UNIVERSIDADE DE ÉVORA

ESCOLA DE CIÊNCIAS E TECNOLOGIAS

DEPARTAMENTO DE BIOLOGIA

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

O efeito da redução do habitat causado por estradas no uso do espaço e padrões de movimento de um roedor ameaçado

Nelson Horta Fernandes

Orientação: Dra. Sara Maria Lopes Santos
Dr. Ricardo Pita
Prof. António Mira

Mestrado em Biologia da Conservação

Dissertação
Évora, 2019

UNIVERSIDADE DE ÉVORA

ESCOLA DE CIÊNCIAS E TECNOLOGIA
DEPARTAMENTO DE BIOLOGIA

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

Nelson Horta Fernandes

Orientação: Doutora Sara Maria Lopes Santos
Doutor Ricardo Pita
Professor António Mira

Mestrado em Biologia da Conservação

Dissertação

Évora, 2019

Júri

Presidente de Júri: Prof. Paulo Sá Sousa

Orientador: Drª Sara Santos

Arguente: Drª Inês Teixeira do Rosário

Index

Research article

Figure index

Table index

Annex index

O efeito da redução do habitat causado por estradas no uso do espaço e padrões de movimento de um roedor ameaçado

Resumo

Nesta dissertação pretendo avaliar como a redução do habitat devido à presença de estradas afecta o uso do espaço e padrões de movimento de um roedor ameaçado. A espécie alvo é o rato de Cabrera (*Microtus cabrerae*), um endemismo Ibérico com estatuto Nacional de "Vulnerável". Foram selecionadas duas parcelas de habitat com diferentes níveis de influência de estradas: uma mais pequena e exposta a estradas (a Berma) e outra maior e menos exposta a estas infra-estruturas (Prado). Os resultados mostraram que os indivíduos da Berma tiveram áreas vitais mais reduzidas e menos complexas que as dos indivíduos do Prado. Os padrões de movimento foram influenciados pelo período do dia apenas na Berma. Os dados mostraram um efeito barreira das estradas em ambas as parcelas de habitat, embora mais forte na Berma. Este estudo mostra que o uso do espaço e os padrões de movimento poderão ser afectados pela redução de habitat causada pela presença das estradas.

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

Abstract

In this dissertation I intend to assess how the habitat encroachment by roads affects the space use and movement patterns of an endangered vole. The target species is the Cabrera vole (*Microtus cabrerae*), an Iberian endemism considered "Vulnerable" at the national level. Two habitat patches with different degrees of encroachment by roads were selected: one more encroached (Verge) and one less encroached (Meadow). Results showed that individuals from the Verge had smaller and less complex home ranges than those of individuals from the Meadow. Movement patterns were influenced by the day period only in the Verge patch. The data showed a barrier effect on both habitat patches, although stronger in the Verge. The present study shows that space use and movement patterns might be affected by the habitat encroachment by roads.

Introduction

Roads are a widespread sign of human presence throughout the world. Roads were documented to provide resources such as food and refuge (Forman and Alexander 1998). However the negative effects far outnumber the positive ones (Fahrig and Rytwinski 2009). Roads fragment the landscape, impose barriers to wildlife movement thus disturbing gene flow and hindering resource acquisition and may even lead to death by roadkill (Forman 1998).

Despite the positive effects that roads may have on wildlife, the negative effects put at risk the persistence of many species. Landscape fragmentation happens when the available habitat is partitioned, for instance by roads, into smaller and isolated fragments (European Envoironment Agency, 2011). This process is often accompanied by habitat loss (Bennett 2003), being responsible for the decline or even extinction of many species worldwide (Fahrig 2013). The brown hare (*Lepus europaeus*) in the Switzerland is an example of this, since the species decline is aggravated by the road presence and habitat loss according to Roedenbeck and Voser (2008).

Local population extinction may happen when the remaining natural habitat can no longer sustain individuals. Not only road construction requires the clearing of vegetation, but also roads facilitate human access to otherwise unreachable areas, thus promoting further development of such areas and leading to further habitat destruction (van der Ree et al 2015 p4). Road presence also leads to habitat loss through habitat degradation. This degradation can be a product of the disturbance and pollution caused by traffic which can affect a wider area than the one occupied by the road surface, making it unfavorable to many fauna and flora species (Seiler 2001). Habitat patches created by fragmentation often tend to become smaller throughout time (Bennett 2003). This slow process of overall habitat loss and patch size reduction transforms the landscape from a once favorable large area to few and small ephemeral patches. Roads then may exert a barrier effect on the movements of animals between these patches.

Barrier effects may hinder inter-patch movement and may be reflected in a decrease or interruption in the *apport* of new individuals to the patch (e.g. Wilson et al. 2007), or in increased isolation of the individuals living therein (Bennett 2003). This phenomenon was observed for small mammals in a highway from Canada (McDonald and St Clair, 2004). Also, Van Dyke et al. (1986) observed that mountain lions (*Felis concolor*) avoided crossing roads, being roads much less likely to occur within animals' home range. It was shown by Ascenção and collaborators (2016) that road clearance avoidance may be responsible for the barrier effects in wood mouse (*Apodemus sylvaticus*) which can lead to a reduced gene flow. Thus, some species when confined to habitat patches too small to support them may be driven to extinction (Andrén 1994).

These negative effects can be minimized by promoting landscape connectivity. Landscape connectivity is the extent to which the landscape allows movement among patches, thus allowing the flow of individuals through the landscape (Taylor et al. 2006). Landscape connectivity is species-specific as different species perceive the landscape differently (Taylor et al. 2006). This way landscape connectivity is essential for wildlife survival, as it allows for food acquisition, mate location, dispersal movements and gene flow. As Wilson and collaborators (2016) observed, caribou movement patterns during migrations were affected by the distance to road. Based on observations made in southern Spain regarding three small mammal species, it was suggested that even though habitat generalists may cross roads more easily, habitat specialists may be restricted by their presence, showing lower crossing capabilities (Grilo et al. 2018). This highlights the importance of maintaining landscape connectivity to allow species movement through the landscape. On the other hand, while mitigation measures can be taken to re-establish landscape connectivity these may not be enough since they will not be effective if there is no habitat left for wildlife (Fahrig 2002).

Species with metapopulation structures can be particularly affected by landscape fragmentation, as increased local extinctions rates will reduce the chances of overall metapopulation long-term persistence it (Hanski 1999; Forman et al., 2003).

An example of a species with a metapopulation structure is the Cabrera vole (*Microtus cabrerae* Thomas 1906) (Pita et al 2011). This vole is one of the largest *Microtus* species (Fernández-Salvador 1998). The Cabrera vole is an Iberian endemism with a fragmented distribution (Pita et al 2014). The species has suffered a population decline in the recent past, in part due to the agricultural intensification (Pita et al 2014). Currently the species is classified as "Vulnerable" in Portugal, and is listed in the Habitats directive (92/43/EEC; Queiroz et al. 2005).

