

# The effect of habitat reduction by roads on space use and movement patterns of an endangered species, the Cabrera vole *Microtus cabreræ*

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## Abstract

Roads are among the most widespread signs of man's presence around the globe. From simple low traffic trails to wide and highly used highways, roads have a wide array of effects on wildlife. In the present study, we tested how habitat reduction by roads may affect the space use and movement patterns of the Cabrera vole (*Microtus cabreræ*), a near-threatened Iberian endemism, often living on road verges. A total of 16 voles were successfully radio-tracked in two habitat patches with different size and proximity to roads. Results showed that individuals from the smaller patch (Verge patch) had smaller and less complex home-ranges than those from the larger patch (Meadow patch). Movement patterns were significantly influenced by the day period but only in individuals from the Verge patch. There was evidence of a barrier effect in both habitat patches, being this effect much more noticeable in the verge population. Overall, this study shows that space use and movement patterns of Cabrera voles near roads may be affected by the degree of habitat reduction imposed by these infrastructures. This suggests that species space use and movement patterns at fine-scale should be accounted for in road planning, even for species that may benefit from road verge habitats as refuges.

## Keywords

Barrier effect, Cabrera vole, fragmentation, road ecology, small mammals

## Introduction

Roads are a widespread sign of human presence across the globe, imposing several contrasting effects on wildlife species, from positive to negative (Forman 2000). Positive effects of roads include increased availability of foraging habitat and food supplies, low predation pressure, hunting areas for avian predators, or even attractive microclimate conditions of road surface (Morelli et al. 2014). Road verges are frequently the only remaining favourable habitat for many species in human-modified landscapes, providing refuge, habitat, or dispersal corridors (Way 1977; Porto-Peter et al. 2013; Redon et al. 2015). This is probably the case for many small prey species that find in road verge habitats protection from predators, which have been shown to be negatively affected by roads (Fahrig and Rytwinski 2009). This is supported by several studies showing a higher abundance of small mammals in road verges when compared with the surrounding habitats (e.g. Adams and Geis 1983; Sabino-Marques and Mira 2011; Porto-Peter et al. 2013; Redon et al. 2015).

The negative effects of roads are however more frequent than positive effects, being mainly related to direct mortality, habitat fragmentation, disturbance, and chemical pollution (Forman and Alexander 1998; Trombulak and Frissell 2000; Seiler 2001). Roads also act as barriers to movement for many species, thereby decreasing their access to mates, water, food or other resources (Trombulak and Frissell 2000; Brown et al. 2006), with both genetic and demographic costs to populations, increasing local extinction risk (Shepard et al. 2008). For instance, it has been shown that road proximity can have negative impacts on mammal species abundance or activity (Kozel and Fleharty 1979; Garland and Bradley 1984; Clark et al. 2001), this effect decreasing with the distance to the road (Benítez-López et al. 2010; Medinas et al. 2019). Roads have been also shown to decrease edge permeability for some small mammal species such as the montane akodont *Akodon montensis* (Ascensão et al. 2017). Habitat fragmentation caused by road development might therefore result in high risk of extinction (Crooks et al. 2017), due to associated habitat loss and increased patch isolation (Bennett 2003), reducing the chances of local (re)colonization (McGregor et al. 2008). Besides, in addition to the reduction in animal populations, species movement behaviour may be impacted near roads (Coffin 2007).

Although the negative effects of roads on wildlife are well-documented for many species (Forman et al. 2003; Shepard et al. 2008), few studies have focused on the behavioural consequences of roads to individual animals or their populations (see Shepard et al. 2008). Understanding behavioural responses of animals to roads provides insights into the causes and mechanisms of the effects of linear infrastructures on wildlife, allowing more informed mitigation and conservation planning (Roedenbeck et al. 2007). Existing evidence suggests that responses vary considerably across species (Goosem 2001; Bissonette and Rosa 2009; Rytwinski and Fahrig 2012; Porto-Peter et al. 2013; Grilo et al. 2018) and depending on the landscape context. Galantinho et al. (2017) found that in *montado* systems, wood mouse (*Apodemus sylvaticus*) populations living near roads have a lower fitness than those living far from roads. Moreover, small mammals with

high site fidelity and slow movements are more susceptible to the negative effects of roads (Coffin 2007). This was documented by Rico et al. (2007) that observed a lower crossing rate in less mobile rodent species. Even dirt roads may confine individual home ranges and inhibit their movements, as shown for the Abert's squirrel (*Sciurus aberti*) (Chen and Koprowski 2016). In general, terrestrial species with small and fragmented populations, and specific habitat or environmental requirements should be particularly vulnerable to impacts of road barrier effects, though compelling evidence supporting this idea is still scarce (Goosem 2001; McDonald and St Clair 2004).

