Universidade de Évora - Escola de Ciências e Tecnologia

Mestrado em Biologia da Conservação

Dissertação

Comparação de métodos de amostragem não-invasiva para detetar pequenos mamíferos a largas escalas espaciais: O caso do rato de Cabrera (*Microtus cabrerae*) no sul de Portugal

Alexandra Quitoles de Oliveira

Orientador(es) | Ricardo Pita
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A dissertação foi objeto de apreciação e discussão pública pelo seguinte júri nomeado pelo Diretor da Escola de Ciências e Tecnologia:

Presidente	|	Paulo Sá-Sousa (Universidade de Évora)
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Comparação de métodos de amostragem não-invasiva para detetar pequenos mamíferos a largas escalas espaciais: O caso do rato de Cabrera (*Microtus cabrerae*) no sul de Portugal

Resumo

A monitorização da biodiversidade a largas escalas espaciais é fundamental para inferir as respostas das espécies às alterações globais. No entanto, a implementação deste tipo de estudos impõe importantes desafios relacionados com a amostragem e detetabilidade das espécies, sobretudo daquelas com reduzida dimensão e pouco abundantes. Neste trabalho pretendeu-se abordar esta questão usando o rato-de-cabrera no sul de Portugal. Com base em histórias de deteção da espécie em quadrículas 10×10km^2 amostradas por análise de regurgitações de coruja-das-torres e procura de indícios de presença, foram implementados modelos de ocupação para estimar a detetabilidade da espécie associada a cada um dos métodos. Os resultados indicam maior eficácia da amostragem de indícios, sugerindo que este método será uma boa alternativa para estudos focados apenas nesta espécie. Apesar de amplamente usada em estudos dirigidos a múltiplas espécies, a análise de regurgitações deverá ser complementada com outros métodos que permitam maior detetabilidade de determinadas espécies.

Palavras chave: Conservação; Métodos não-invasivos; Modelação; Monitorização; Roedores.

Comparison of non-invasive sampling methods to detect small mammals at large spatial scales: The case of the Cabrera's vole (*Microtus cabrerae*) in southern Portugal

Abstract

Monitoring biodiversity at large spatial scales is essential to infer species responses to global change. However, the implementation of such studies imposes important challenges related to the sampling and detectability of species, especially those that are small and not very abundant. In this work we address this issue using the Cabrera vole in southern Portugal. Based on detection histories of the species in $10\times10km^2$ grids sampled through barn owl pellet analysis and presence sign searches, occupancy models were implemented to estimate the detectability of the species associated with each of these sampling methods. Results indicated a greater efficiency of sign survey, suggesting that this method should be a suitable alternative for monitoring studies focused on this species only. On the other hand, despite being widely used in multi-species studies, owl pellet analysis should be complemented with other methods in order to increase the detectability of certain species.

Keywords: Conservation; Modeling; Monitoring; Non-invasive methods; Rodents.

Conteúdo

Capítulo 1. Introdução Geral

Atualmente a biodiversidade enfrenta grandes ameaças derivadas da ação antrópica, tais como a destruição, degradação e fragmentação dos habitats naturais, as alterações climáticas, e a expansão de espécies exóticas invasoras (ICNF, 2022). Esta perda da biodiversidade implica o desequilibro dos ecossistemas, influenciando as interações entre as espécies, sendo que a perda de uma espécie pode implicar alterações na composição de toda a comunidade (Banavar & Maritan, 2009). Assim, são necessárias medidas de conservação para contrariar o declínio das espécies, estando a aplicação e eficiência dessas medidas dependente da informação acerca da distribuição e abundância das espécies alvo. Para tal, é necessário documentar de forma consistente o conhecimento sobre as espécies e identificar as ameaças relacionadas.

A amostragem e monitorização da biodiversidade a larga escala são de grande importância para a compreensão da ecologia das espécies, incluindo aspectos como a distribuição, abundância, e riqueza específica (Araujo *et al.*, 2005). Os dados adquiridos em amostragens de larga escala fornecem informações essenciais para a produção de atlas que representam a ocorrência das espécies em quadrículas de áreas idênticas nos mapas (Araujo *et al.*, 2005). No entanto, este tipo de amostragem é sensível ao método utilizado, sugerindo que haja um esforço adequado a cada quadrícula, o que pode ser difícil quando há muitas quadrículas que necessitam de ser estudadas num curto espaço de tempo e com recursos limitados (Joseph & Possingham, 2008). Os dados obtidos em estudos a larga escala fornecem informações cruciais para as revisões das listas vermelhas de espécies, e a sua classificação em categorias ameaçadas, com base no risco de extinção que apresentam. Esta classificação é feita de acordo com os critérios da União Internacional para a Conservação da Natureza e dos Recursos Naturais (IUCN, do inglês *International Union for Conservation of Nature*), especialmente nos parâmetros avaliados pelo critério B1 (extensão de ocorrência) e B2 (área de ocupação), além de vários outros subcritérios (IUCN Standards and Petitions Committee, 2022). A monitorização da biodiversidade a larga escala também é importante para as espécies protegidas incluídas nas regulamentações ambientais, como a Diretiva Habitats da UE (Diretiva 92/43/EEC). Estas espécies protegidas agem como "guarda-chuva", sendo que a sua conservação protege/engloba outras espécies semelhantes na comunidade, tornando-se de maior interesse para a contínua monitorização e conservação da biodiversidade (Lisón *et al.*, 2015).