Given its conservation status and population trends, it is important to understand the effects that road encroachment may have on the Cabrera vole. Harding (2002) have shown, using *Microtus californicus*, that movement between source and sink habitats can have determinant effects on long term population growth or maybe even persistence. Also Rico and collaborators (2007) found a lower small mammal species diversity and abundance in road verges (but see McGregor et al 2008). In addition, McDonald and St. Clair (2004) observed that voles (*Microtus ssp.*) had decreased crossing rates when crossing an artificial barrier (highway) and their movement patterns changed. Bender and Fahrig (2005) also showed that in a homogeneous matrix, patch size and isolation may be a determinant factor in immigration rates. In the case of roads it was even hypothesized that recolonization events of road verge habitats could be hampered (McGregor et al 2008). Therefore, since the Cabrera vole presents a metapopulation structure (Pita et al. 2007), habitat isolation caused by roads may pose a threat to the species population persistence. Debinski and Holt (2000) found that in the case of small mammals there are contradictory results from a plethora of studies that could be caused, among other factors, by species-specific characteristics. This means that the Cabrera vole may respond differently to what was observed in other species in similar situations, which highlights the importance of this study for improving conservation efforts for this species.

Looking at these impacts is the project “POPCONNECT - Combining genetic and field-based data to assess the effects of roads on landscape functional connectivity and population viability (ref PTDC/AAG-MAA/0372/2014)”. This research project aims to assess the importance of road mortality and barrier effect on landscape connectivity and effects in long term population viability. The project uses as target species the lesser horseshoe bat (*Rhinolophus hipposideros*), the common genet (*Genetta genetta*), the wood mouse (*Apodemus sylvaticus*) and the Cabrera vole (*Microtus cabrerae*).

It is within the Popconnect framework that my dissertation takes place, where I intend to assess how the Cabrera vole (*Microtus cabrerae*) responds to the road presence. Specifically, I aimed to assess if habitat encroachment by roads influences space use and movement patterns of Cabrera voles in southern Portugal. I studied individuals sampled in two habitat patches with different levels of habitat encroachment by roads, using radio-telemetry data.

Bibliography

Andrén H (1994) Effects of Habitat Fragmentation on Birds and Mammals in Landscapes with Different Proportions of Suitable Habitat: A Review. Oikos 71:355.

Ascensão F, Mata C, Malo JE, et al (2016) Disentangle the causes of the road barrier effect in small mammals through genetic patterns. PLoS One 11:1–23.

Bender DJ, Fahrig L (2005) Matrix Structure Obscures the Relationship between Interpatch Movement and Patch Size and Isolation. 86:1023–1033.

Bennett AF (2003) Linkages in the landscape: The role of corridors and connectivity in wildlife conservation

Debinski DM, Holt RD (2000) A Survey and Overview of Habitat Fragmentation Experiments. Conserv Biol 14:342–355.

European Envoironment Agency. (2011). *Landscape fragmentation in Europe*. Copenhagen.

Fahrig L (2013) Rethinking patch size and isolation effects: The habitat amount hypothesis. J Biogeogr 40:1649–1663.

Fahrig L (2002) Effect of Habitat Fragmentation on the Extinction Threshold : A Synthesis. Ecol Appl 12:346–353

Fahrig L, Rytwinski T (2009) Effects of roads on animal abundance: An empirical review and synthesis. Ecol Soc 14.

Fernández-Salvador R (1998) Topillo de Cabrera, *Microtus cabrerae* THOMAS, 1906. Galemys 3:5–18.

Forman RTT (1998) Road ecology: A solution for the giant embracing us. Landsc Ecol 13.

Forman RTT, Alexander LE (1998) Roads and Their Major Ecological Effects. Annu Rev Ecol Syst 29:207–231.

Forman RTT, Sperling D, Bissonette JA, et al (2003) Road ecology - Science and solutions. Island Press, Washington, DC

Grilo C, Molina-Vacas G, Fernández-Aguilar X, et al (2018) Species-specific movement traits and specialization determine the spatial responses of small mammals towards roads. Landsc Urban Plan 169:199–207.

Hanski I (1999) Metapopulation ecology. Oxford Univ Press 313

Harding EK (2002) Modelling the influence of seasonal inter-habitat movements by an ecotone rodent. Biol Conserv 104:227–237.

McDonald WR, St Clair CC (2004) The effects of artificial and natural barriers on the movement of small mammals in Banff National Park, Canada. Oikos 105:397–407.

McGregor RL, Bender DJ, Fahrig L (2008) Do small mammals avoid roads because of the traffic? J Appl Ecol 45:117–123.

Pita R, Beja P, Mira A (2007) Spatial population structure of the Cabrera vole in Mediterranean farmland: The relative role of patch and matrix effects. Biol Conserv 134:383–392.

Pita R, Mira A, Beja P (2011) Assessing habitat differentiation between coexisting species: The role of spatial scale. Acta Oecologica 37:124–132.

Pita R, Mira A, Beja P (2014) *Microtus cabrerae* (Rodentia: Cricetidae). Mamm Species 912:48–70.

Queiroz AI (coord.), Alves PC, Barroso I, Beja P, Fernandes M, Freitas L, Mathias ML, Mira A, Palmeirim JM, Prieto R, Rainho A, Rodrigues L, Santos-Reis M, Sequeira M (2005). Microtus cabrerae *Rato de Cabrera* Pp 481-482 in Livro Vermelho dos Vertebrados de Portugal (Cabral MJ et al. (eds.)). Instituto da Conservação da Natureza. Lisboa.

Rico A, Kindlmann P, Sedláček F (2007) Barrier effects of roads on movements of small mammals. Folia Zool 56:1–12

Roedenbeck IA, Voser P (2008) Effects of roads on spatial distribution, abundance and mortality of brown hare (Lepus europaeus) in Switzerland. Eur J Wildl Res 54:425–437.

Seiler A (2001) Ecological effects of roads: a review. Introd. Res. essay 9:1–40

Taylor PPD, Fahrig L, With KK a (2006) Landscape connectivity: a return to the basics. Connect Conserv 29–43.

van der Ree, R., Smith, D. J., & Grilo, C. (2015) The ecological effects of linear infrastructure and traffic: Challenges and opportunities of rapid global growth. IN: van der Ree, R., Smith, D. J., & Grilo, C.. Handbook of Road Ecology. Wiley. Chichester: Wiley Blackwell.

Van Dyke FG, Brocke RH, Shaw HG (1986) Use of road track counts as indices. J Wildl Manage 50:102–109

Wilson RF, Marsh H, Winter J (2007) Importance of canopy connectivity for home range and movements of the rainforest arboreal ringtail possum (Hemibelideus lemuroides). Wildl Res 34:177–184.

Wilson RR, Parrett LS, Joly K, Dau JR (2016) Effects of roads on individual caribou movements during migration. Biol Conserv 195:2–8.

Research article

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

Abstract

Roads are the most widespread sign of man 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, I tested how habitat encroachment by roads may affect the space use and movement patterns of the Cabrera vole (*Microtus cabrerae*), a near-threatened, Iberian endemic rodent, which is often associated to road verge habitats. A total of 18 voles were radio-tracked in two habitat patches with different degrees of encroachment by roads. Results showed that individuals from the patch more heavily encroached by roads (Verge patch) had smaller and less complex home-ranges than those from the less encroached 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 patch Overall, the present study shows that space use and movement patterns of Cabrera voles near roads may be affected by the degree of habitat encroachment imposed by these infrastructures. This suggests that species space use and movement patterns at fine-scales should be accounted for in road construction planning, even for species that are known to benefit to some extent from road verge habitats.

Key words: Small mammals; Cabrera vole, barrier effect; fragmentation; road ecology.