The Cabrera vole (*Microtus cabreræ* Thomas, 1906) is an Iberian endemism with a patchy distribution across all its range. It is considered "Vulnerable" both in Portugal (Queiroz et al. 2005) and in Spain (Fernández-Salvador 2007) and shows a spatial pattern consistent with a metapopulation structure, with frequent local extinctions and colonizations (Pita et al. 2014), and with home ranges typically < 1000 m<sup>2</sup> (Fernández-Salvador et al. 2001; Pita et al. 2010, 2014). In highly modified landscapes, the specific habitats selected by the species (tall and dense wet herbaceous patches) are often restricted to marginal areas, including along road verges (Fernández-Salvador 1998; Pita et al. 2006, 2007; Santos et al. 2007). Despite the exposition to traffic noise and increased mortality risk by roadkill (Santos et al. 2007; Valerio et al. 2020), road verge habitats may still provide important resources for species living on them. However, the behavioral consequences of roads to the species are still largely unknown, even though these may impact local population viability.

In the present study we evaluated how living in road verges influences space use and movement patterns of Cabrera voles in southern Portugal. Specifically, we assessed whether space use of Cabrera voles may change when occupying road verge patches that are spatially limited and linearly shaped, with individuals exhibiting less complex home range boundaries (Ford 1983; Hiller et al. 2016) or increasing intrasexual overlap (Madison 1980; Ims et al. 1992; Collins and Barrett 1997). We also assessed whether home ranges in road verges are smaller and more linear, as predicted for other small mammals (Stumpf and Mohr 1962), due to the higher availability of food and shelter, potentially attracting a high number of individuals compared to the surrounding matrix habitats. In addition, we assessed whether movement paths are shorter and more linear when compared with those of more extensive habitat patches (Maclagan et al. 2019). Furthermore, because vehicles pass closer to animals in road verge habitats, we assessed whether individuals may adjust their movement periods to avoid higher traffic hours as observed for other mammals (Chen and Koprowski 2016; Kušta et al. 2017). Finally, we assessed whether voles living in road verges might cross the road more often due to the recolonization dynamics of metapopulations (Pita et al. 2007) and to increased intraspecific competition typical of small habitat patches (Ims et al. 1992). In order to test these predictions, we derived the following hypotheses: i) individuals occupying road verges have smaller home ranges with lower shape complexity, smaller and lower number of core areas and/or higher intrasexual spatial overlap; ii) have shorter movement paths, iii) have more linear movement paths, iv) make shorter movements during high traffic periods, and v) cross the road more frequently than those living in larger habitats.

Overall, we expect our study will contribute for a better understanding of the behavioral consequences of roads to small mammals, which should be critical for species management planning and road impacts mitigation.

## Materials and methods

### Study area

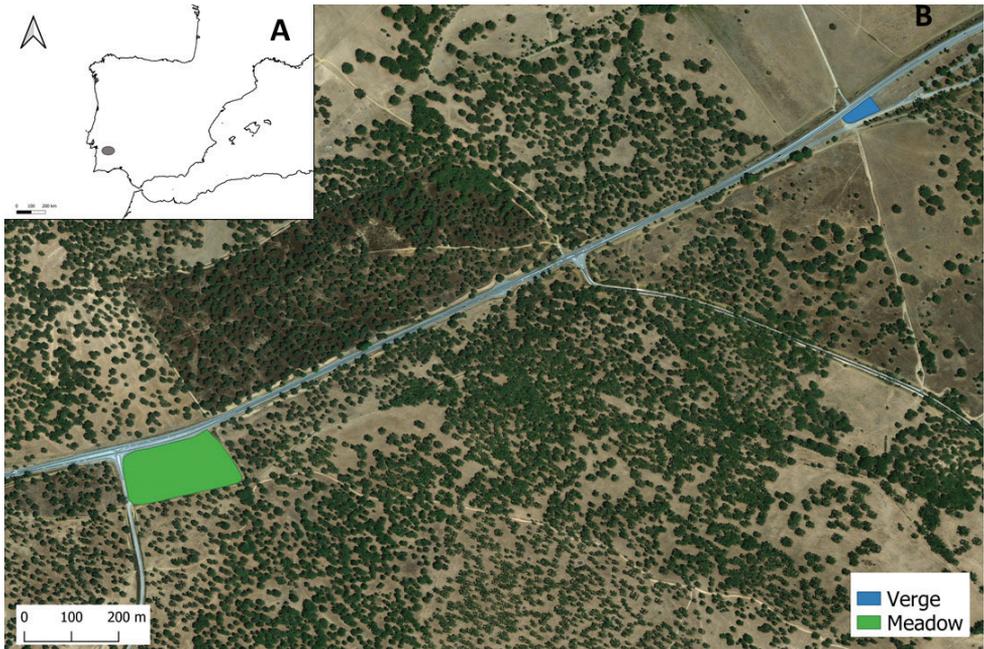
The present study took place in Alentejo, southern Portugal (38°41'42"N, 08°04'46"W; Figure 1A). The climatological normal mean (1981–2010) varied between 14.3 °C and 21.4 °C for the study area (IPMA 2018). The landscape is mainly characterized by the agroforestry system commonly known as “montado”. It is characterized by an open tree layer with Cork oak (*Quercus suber*) and/or Holm oak (*Quercus rotundifolia*), with sclerophyll shrubs and annual grasses (Pinto Correia et al. 2011). There is one main road in the study area (N4 national road) with an annual traffic of 3882 motorized vehicles (3424 during daytime and 458 during night-time) and connecting Lisbon to Spain (EP 2005) (Figure 1B).