A monitorização de pequenos mamíferos a larga escala pode ser desafiante, levando a muitas questões em relação à precisão dos dados, uma vez que o uso de técnicas de amostragem, como por exemplo as armadilhas, requerem bastantes recursos (van Strien *et al.*, 2015). Estas limitações contribuem para erros na detetabilidade de espécies, levando a interpretações enviesadas sobre os padrões macroecológicos e variações ao longo do tempo (Lahoz-Monfort *et al.*, 2014). Assim, para estudos em larga escala, espécies com hábitos elusivos ou raras e que estão distribuídas de forma desigual devem ser estudadas por métodos expeditos, e sempre que possível não invasivos, que possam maximizar a detetabilidade de tais espécies e minimizar possíveis conflitos com o bem-estar animal (Gaines & McClenaghan, 1980; Zemanova, 2020). Deste modo, é imprescindível a criação, utilização e melhoramento de métodos de amostragem não invasivos que sejam eficazes e de baixo custo quando implementados a largas escalas espaciais.

Um exemplo de espécie com hábitos elusivos é o rato-de-cabrera (*Microtus cabrerae*), cuja captura é relativamente difícil, dificultando a realização de amostragens a larga escala. Por conseguinte, a maioria dos estudos focados em espécies como o rato-de-cabrera, têm sido baseados na análise de regurgitações de aves de rapina, como é o caso da coruja-das-torres (*Tyto alba*), e amostragem por indícios de presença específicos da espécie. Estes últimos incluem vestígios na superfície, como túneis na vegetação, pedaços de erva cortada e dejetos, que são característicos desta espécie (Pita *et al.*, 2014).

O rato-de-cabrera (*Microtus cabrerae* Thomas, 1906), o foco deste estudo, é um pequeno mamífero pertencente à Ordem Rodentia endémico da Península Ibérica (ICNB, n.d.; LVMPC, 2022; Mira *et al.*, 2008; Pita *et al.*, 2014; Valerio *et al.*, 2020). Segundo a IUCN está globalmente classificado como "Quase Ameaçado" (NT), no entanto é considerado ''Vulnerável'' em Portugal e Espanha, estando incluído nos

Anexos II e IV da Diretiva Habitats (92/43/CEE) e no Anexo II da Convenção de Berna (82/72/CEE) (ICNB, n.d.; Mira *et al.*, 2008; Pita *et al.*, 2014; Valerio *et al.*, 2020). Esta espécie tem uma distribuição bastante fragmentada sendo conhecida no centro e sul de Portugal, e centro e sudeste de Espanha (Mira *et al.*, 2008). Ocupa preferencialmente zonas de gramíneas perenes, prados, policulturas, montados de sobro e azinho, podendo também ser encontrado em áreas com um grau significativo de humidade no solo, como margens de ribeiras temporárias, juncais, ou zonas de mato junto a pequenos ribeiros com uma cobertura herbácea abundante e alta (ICNB, n.d.; Mira *et al.*, 2008; Valerio *et al.*, 2020). A destruição destes habitats devido às práticas agrícolas, pastoreio e remoção de cortiça, podem provocar mortalidade, dispersão forçada e causar a fragmentação da população (Valerio *et al.*, 2020).

Este trabalho está incluído no projeto "Inventariação de mamíferos de pequeno porte (Ordens *Eulipotyphla, Rodentia* e *Lagomorpha*) por análise de dieta de predadores e identificação de indícios de presença, no âmbito da revisão do Livro Vermelho dos Mamíferos de Portugal Continental e contributo para a avaliação do estado de conservação das espécies", cujo trabalho de campo decorreu entre 2019 e 2020 e focou-se no uso de dois métodos não-invasivos: análise de regurgitações de coruja-das-torres e amostragem de indícios de presença em transectos. Em ambos os métodos foram analisadas amostras recolhidas em quadrículas de 10×10 km^2, onde se registou a presença de *M. cabreare*.

As corujas oferecem uma metodologia importante e económica, uma vez que são caçadoras eficientes que conseguem capturar espécies de pequenos mamíferos difíceis de avistar ou capturar (Johnson & St George, 2020; van Strien *et al.*, 2015). A sua dieta é composta principalmente de pequenos roedores e musaranhos. Engolem as suas presas por inteiro, e posteriormente regurgitam em bolas compactadas as partes não digeridas, como ossos e pêlos, que se denominam de regurgitações ou egagrópilas (ver Fig. 1A e 1B) (Roulin, 2004). O método da análise de regurgitações de coruja-das-torres consiste em pesquisar poisos e/ou ninhos dessa espécie, frequentemente em edifícios abandonados e torres de igreja (ver Fig. 1C e 1D), recolher as regurgitações e posteriormente, em laboratório, separar os crânios e mandíbulas (ver Fig. 1E) para depois serem analisados e identificados com base em bibliografia e chaves dicotómicas específicas.