Introduction

Roads are a widespread sign of human presence across the globe, imposing a number of 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). Actually, road verges are frequently the only remaining favourable habitat for many species in highly humanized landscapes, providing refuge, habitat or dispersal corridors (Way 1977; Redon et al. 2015). This is probably the case of many 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. Sabino-Marques and Mira 2011; Redon et al. 2015).

The negative effects of roads are more important and widespread 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). In many species roads also act as barriers to movement, 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 the local extinctions risks (Shepard et al. 2008). For instance, it has been shown that road proximity can have negative impacts on mammal species abundance or activity, this effect decreasing with the distance to the road (Benítez-López et al. 2010; Medinas et al. 2019). This suggests that mammals, and particularly large mammals such as carnivores, should prefer areas with low road density (Ceia-Hasse et al. 2017), as confirmed for instance for wolves (Mladenoff et al. 1999). Likewise, 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 due to road development should 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 behaviour may be changed 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 that roads may impose to individuals living near roads (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, I.A. et al. 2007). It is known that responses to roads vary considerably across species (Bissonette and Rosa 2009; Rytwinski and Fahrig 2012). Grilo and collaborators (2018) also pointed out that road crossing rates decreased in wider roads, despite the behavioural responses to roads being species-specific. Galantinho and collaborators (2017) found that *Apodemus sylvaticus* populations living in road surroundings have a lower fitness than those occurring 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 and collaborators (2007) that observed a lower crossing rate in less mobile rodent species and by Chen and Koprowski (2016) which have shown that even dirt roads confine home ranges and inhibited Abert's squirrel (*Sciurus aberti*) movements. Terrestrial species with small and fragmented populations, and specific habitat or environmental conditions should be particularly vulnerable to impacts of road barrier effects (McDonald and St Clair 2004).

The Cabrera vole (*Microtus cabrerae* 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 Spain (Fernández-Salvador 2007) and is present in the Annexes II and IV of the Habitats Directive (92/43/EEC; Queiroz et al. 2005). The Cabrera vole is a habitat specialist, requiring patches with high herbaceous cover associated with high humidity and temperature conditions (Ventura et al. 1998; Santos et al. 2005; Pita et al. 2011a). In addition, this species shows a spatial pattern consistent with a metapopulation structure, with frequent local extinctions and colonisations. The species displays a monogamous matting system, and home ranges are typically <1000 m^2 (Fernández-Salvador et al. 2001; Pita et al. 2010, 2014). Suitable habitats for this species are usually restricted to areas without intensive agriculture or overgrazing, which allows the growing of tall and dense grasses (Fernández-Salvador 1998; Pita et al. 2006, 2007). Cabrera vole is frequently found in association with road verge habitats, despite the lower nutritional value of the grasses found there which may lead to an adjustment in its diet (Santos et al. 2007; Rosário et al. 2008). This way space use may change to accommodate for a less nutritional diet by adopting a less complex home range boundaries (Ford 1983; Hiller et al. 2016) or by increasing the intrasexual overlap (Madison 1980). Also by occupying road verges animals are more exposed to mortality by roadkill, however in verges of dirt roads Cabrera voles have been documented to survive longer than those that occupied other habitat types (Proença-Ferreira et al. 2019). In addition, road verges are subject to periodic vegetation removal (by road companies), are linearly shaped, and are bordered by the road surface and unsuitable matrix habitat, thus providing particular challenging conditions for populations establishment and persistence. This underlines the importance of fully understanding how road verges effect the species, particularly its behavioral patterns such as space use and movement patterns.

In the present study I aimed to assess for the first time if habitat encroachment by roads influences space use and movement patterns of Cabrera voles in southern Portugal. I used radio-telemetry data from individuals sampled in two habitat patches with different levels of habitat encroachment by roads. The term encroachment is here used to describe the advancement of roads into natural habitats, involving embankment and fill operations, removal of vegetation, and alteration of topography into such areas. This process is known to cause impacts to the function and value of those natural areas, such as changes in hydrology, decline in water quality, habitat loss, and eventual disruption of equilibrium conditions of ecological process that sustain wildlife species using road verge habitats, such as the Cabrera vole. To my knowledge, no study has ever determined how habitat encroachment by roads may affect space use and movement patterns of Cabrera voles, even though such information should be critical for species management plans.

Small mammals' home range size is inversely related to food availability (Taitt 1981; Taitt and Krebs 1981) and population density (Getz 1961; Erlinge et al. 1990). Therefore, in roads crossing highly modified landscapes, home ranges in road verges should be smaller and more linear, as it was predicted by Stumpf and Mohr (1962), due to the higher availability of food and shelter, attracting a high number of individuals compared to the surrounding matrix habitats.

Movement patterns may also change in such small and linear habitat patches. Movement paths might be shorter, more linear and more frequently along the road when compared with more extensive habitat patches. Also, because, the vehicles pass closer to animals in road verge habitats, the animals may adjust their typical diurnal activity (Fernández-Salvador 1998) to other periods of the day with less traffic (nightime). In addition, even if the animals can use effectively food resources of road verges, they should cross the road more often because those resources are spatially limited by the roads.

Accordingly to these predictions, I tested if individuals occupying areas more encroached by roads: i) 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 movements more frequently along the road, v) make less movements during high traffic periods (daytime), and vi) cross the road more frequently than those in areas less encroached by roads.

Materials and methods

The present study took place in Alentejo, southern Portugal. The climatological normal mean (1981-2010) varied between 14.3º C and 21.4º C for the study area (IPMA 2018). The landscape is mainly characterized by the agroforesty system commonly known as "montado", an open tree layer with Cork oak (*Quercus suber*) and/or Holm oak (*Quercus rotundifolia*), with sclerophyll shrubs and annual grasses, adapted to endure the harsh dry season (Pinto Correia et al. 2011). The four main roads in the region are one highway (A6) and three national roads (N4, N114 and N18; Figure 1 Panel B).