### Study design

We used radio-telemetry data from individuals captured in two habitat patches with different size and road proximity (Meadow and Verge; Figure 1B). The patches were identified after previous searches for species presence signs in areas near the main roads of the study region (October 2016 to February 2017). The two patches presented abundant and conspicuous pathways among grasses, latrines of dark-green droppings and the fresh cut grasses, typical and undoubtedly recognizable as Cabrera voles presence signs (Pita et al. 2006). Both patches were located along the same N4 road and separated by 1.5 km from each other (Figure 1B).

The Meadow patch (38°41'30.76"N, 08°05'14.33"W) is a large patch (24 589 m<sup>2</sup>) with high habitat availability for the species. Dominant vegetation here is sedge/rush and tall perennial grass communities with isolated cork trees in the periphery. The Meadow patch is also crossed by a very small stream, only flooding after abundant rainfall. This area is separated from the road by a fence and a fire break. This patch is flanked by N4 road at North side and by a smaller dead-end road at the West side, with very low traffic. Vole presence signs suggested high local population abundance and were all located outside the road verge habitat (SM Santos, pers. observ.).

The Verge patch (38°41'54.94"N, 08°04'14.26"W) is a small patch (2 021 m<sup>2</sup>) spatially constrained between two paved roads, N4 at North, and a smaller and less used road at South providing access to private property. The dominant vegetation in this patch is mainly annual grass communities with isolated shrubs (*Cytisus* spp., *Genista* spp.), typical of road verge communities (Santos et al. 2007). This patch is closer to the road, without a physical separation, such as a fire break or a fence. This means that the Verge patch is adjacent to the road pavement.



**Figure 1.** Panel **A** study area location within the Iberian Peninsula; Panel **B** selected habitat patches along the N4 road for the study of space use and movement patterns of Cabrera voles in southern Portugal (green- Meadow patch; blue – Verge patch).

Both patches are bordered by the same road, but the habitat is much more reduced at the Verge patch when compared to Meadow patch. While the centroid of Verge patch is 14 m from the nearest road, the centroid of the Meadow patch is 60 m away. Therefore, the road effects are expected to be much more evident in the Verge patch. Given the mean home ranges of 300–400 m<sup>2</sup> for Cabrera voles in Mediterranean areas (Pita et al. 2014), we assume that the Meadow patch is a control area for assessing the effects of roads on Cabrera voles living in the road verges.

### Capture and radio-telemetry

Voles were captured with Sherman live traps (7×23×9 cm) laid in clusters where the species signs were more concentrated and fresher. Apple and carrot were used as bait, and hydrophobic cotton and grass were provided as bedding (Pita et al. 2011). A total of 14 trapping sessions were conducted (April to June 2017). The sampling period corresponded to the end of the wet season, which is when reproduction should be higher (Pita et al. 2014). The traps were set in the morning at 7:00 a.m. and disabled at 1:00 p.m. to avoid prolonged time of animals inside traps. The average trapping effort was 58 traps per day. Animals from other species were released immediately at the site of capture with no further manipulation or intervention.

All Cabrera voles captured were weighed and sex determined in the field to immediately exclude animals with low weight, and pregnant or lactating females, to avoid any negative impacts on local populations. Voles with conditions to be radio-collared (good physical condition and body weight > 36g) were sedated with a subcutaneous injection of Dormitor (0.5 mg/kg) combined with Clorketam (40 mg/kg) to reduce handling stress during collar fitting, following all animal welfare conditions for animals used in research. During sedation, the reproductive status was confirmed based on the presence of descendent testes or perforated vulva and nipple development. Radio transmitters (SOM-2018; Wildlife Materials, Inc., Murphysboro, IL, USA) were attached with collars to voles. The transmitters weighed 2.0 g and represented an average 4.2% (range: 3.1–5.3%) of voles' body mass (range: 38 – 65 g) in order to ensure that additional energetic costs were kept to a minimum (Sikes et al. 2011). Voles were additionally fitted with PIT tags to easily identify them in case of future recaptures. Voles were then induced out of sedation with Antisedam (0.2 mg/kg). Before release in the field, collared animals were kept a few hours for observation, ensuring that they were wide awake during their release. Animals were released close to their place of capture and radio tracking begun at least 4 h after their release (adapted from Pita et al. 2011).

