Distance sampling as a tool for estimating active burrow density in the European ground squirrel (*Spermophilus citellus*): a case study from western Romania

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Abstract:
The European ground squirrel (*Spermophilus citellus*), an endangered small mammal endemic to Central and Southeastern Europe, has an unfavourable-inadequate conservation status in Romania. This study assessed the species density of active burrow openings within six areas of the Natura 2000 site ROSAC0108 Lunca Mureșului Inferior in Western Romania. Distance sampling line transect counts of active burrow openings were conducted for two consecutive seasons. Survey design resulted in interannual difference in effective strip width, encounter rate and detection probability. Higher densities were obtained using systematic parallel transects with complete spatial coverage of known colonies than using random line transects in potentially suitable habitat. This study provides insights into methods for estimating population density by using distance sampling approach to counting active burrow openings.

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Short title
Distance sampling of European ground squirrel density

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Abstract
The European ground squirrel (*Spermophilus citellus*), an endangered small mammal endemic to Central and Southeastern Europe, has an unfavourable-inadequate conservation status in Romania. This study assessed the species density of active burrow openings within six areas of the Natura 2000 site ROSAC0108 Lunca Mureşului Inferior in Western Romania. Distance sampling line transect counts of active burrow openings were conducted for two consecutive seasons. Survey design resulted in interannual difference in effective strip width, encounter rate and detection probability. Higher densities were obtained using systematic parallel transects with complete spatial coverage of known colonies than using random line transects in potentially suitable habitat. This study provides insights into methods for estimating population density by using distance sampling approach to counting active burrow openings.

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The European ground squirrel (hereafter as EGS; *Spermophilus citellus*) is listed as endangered on the IUCN Red List (Ćosić et al. 2024) and the Red Book of Vertebrates of Romania (Murariu 2005). It inhabits open short grass steppe, degraded fields with low herbaceous vegetation, earth dikes or dams, and alfalfa crops among cultivated landscapes (Kryštufek 1999, Kryštufek and Vohralik 2012, Ramos-Lara et al. 2014, Ružić 1978). It continues to face population declines despite legal protection (the Bern Convention and the Habitat Directive), and the conservation status remains unfavourable-inadequate, as indicated by two consecutive assessments under Article 17 of the Habitat Directive/Romania (E.E.A 2025). Assessing the population size is essential for prioritizing appropriate conservation actions (Rammou et al. 2021), although acquiring reliable estimates of wildlife population density through statistically robust sampling techniques remains one of the most significant challenges for field ecologists (Jacquier et al. 2021, Luikart et al. 2010, Thompson 2004). The species is suitable for population studies using indirect methods like counting active burrow openings (Katona et al. 2002), however, comprehensive and standardized studies in Romania remain limited (Hegyeli et al. 2012), in the absence of a standardised method for estimating EGS abundance across its range (Gedeon et al. 2022, Koshev 2008).
The main objective of this study was to implement a robust methodology for estimating the density of EGS active burrow openings by employing distance sampling techniques and using two different field sampling designs.

The study was conducted within the Natura 2000 site ROSAC0108 Lunca Mureşului Inferior (western Romania), along the left bank of the Mureș River (90 and 200 m a.s.l.). One of the protected species for which the site was designated is EGS. The site comprises a mosaic of grazed and ungrazed grasslands embedded within an agricultural–forest matrix. Grasslands, representing suitable EGS habitat, cover a total of 2,177 ha, most of which are currently used as pastures, predominantly grazed by sheep, with local signs of overgrazing and abandonment (MMAP, 2016).
Line transect surveys were conducted in August–September 2022 and June 2023. Each transect was surveyed once by a single observer, and all visible active burrow openings were recorded, measuring the perpendicular distance from the transect line to each detected opening, following standard distance sampling protocols (Buckland et al., 2001). An active burrow opening was identified based on fresh signs of activity, including freshly excavated soil, clear entrances, scrapes at the burrow entrance, fresh droppings and feeding signs (Rammou et al. 2021).

In 2022, surveys targeted known EGS colonies based on past surveys: Sânpetru German (SG), Felnac (FC), and Zădăreni (ZD) (Fig. 1). Within each location, systematic parallel line transects were established, spaced 20 metres apart, corresponding to twice the nominal survey width of the national methodology (Ionescu et al., 2013). The whole areas of the colonies were surveyed, without overlap between adjacent transect detection areas. Individual transect lengths varied depending on local topography, totalling 11.5 km.