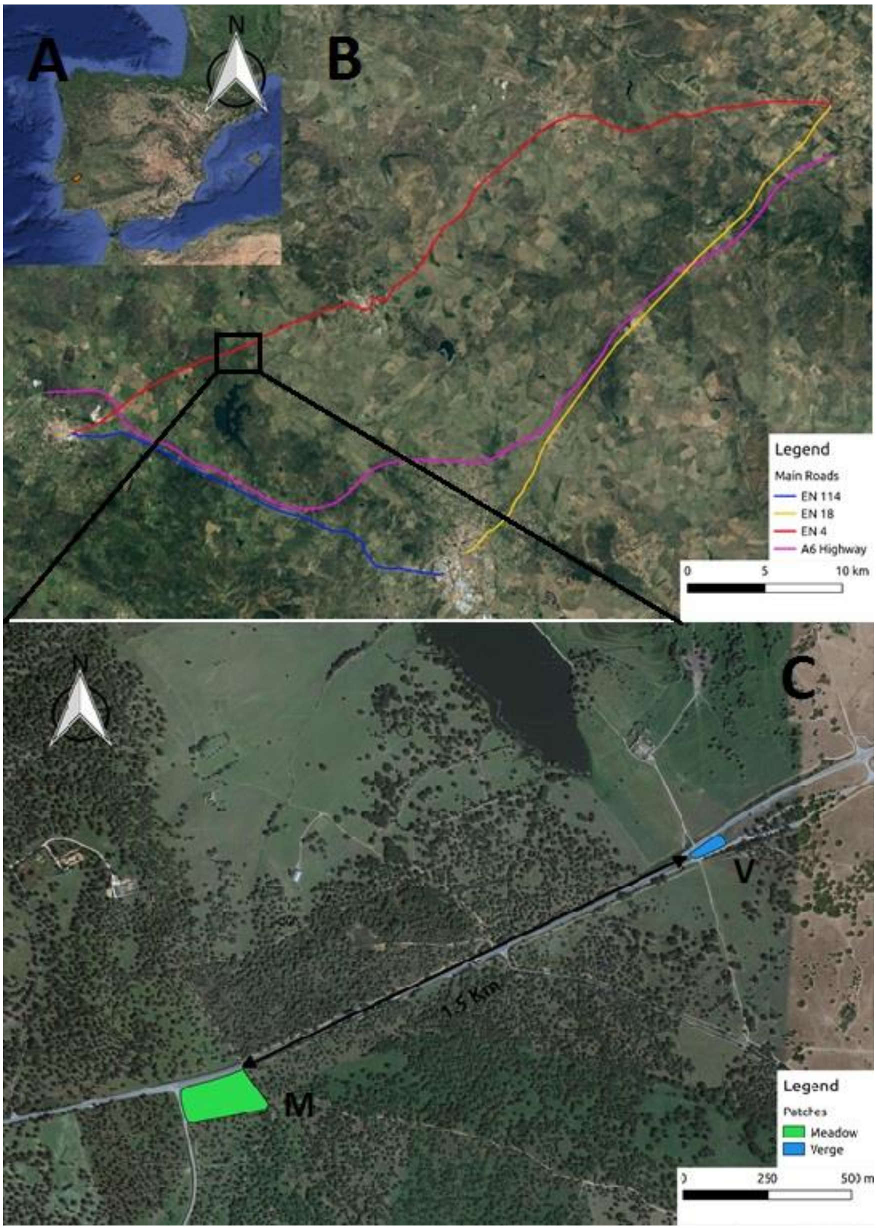

Figure 1: Panel A- Study area location within the Iberian Peninsula; Panel B- Main roads location in the region; Panel C- Habitat patches along the N4 road (M- Meadow patch; V – Verge patch).

Study design

Two study patches were established (Meadow and Verge; Figure 1, Panel C) after previous searches for species presence signs in areas near the main roads of the study area (October 2016 to February 2017; Figure 1, Panel B). The two patches presented abundant conspicuous pathways among grasses, the typical dark-green droppings and the fresh cut grasses (Pita et al. 2006). Both patches were located along the N4 road and separated by 1.5 km from each other (Figure 1, Panel C).

The Meadow patch (38.692015 N, -8.087678 W) is a large patch (24 589 m^2) 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 two paved roads: N4 at North and by a smaller dead-end road at the West side.

The Verge patch (38.698661 N, -8.070548 W) is a small patch (2 021 m^2) spatially constrained between two paved roads, N4 at North, and a smaller and less used road at South. The dominant vegetation in this patch is mainly annual grass communities with isolated shrubs (*Cytisus spp*., *Genista spp*.). 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 surface.

Road N4 has an annual traffic of 3882 motorized vehicles (3424 during day time and 458 during night time) and connects Lisbon to Spain (IP 2005).

Capture and radio-telemetry

Voles were captured with Sherman live traps (7x23x9 cm) laid in clusters where the species signs were more evident and fresh. Apple and carrot were used as bait, and hydrophobic cotton and grass were provided as bedding (Pita et al. 2011b). A total of 14 trapping sessions were conducted (April to June 2017) in order to sample enough animals, while allowing simultaneous tracking of voles in each trapping session. 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 (Pita et al. 2011b).

All Cabrera voles captured were weighed and sexed in the field in order to immediately exclude animals with low weight, and pregnant or lactating females, so as to avoid any negative impacts on local populations. Voles with conditions to be radio-collared 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. During sedation, the weight, sex and 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 low (Gannon et al. 2007). 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 so 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 (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; Fernández-Salvador et al. 2005; Pita et al. 2010).

From 7th April to 14th 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).

To access the movement paths and due to the short battery life, it was decided to use a clustered sampling scheme, with discontinuous tracking at 15min intervals (Pita et al. 2010; Santos et al. 2010). Hence tracking was done in six 4-h sessions, comprising 16 position fixes and separated at least 4h from the next session in order to sample all the 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. 2011b). This allowed to optimize sampling to the periods of higher activity. Voles were seen in 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 in order to remove the collars from any recaptured animal.

Data analysis

Response variables

In order 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, step direction 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 (Lmin). The maximum step duration for defining successive relocations was defined as 4h (Tmax) and the minimum smoothing parameter was set to 1.2 in all animals (hmin). 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 $C=L / (2*\sqrt{(A\pi)})$, 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 individuals (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, three responses were calculated from radio-telemetry data: path length, path linearity index, and step direction to the main road (N4).

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 x 16). Previous to 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), divided 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).

The absolute angle of each step was standardized so that 0 and π were aligned with the main road bearing (N4). Thus, road angles of 0 or π represent movement steps parallel to the road, π /2 represent movement steps toward the road and - π /2 represent movement steps away from the road (adapted from Brown et al. 2006). Each step was then classified as 1 (parallel to the road) or 0 (moves away or towards the road). Step direction in relation to the road is also used to describe movement patterns as it expresses the species movement near the road, under a potential constrain in the verge. In linear habitats, resources should distribute along the road direction, and thus the movements should be more frequent along the road when compared with perpendicular movements.

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, it was also registered the time at which the fix was taken, together with several variables describing microhabitat composition and structure (Annex, Table 1). A detailed digital elevation model (pixel: 1 x 1 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 it was 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 (Annex, Table 1).

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 (Annex, Table 1).

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. The variables concerning angles (relative angle and absolute angle) were handled with appropriate circular statistics or transformed with sine and cosine (Murray and St. Clair 2015). For the movement pattern analyses, the paths and steps with zero length were removed.

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) in order to assess differences between patches in space use parameters (Sokal and Rohlf 1997).

There were no effects of sex on home range (95% BRBK; W=36, p=0.5622), on core area (50% BRBK; W=28, p=0.8749), on number of core areas (No BRBK50; W=35, p=0.5870) and on shape complexity (W=46, p=0.0934) and therefore sexes were combined in space use analyses.

To assess the influence of explanatory variables (including the habitat patch and day period) in movement patterns, three mixed models were built. Voles' path length and path linearity index were modelled with Linear Mixed Models (LMM), while step direction (binary response) was modelled with Generalized Linear Mixed Model with binomial link (GLMM) (Zuur and Ieno 2016). The three response variables were modelled as a function of explanatory variables, with individual voles as a random intercept to avoid pseudo-replication among individuals (Zuur and Ieno 2016). Model selection was based on Akaike's Information Criterion (AIC; Burnham and Anderson 2002).

Previous to model building, the collinearity among explanatory variables was verified. Thus, for variable pairs showing high collinearity (Pearson correlation: $r > 0.7$), the one with strongest correlation with response variables was retained. In order 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 the produced 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 LMM or GLMM.

Mixed models showing an AIC within two units of the best model ($\Delta AIC<2$) were considered to have substantial support (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 a $\Delta AIC > 2$ relative to the Null model were considered valid.

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 globally for 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 the packages adehabitatHR, adehabitatLT, MuMIn, lme4 and nlme.