Eighteen voles were fitted with collar radio-transmitters: 9 voles in Meadow patch (7 females; 2 males) and 9 in Verge patch (4 females; 5 males). All voles tracked were non-juveniles (> 28g), as recommended elsewhere (Fernández-Salvador et al. 2005; Pita et al. 2010).

From 7<sup>th</sup> April to 14<sup>th</sup> June 2017 the collared voles were tracked on foot using the “homing-in” method (White and Garrott 1990) and by multiple triangulations when the observer was close to the animals, with a hand-held 2-element Yagi antenna and a SIKA radio receiver (Biotrack, United Kingdom).

Due to the short battery life, it was decided to use a clustered sampling scheme, with discontinuous tracking at 15 min intervals, to access space use and movement patterns (Pita et al. 2010; Santos et al. 2010). Hence tracking was done in six 4-h sessions, comprising 16 position fixes each and separated at least 4h from the next session in order to sample the entire 24h cycle (05–09h, 09–13h, 13–17h, 17–21h, 21–01h, 01–05h). The nocturnal session (01–05h) was sampled only once per animal as Cabrera voles are more active during the daytime (Fernández-Salvador et al. 2005; Pita et al. 2011). This allowed to optimize sampling to the periods of higher activity. Voles were seen on several occasions during tracking, and appeared little affected by the presence of the observer. At each position fix, a coordinate was recorded using a Garmin eTrex handheld GPS. Mean fix error was 1.2 m ( $n = 35$ ; 0.2 – 3.1m).

Whenever possible, tracking was carried out until at least a minimum of two session replicates were reached for each individual (excepting the nocturnal session), corresponding to 176 location fixes. At the end of field work a new trapping session took place to remove the collars from tracked animals, though this was only possible for a few of them ( $n = 4$ ) due to the low recapture rates.

## Data analysis

### Response variables

To assess differences in animals' space use between habitat patches, the individual home ranges, shape complexity index, extension and number of core areas, and the female spatial overlap were estimated. Movement patterns were assessed through path length and linearity, and road crossing rates.

Individual home ranges were estimated using biased random bridge kernel (BRBK) at 95% (where animals spend 95% of their time) and 50% utilization distribution contour (core areas). The BRBK estimator is based on the biased random walk model and deals with serial autocorrelation of the fixes (Millspaugh et al. 2006; Benhamou 2011). Movement step distances of less than the average location error (1.2 m) were assumed as non-movement ( $L_{min}$ ). The maximum step duration for defining successive relocations was defined as 4h ( $T_{max}$ ) and the minimum smoothing parameter was set to 1.2 in all animals ( $h_{min}$ ). The contours of utilization distribution (UD) were adjusted to the road limit whenever necessary. All BRBK estimates were based on more than 140 location fixes.

The shape complexity index ( $C$ ) was calculated for each animal to infer differences in resource use between patches as  $C = L / (2 \cdot \sqrt{A \cdot \pi})$ , where  $L$  is the UD contour perimeter length (m) and  $A$  is the area ( $m^2$ ) of contour UD. A perfectly circular contour has  $C = 1$  (Hiller et al. 2016).

Differences in the degree of spatial interactions were examined calculating home range overlap between females for 95% BRBK (Frère et al. 2010). The utilization distribution overlap index (UDOI) was used to measure space-use sharing between two females (Fieberg and Kochanny 2005). The UDOI ranges from 0 when two home ranges do not overlap and equals 1 if both home ranges are uniformly distributed and have 100% overlap (Fieberg and Kochanny 2005).

To assess differences in movement patterns between individuals from the two habitat patches, two responses were calculated from radio-telemetry data: path length and path linearity index.

In the present study, a step is assumed as the movement measured in 15 min, and the path is the group of 16 steps measured during a period of 4 h (15 min  $\times$  16). Before these calculations, telemetry data was converted into a time-regular trajectory data from which standard parameters were extracted for each telemetry session: step length, step absolute angle and step relative angle (i.e., turning angle) (Calenge et al. 2009). Step lengths lower than 3 m (maximum fix error) were corrected to zero (along with the respective absolute and relative angles) and classified as no movement.

The path length expresses how active an individual was in each session, and it allows to monitor the periods of activity and behavioural patterns (e.g. nocturnal species will have higher path lengths during the night) (Edelhoff et al. 2016).

The linearity index was calculated for each observed path as the net displacement distance (the Euclidean distance between the start and the final point of a path), di-

vided by the total length of the path (Almeida et al. 2010). This index varies from 0 to 1 and quantifies the searching efficiency of the animal while adjusting its path to the most profitable route in terms of resource acquisition (Benhamou 2004). Linearity indices closer to 1 are indicative of higher search efficiency (Almeida et al. 2010).