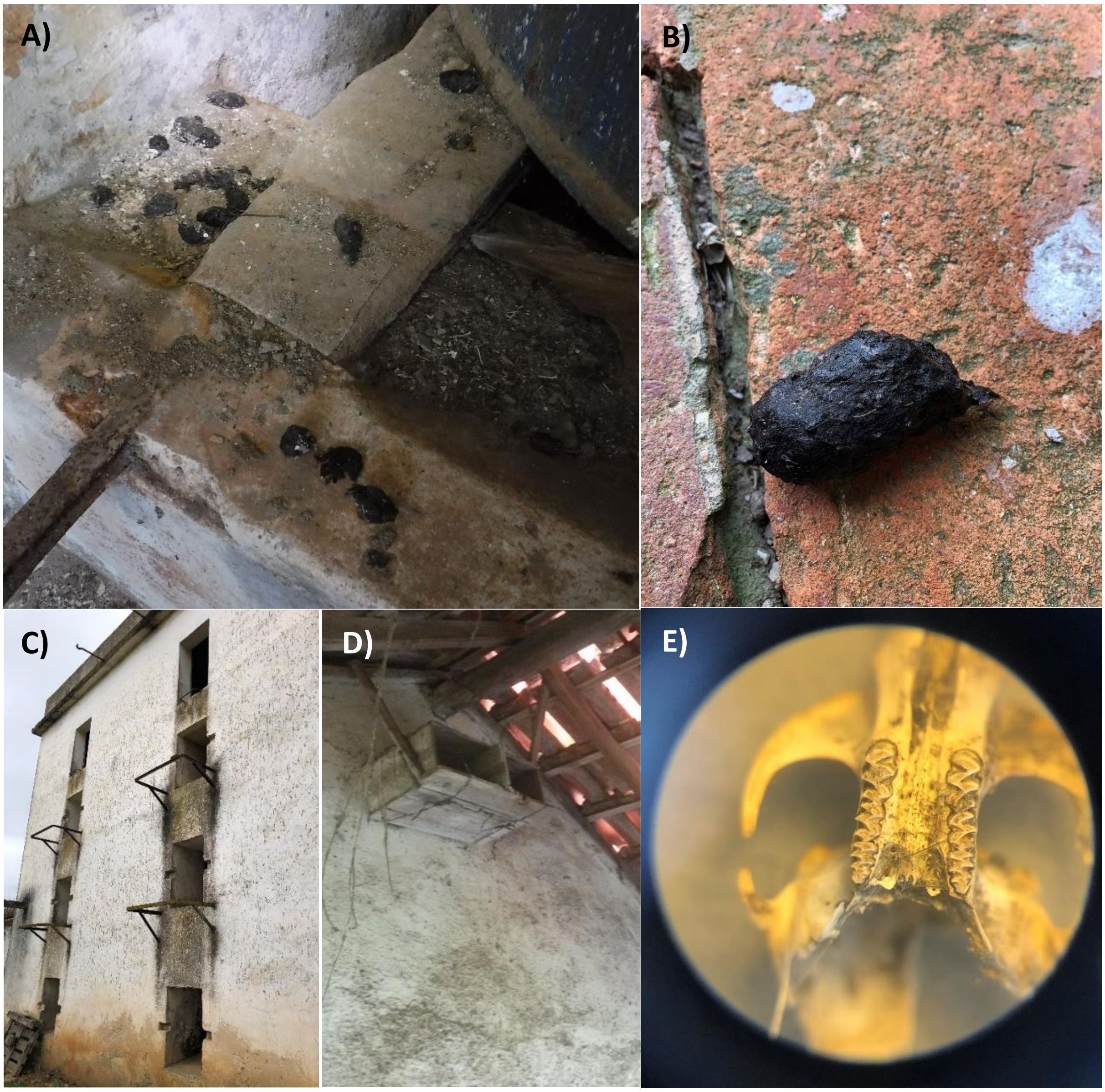

Figura 1 – (A) Exemplares de regurgitações de coruja-das-torres. Fotografia com autoria de Denis Medinas, 2021. (B) Exemplo de regurgitação de coruja-das-torres. Fotografia com autoria de Catarina Milhinhas, 2021. (C) Exemplo de um edifício abandonado presente na quadrícula NB36. Fotografia com autoria de Catarina Milhinhas, 2021. (D) Exemplo de um ninho de coruja-das-torres presente na quadrícula NC06. Fotografia com autoria de ICNF, 2021. (E) Exemplo de um crânio e molares de *M. cabrerae*, retirados de um regurgito de coruja-das-torres, visto através de uma Lupa. Fotografia com autoria de Catarina Milhinhas, 2021.

Por outro lado, a amostragem de indícios de presença em transectos consiste na utilização de trilhos estabelecidos em habitat favorável para a espécie para registo e contagem dos seus túneis e dejetos (ver Fig. 2A e 2B). As parcelas de habitat são selecionadas numa primeira fase com base na interpretação de imagem aérea e posteriormente com confirmação no campo. Este método oferece um tipo de amostragem confiável, especialmente se os indícios forem facilmente reconhecidos e não existirem espécies simpátricas com indícios semelhantes (Valerio *et al.*, 2020), o que não é um problema neste estudo uma vez que a área de estudo se situa no Sul de Portugal, onde os indícios para a identificação da presença de rato-de-cabrera não são confundíveis com os de outras espécies simpátricas, como acontece por exemplo no Nordeste de Portugal com o rato-de-campo-lusitano, *Microtus rozianus*.

Figura 2 – (A) Exemplo túneis na vegetação caracteristicos da espécie *M. cabrerae* presentes na NB36. Fotografia com autoria de Denis Medinas, 2021. (B) Exemplos de dejetos característicos da espécie. Fotografia com autoria de Dinora Peralta, 2017.

O objetivo principal da presente dissertação de mestrado é comparar a eficiência de dois métodos de amostragem não-invasivos na deteção do rato-de-cabrera em larga escala, nomeadamente, a análise de regurgitações de coruja-das-torres e a amostragem de indícios de presença registados em transectos.

Com base nos resultados obtidos discute-se o contributo de cada método para a avaliação da distribuição do rato-de-cabrera e a sua utilidade na elaboração de atlas, Livros Vermelhos, entre outros, bem como no delinear de planos de monitorização de tendências na distribuição da espécie a larga escala.