Results

General results

Not all collared voles were successfully tracked. Three batteries failed before the end of the study, one vole was predated by a snake, and another possibly removed the collar. However, it was decided to include in the analysis animals with at least a full 24-h period sampled (16 voles). The maximum number of voles tracked simultaneously was four. Radio-telemetry provided 3886 position fixes collected over 904h for 16 animals. Mean ± SE fixes per animal was 217.8 ± 48.3.

Cabrera voles showed home ranges (95% BRBK) between 175 and 815 m^2 (mean ± SD: 352 ± 163m^2). Core areas (50% BRBK) varied between 37 and 175 m^2 (mean ± SD: 62 ± 34 m^2).

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 ± SD: 5.8 ± 3.5 m), while path length (movement within 4h) varied between 3 and 94.8 m (mean ± SD: 27.4 ± 21.3 m).

Space use patterns

Voles from the Verge patch showed significant (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). Mean home range in the Meadow was 451 m^2, while in the Verge patch was 255 m^2. The extent of core areas (50%BRNK), the number of core areas (No BRBK50) and female spatial overlap (UDOI index) was not statistically different (P-value> 0.05) between patches (Table 2). These results seem to support the first hypothesis (i), that individuals from areas more encroached by roads have smaller home ranges and lower shape complexity.

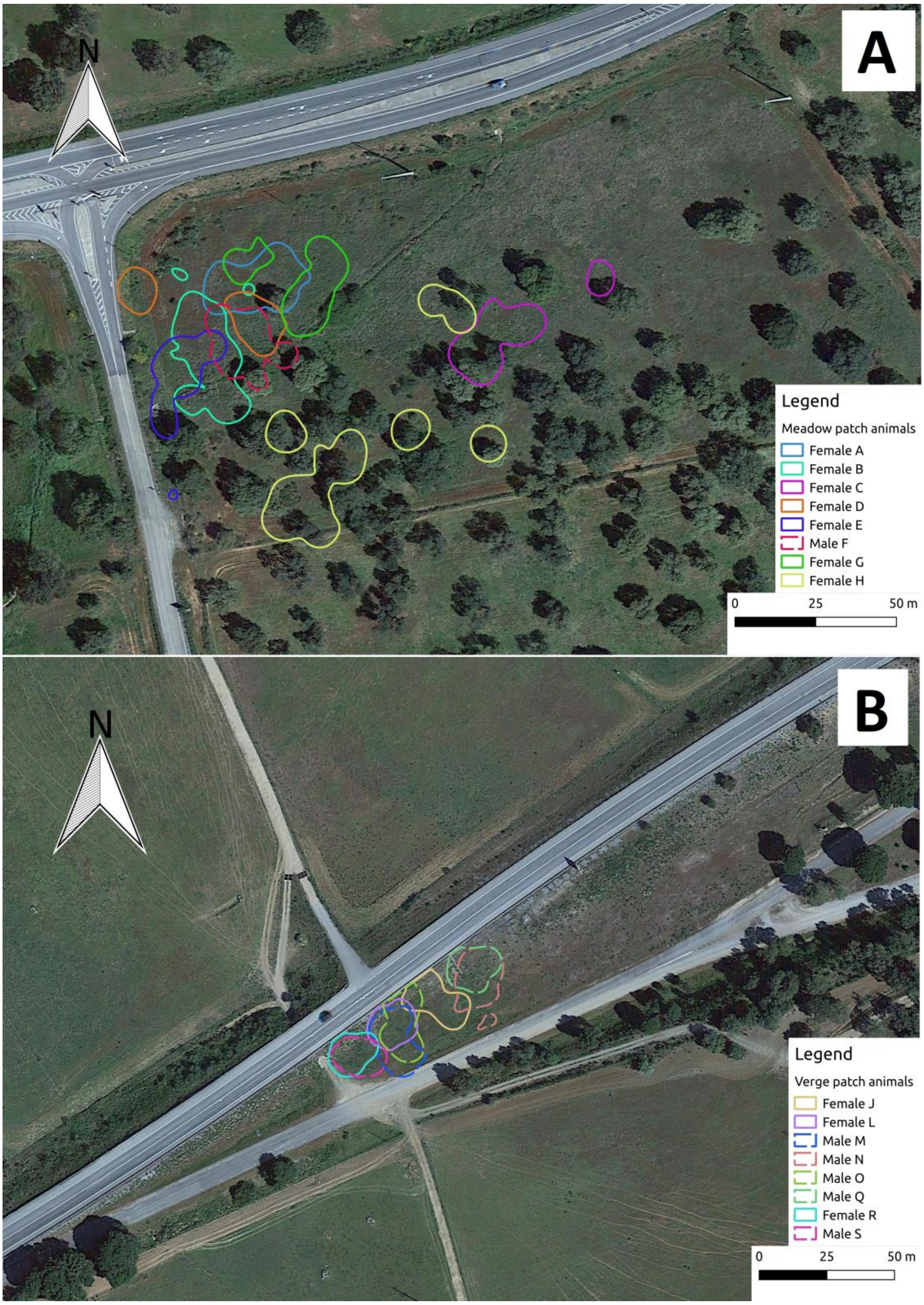

Figure 2: Homerange (BRB Kernel 95%) of each animal; Pannel A- Meadow patch; Pannel B- Verge patch.

Table 2: Summary results of Wilcoxon rank tests applied to the space use parameters of Cabrera voles with observed values for the two habitat patches (mean ± SD).

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

a) log transformed

b) square root transformed

Differences in movement patterns between patches

The length of movement paths was explained by a group of five models (ΔAICc<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 best model, there were differences in path length between day periods, according to the patch type: paths were longer for the period 5-9h when compared with 9-21h in Verge patch (but not in Meadow patch). This interaction effect is more noticeable between the periods 5-9h (dawn) and 17-21h (sunset; Figure 3; Annex, Table 3). In addition, paths were longer during lower ambient temperatures in both habitat patches.