In 2023, surveys aimed to estimate the density of active burrow openings across potentially suitable EGS habitat beyond known colonies. Potentially suitable habitat was defined as grazed and ungrazed grasslands characterized by low- to medium-height herbaceous vegetation, excluding crops and forested areas, wetlands, and built-up surfaces. Habitat for each location was delineated based on GPS field data and digitised in ArcGIS to map potentially suitable habitat areas, which were used to guide the placement and spatial extent of random transects. A stratified random sampling design was applied, resulting in 62 line transects with randomly determined starting points and orientations. Habitat surface areas were not used as input parameters in the distance sampling analyses, which were based solely on transect length and perpendicular distance data. Transect lengths varied between 120 and 500 m, covering a total surveyed length of 18.7 km. Fieldwork included broader areas of two of the previously surveyed locations (FC and ZD) and three new: Igriş (IG), Secusigiu (SC), and Munar (MN), where no prior information on EGS colonies was available. The SG location was excluded as all potential suitable habitat had been surveyed in 2022.

Perpendicular distances to active burrow openings were analysed by means of conventional distance sampling, using Distance 8.0 Release 1 (Thomas et al. 2010). Data were analysed separately for each location within each year, and subsequently pooled at the year level to estimate a single detection function and an annual density estimate. The maximum observed perpendicular distance to an active burrow opening was used as the integration limit for detection function fitting. Detection functions were constrained to be monotone non-increasing with distance, and density estimation was performed without truncation. Several candidate models were fitted, including half-normal and hazard-rate key functions with various adjustment terms. Model selection was based on Akaike's information criterion (AIC) and the corrected Akaike Information Criterion (AICc), with the model obtaining the lowest score selected as optimal (Burnham and Anderson 2004). The effective strip width (ESW) for each year was derived from the fitted detection function. Density was calculated by dividing the number of detected active burrow openings by the total effective surveyed area (twice the ESW multiplied by the total transect length), thereby accounting for imperfect detection with distance. The total area covered by transects for each location and year was calculated as twice the maximum observed perpendicular distance from transect to active burrow entrances.

In both years, a half-normal key function with cosine adjustment was selected.

In 2022 a total of 437 active burrow openings were recorded, corresponding to an encounter rate of 37.4/km. The estimated detection probability was 0.54129 (%CV = 8.07; df = 434.00; 95% CI = 0.46199 - 0.63420). The maximum observed perpendicular distance to active burrow entrances was 10 m. The total area surveyed by transects was 23.38 ha. The effective strip width (ESW) was 5.4 m (CV = 8.1%, 95% CI = 4.6–6.3 m). The density of active burrow openings was 34.53 per hectare (SE = 3.44, %CV = 9.95).

The 2023 survey yielded 162 active burrow openings observations, with an encounter rate of 8.67/km. The detection probability was estimated at 0.24527 (%CV = 12.95%; df = 158; 95% CI = 0.19011-0.31645) and 29 transects registered no observations. The maximum observed perpendicular distance to active burrow entrances was 7.5 m and the total area surveyed was 28.03 ha. The effective strip width (ESW) was 1.8 m (CV = 13.0%, 95% CI = 1.4–2.3 m). The density of active burrow openings was 23.55 per hectare (SE = 5.45, %CV = 23.14).

Density data for each location and year are presented in Table 1.

The effective strip width (ESW) estimated in 2023 was substantially lower than in 2022, indicating a steeper decline in detectability with increasing distance from transect lines. This difference may reflect surveys being conducted earlier in the vegetation season in 2023, when herbaceous cover is generally denser, potentially reducing visibility of burrow entrances at greater distances relative to late summer conditions in 2022. However, vegetation structure was not quantified directly, and this interpretation should be regarded as contextual rather than causal.
Interannual differences in encounter rate and detection probability further reflects differences in sampling strategies. In 2022, surveys intentionally focused within known colonies, where burrow entrances are spatially clustered (Gedeon et al. 2022, Kachamakova et al. 2022), resulting in high encounter rates. In contrast, the 2023 surveys employed random transects distributed across a broader landscape, including areas with low or no burrow presence, which led to reduced encounter rates and a higher proportion of transects without detections. These design differences are reflected in lower detection probability estimated for 2023.
Density estimates mirrored these methodological contrasts. The systematic, colony-focused design used in 2022 yielded more precise density estimates, but it primarily characterizes conditions within occupied colonies. In contrast, the random transect design applied in 2023 provides density estimates that are more representative of the broader habitat matrix, albeit with increased variability. The higher coefficient of variation observed in 2023 indicates greater uncertainty, consistent with the inclusion of sparsely occupied habitat and a lower number of detections contributing to detection-function estimation. When analysed separately by location and year, observations were often too few, resulting in wide confidence intervals and imprecise density estimates. These results are reported for completeness but should be interpreted cautiously due to low sample sizes in some cases. To enable meaningful interpretation and improve data reliability in future surveys, it is recommended to increase survey effort.
Distance sampling offers a robust statistical framework for estimating densities of EGS active burrow openings by explicitly modelling detectability as a function of distance (Buckland et al. 2001). Empirical studies have shown that counts of active burrow entrances are positively correlated with individual abundance in this species (Katona et al. 2002; Stoeva et al. 2016; Gedeon et al. 2017), supporting the use of burrow-based distance sampling as a reliable approach for population assessment.