Capítulo 2. Large-scale grid-based detection and monitoring of an elusive small mammal: A comparison of two non-invasive sampling methods

Abstract

Monitoring biodiversity is key to assess the status and trends of wildlife, as well as for understanding its response to threats derived from human activities, and for evaluating and improving conservation planning and management. Large-scale, grid-based assessment of species distribution, abundance, and population trends over time, is an important part of these processes, though it still presents 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, a globally near-threatened small mammal, listed in the Habitats Directive protected species list, 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 km^2) surveys relying on two non-invasive sampling techniques: sign surveys and owl pellet analysis. Results provided evidence for a greater effectiveness of signs surveys compared to owl pellet analysis for detecting the species, suggesting that large-scale population monitoring of Cabrera voles (or other species also producing identifiable signs of their presence) may fairly rely on sign-surveys alone, with owl pallet analysis providing a less effective and more costly alternative. Overall, our study supported the view that while owl pellet analysis provides a valuable option if the aim is to assess multiple species or the whole small mammal community in a region, other complementary methods may be required to increase the detection probability of certain species that for some reason (*e.g.* rarity, size, habitat use) remain less predated by owls. We thus argue that choice of the sampling method should depend on the study aims, the study area, the target species and the available resources, and recommend that researchers and managers explore survey-designs trade-offs to ensure the proposed designs has sufficient power to detect real population trends.

2.1 *Introduction*

There has been an increasing concern over global biodiversity declines resulting from habitat destruction and fragmentation, climate change, and introduction of exotic species (Sala *et al.*, 2000). Despite the implementation of international conservation conventions and measures (Hill *et al.*, 2018), biodiversity continues to decline at unprecedented rates (Powers & Jetz, 2019). To counteract biodiversity loss, it is pivotal to implement regional to global-scale biodiversity assessments, based on methods that provide an accurate picture of species distribution, while also allowing to identify changes over time (Gaston & Blackburn, 2003).

Large-scale biodiversity sampling and monitoring have major significance for understanding macroecological patterns and their mechanistic drivers, including species distribution, abundance, richness, rarity, turnover, assemblage composition, and reserve selection (Araujo *et al.*, 2005). Notably, large-scale data on species occurrence provide the baseline information for producing atlases representing species occurrence in near-equal-area grid cells on maps (Araujo *et al.*, 2005). Such grid-based data also provide an important source of information for revising species red lists and allocating them to threatened categories, based on their risk of extinction, according to the IUCN criteria, particularly as regards to the parameters assessed under criteria B1 (extent of occurrence) and B2 (area of occupancy), as well as several other subcriteria (IUCN Standards and Petitions Committee, 2022). Large-scale, grid-based biodiversity monitoring is also a major commitment request as regards to those species that are protected by 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 Anexes II and IV. These species are often referred to act as effective umbrella species for other similar species in the community, being therefore of highest interest for biodiversity conservation monitoring (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 as regards to data accuracy (Marta *et al.*, 2019). Grid-based survey data are sensitive to the sampling method and best practice suggests that an adequate effort should be allocated to each cell, which may be difficult when too many grids are required to be surveyed in limited amount of time (Joseph & Possingham, 2008). Because most monitoring and conservation programs are underresourced, this places constraints in the target species, the kind of sampling methods, and the sampling effort used to detect target species (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 and for which confirming a presence or an absence may require labour intensive and time consuming field methods often involving invasive sampling of animals potentially affecting individuals and populations (Jeliazkov *et al.*, 2022). Overall, 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 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 conservation of other similar species, while regular assessment of its population status is legally required to accomplish international commintments. Specifically, we use multi-method occupancy models (MacKenzie *et al.*, 2002; Zamora-Marín *et al.*, 2021) to assess the detection probability of Cabrera voles at large spatial scales (10×10 km^2 grids) provided by two non-invasive sampling methods: owl pellet analysis and sign surveys. Owl pellets are referred to offer a powerful, cost-effective mean for sampling small mammals across broad scales, as owls are often considered habitat 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 (Pita *et al.*, 2007; Valerio *et al.*, 2020). Our specific aims, therefore, 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.

2.2 *Methods*

2.2.1 Study area and survey grids

The study was carried out in southern Portugal (provinces of Alentejo, Algarve, and part of Extremadura, Ribatejo and southern Beira interior), covering about 5600 km^2 (Fig. 3A). The region is included in the Meso-Mediterranean and Thermo-Mediterranean bioclimatic belts, characterised by a hot, dry Mediterranean climate (Alcoforado *et al.*, 2009; Asensi Marfil *et al.*, 2005; Rivas-Martínez *et al.*, 2002). The landscape is dominated by plains or low hills with elevation ranging from the sea level to 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.* Aguiar *et al.*, 2008). During the past decades there has been an increasing shift from traditional farming to intensive agriculture, which may have negative impacts on overall biodiversity (Auriault, 2012). Within the study region, 56 grids of $10\times10km^2$ were considered for the study (Fig. 3A). These grids are part of a larger set included in a nationwide research program designed to survey small mammal species (rodents and eulipotyphla) and to aid the revision of their category of threat and conservation status (project POSEUR-03-225-FC-000097). For this study, each selected grid was sampled for Cabrera voles between November 2019 and July 2020, as described below.