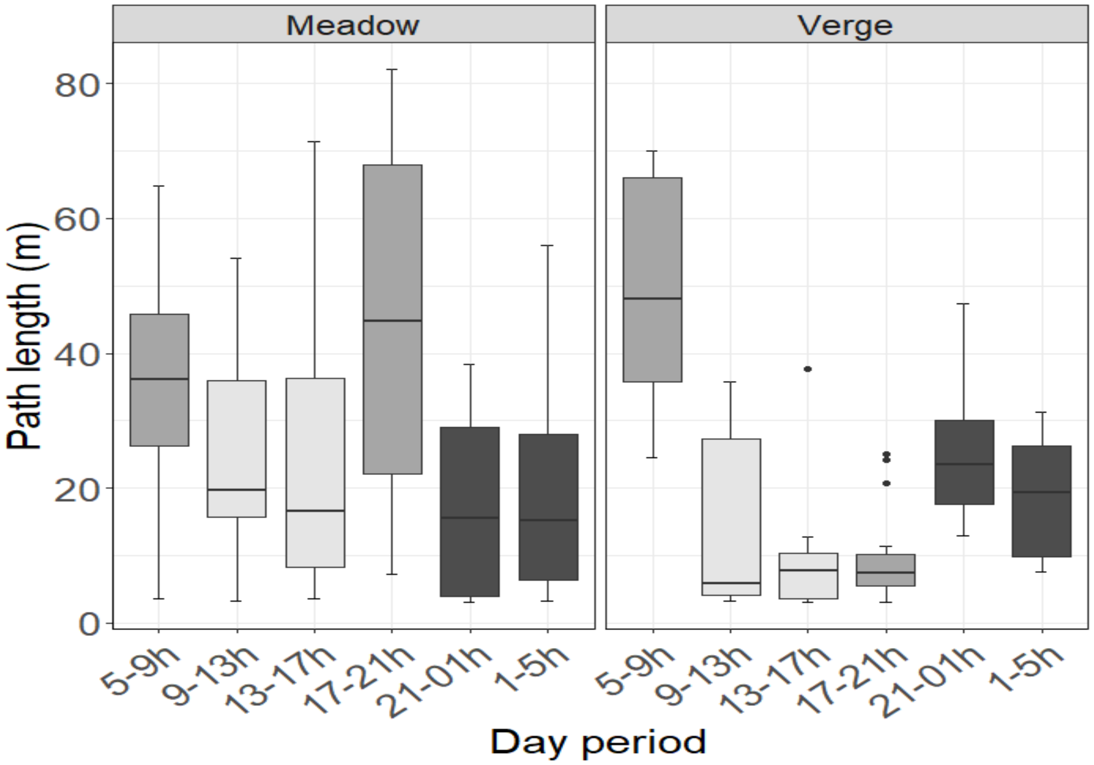

Figure 3: Variation of path length according to habitat patch (Meadow and Verge) and day period (dark grey: nocturnal periods; medium grey: crepuscular and dawn periods; light grey: diurnal periods).

None path linearity model was significant as none had a fit superior to the Null model (AIC=56.4), and then its results will not be discussed further.

Step directions were explained by a group of seven models (ΔAIC<2), the first model showing a weight of 0.23 and an AIC improvement relatively to the Null model of 2.5. (Null model AIC=913.5; best model AIC=911).

According to the best model, movement steps along the main road (parallel) were more frequent during the 5-9h period when compared with 13-17h period, and only in the Verge patch (Annex, Table 4; Figure 4).

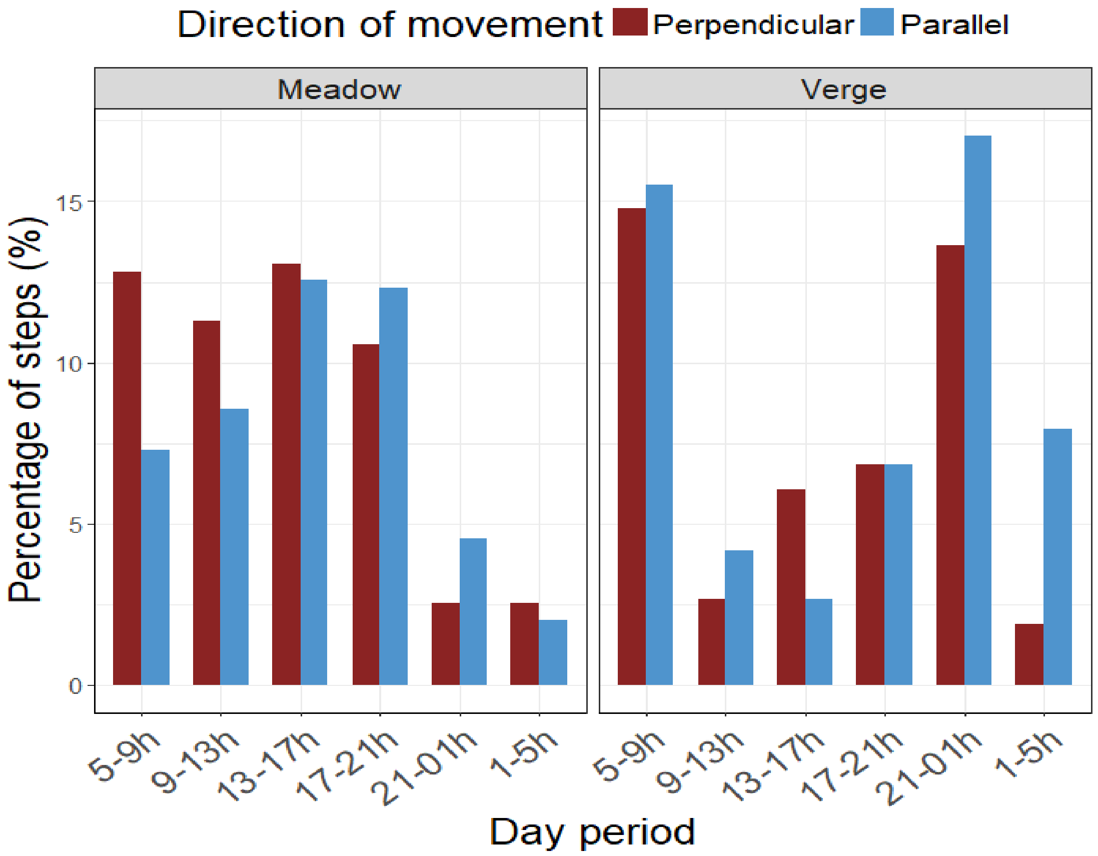

Figure 4: Variation of step direction according to habitat patch (Meadow and Verge) and day period.

The results from the mixed models did not support neither hypotheses that individuals in the more encroached area have shorter paths (hypothesis ii), more linear paths (hypothesis iii) and make movements more frequently along the road (hypothesis iv). These results suggest that movement patterns are not affected by the degree of road encroachment. Indeed, there were no significant differences in the three measured parameters (path length, path linearity and step direction) between the two habitat patches. However, there was an interaction effect between habitat patch and day period for path length and step direction, which partially supports the hypothesis that individuals in more encroached areas make less movements during high traffic periods (daytime) when compared with animals occupying areas less encroached by roads (hypothesis v). Path length along the different day periods differed between habitat patches, with a tendency for shorter paths during the 17-21h period in the Verge patch when compared with the same period in the Meadow patch (Figure 3). Moreover, although not significant, a high proportion of the diurnal period (9-21h) had shorter paths in the Verge patch, a pattern not registered in the Meadow patch. Regarding step direction, there was a higher prevalence of movements along the road (parallel steps) during the 5-9h period when compared with the 13-17h period in Verge patch, which was not observed in the Meadow patch.

Frequency of road crossing by animals

No crossing events were registered in any of the radio-tracked voles, neither in the Meadow nor the Verge patch.

It was verified that the global expected road crossing percentage in the Meadow patch is 10.2% (Pearson chi-square = 12.25; p-value=0.0005) while the expected value for the Verge patch is 54.2% (Pearson chi-square = 101.79; p-value=0.0000; Table 5). This shows that, for both habitat patches, the observed crossing rates were significantly lower than 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 5). These results do not support the hypothesis that individuals in more encroached areas cross the road more frequently than those in the less encroached area (hypothesis vi), as none of the individuals crossed the road during the study period. In addition animals from the Verge patch should have crossed the road more often when compared with voles from the Meadow patch, when considering the randomly generated data, suggesting a stronger barrier effect in the Verge patch.

Table 5: 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

When analyzing 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 6). 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 animal E (Pearson chi-square=4.49; p-value=0.034) which occupied a home range that included the road verge (Table 6). 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 animals (all p-values<0.05).

Table 6: 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

Discussion

Despite the existence of previous studies regarding the effect of roads on space use and movement patterns on different taxa (Shepard et al. 2008; Chen and Koprowski 2016b; Wilson et al. 2016), no previous study documented the road encroachment effect on *Microtus cabrerae*. The results support that there are differences in space use between patches (hypothesis I). Although there were no differences in movement patterns between patches (hypotheses ii, iii and iv), our results show that there are less movements in Verge patch during daytime (partially supporting hypothesis v); and that road crossings seem to be a rare event, which doesn't support hypothesis vi, but suggests the existence of a strong road-barrier effect for individuals living in road verges.