In the present study it was assumed that all movements were routine daily movements as the individuals were adults and never abandoned their home range.

## Explanatory variables

For each individual the sex and patch where the tracking took place were registered. For each position fix recorded in the field, we also registered the time at which the fix was taken, together with several variables describing microhabitat composition and structure (Suppl. material 1: Table S1). A detailed digital elevation model (pixel:  $1 \times 1 \text{ m}^2$ ) was built for the two habitat patches based on a detailed topographic field measurement (CL Topografia, Lda) from which elevation was extracted for each position fix. Because each patch is at a different elevation, we calculated the difference between the elevation in each fix and the lowest elevation in the respective patch. Regional meteorological conditions at each hour (air temperature, relative humidity and amount of rainfall) were obtained from Centro de Geofísica de Évora (University of Évora; Mitra station) and later added to the dataset.

A total of 23 explanatory variables were initially considered for movement pattern analyses: 9 in the step dataset and 17 in the path dataset. The explanatory variables of path dataset are a summary (sum, average, median or mode) of steps variables comprising each path (Suppl. material 1: Table S1).

## Statistical analyses

All defined response variables were screened for their distribution and the need of transformations. Path length, BRBK (95% and 50%), and Number of core areas were log transformed. For the movement pattern analyses, the paths and steps with zero length were discarded.

The area of individual home ranges (95% BRBK), core areas (50%BRBK), the number of core areas (No BRBK50), the shape complexity index, and female overlap index (UDOI) were compared between the two habitat patches with a Wilcoxon rank-sum test ( $W$ ) to assess differences between patches in space use parameters (Sokal and Rohlf 1997). Because there were no effects of sex on home range and core area sizes, neither on the number of core areas and shape complexity (Suppl. material 1: Table S2), sexes were combined in space use analyses.

To assess the influence of explanatory variables (including the habitat patch and day period) in movement patterns, Linear Mixed Models (LMM) were applied to path length and path linearity index (Zuur and Ieno 2016). The two response variables were modelled as a function of explanatory variables, with individual voles as a random intercept to deal with pseudo-replication arising from repeated measures made on the

same individual (Zuur and Ieno 2016). Model selection was based on Akaike's Information Criterion (AIC; Burnham and Anderson 2002).

Before model building, the collinearity among explanatory variables was verified. Thus, for variable pairs showing high collinearity (Pearson correlation:  $r > 0.7$ ), only the one with strongest correlation with response variables was retained for further analysis. To reduce the number of competing candidate models and avoid spurious effects, each non-collinear explanatory variable was individually tested against the response variable with a Generalised Linear Model (GLM) and this model AIC compared with the respective Null model (a GLM with only the intercept). Explanatory variables that produced models with an AIC higher than the Null model were not considered in mixed models.

Mixed models showing an AIC within two units of the best model ( $\Delta\text{AIC} < 2$ ) were considered to be equally supported by the data (Burnham and Anderson 2002). In these circumstances we performed model averaging accounting for the average parameters on the group of models with  $\Delta\text{AIC} < 2$  (Burnham and Anderson 2002). Explanatory variables included in these models were considered significant if their confidence intervals did not overlap zero (Burnham and Anderson 2002). Models were also globally evaluated through the comparison of their AIC with the AIC of the Null model. Models with an  $\Delta\text{AIC} > 2$  relative to the Null model were assumed to have considerable support.

To assess road barrier effect, the number of observed road crossings was compared to the expected number of road crossings through Pearson chi-square test. The expected number of road crossings was generated with correlated random walk (CRW) models (Calenge et al. 2009). CRW models (Kareiva and Shigesada 1983) were parameterized using observed telemetry data as follows: the concentration parameter ( $r$ ) was obtained using the Wrapped Normal Maximum Likelihood estimate for observed turning angles; the scaling parameter ( $h$ ) was calculated from each observed path; and the spatial coordinate to start from. A total of 100 simulated paths were produced for each observed path from which the number of times each path crossed a road were extracted (Rondinini and Doncaster 2002). If a vole significantly avoided roads, then the number of observed road crossings should be below the 95% of the distribution of predicted crossings (i.e. one-tailed  $P < 0.05$ ) generated from the individual's simulated movement paths (Shepard et al. 2008). The expected number of road crossings was generated for all voles together in each habitat patch, and then for individual voles.

Analyses were performed in QGIS (2.18 Las Palmas) software and R environment, version 3.4.4 (R Development Core Team 2017), and using the packages `adehabitatHR`, `adehabitatLT`, `MuMIn`, `lme4` and `nlme`.

