 2017), we also assumed that every spatial replicate within an occupied grid cell should have a given probability of being occupied by voles, and hence that our approach should not result in significant bias in detection-occupancy estimates (Guillera-Arroita, 2011). Regarding the data from barn owl pellet analyses considering the 44 grid cells with >100 prey items identified, we followed the 'half batches' approach for defining replicates (as in van Strien et al., 2015), which consists in splitting the entire batch data within each grid cell into equal parts, such that two replicates are created out of each batch, with the detection/non-detection of Cabrera vole skulls/mandibles in each 'half batch' being recorded as 1 and 0 respectively.

In model building, we considered only those grid cells for which at least two replicates were available (either from sign sampling or owl pellet sampling), which totalled 48 grid cells included in the analysis (see Table S1 in Supplementary material). Based on these data, we implemented two competing models for testing the hypothesis that the sampling method affected the detectability of Cabrera voles. Specifically, we built one model including the fixed effect of the sampling method on the estimates of detection probability (multi-method model), and one without these effects (null model). We then used R package 'AICcmodavg' (Mazerolle, 2020) to test the support of each of these models based on the Akaike Information Criteria corrected for small sample sizes (AICc, Burnham & Anderson, 2002), with $\Delta$AICc < 2 indicating equally supported models (Burnham & Anderson, 2002). We also assessed the support of the model including sampling intensity as fixed effect by estimating the respective AICc-weigh relative to the null model (Burnham & Anderson, 2002). Furthermore, we computed the goodness-of-fit (GOF) of the best model based on Pearson's chi-square for single season occupancy models, using 1000 bootstrapped iterations to estimate the overdispersion parameter (c-hat), for which values close to 1 are indicative of no overdispersion (MacKenzie & Bailey, 2004).

In addition, in order to confirm that no effect related to survey effort employed within each sampling method could be affecting the results, we implemented additional occupancy models considering each method in separate datasets. Regarding sign survey method, we considered only those grid cells where at least two transects were conducted (i.e., 34 grids, see Table S1 in Supplementary material), implementing one model considering the effect of transect length on sign detectability and

another without this effect. For the owl pellet sampling method, we considered the 44 grids where at least 100 prey items were identified, and followed the same approach, implementing one model including the number of small mammal prey items identified in each grid as a covariate of detectability, and one model without this effect. In each case, AICc-based model comparison was applied to identify the best supported model.

## 3. Results

Overall, we conducted a total of 93 transects summing up ca. 53.96 km walked across the 55 grid cells surveyed for Cabrera voles signs. From these, we detected the species in a total of 51 transects allocated to 33 grid cells (Fig. 1a and 2). Regarding owl pellets sampling, we detected 107 Cabrera vole individuals among a total of 10 435 individual prey items analysed from 94 nests located in a total of 49 grid cells, with 5 grid cells summing up<100 small mammal prey items (Fig. 1a). Overall, Cabrera vole skulls were detected in 33 'half batches' from a total of 23 grid cells (see Fig. 2). Sign surveys allowed the detection of Cabrera voles in 14 grid cells where owl pellet analysis
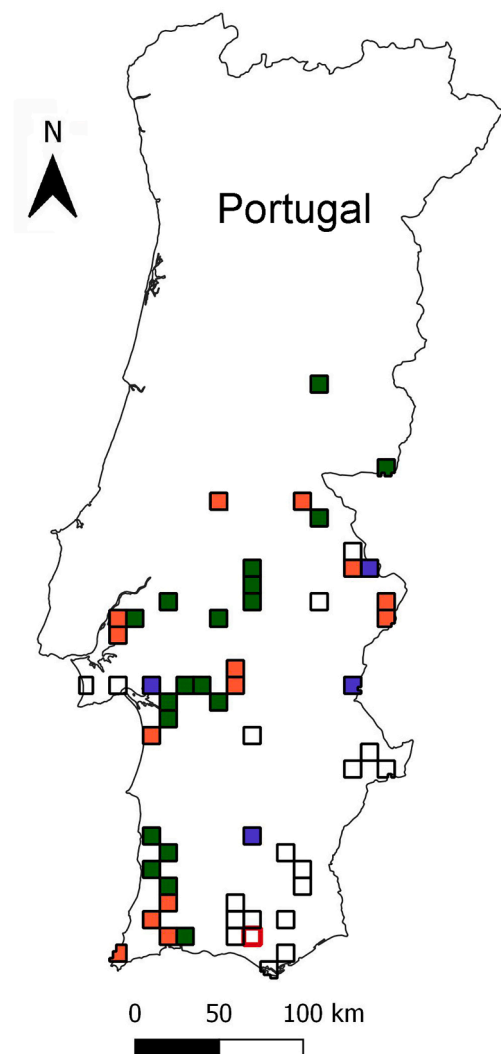