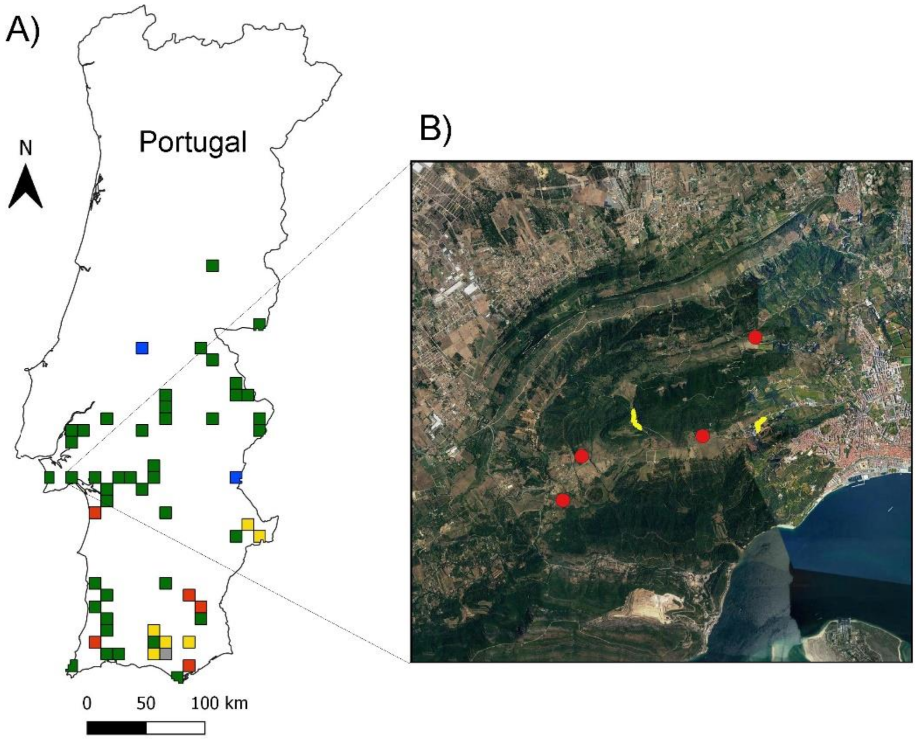

Figure 3 – (A) Study area and location of the 56 grids of 10×10 km^2 in southern Portugal, selected to sample Cabrera voles based on sign surveys and owl pellet analyses. Each color indicates the sampling method(s) employed (see text for details): Green – Sign surveys and owl pellet data totaling ≥ 100 small mammal prey items; Orange – Sign surveys and owl pellets with < 100 prey items; Yellow – Sign surveys only; Blue – Owl pellet data only (≥ 100 small mammal prey items identified); Grey – not sampled with either method. (B) Example of a 10×10 km^2 grid showing the location of two transects for surveying vole signs (yellow lines) and 4 barn owl nests where pellets were collected (red circles).

2.2.2 Vole sign sampling

In each selected grid, up to 3 transects were considered to sample Cabrera vole signs, with a mean ± SD of 1.75 ± 0.64 transects per grid. Transects had a mean ± SD length of 626.8 ± 82.9 (range 484-926 m) 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 separators (Luque-Larena & López, 2007; Pita *et al.*, 2007; Santos *et* *al.*, 2006). In 3 of the selected grids no sign transects were defined due to the difficulty of identifying suitable areas for voles. Therefore, a total of 53 grids were considered for vole sign sampling (see Fig. 3A). Along each established transect (*e.g.* Fig. 3B), we carefully searched for the typical 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, 2014; Luque-Larena & López, 2007; Pita *et al.*, 2007; Santos *et al.*, 2006). In southern Portugal there is no possible misidentification of these signs with those of other species, as signs left by the sympatric southern water vole (*Arvicola sapidus*) are much larger. On the other hand, the Portuguese field vole (*Microtus rozianus*), which may produce similar signs to those of Cabrera voles, is only found in the north of the country (Paupério *et al.*, 2012). All transects were thoroughly walked by trained observers from the Conservation Biology Lab of University of Évora (2 observers/transect), and each detection of Cabrera voles based on presence signs was recorded.

2.2.3 Owl pellet sampling

Barn owl (*Tyto alba*) nests were searched within each grid, by visiting abandoned farm houses that could potentially provide nesting or roosting sites for owls (van Strien *et al.*, 2015). These were firstly identified based on information previously provided by the national authority for nature conservation (ICNF, Instituto da Conservação da Natureza e Florestas), and on google earth imagery, followed by ground confirmation. In 7 of the selected grids, we could not find any active barn owl nest, so these grids were not surveyed with this method (see Fig. 3A). Fresh pellets detected in each site 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; Alcantara, 1998; Blanco, 1998a, 1998b; Moreno & Balbotín, 1998; Turón, 2012). 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 identify the species (see Cuenca-Bescós *et al.*, 2014). We considered only those grids for which a minimum of 100 small mammal prey items were available for the analyses (Fig. 3A), which often implied the inclusion of data from different nests within each grid (mean±SD = 1.63±1.32 nests per grid, range: 0-7) (*e.g.* Fig. 3B), and that some nests were revisited in an additional occasion within the study period. Otherwise, the grid 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). Overall, therefore, a total of 44 grids met these criteria and were considered in the analyses of pellet owl data (Fig. 3A).

2.2.4 Data Analysis

In order to assess the detection probability provided by each method, 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 10×10 km^2 grid). 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). As regards to the data from barn owl pellet analyses, we followed the 'half batches' approach for defining replicates (van Strien *et al.*, 2015), which consists in splitting the entire batch data 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 batch recorded as 1 and 0 respectively.

In model building, we considered only those grids for which at least to two replicates were available (either from sign sampling or owl pellet sampling), which totalled 47 grids included in the analysis (see Appendix, Table A1). Based on these data, we implemented two competing models for testing the hypothesis that the sampling method affects 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 ΔAICc<2 indicating equally supported models (Burnham & Anderson, 2002). We also assessed the probability that the model including sampling intensity as fixed effect was correct 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 bootstarpped 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. As regards to sign sampling method, we considered only those grids where at least two transects were conducted (*i.e.* 32 grids), implementing one model considering the effect of transect length on sign detectability and another without this effect. For the owl pallet 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 most supported model.