Differences in space use in the Verge patch

As expected individuals occupying the more encroached area (Verge patch) showed smaller home ranges with lower shape complexity than those in the less encroached 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 through different time and space (e.g. Madinson 1990, p25). This seems to indicate that road encroachment may hinder individual's home ranges, but the characteristics of core areas and social structure are maintained. This way, habitat encroachment may have forced the animals to increase the efficiency of resource use within a smaller area. According to the results of Rosário and collaborators (2008), it is possible that individuals occupying verge habitats learn to adjust their diet to the available plants. In line with this, other adjustments may be required for voles living in these habitats, which might reflect (or not) in the physical condition and reproduction parameters of those populations.

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. Since traffic intensity is higher during the day, animals in Verge patch may have decreased their path length in response to increased traffic as was observed by Chen and Koprowski (2016a) in Arizona (USA) with Mount Graham red squirrels (*Tamiasciurus hudsonicus grahamensis*). The higher proximity of animals to the road in the Verge patch, when compared to the Meadow patch, may explain the difference in path lengths between the two patches. By being restrained in the more encroached patch, voles may be forced to adapt their behavior by decreasing their activity during the day. There was also an influence of temperature on movement length, with longer path lengths 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. 2011b). This could explain why path length was shorter during the day 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 aerial diurnal predation (e.g., buzzards and kites that are frequently observed along the studied road).

Step directions were similar among the two habitat patches. This result might be expected because all estimated home ranges on the Verge patch were circle-shaped, and thus, a higher proportion of parallel versus other movement directions was not expected. Similarly to what was observed regarding path length, there was an interaction between the day period and habitat patch. The higher prevalence of parallel steps in the Verge patch during the sunrise when compared with the 13-17h period may be explained (as for path length) by daytime-related factors such as traffic, predation or temperature. Individuals may have avoided parallel movements during the day in order to avoid perturbation from road. This behavior was only expressed in the Verge patch, probably because animals in this area are closer to the road and consequently more exposed to it than animals in Meadow patch.

Due to their poor ability to move further away from the road, animals seem to have adapted their movement patterns in order to accommodate the exposure to the road disturbances. This way road encroachment appears to force the voles in this area to change their behaviour and daily activity. 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 period). This agrees with observations for mooses (*Alces alces*), which remain further away from roads during high traffic periods (daytime) (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 this 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 from the road than in Verge patch. As individuals in Verge patch are compressed in a more restricted area it is more likely that an expected path would cross the road, whereas in Meadow patch, by being further from the road, it is less likely. This could mean 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. Grilo et al. 2018), showing that roads can have influence on species 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 more uniform vegetation structure in the Verge, which may offer less protection against aerial diurnal predators, and therefore may promote a different response from the voles during certain periods of the day. Due the low number of habitat patches considered, the study only allows to assign these conclusions to the specific studied patches. More data is therefore required to verify if these conclusions hold true for this species in other regions of its occurrence and in different seasons. Despite these limitations, this study highlights the need to recognize in future studies the importance of road encroachment on the space use and movement patterns of this and other species that are often associated to road verge habitats.

Main Conclusions

The present study suggests that road encroachment may restrict Cabrera vole space use and movement patterns and even act as a barrier to vole road crossings in southern Portugal. Despite the extensive number of studies about the effect of roads on small mammals, none focused the Cabrera vole, an endangered species often living on road verges. 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 wide and unrestricted verges to 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. The present study also opens the possibility for further studies regarding the road effect on Cabrera voles, such as the effect of traffic and day periods on movement patterns and the distance to road on the species space use.

Acknowledgements

Prof. Joana Reis from Veterinary Hospital of Évora University (HVUE) gave invaluable support in anesthetizing and handling of the captured voles.

This study was supported by the projects POPCONNECT (PTDC/AAG-MAA/0372/2014) and LIFE LINES (LIFE14 NAT/PT/001081).

Bibliography

Almeida PJAL, Vieira M V., Kajin M, et al (2010) Indices of movement behaviour: conceptual background, effects of scale and location errors. Zool 27:674–680.

Ascensão F, Lucas PS, Costa A, Bager A (2017) The effect of roads on edge permeability and movement patterns for small mammals: a case study with Montane Akodont. Landsc Ecol 32:781–790.

Benhamou S (2011) Dynamic approach to space and habitat use based on biased random bridges. PLoS One 6.

Benhamou S (2004) How to reliably estimate the tortuosity of an animal's path: Straightness, sinuosity, or fractal dimension? J Theor Biol 229:209–220.

Benítez-López A, Alkemade R, Verweij PA (2010) The impacts of roads and other infrastructure on mammal and bird populations: A meta-analysis. Biol Conserv 143:1307–1316.

Bennett, A.F. (1998, 2003). Linkages in the Landscape: The Role of Corridors and Connectivity in Wildlife Conservation. IUCN, Gland,Switzerland and Cambridge, UK. xiv + 254 pp.

Bissonette JA, Rosa SA (2009) Road Zone Effects in Small-Mammal Communities. Ecol Soc 14.

Brown GP, Phillips BL, Webb JK, Shine R (2006) Toad on the road: Use of roads as dispersal corridors by cane toads (Bufo marinus) at an invasion front in tropical Australia. Biol

Conserv 133:88–94.

Burnham, K. P. and Anderson, D. R. (2002). Model selection and multimodel inference. A practical information-theoretic approach, 2nd ed. Springer.

Calenge C, Dray S, Royer-Carenzi M (2009) The concept of animals’ trajectories from a data analysis perspective. Ecol Inform 4:34–41.

Ceia-Hasse A, Borda-de-Água L, Grilo C, Pereira HM (2017) Global exposure of carnivores to roads. Glob Ecol Biogeogr 26:1–9.

Chen HL, Koprowski JL (2016a) Differential effects of roads and traffic on space use and movements of native forest-dependent and introduced edge-tolerant species. PLoS One 11:1–18.

Chen HL, Koprowski JL (2016b) Barrier effects of roads on an endangered forest obligate: Influences of traffic, road edges, and gaps. Biol Conserv 199:33–40.

Coffin AW (2007) From roadkill to road ecology: A review of the ecological effects of roads. J Transp Geogr 15:396–406.

Crooks KR, Burdett CL, Theobald DM, et al (2017) Quantification of habitat fragmentation reveals extinction risk in terrestrial mammals. Proc Natl Acad Sci.

Edelhoff H, Signer J, Balkenhol N (2016) Path segmentation for beginners: an overview of current methods for detecting changes in animal movement patterns. Mov Ecol 4:1–21.

EP (2005) Recenseamento do tráfego – É vora. Estradas de Portugal, E.P.E

Erlinge S, Hoogenboom I, Agrell J, et al (1990) Density-Related Home-Range Size and Overlap in Adult Field Voles (Microtus agrestis) in Southern Sweden. J Mammal 71:597–603.

Fahrig L, Rytwinski T (2009) Effects of roads on animal abundance: An empirical review and synthesis. Ecol Soc 14.

Fernández-Salvador R (2007) Microtus cabrerae. Atlas y Libr Rojo los Mamíferos Terr España 112:429–433.