## Results

### General results

A total of 16 voles were successfully tracked. Radio-telemetry provided 3886 position fixes collected over 904h for 16 animals. Mean  $\pm$  SE fixes per animal was  $217.8 \pm 48.3$ . Three



**Figure 2.** Home range (BRB Kernel 95%) of each radio-tracked Cabrera vole in southern Portugal; Panel **A** meadow patch; Panel **B** verge patch. Females are represented with continuous home range outline, while males are represented with discontinuous home range outline.

batteries failed before the end of the study, one vole was predated by a snake, and another possibly removed the collar. The animals included in analyses have at least a full 24-h period sampled (16 voles). The maximum number of voles tracked simultaneously was four.

Cabrera voles showed home ranges (95% BRBK) between 175 and 815 m<sup>2</sup> (mean  $\pm$  SD: 352  $\pm$  163m<sup>2</sup>). Core areas (50% BRBK) varied between 37 and 175 m<sup>2</sup> (mean  $\pm$  SD: 62  $\pm$  34 m<sup>2</sup>).

Steps and paths of zero length were calculated as 73.1% and 14.3% of observations respectively, and were not included in the analyses of movement patterns. Thus, steps length (movement within 15 min) varied between 3 and 28 m (mean  $\pm$  SD: 5.8  $\pm$  3.5 m), while path length (movement within 4h) varied between 3 and 94.8 m (mean  $\pm$  SD: 27.4  $\pm$  21.3 m).

### Space use patterns

Voles from the Verge patch showed significantly ( $P$ -value < 0.05) smaller home ranges (95%BRBK) and lower shape complexity index (*sh\_complex*) when compared with voles from the Meadow patch (Figure 2; Table 1). Mean home range in the Meadow was 451 m<sup>2</sup>, while in the Verge patch was 255 m<sup>2</sup>. The extent of core areas (50%BRNK), the number of core areas (No BRBK50) and female spatial overlap (UDOI index) were not statistically different ( $P$ -value > 0.05) between patches (Table 1).

### Differences in movement patterns between patches

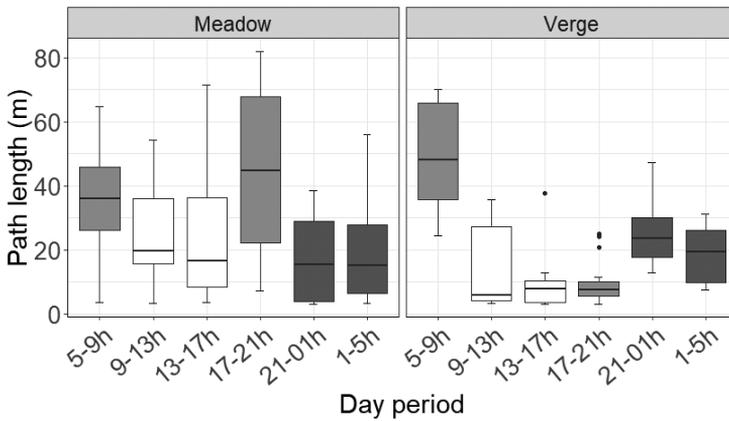
The length of movement paths was explained by a group of five models ( $\Delta$ AIC<sub>c</sub> < 2), the first model had a weight of 0.37 and an AIC improvement relatively to the Null model of 38. (Null model AIC = 422; best model AIC = 384).

According to the average model, there were differences in path length between day periods, according to the patch type: paths were longer for the 5–9h period (dawn) when compared with the three following periods (9–13h, 13–17h, 17–21h) in Verge

**Table 1.** Summary results of Wilcoxon rank tests applied to the space use parameters of Cabrera voles with observed values for the two habitat patches (mean  $\pm$  SD), also showing the value of the test statistic (W) and the p-value for the test (P-value); 95%BRBK: biased random bridge kernel (BRBK) at 95% utilization distribution contour; 50%BRBK: biased random bridge kernel (BRBK) at 50% (core areas); No BRBK50: number of core areas; sh\_complex: shape complexity index; UDOI: utilization distribution overlap index.

	Meadow	Verge	W	P-value
95%BRBK <sup>a</sup>	450.5 $\pm$ 178.4 (m <sup>2</sup> )	254.8 $\pm$ 60.6 (m <sup>2</sup> )	56	0.01
50%BRBK <sup>a</sup>	71.2 $\pm$ 45.7 (m <sup>2</sup> )	52.2 $\pm$ 12.2 (m <sup>2</sup> )	39	0.505
No BRBK50 <sup>a</sup>	2.1 $\pm$ 2.0	1.6 $\pm$ 0.9	34.5	0.815
sh_complex	1.7 $\pm$ 0.4	1.1 $\pm$ 0.1	61	0.001
UDOI <sup>b</sup>	0.013 $\pm$ 0.033	0.000011 $\pm$ 0.000017	152	0.375

a) log transformed. b) square root transformed



**Figure 3.** Variation of path length according to habitat patch (Meadow and Verge) and day period of Cabrera voles in southern Portugal (dark gray: nocturnal periods; medium gray: sunrise and sunset periods; white: diurnal periods).

patch (but not in Meadow patch). This interaction effect is more noticeable between the periods 5–9h (dawn) and 17–21h (sunset; Figure 3; Suppl. material 1: Table S3). In addition, paths were longer during lower ambient temperatures in both habitat patches (Suppl. material 1: Table S3).