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Table 1. Location and year-specific density estimate of active burrow openings derived from conventional distance sampling and the mapped suitable habitat area (SE - Standard error; %CV - Coefficient of variation; 95% CI - Confidence interval)

Year	Location	Density (active burrow openings/ha)	SE	%CV	95% CI	Suitable habitat area (ha)
2022	Sânpetru German (SG)	34.184	4.5697	13.37	26.283 - 44.462	
	Felnac (FC)	32.208	3.5814	11.12	25.795 - 40.214	
	Zădăreni (ZD)	27.927	6.4622	23.14	17.574 - 44.378	
2023	Igriş (IG)	31.827	18.319	57.69	9.6905 -104.53	52
	Secusigiu (SC)	43.591	16.498	37.35	19.834 - 95.805	229
	Munar (MN)	*Not estimated, not enough observation data*				256
	Felnac (FC)	19.966	5.8869	29.48	11.192 - 35.620	267
	Zădăreni (ZD)	12.916	5.9697	46.22	4.8728 - 34.236	60.2

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Figure 1. Location of the six areas where density data of EGS were collected within the Natura 2000 site ROSAC0108 Lunca Mureșului Inferior: IG - Igriş, SC - Secusigiu, MN - Munar, SG - Sânpetru German, FC - Felnac, ZD - Zădăreni, shown in relation to its geographic distribution according to the IUCN Red List.

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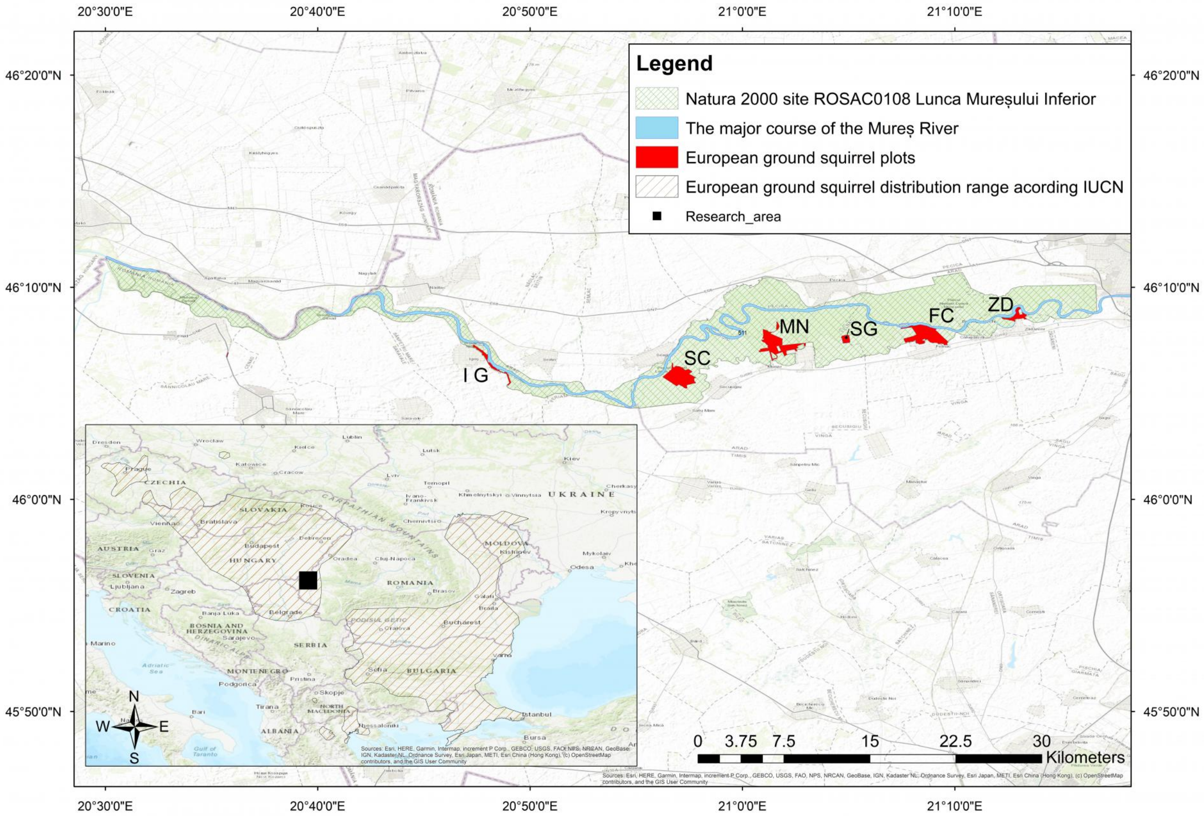

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