**Fig. 2.** Map showing the grid cells where Cabrera voles were detected from sign surveys only (orange grids), from owl pellet data alone (purple), and from both methods (green). White grid cells indicate those where no Cabrera vole skulls or presence signs were detected (black contours) or surveyed with either methods (red contours). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Table 1**

AICc-based comparison of occupancy models built to test the effect of the sampling method on the probability of detection of Cabrera voles at large spatial scales in central amd southern Portugal. K - number of model parameters; AICc - Akaike Information Criteria corrected for small sample sizes; ΔAICc - difference between models AICc. AICc-Wt – weighted AICc.

|  | K | AICc | ΔAICc | AICc-Wt |
|---|---|---|---|---|
| Multi-method Model | 4 | 234.06 | 0.00 | 0.91 |
| Null Model | 2 | 238.51 | 4.45 | 0.09 |

failed to detect it. Conversely owl pellet analysis allowed the detection of voles in 4 grid cells where sign abundance did not detect the species.

Occupancy modelling based on the data from the 48 grid cells with at least two survey replicates (either from transects or from pellet analysis) revealed that the model including the effect of the sampling method on species detectability (Multi-method Model) showed a lower AICc (>4 units lower) than the Null Model, being therefore the best supported one (Table 1). This is also shown by the weighted AICc scores of models, indicating that the Multi-method Model has a 91 % probability of being the best model (Table 1). The GOF test indicated a c-hat = 1.19, suggesting no major problems of model overdispersion (Mazerolle, 2015). Multi-method Model results suggested that the sign sampling method provided much higher detectability than the owl pellet sampling method, with a mean (95 % confidence interval) detection probability of 0.70 (0.57, 0.80) and 0.46 (0.34, 0.58) (Fig. 3), respectively. This model estimated a mean [95 % confidence interval] occupancy of 0.83 [0.66, 0.93], which is above the naïve occupancy estimate of 0.77 [i.e. the number of grid cells where Cabrera voles were detected at least once independently of the sampling method (n = 37, see Fig. 2), divided by total number of grid cells considered in the analysis (n = 48)].

When testing whether the survey effort employed within each sampling method affected Cabrera vole's detectability, we found no support for such effects for both the sign sampling and the owl pellet data (see Tables S2 and S3 in Supplementary material), thus supporting our finding that sign surveys provide better detectability than owl pellet analysis.
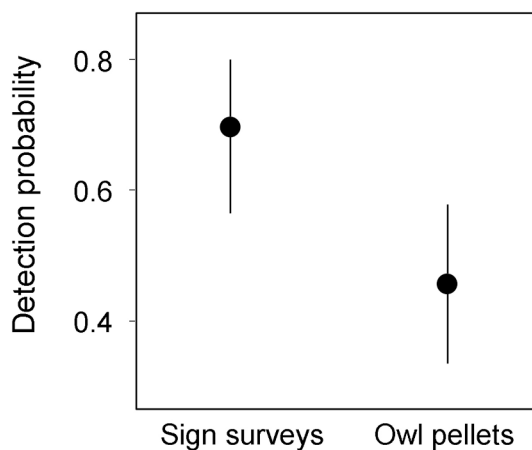


**Fig. 3.** Cabrera vole detection probabilities (mean and 95 % confidence intervals) obtained from the multi-method occupancy model descriminating estimates for sign surveys and owl pellet analysis conducted in $10 \times 10 \ km^2$ grid cells across southern Portugal between November 2019 and July 2020.

## 4. Discussion

Effective large-scale biodiversity monitoring is critical to evaluate species population status and trends across their distribution range (Lindenmayer et al., 2012) but standards to improve species detectability are needed for delivering relevant information to conservation practice (Einoder et al., 2018; Morelli et al., 2022), particularly among small and elusive species legally requiring regular assessment of their populations (Legge et al., 2018). Our multi-method occupancy modelling approach focused on assessing Cabrera's vole detectability associated to large-scale, grid-based non-invasive sampling through sign surveys and the analysis of owl pellets highlighted that, as expected, both methods suffer from imperfect detection. Results point also to a greater cost-effectiveness of sign surveys compared to owl pellet analysis for detecting the species. Because the choice of the sampling method is critical to obtain sufficiently accurate data on species occurrence (Zamora-Marín et al., 2021), we believe these results may help setting most suitable study designs aiming to maximise species detectability in monitoring programs compulsory for EU member states, and hence improve conservation planning over large spatial scales.