2.3 *Results*

Overall, we conducted a total of 82 transects summing up ca. 51.4 km walked across the 53 grids surveyed for Cabrera voles signs. From these, we detected the species in a total of 50 transects allocated to 32 grids (see Fig. 4). As regards to owl pellet sampling, we detected 107 Cabrera voles individuals among a total of 10435 individual prey items analysed from 94 nests located in a total of 49 grids, with 5 grids summing up less than 100 small mammal prey items (Fig. 3A). Overall, Cabrera vole skulls were detected 33 times in a total of 23 grids (see Fig. 4).

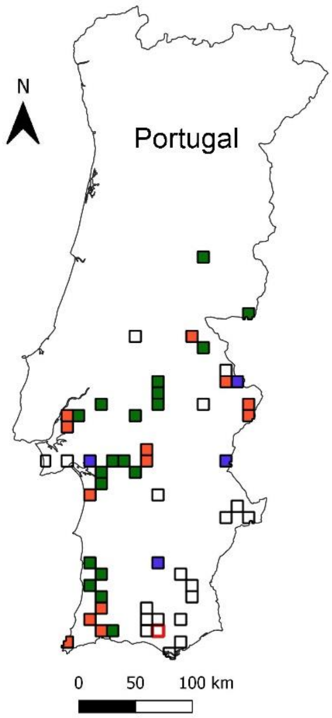

Figure 4 – Map showing the grids where Cabrera voles were detected from sign surveys only (orange grids), from owl pellet data alone (purple), and from both methods (green). White grids indicate those where no Cabrera vole skulls or presence signs were detected (black contours) or surveyed with either methods (red contours).

Occupancy modelling based on the data from the 47 grids with at least two replicate surveys (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 (more than 4 units lower) than the Null Model, being therefore the most supported one (Table 1). This is also shown by the weighted AICc scores of models, indicating that the Multi-method Model has a 92% probability of being the best model (Table 1). The GOF test indicated a c-hat = 1.21, 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 [0.58, 0.81] and 0.45 [0.33, 0.57] (Fig. 5), respectively. This model estimated a mean [95% confidence interval] occupancy of 0.83 [0.65, 0.93], which is above the naïve occupancy estimate of 0.76 [i.e. the number of grids where Cabrera voles were detected at least once independently of the sampling method (n=36, see Fig. 4), divided by total number of grids considered in the analysis (n=47)].

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 southern Portugal. Nr Param – number of model parameters; AICc – Akaike Information Criteria corrected for small sample sizes; ΔAICc – difference between models AICc. AICc-Wt – weighted AICc.

	Nr Param	***AICc***	***ΔAICc***	***AICc-Wt***
Null Model	2	231.99	4.78	0.08
Multi-method Model	4	227.22	0.00	0.92

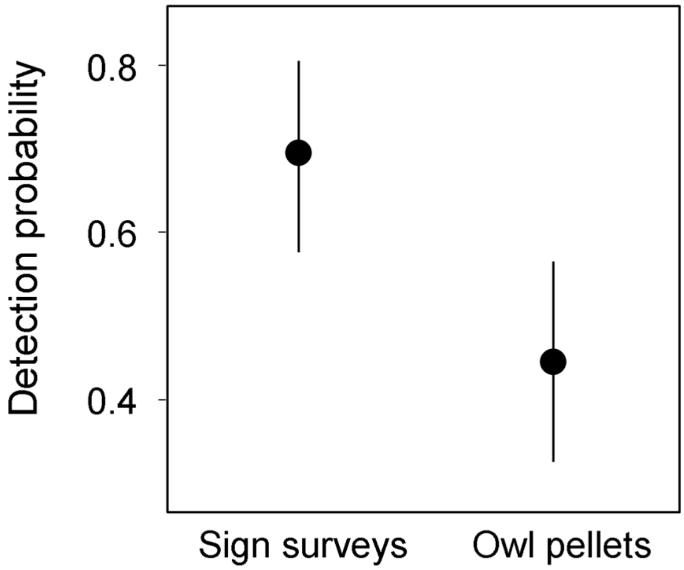

Figure 5 – Cabrera vole detection probabilities (mean and 95% confidence intervals) obtained from the multi-method occupancy model descriminating estimates for sign surveys and owl pellet analysis conducted in 10×10 km grids across southern Portugal between November 2019 and July 2020.

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 detailed results in Appendix, Tables A2 and A3), thus supporting our finding that sign surveys provide better detectability than owl pellet analysis.

2.4 *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 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 appropriate study designs aiming to maximise species detectability in monitoring programs, 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, 2014; 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 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.* Barão, 2022; Barbosa *et al.*, 2013; Mestre *et al.*, 2015), no further procedures are necessary for data completion. Conversely, owl pellet analyses require more personal and time demanding lab work for pellet dissection and species identification, which also require considerable expertise (Yalden & Morris, 1990). In addition, sign surveys are not constrained by any kind of restriction beyond the ability of identifying 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 given the barn owl might be declining in many regions (*e.g.* Martínez & Zuberogoitia, 2004), including in Portugal (Lourenço *et al.*, 2015). A further advantage of sign surveys over owl pellet analysis to monitor 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 complicated by the fact that 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 pallet analysis is undeniable when focusing or attempting to sample and monitoring the entire small mammal community in 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 increase the chances of detecting particular species that for some reason (*e.g.* rarity, size, habitat use) remain less predated by owls (van Strien *et al.*, 2015). 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) may be possibly 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). 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 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. Notably, our results also provide support for the importance of multi-method approaches in studies involving multiple small mammal species, so as to ensure target species are detected if present (Nichols *et al.*, 2008). This is particularly critical, given the growing demand for accurate data on species distribution patterns and dynamics, such as for instance, in atlases production, species extinction risk evaluations, red lists preparation, and assessment of species conservation status under international legislation (*e.g.* Habitats Directive).