None of the path linearity models had a fit superior to the Null model (AIC = 56.4).

### Frequency of road crossing by animals

There were no crossing events recorded for any of the radio-tracked voles. The overall expected road crossing percentage in the Meadow patch was 10.2% (Pearson chi-square = 12.25; p-value = 0.0005) while the expected value for the Verge patch was 54.2% (Pearson chi-square = 101.79; p-value = 0.0000; Table 2). This shows that, for both habitat patches, the observed crossing rates were significantly lower than

**Table 2.** Comparison between the observed and the expected paths through a Pearson chi-square test by patch; for each comparison is also presented the crossing estimate, Chi-square and P-Value.

	Positive Observed crossings	Negative Observed crossings	Positive Expected crossings	Negative Expected crossings	Estimate	Chi-square	P-Value
Meadow	0	108	1190	10485	0.102	12.245	<0.001
Verge	0	83	5640	4485	0.542	101.79	<0.001

**Table 3.** Comparison between the observed and the expected paths through a Pearson chi-square test by individual with the crossing estimate, Chi-square and P-Value.

Patch	Animal	Positive Observed crossings	Negative Observed crossings	Positive Expected crossings	Negative Expected crossings	Estimate	Chi-square	P-Value
Meadow	A	0	18	254	1641	0.134	2.782	0.095
	B	0	16	275	1370	0.167	3.205	0.073
	C	0	17	1	1644	0.000	0.010	0.919
	D	0	7	147	853	0.147	1.205	0.272
	E	0	14	400	1245	0.243	4.486	0.034
	F	0	14	49	1451	0.033	0.473	0.492
	G	0	13	51	1194	0.041	0.555	0.456
	H	0	9	13	1087	0.012	0.108	0.743
Verge	J	0	12	833	912	0.477	10.893	0.001
	L	0	12	849	596	0.587	16.896	<0.001
	M	0	14	980	520	0.653	25.933	<0.001
	N	0	9	471	774	0.378	5.453	0.02
	O	0	11	626	474	0.569	14.340	<0.001
	Q	0	7	345	500	0.408	4.803	0.028
	R	0	9	843	257	0.766	28.756	<0.001
	S	0	9	693	452	0.605	13.636	<0.001

predicted by chance, although the difference between observed and expected was much higher in the Verge patch (-0.102 for Meadow and -0.542 for Verge; Table 2).

When analyzing crossing events for individual animals, all voles presented road crossing rates lower than expected, although the differences were not statistically significant for most voles from the Meadow patch (Table 3). Accordingly, the expected crossing percentage of paths of individual voles from the Meadow varied between 0.0006% and 24% (mean of 9.7%) with only statistical significance for one individual (Pearson chi-square = 4.49; p-value = 0.034) which occupied a home range near the road verge of the Meadow patch (vole E; Table 3). The expected crossing percentage of paths of individual voles from the Verge patch varied between 37.8% and 76.6% for each vole (mean of 55.7%) with statistical significance for all individuals (all p-values < 0.05; Table 3).

## Discussion

Despite the potential positive role of vegetated road verges for biodiversity conservation, they are subject to periodic vegetation removal (by road companies), are linearly

shaped, and are bordered by the road surface and, often unsuitable matrix habitat, thus providing challenging conditions for population establishment and persistence. This underlines the importance of fully understanding how road verges affect species of conservation concern, particularly its behavioral patterns such as space use and movements. Our results seem to support the first hypothesis (i), that individuals occupying road verges have smaller home ranges with lower shape complexity. As for movement patterns, the model results did not support the hypotheses that individuals living in the road verge have shorter paths (hypothesis ii), and more linear paths (hypothesis iii). These results suggest that movement behaviour is little affected by the degree of habitat reduction. However, there was an interaction effect between habitat patch and day period for path length, which partially supports the hypothesis that individual movements during high traffic periods (daytime and sunset) are more constrained in smaller habitat patches adjacent to the road (hypothesis iv). Road crossing results do not support the hypothesis that individuals living in smaller habitat patches cross the road more frequently than those in larger patches (hypothesis iv), although it suggests the existence of a strong road-barrier effect for individuals living in road verges.