Owl pellets have been extensively used to characterise small mammal communities at broad-scales and evidence suggests that, comparing for instance to conventional live-trapping, this method may provide a more effective alternative to monitor small mammals (Heisler et al., 2015; Viteri et al., 2021) and even to overcome the difficulties associated with the detection of rare or elusive species over large geographic areas (Biedma et al., 2019). However, problems related to imperfect detection are rarely addressed in studies using owl pellets to monitor small mammals (e.g., van Strien et al., 2015), so it is largely unknown how ignoring this may have impacted the results from such studies. In the case of the Cabrera vole, we found moderate detection probability from owl pellet surveys, suggesting that accurate monitoring of the species over large scales may require the application of complementary sampling methods, such as for instance sign surveys. In fact, our study suggests that for large scale monitoring programs focused on specific target species producing identifiable signs such as the Cabrera vole (e.g., Garrido-García & Soriguer, 2015; Pita et al., 2007; Santos et al., 2006; Valerio et al., 2020), methods uniquely based on sign survey should provide a cost-effective alternative to owl pellet analysis. Although our inferences on sign survey detectability were based on spatial replication rather than temporal replication, we believe our approach was appropriate, given the specificities of our study species and system, for which a constant proportion of occupied subunits within a grid cell is mostly unlikely (Guillera-Arroita, 2011). However, even considering any potential shortcoming related to the use of spatial replicates, we believe this would have minimal impact on our assertion that sign surveys should provide higher detectability of Cabrera voles than owl pellet analysis over large scales. In fact, eventual bias induced by spatial replicates typically results in underestimation of detectability (Kendall & White, 2009), which in our case would suggest that the difference in detectability between sign surveys and owl pellet analysis could be even greater than that reported here.

Although sign surveys may possibly require some level of expertise (e.g., Peralta et al., 2016), they also require relatively low sampling effort and, except when genetic confirmation is required due to uncertainties in species identification (e.g. regions of sympatry with *M. rozianus*; Barão et al., 2022; Barbosa et al., 2013; Mestre et al., 2015), no further procedures are necessary for data completion. Conversely, owl pellet analyses require more human and logistic resources for pellet dissection and species identification, which demand even more training and expertise than that involved in sign surveys (Yalden & Morris,

1990). Moreover, sign surveys are not constrained by any kind of restriction beyond the ability to identify suitable habitats for field sampling, while owl pellets are obviously limited to areas in which barn owls occur (Biedma et al., 2019). This may be of concern as barn owls are declining in many regions (e.g. Martínez & Zuberogoitia, 2004; Regan et al., 2018), including in Portugal (Lourenço et al., 2015, 2019), and they do not occur in mountainous areas or dense forests (Taylor, 2004), thus limiting the detection of prey species associated to those environments. Moreover, some barn owl nests or roosting sites can be placed in private buildings or inaccessible holes, making them unavailable for data collection. A further advantage of sign surveys over owl pellet analysis to monitor Cabrera voles or targeted small mammal species is that they provide information on the exact location of potentially important habitats or local populations within monitored areas, which may be useful for conservation practice. In the case of owl pellet analysis, this is challenging given that home-ranges and hunting areas of owls are large and often include a mosaic of several habitat types (Heisler et al., 2015).

Therefore, while the usefulness of owl pellets analysis is undeniable when focusing or attempting to sample and monitor multiple small mammal species within a region (Heisler et al., 2015; Viteri et al., 2021), according to our results, multi-method approaches involving other sampling techniques may be needed to maximize the chances of detecting particular elusive species that for some reason (e.g. rarity, body size, habitat use) remain less predated by owls (van Strien et al., 2015). Complementary methods may include sign surveys, as suggested in our study focused on Cabrera voles, but other non-invasive techniques (e.g. hair trapping, camera trapping, environmental DNA/metabarcoding, acoustic sampling) may be considered, depending on the target species. The use of multiple detection methods or devices should thus be encouraged in multi-species surveys, as no single method is expected to detect unbiasedly all species of any given community (e.g., Torre et al., 2018). This is supported by the fact that the data from each specific method allowed the confirmation of Cabrera voles in grid cells where the concurrent method failed to detect the species. Because targeting is a key aspect of monitoring schemes relying on proxies of conservation interest (e.g., Lisón et al., 2015), it is clear that the choice of the most appropriate sampling method(s) to employ in a given study or monitoring program will depend on the main objectives, the target species, the study area, and the available resources. We thus recommend rigorous evaluation of the most suitable sampling methods prior to implementing a monitoring program, based on well-conceived and designed sampling schemes, ideally providing information on the observation process, to account with the uncertainties associated with the data collected during surveys (Haynes et al., 2013; MacKenzie et al., 2002).