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Capítulo 3. Conclusão

A amostragem e monitorização da biodiversidade a largas escalas espaciais é crucial na avaliação do estado das populações bem como na compreensão das tendências das espécies dentro da comunidade (Araujo *et al*., 2005; Lindenmayer *et al*., 2012). No entanto, a amostragem e monitorização de espécies a larga escala acarretam desafios importantes que muitas vezes levantam preocupações em relação à precisão dos dados, sobretudo quando se referem a espécies elusivas e de pequenas dimensões (Legge *et al*., 2018).

Assim, o foco deste trabalho consistiu em avaliar a detectabilidade do rato-de-cabrera, uma espécie pequena e elusiva, utilizando métodos de amostragem não invasiva, nomeadamente a análise de regurgitações de coruja-das-torres e a amostragem de indícios de presença, registados em transectos realizados nos habitats típicos de ocorrência da espécie. Desta forma, foram usados modelos de ocupação "multi-method" para comparar a eficácia destes dois métodos de amostragem e a sua capacidade de detectar a ocorrência do rato-de-cabrera a larga escala (quadrículas de 10×10 km^2 no centro e sul de Portugal). Foram consideradas 47 quadrículas para análise, construindo um modelo que inclui o efeito do método de amostragem nas estimativas de probabilidade de detecção (*i.e.,* modelo "multi-method"), e outro sem considerar esse efeito (*i.e.,* modelo nulo), utilizando modelos de ocupação baseados em históricos de detecção/não detecção da espécie (1/0).

O modelo "multi-method" foi o mais suportado, uma vez que o seu valor de AICc foi menor (em mais de 4 unidades) que o AICc do modelo nulo, o que reflecte a importância de considerar o efeito dos métodos de amostragem na estimativa da detectabilidade da espécie.

Para além disso, os resultados da análise referentes ao modelo "multi-method" sugerem que o método de amostragem de indícios de presença por transectos forneceu uma detectabilidade superior do que o método de amostragem de regurgitações de coruja-das-torres, tendo uma probabilidade de 70% em detectar corretamente a espécie em estudo, ao contrário do segundo método de amostragem de apenas 45%. Estes resultados permitem inferir acerca da influência que a escolha do método de amostragem tem sobre a precisão dos dados obtidos referentes à ocorrência das espécies (Zamora-Marín *et al.*, 2021). Deste modo, a detectibilidade das espécies incluídas nos programas de monitorização pode ser maximizada através da escolha mais adequada do método de amostragem, e consequentemente, ajudar a melhorar o planeamento de conservação da biodiversidade a largas escalas espaciais.

Apesar da análise de regurgitações de corujas ser bastante económica e eficaz na monitorização de pequenos mamíferos, até mesmo em caso de espécies raras e elusivas (Biedma *et al.*, 2019; Heisler *et al.*, 2015; Viteri *et al.*, 2021), a inexistência de estudos realizados à sua detecção imperfeita condiciona o conhecimento sobre as implicações em relação aos estudos que utilizam esta metodologia (van Strien *et al.*, 2015). Adicionalmente, este tipo de metodologia encontra-se sujeita à ocorrência de corujas-das-torres, por isso sensível ao seu declínio (Martínez & Zuberogoitia, 2004), e, extende-se para o trabalho em laboratório onde é necessário dissecar e identificar as espécies presentes (Yalden & Morris, 1990). O presente estudo indica que para espécies como o rato-de-cabrera, que produz sinais identificáveis, o uso da metodologia da análise de regurgitações pode não ser suficiente e, por isso, necessitar de amostragens complementares como os métodos baseados exclusivamente em indícios de presença que fornecem informações exactas de habitats e populações locais importantes para a sua conservação (Heisler *et al.*, 2015). Portanto, o método de amostragem por indícios de presença representa uma alternativa mais económica e eficaz, sendo no entanto de referir, que este método pode exigir algum nível de especialização, e em certos casos estar sujeito a confirmação por métodos de análise genética (*e.g.*, Mestre *et al.*, 2015).

Não obstante, a amostragem por análise de regurgitações de coruja-das-torres continua a ser um método válido, e útil, sobretudo quando o estudo se concentra em múltiplas espécies numa comunidade. Os resultados obtidos reforçam a utilização de múltiplos métodos de amostragem a fim de aumentar não só a detetabilidade de espécies predadas pelas corujas, mas também de espécies que, de alguma forma, são menos predadas, inviabilizando a precisão da análise de regurgitações de coruja-das-torres.

Em resumo, a amostragem por indícios de presença em transectos consiste numa melhor metodologia para amostrar, a larga escala, pequenos mamíferos em grandes áreas e com indícios facilmente identificáveis, como o caso da espécie *Microtus cabrerae*. Porém, este método requer um planeamento cuidadoso, sendo importante conhecer os habitats de ocorrência da espécie e integrar observadores que tenham experiência na identificação dos indícios. Além disso, este método é confiável quando a área de estudo não inclui espécies que produzam indícios semelhantes, e, na eventualidade de incluir, o método poderá ser utilizado apenas para alguns tipos de indícios (sobretudo excrementos, preferencialmente frescos), cuja identificação específica possa ser feita posteriormente recorrendo a análises genéticas.