### Differences in space use in the Verge patch

As predicted, individuals occupying the Verge patch showed smaller home ranges with lower shape complexity than those in the larger area (Meadow patch). However, there were no significant differences in core areas, number of core areas and female overlap, as observed in previous studies with other vole species testing social organization over time and space (e.g. Madison 1990, Ims et al. 1992). This seems to indicate that habitat reduction by roads may hinder individual's home ranges, but the characteristics of core areas and social structure are maintained. Although road verges have been associated to a lower nutritional quality of food resources (Santos et al. 2007, Rosário et al. 2008), home ranges with circular shapes (i.e., lowest shape complexity) suggest that road verges might present evenly distributed resources, which tend to minimize energy expenditure, contrasting with heterogeneous distribution of resources in larger patches that originate more complex home ranges (Hiller et al 2016).

### Differences in movement patterns in the Verge patch

The path length was similar among both patches. However, there was an interaction between the day period and the habitat patch. This interaction points to longer paths in the period of 5–9h (sunrise) in the Verge patch when compared with the 9–21h period. This has not happened in the Meadow patch. Since traffic intensity is higher during the day (and sunset), animals in Verge patch may have decreased their path length in response to increased traffic as was observed by Chen and Koprowski (2016) in Arizona (USA) with Mount Graham red squirrels (*Tamiasciurus hudsonicus grahamensis*). The higher proximity of animals to the road pavement in the Verge patch, when compared to the Meadow patch, may explain this interaction effect. By being

restrained in a smaller habitat patch, voles may be forced to adapt their behavior by decreasing their activity during some periods of the day. There was also an influence of temperature on movement length, with longer path lengths occurring during coolest hours of the day, for both habitat patches. This is confirmed by previous studies on activity rhythms of this species (Pita et al. 2011; Grácio et al. 2017) and can explain why path length seems shorter during midday, since it coincides with higher temperature period. Also, the differences in the vegetation structure between habitat patches (lower abundance of shrubs in the Verge patch) could be another possible explanation for the differences in path length, as Verge patch might offer less protection from avian diurnal predation (e.g., buzzards and kites that are frequently observed along the studied road).

Due to their poor ability to move further away from the road, voles seem to have adapted their movement patterns to accommodate the exposure to the road disturbance. While animals in the Meadow patch showed no significant differences in movement patterns throughout the daily cycle (beyond what would be expected in diurnal animals), in the Verge patch, movement patterns may have changed or even been hindered during at least part of the day. Traffic disturbance could be the reason for the disparity of results between habitat patches, as the changes in the movement patterns coincided with the period of increased traffic (day and sunset periods). This agrees with observations for moose (*Alces alces*), which remain further away from roads during high traffic periods (Neumann et al. 2013).

### Road barrier effects

When analyzed at the patch scale, there were significant differences between observed and random paths in both patches. Although results indicates that the voles from both habitat patches avoided the road, this avoidance signal was 5 times stronger in the Verge patch. This explains why most animals from the Meadow patch showed individually non-significant differences in crossing estimates. Thus, the disparity between crossing estimates by animals in the different patches may be explained by the spatial location of home ranges in Meadow patch being further away from the road than in Verge patch. As individuals in Verge patch are restricted to a smaller area, it is more likely that any expected path would cross the road, whereas in Meadow patch, by being further away from the road, this is less likely. This could suggest that voles in Verge patch are more exposed to the barrier effect and thus more prone to local extinction events (Seiler 2001).

Overall, the present study is in accordance with other studies (e.g. McDonald and St. Clair 2004; Grilo et al. 2018), showing that roads can have influence on small mammal space use and movement patterns. The difference in space use and movement patterns between habitat patches may have been caused by traffic disturbance or by the less heterogeneous vegetation structure in the Verge, which may offer less protection against avian diurnal predators, and therefore may promote a different response from the voles during certain periods of the day. While we acknowledge that the number

of study patches and individuals, and the particular period of the year considered may limit our inferences to other geographical areas and seasons, we believe that our study highlights the need to recognize in future studies the importance of road effects on the space use and movement patterns of the Cabrera vole and other species that are often associated to road verge habitats.

## Main Conclusions

This study suggests that, although road verges can have several potential advantages for Cabrera voles, the small habitat patches typical of verges may restrict vole space use and movement patterns, and even act as a behavioural barrier to vole road crossings. Despite the extensive number of studies about the effect of roads on small mammals, few have focused on the behavioural traits related to individual space use and movement of an endangered species that often occur on road verges, such as the Cabrera vole. Due to the “Vulnerable” status of this species, the present study should be particularly relevant in terms of conservation. The results point to the importance of promoting wide and unrestricted verges for the species conservation. In the present case, it is possible that road crossing structures, such as small culverts, could soften the road-barrier effect, especially in the Verge patch.

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## Supplementary material I

### **The effect of habitat encroachment by roads on space use and movement patterns of an endangered vole**

Authors: Nelson Fernandes, Eduardo M. Ferreira, Ricardo Pita, António Mira, Sara M. Santos

Data type: Docx file.

Explanation note: Details of predictors analysed and detailed tests and model results.

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