In summary, while we acknowledge that our results should be complemented with further studies considering other detection methods (e.g., hair or camera trapping) or other sources of variation in detectability within each method (e.g., seasonality, breeding status), we believe our findings represent a piece of information that could be useful for designing more effective survey schemes for monitoring Cabrera voles over large scales. This is of particular interest for Portugal and Spain reporting under Habitats Directive concerning the species occupancy and population trends. In fact, the Cabrera vole is one of the few Cricetidae species whose assessment of conservation status is compulsory under international legislation. Notably, our results also provide support for the importance of multi-method approaches in studies involving multiple small mammal species, allowing to maximize detection probabilities of target species (Nichols et al., 2008). This is particularly critical in conservation practice due to the growing demand for accurate data on species distribution patterns and dynamics to inform an effective management of protected areas, species extinction risk assessments, and national/regional red lists updating.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jnc.2023.126362.

## References

Alcántara, M. (1998). Orden Insectívoros. In J. C. Blanco (Ed.), *Mamíferos de España I* (pp. 39–113). S.A., Barcelona: Editorial Planeta.

Araújo, M. B., Thuiller, W., Williams, P. H., & Reginster, I. (2005). Downscaling European species atlas distributions to a finer resolution: Implications for conservation planning. *Global Ecology and Biogeography, 14*(1), 17–30. https://doi.org/10.1111/j.1466-822X.2004.00128.x

Avenant, N. L. (2005). Barn owl pellets: A useful tool for monitoring small mammal communities? *Belgian Journal of Zoology, 135*(supplement), 39–43.

Barão, I., Queirós, J., Vale-Gonçalves, H., Paupério, J., & Pita, R. (2022). Landscape characteristics affecting small mammal occurrence in heterogeneous olive grove agro-ecosystems. *Conservation, 2*(1), 51–67. https://doi.org/10.3390/conservation2010005

Barbosa, S., Pauperio, J., Searle, J. B., & Alves, P. C. (2013). Genetic identification of Iberian rodent species using both mitochondrial and nuclear loci: Application to noninvasive sampling. *Molecular Ecology Resources, 13*(1), 43–56. https://doi.org/10.1111/1755-0998.12024

Biedma, L., Román, J., Godoy, J. A., & Calzada, J. (2019). Using owl pellets to infer habitat associations and clarify the regional distribution of a cryptic shrew. *Journal of Zoology, 308*(2), 139–148. https://doi.org/10.1111/jzo.12660

Blanco, J. C. (1998a). *Mamíferos de España I (Insectivoros, Primates y Carnívoros)*. Barcelona: Editorial Geoplaneta.

Blanco, J. C. (1998b). *Mamíferos de España II (Cetáceos, Artiodáctilos, Roedores y Lagomorfos)*. Barcelona: Editorial Geoplaneta.

Burnham, K. P. & Anderson, D. R. (2002). *Model selection and multimodel inference: A practical information-theoretic approach*. Springer. Springer New York. 10.1007/b97636.

Cabral, M. J., Almeida, J., Almeida, P. R., Delliger, T., de Almeida, N. F., Oliveira, M. E., … Santos-Reis, M. (2005). *Livro Vermelho dos Vertebrados de Portugal*. Lisboa: Instituto da Conservação da Natureza.

Cuenca-Bescós, G., López-García, J. M., Galindo-Pellicena, M. A., García-Perea, R., Gisbert, J., Rofes, J., & Ventura, J. (2014). Pleistocene history of *Iberomys*, an endangered endemic rodent from southwestern Europe. *Integrative Zoology, 9*(4), 481–497. https://doi.org/10.1111/1749-4877.12053

Einoder, L. D., Southwell, D. M., Lahoz-Monfort, J. J., Gillespie, G. R., Fisher, A., & Wintle, B. A. (2018). Occupancy and detectability modelling of vertebrates in northern Australia using multiple sampling methods. *PLOS ONE, 13*(9), e0203304.

Fiske, I. J., & Chandler, R. B. (2011). Unmarked: An R package for fitting hierarchical models of wildlife occurrence and abundance. *Journal of Statistical Software, 43*(10), 1–23. https://doi.org/10.18637/jss.v043.i10

Fernandes, M., Pita, R. & Mira, A. 2019. *Microtus cabrerae. The IUCN Red List of Threatened Species* 2019: e.T13418A90931498. https://dx.doi.org/10.2305/IUCN.UK.2019-1.RLTS.T13418A90931498.en (Assessed 06 July 2022).