É de referir que como perspetiva futura deverão ser realizados estudos adicionais, considerando diferentes métodos de amostragem, preferencialmente não-invasivos, para, não só comparar com a amostragem por indícios de presença, assim como a influência de diferentes fontes de variação, como por exemplo a sazonalidade.

A escolha da metodologia mais adequada dependerá sempre dos objetivos principais do estudo, espécies-alvo, área de estudo e recursos disponíveis. Assim, estas comparações e utilizações de vários métodos de amostragem ajudam a entender o quão importante são para garantir uma deteção mais precisa de espécies-alvo (Nichols *et al.*, 2008). Estes dados são especialmente críticos aquando a produção de atlas e avaliações do risco de extinção, bem como na avaliação do estado de conservação das espécies presentes nas listas vermelhas e que estão sob a legislação internacional (por exemplo, a Diretiva Habitats).

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Appendix

Table A1 – Resume of the data used to assess the effects of the sampling intensity on the probability of detection of Cabrera voles based on sign sampling at large spatial scales in southern Portugal.

Grid UTM	Sign surveys		Owl pellets			Total nº of Replicates ≥2 (0/1)	Detection of voles (0/1)		
	Surveyed (0/1)	Nº Replicates	Surveyed (0/1)	> 100 prey items (0/1)	Nº Replicates		Transects	Owl pellets	Combined
MC86	1	2	1	1	2	1	0	0	0
NA99	1	1	1	1	2	1	0	0	0
NB00	1	2	1	1	2	1	1	0	1
NB22	1	3	1	0	0	1	1		1
NB25	1	2	1	1	2	1	1	1	1
NB27	1	2	1	1	2	1	1	1	1
NB31	1	2	1	1	2	1	1	0	1
NB33	1	1	1	1	2	1	1	0	1
NB34	1	2	1	1	2	1	1	1	1
NB36	1	2	1	1	2	1	1	1	1
NB41	1	3	1	1	2	1	1	1	1
NB71	1	1	0	0	0	0			
NB72	1	1	1	1	2	1	0	0	0
NB73	1	1	0	0	0	0			
NB81	0	0	0	0	0	0			
NB82	1	1	0	0	0	0			
NB87	1	2	1	1	2	1	0	1	1
NC06	1	2	1	1	2	1	0	0	0
NC09	1	2	1	1	2	1	1	0	1
NC23	1	2	1	0	0	1	1		1
NC26	1	3	1	1	2	1	0	1	1
NC34	1	2	1	1	2	1	1	1	1
NC35	1	1	1	1	2	1	1	1	1
NC46	1	2	1	1	2	1	1	1	1
NC56	1	2	1	1	2	1	1	1	1
NC65	1	2	1	1	2	1	1	1	1
NC76	1	1	1	1	2	1	1	0	1
NC77	1	2	1	1	2	1	1	0	1
NC83	1	2	1	1	2	1	0	0	0
ND00	1	2	1	1	2	1	1	0	1
ND10	1	1	1	1	2	1	1	1	1
ND31	1	2	1	1	2	1	1	1	1
ND60	1	2	1	1	2	1	1	1	1
ND67	0	0	1	1	2	1		0	0

ND81	1	2	1	1	2	1	1	1	1
ND82	1	1	1	1	2	1	1	1	1
ND83	1	3	1	1	2	1	1	1	1
PB00	1	1	1	0	0	0			
PB02	1	1	1	0	0	0			
PB06	1	1	1	0	0	0			
PB14	1	1	1	1	2	1	0	0	0
PB15	0	0	1	0	0	0			
PC41	1	1	1	1	2	1	0	0	0
PC46	1	1	1	1	2	1	0	1	1
PC52	1	2	0	0	0	1	0		0
PC61	1	1	0	0	0	0			
PD17	1	2	1	1	2	1	1	0	1
PD21	1	1	1	1	2	1	0	0	0
PD26	1	2	1	1	2	1	1	1	1
PD43	1	2	1	1	2	1	1	0	1
PD44	1	1	1	1	2	1	0	0	0
PD53	1	1	1	1	2	1	0	1	1
PD60	1	2	1	1	2	1	1	0	1
PD61	1	1	1	1	2	1	1	0	1
PD69	1	2	1	1	2	1	1	1	1
PE24	1	2	1	1	2	1	1	1	1
	53	***89***	***50***	***44***	***88***	***47***	***32***	***23***	***36***

Table A2 – AICc-based comparison of occupancy models built to test the effect of the sampling intensity on the probability of detection of Cabrera voles based on sign sampling at large spatial scales in southern Portugal. Nr Param – number of model parameters; AICc – Akaike Information Criteria corrected for small sample sizes; ΔAICc – difference between models AICc. AICc-Wt – weighted AICc. C-hat of the best supported model (Null Model) was 0.63, indicating good model fit.

	Nr Param	***AICc***	***ΔAICc***	***AICc-Wt***
Null Model	2	109.39	0.00	0.97
Survey Effort Model	3	116.34	6.94	0.03

Table A3 – AICc-based comparison of occupancy models built to test the effect of the sampling intensity on the probability of detection of Cabrera voles based on owl pellet sampling at large spatial scales in southern Portugal. Nr Param – number of model parameters; AICc – Akaike Information Criteria corrected for small sample sizes; ΔAICc – difference between models AICc. AICc-Wt – weighted AICc. C-hat of the best supported model (Null Model) was 1.04, indicating good model fit.

	Nr Param	***AICc***	***ΔAICc***	***AICc-Wt***
Null Model	2	114.71	0.51	0.44
Survey Effort Model	3	114.21	0.00	0.56