Garrido-García JA, Soriguer RC (2015) Topillo de Cabrera *Iberomys cabrerae* (Thomas, 1906). *Guía de indícios de los mamíferos de España.* SECEM. http://www.secem.es/wp-content/uploads/2015/07/020.-Iberomys-cabrerae.pdf (Assessed 17 April 2022).

Gaston, K.J. & Blackburn, T.M. (2003) Macroecology and conservation biology. In T.M. Blackburn and K.J. Gaston (Eds.), *Macroecology: Concepts and consequences* (pp. 345–367). Blackwell Scientific Publications, Oxford.

Guillera-Arroita, G. (2011). Impact of sampling with replacement in occupancy studies with spatial replication. *Methods in Ecology and Evolution, 2,* 401–406. https://doi.org/10.1111/j.2041-210X.2011.00089.x

Haynes, T. B., Rosenberger, A. E., Lindberg, M. S., Whitman, M., & Schmutz, J. A. (2013). Method- and species-specific detection probabilities of fish occupancy in Arctic lakes: Implications for design and management. *Canadian Journal of Fisheries and Aquatic Sciences, 70*(7), 1055–1062. https://doi.org/10.1139/cjfas-2012-0527

Heisler, L. M., Somers, C. M., & Poulin, R. G. (2015). Owl pellets: A more effective alternative to conventional trapping for broad-scale studies of small mammal communities. *Methods in Ecology and Evolution, 7*(1), 96–103. https://doi.org/10.1111/2041-210X.12454

Hill, M. J., Hassall, C., Oertli, B., Fahrig, L., Robson, B. J., Biggs, J., … Wood, P. J. (2018). New policy directions for global pond conservation. *Conservation Letters, 11*(5), e12447.

Hines, J. E., Nichols, J. D., Royle, A. J., Mackenzie, D. I., Gopalaswamy, A. M., Kumar, N. S., & Karanth, K. U. (2010). Tigers on trails: Occupancy modeling for cluster sampling. *Ecological Applications, 20,* 1456–1466. https://doi.org/10.1890/09-0321.1

IUCN Standards and Petitions Committee (2022). *Guidelines for Using the IUCN Red List Categories and Criteria. Version 15.1.* https://www.iucnredlist.org/documents/RedListGuidelines.pdf (Assessed 21 June 2022).

Jeliazkov, A., Gavish, Y., Marsh, C. J., Geschke, J., Brummitt, N., Rocchini, D., … Henle, K. (2022). Sampling and modelling rare species: Conceptual guidelines for the neglected majority. *Global Change Biology, 28*(12), 3754–3777. https://doi.org/10.1111/gcb.16114

Joseph, L. N., & Possingham, H. P. (2008). Grid-based monitoring methods for detecting population declines: Sensitivity to spatial scale and consequences of scale correction. *Biological Conservation, 141*(7), 1868–1875. https://doi.org/10.1016/j.biocon.2008.05.003

Kendall, W. L., & White, G. C. (2009). A cautionary note on substituting spatial subunits for repeated temporal sampling in studies of site occupancy. *Journal of Applied Ecology, 46,* 1182–1188. https://doi.org/10.1111/j.1365-2664.2009.01732.x

Kiamos, N., Lymberakis, P., Rallis, G., & Poulakakis, N. (2019). Barn owl (*Tyto alba*) prey in Evros (Greece) and the discovery of a new mammal for the Greek fauna. *Journal of Natural History, 53*(27–28), 1691–1705. https://doi.org/10.1080/00222933.2019.1658820

Lahoz-Monfort, J. J., Guillera-Arroita, G., & Wintle, B. A. (2014). Imperfect detection impacts the performance of species distribution models. *Global Ecology and Biogeography, 23*(4), 504–515. https://doi.org/10.1111/geb.12138

Legge, S., Lindenmayer, D., Robinson, N., Scheele, B., Southwell, D., & Wintle, B. (2018). *Monitoring threatened species and ecological communities.* Melbourne: CSIRO Publishing.

Lindenmayer, D. B., Gibbons, P., Bourke, M., Burgman, M., Dickman, C. R., Ferrier, S., … Robinson, D. (2012). Improving biodiversity monitoring. *Austral Ecology, 37*(3), 285–294. https://doi.org/10.1111/j.1442-9993.2011.02314.x

Lisón, F., Sánchez-Fernández, D., & Calvo, J. F. (2015). Are species listed in the Annex II of the Habitats Directive better represented in Natura 2000 network than the remaining species? A test using Spanish bats. *Biodiversity and Conservation, 24,* 2459–2473. https://doi.org/10.1007/s10531-015-0937-1

Lourenço, R., Roque, I., Tomé, R., Sepúlveda, P., Atlas, E., Melo, C., & Pereira, C. (2015). Current status and distribution of nocturnal birds (Strigiformes and Caprimulgiformes) in Portugal. *Airo, 23,* 36–50.

Lourenço, R., Tomé, R., & Roque, I. (2019). NOCTUA Portugal, 2010–2019. In H. Alonso, J. Teodósio, & D. Leitão (Eds.), *O estado das aves em Portugal, 2019* (pp. 17–19). Lisboa: Sociedade Portuguesa para o Estudo das Aves.

Love, R. A., Webben, C., Glue, D. E., & Harris, S. (2000). Changes in the food of British barn owls (*Tyto alba*) between 1974 and 1997. *Mammal Review, 30,* 107–130. https://doi.org/10.1046/j.1365-2907.2000.00060.x

Luque-Larena, J. J., & López, P. (2007). Microhabitat use by wild-ranging Cabrera voles *Microtus cabrerae* as revealed by live trapping. *European Journal of Wildlife Research, 53*(3), 221–225. https://doi.org/10.1007/s10344-006-0084-9

MacKenzie, D. I., & Bailey, L. L. (2004). Assessing the fit of site-occupancy models. *Journal of Agricultural, Biological, and Environmental Statistics, 9*(3), 300–318. https://doi.org/10.1198/108571104X3361

MacKenzie, D. I., Nichols, J. D., Lachman, G. B., Droege, S., Royle, A. A., & Langtimm, C. A. (2002). Estimating site occupancy rates when detection probabilities are less than one. *Ecology, 83*(8), 2248–2255. https://doi.org/10.1890/0012-9658(2002)083[2248:ESORWD]2.0.CO;2

MacKenzie, D. I., Nichols, J. D., Royle, J. A., Pollock, K. H., Bailey, L. L., & Hines, J. E. (2006). *Occupancy estimation and modeling: Inferring patterns and dynamics of species occurrence.* New York: AcademicPress.

Madureira, M. L. (1983). On the identification of west-European *Microtus* of the sub-genera *Microtus* and *Chionomys* (Mammalia, Rodentia). A principal component analysis. *Revista de Biologia, 12,* 615–632.

Marta, S., Lacasella, F., Romano, A., & Ficetola, G. F. (2019). Cost-effective spatial sampling designs for field surveys of species distribution. *Biodiversity and Conservation, 28*(11), 2891–2908. https://doi.org/10.1007/s10531-019-01803-x

Martínez, J., & Zuberogoitia, I. (2004). Habitat preferences and causes of population decline for barn owls *Tyto alba*: A multi-scale approach. *Ardeola, 52*(12), 303–317.

Mazerolle, M. J. (2015). Estimating detectability and biological parameters of interest with the use of the R environment. *Journal of Herpetology, 49*(4), 541–559. https://doi.org/10.1670/14-075

Mazerolle, M. J. (2020). *AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). R Package Version 2.3-1.* https://cran.r-project.org/package=AICcmodavg (Assessed 31 May 2022).

McDonald, K., Burnett, S., & Robinson, W. (2013). Utility of owl pellets for monitoring threatened mammal communities: An Australian case study. *Wildlife Research, 40*(8), 685. https://doi.org/10.1071/WR13041

Mestre, F., Pita, R., Paupério, J., Martins, F. M. S., Alves, P. C., Mira, A., & Beja, P. (2015). Combining distribution modelling and non-invasive genetics to improve range shift forecasting. *Ecological Modelling, 297,* 171–179. https://doi.org/10.1016/j.ecolmodel.2014.11.018

Mestre, F., Risk, B. B., Mira, A., Beja, P., & Pita, R. (2017). A metapopulation approach to predict species range shifts under different climate change and landscape connectivity scenarios. *Ecological Modelling, 359,* 406–414. https://doi.org/10.1016/j.ecolmodel.2017.06.013

Mira, A., Marques, C. C., Santos, S. M., Rosário, I. T., & Mathias, M. L. (2008). Environmental determinants of the distribution of the Cabrera vole (*Microtus cabrerae*) in Portugal: Implications for conservation. *Mammalian Biology, 73*(2), 102–110. https://doi.org/10.1016/j.mambio.2006.11.003

Morelli, F., Brlík, V., Benedetti, Y., Bussière, R., Moudrá, L., Reif, J., & Svitok, M. (2022). Detection rate of bird species and what it depends on: Tips for field furveys. *Frontiers in Ecology and Evolution, 9*(671492). https://doi.org/10.3389/fevo.2021.671492

Moreno, S., & Balbotín, J. (1998). Orden Roedores. In J. C. Blanco (Ed.), *Mamíferos de España II* (pp. 166–273). S.A., Barcelona: Editorial Planeta.

Nichols, J. D., Bailey, L. L., O'Connell, A. F., Jr., Talancy, N. W., Campbell Grant, E. H., Gilbert, A. T., … Hines, J. E. (2008). Multi-scale occupancy estimation and modelling using multiple detection methods. *Journal of Applied Ecology, 45*(5), 1321–1329. https://doi.org/10.1111/j.1365-2664.2008.01509.x

Palomo, L. J., Gisbert, J., & Blanco, J. C. (2007). *Atlas y Libro Rojo de los Mamíferos Terrestres de España.* Madrid: Dirección General para la Biodiversidad-SECEM-SECEMU.

Paupério, J., Herman, J. S., Melo-Ferreira, J., Jaarola, M., Alves, P. C., & Searle, J. (2012). Cryptic speciation in the field vole: A multilocus approach confirms three highly divergent lineages in Eurasia. *Molecular Ecology, 21*(24), 6015–6032. https://doi.org/10.1111/mec.12024

Peralta, D., Leitão, I., Ferreira, A., Mira, A., Beja, P., & Pita, R. (2016). Factors affecting southern water vole (*Arvicola sapidus*) detection and occupancy probabilities in Mediterranean farmland. *Mammalian Biology, 81*(2), 123–129. https://doi.org/10.1016/j.mambio.2015.10.006

Peralta, D., Vaz-Freire, T., Ferreira, C., Mendes, T., Mira, A., Santos, S., … Pita, R. (2023). From species detection to population size indexing: The use of sign surveys for monitoring a rare and otherwise elusive small mammal. *European Journal of Wildlife Research, 69,* 9. https://doi.org/10.1007/s10344-022-01634-2

Pinto-Correia, T. and Vos, W. (2004) Multifunctionality in Mediterranean landscapes: past and future. In R. H. Jongman (Ed.), *The New Dimensions of the European Landscapes* [pp. 135–164]. Springer, Dordrecht.

Pita, R., Beja, P., & Mira, A. (2007). Spatial population structure of the Cabrera vole in Mediterranean farmland: The relative role of patch and matrix effects. *Biological Conservation, 134*(3), 383–392. https://doi.org/10.1016/j.biocon.2006.08.026

Pita, R., Mira, A., & Beja, P. (2014). *Microtus cabrerae* (Rodentia: Cricetidae). *Mammalian Species, 46*(912), 48–70. https://doi.org/10.1644/912.1

Pita, R., Mira, A., & Beja, P. (2010). Spatial segregation of two vole species (*Arvicola sapidus* and *Microtus cabrerae*) within habitat patches in a highly fragmented farmland landscape. *European Journal of Wildlife Research, 56,* 651–662. https://doi.org/10.1007/s10344-009-0360-6

Powers, R. P., & Jetz, W. (2019). Global habitat loss and extinction risk of terrestrial vertebrates under future land-use-change scenarios. *Nature Climate Change, 9*(4), 323–329. https://doi.org/10.1038/s41558-019-0406-z

Proença-Ferreira A., Ferreira C., Leitão I., Paupério J., Sabino-Marques H., Barbosa S., Lambin X., Alves P.C., Beja P., Moreira F., Mira A., Pita R. (2019) Drivers of survival in a small mammal of conservation concern: an assessment using extensive genetic non-invasive sampling in fragmented farmland. Biological Conservation 230, 131-140. https://doi.org/10.1016/j.biocon.2018.12.021.

R Core Team. (2022). *R: A language and environment for statistical computing.* Vienna, Austria: R Foundation for Statistical Computing. https://www.r-project.org/.

Regan, T., McClure, C. J. W., & Belthoff, J. R. (2018). Assessing patterns of barn owl *Tyto alba* occupancy from call broadcast surveys. *Wildlife Biology, 1,* 1–12. https://doi.org/10.2981/wlb.00411

Rivas-Martínez, S., Fernández-González, F., Loidi, J., Lousa, M., & Penas, A. (2002). Vascular plant communities of Spain and Portugal. Addenda to syntaxonomical checklist of 2001, Part II. *Itinera Geobotanica, 15*(1–2), 5–922.

Román, J. (2019). *Manual para la identificación de los cráneos de los roedores de la península ibérica, islas baleares y canarias.* Manuales de Mastozoología, No 2. Sociedad Española para la Conservación y Estudio de Mamíferos (SECEM), Málaga.

Sabino-Marques, H., Ferreira, C. M., Paupério, J., Costa, P., Barbosa, S., Encarnação, C., … Pita, R. (2018). Combining genetic non-invasive sampling with spatially explicit capture-recapture models for density estimation of a patchily distributed small mammal. *European Journal of Wildlife Research, 64*(4), 44. https://doi.org/10.1007/s10344-018-1206-x

Sala, O. E., Stuart Chapin, F. III, Armesto, J. J., Berlow, E., Bloomfield, J., Dirzo … Wall, D. H. (2000). Global Biodiversity Scenarios for the Year 2100. *Science, 287(5459),* 1770–1774. 10.1126/science.287.5459.1770.

Santos, S. M., Simoes, M. P., Mathias, M. L., & Mira, A. (2006). Vegetation analysis in colonies of an endangered rodent, the Cabrera vole (*Microtus cabrerae*), in southern Portugal. *Ecological Research, 21*, 197–207. https://doi.org/10.1007/s11284-005-0104-3

Seidlitz, A., Bryant, K. A., Armstrong, N. J., Calver, M. C., & Wayne, A. F. (2021). Sign surveys can be more efficient and cost effective than driven transects and camera trapping: A comparison of detection methods for a small elusive mammal, the numbat (*Myrmecobius fasciatu*s). *Wildlife Research, 48*(6), 491. https://doi.org/10.1071/WR20020

Simonson, W. D., Allen, H. D., Parham, E., e Santos, E. D. B., & Hotham, P. (2018). Modelling biodiversity trends in the montado (wood pasture) landscapes of the Alentejo, Portugal. *Landscape Ecology, 33*, 811–827. 10.1007/s10980-018-0627-y.

Srivathsa, A., Puri, M., Samba Kumar, N., Jathanna, D., & Ullas Karanth, K. (2018). Substituting space for time: Empirical evaluation of spatial replication as a surrogate for temporal replication in occupancy modeling. *Journal of Applied Ecology, 55*, 754–765. https://doi.org/10.1111/1365-2664.13005

Taylor, I. (2004). *Barn Owls: Predator-Prey Relationships and Conservation*. Cambridge: Cambridge University Press.

Torre, I., Raspall, A., Arrizabalaga, A., & Díaz, M. (2018). SEMICE: An unbiased and powerful monitoring protocol for small mammals in the Mediterranean Region. *Mammalian Biology, 88*, 161–167. https://doi.org/10.1016/j.mambio.2017.10.009

Turón, J. (2012). *Huellas y señales de los micromamíferos ibéricos*. Ediciones Muskari.

Valerio, F., Ferreira, E., Godinho, S., Pita, R., Mira, A., Fernandes, N., & Santos, S. M. (2020). Predicting microhabitat suitability for an endangered small mammal using Sentinel-2 data. *Remote Sensing, 12*(3), 562. https://doi.org/10.3390/rs12030562

van Strien, A. J., Bekker, D. L., La Haye, M. J. J., & van der Meij, T. (2015). Trends in small mammals derived from owl pellet data using occupancy modelling. *Mammalian Biology, 80*(4), 340–346. https://doi.org/10.1016/j.mambio.2015.04.002

Viteri, M. C., Stegner, M. A., & Hadly, E. A. (2021). Assessing the reliability of raptor pellets in recording local small mammal diversity. *Quaternary Research, 106*, 1–10. https://doi.org/10.1017/qua.2021.59

Whittington, J., Heuer, K., Hebblewhite, M., & Lukacs, P. M. (2015). Estimating occupancy using spatially and temporally replicated snow surveys. *Animal Conservation, 18*, 92–101. https://doi.org/10.1111/acv.12140

Yalden, D. W., & Morris, P. A. (1990). The Analysis of Owl Pellets. Occasional publications no. 13. London: The Mammal Society In Mammal Society.

Zamora-Marín, J. M., Zamora-López, A., Calvo, J. F., & Oliva-Paterna, F. J. (2021). Comparing detectability patterns of bird species using multi-method occupancy modelling. *Scientific Reports, 11*(1), 2558. https://doi.org/10.1038/s41598-021-81605-w

Zemanova, M. A. (2020). Towards more compassionate wildlife research through the 3Rs principles: Moving from invasive to non-invasive methods. *Wildlife Biology, 2020*(1), 1–17. https://doi.org/10.2981/wlb.00607