Fernández-Salvador R (1998) Topillo de Cabrera, *Microtus cabrerae* THOMAS, 1906. Galemys 3:5–18.

Fernández-Salvador R, García-Perea R, Ventura J (2001) Reproduction and postnatal growth of the Cabrera vole, *Microtus cabrerae*, in captivity. Can J Zool 79:2080–2085.

Fernández-Salvador R, García-Perea R, Ventura J (2005) Effect of climatic fluctuations on body mass of a Mediterranean vole, Microtus cabrerae. Mamm Biol 70:73–83.

Fieberg J, Kochanny CO (2005) Quanitfying home-range overlap: the importance of the utilization distribution. J Wildl Manage 69:1346.

Ford RG (1983) Home Range in a Patchy Environment : Optimal. Baseline 326:315–326

Forman RTT (2000) Estimate System in of the the Area United Afected States Ecologically by the Road. Conserv Biol 14:31–35.

Forman RTT, Alexander LE (1998) Roads and Their Major Ecological Effects. Annu Rev Ecol Syst 29:207–231.

Forman RTT, Sperling D, Bissonette JA, et al (2003) Road ecology - Science and solutions. Island Press, Washington, DC

Frère CH, Krützen M, Mann J, et al (2010) Home range overlap, matrilineal and biparental kinship drive female associations in bottlenose dolphins. Anim Behav 80:481–486.

Galantinho A, Eufrázio S, Silva C, et al (2017) Road effects on demographic traits of small mammal populations. Eur J Wildl Res 63:22.

Gannon WL, Sikes RS, Animal Care and Use Committee of the American Society of Mammalogists (2007) Guidelines of the American Society of Mammalogists for the use of wild mammals in research. J Mammal 88:235–253.

Getz L (1961) Home Ranges, Territoriality, and Movement of the Meadow Vole. J Mammal 42:24–36

Grilo C, Molina-Vacas G, Fernández-Aguilar X, et al (2018) Species-specific movement traits and specialization determine the spatial responses of small mammals towards roads. Landsc Urban Plan 169:199–207.

Hiller TL, Beringer J, Belant JL (2016) Shape complexity of space used by American black bears influenced by sex and intensity of use. Basic Appl Ecol 18:67–74.

IPMA, 2017. Instituto Português do Mar e da Atmosfera, IP, Portugal. Normais climáticas/Évora 1981-2000. Available: http://www.ipma.pt (accessed 27.08.18).

Kareiva PM, Shigesada N (1983) Analysing insect movement as a correlated random walk. Oecologia 56:234–238

Madinson, D. M. (1990). Social organization modes in models of microtine cycling. In R. H. Tamarin, R. S. Ostfeld, S. R. Pugh, & G. Bujalska (Eds.), *Social Systems and Population Cycles in Voles* (1st ed., pp. 25–34). Springer.

Madison DM (1980) Space use and social structure in meadow voles, *Microtus pennsylvanicus*. Behav Ecol Sociobiol 7:65–71.

McDonald WR, St Clair CC (2004) The effects of artificial and natural barriers on the movement of small mammals in Banff National Park, Canada. Oikos 105:397–407.

McGregor RL, Bender DJ, Fahrig L (2008) Do small mammals avoid roads because of the traffic? J Appl Ecol 45:117–123.

Medinas D, Ribeiro V, Marques JT, et al (2019) Road effects on bat activity depend on surrounding habitat type. Sci Total Environ 660:340–347.

Millspaugh JJ, Nielson RM, McDonald L, et al (2006) Analysis of Resource Selection Using Utilization Distributions. J Wildl Manage 70:384–395.

Mitchell MS, Powell RA (2012) Foraging optimally for home ranges. J Mammal 93:917–928.

Mladenoff DJ, Sickley T a, Wydeven AP (1999) Predicting Gray Wolf Landscape Recolonization : Logistic Regression Models vs . New Field Data. 9:37–44

Morelli F, Beim M, Jerzak L, et al (2014) Can roads, railways and related structures have positive effects on birds? - A review. Transp Res Part D Transp Environ 30:21–31.

Murray MH, St. Clair CC (2015) Individual flexibility in nocturnal activity reduces risk of road mortality for an urban carnivore. Behav Ecol 26:1520–1527.

Neumann W, Ericsson G, Dettki H, Radeloff VC (2013) Behavioural response to infrastructure of wildlife adapted to natural disturbances. Landsc Urban Plan 114:9–27.

Ostfeld R, Lidicker Jr W, Heske E (1985) The relationship between habitat heterogeneity, space use, and demography in a population of California voles. Oikos 45:433–442.

Pinto Correia T, Ribeiro N, Sá Sousa P (2011) Introducing the montado , the cork and holm oak agroforestry system of Southern Portugal. Agroforest Syst 99–104.

Pita R, Beja P, Mira A (2007) Spatial population structure of the Cabrera vole in Mediterranean farmland: The relative role of patch and matrix effects. Biol Conserv 134:383–392.

Pita R, Mira A, Beja P (2011a) Assessing habitat differentiation between coexisting species: The role of spatial scale. Acta Oecologica 37:124–132.

Pita R, Mira A, Beja P (2010) Spatial segregation of two vole species (*Arvicola sapidus* and *Microtus cabrerae*) within habitat patches in a highly fragmented farmland landscape. Eur J Wildl Res 56:651–662.

Pita R, Mira A, Beja P (2014) *Microtus cabrerae* (Rodentia: Cricetidae). Mamm Species 912:48–70.

Pita R, Mira A, Beja P (2006) Conserving the Cabrera vole, *Microtus cabrerae*, in intensively used Mediterranean landscapes. Agric Ecosyst Environ 115:1–5.

Pita R, Mira A, Beja P (2011b) Circadian activity rhythms in relation to season, sex and interspecific interactions in two Mediterranean voles. Anim Behav 81:1023–1030.

Proença-Ferreira A, Ferreira C, Leitão I, et al (2019) Drivers of survival in a small mammal of conservation concern: An assessment using extensive genetic non-invasive sampling in fragmented farmland. Biol Conserv 230:131–140.

Queiroz AI (coord.), Alves PC, Barroso I, Beja P, Fernandes M, Freitas L, Mathias ML, Mira A, Palmeirim JM, Prieto R, Rainho A, Rodrigues L, Santos-Reis M, Sequeira M (2005). Microtus cabrerae *Rato de Cabrera* Pp 481-482 in Livro Vermelho dos Vertebrados de

Portugal (Cabral MJ et al. (eds.)). Instituto da Conservação da Natureza. Lisboa.

R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Redon L, Le Viol I, Jiguet F, et al (2015) Road network in an agrarian landscape: Potential habitat, corridor or barrier for small mammals? Acta Oecologica 62:58–65.

Rico A, Kindlmann P, Sedláček F (2007) Barrier effects of roads on movements of small mammals. Folia Zool 56:1–12

Roedenbeck, I.A., Fahrig, L., Findlay, C.S., et al (2007) The Rauischholzhausen agenda for road ecology. Ecol Soc 12:.

Rondinini C, Doncaster CP (2002) Roads as barriers to movement for hedgehogs. Funct Ecol 16:504–509.

Rosário IT, Cardoso PE, Mathias M da L (2008) Is habitat selection by the Cabrera vole (*Microtus cabrerae*) related to food preferences? Mamm Biol 73:423–429.

Rytwinski T, Fahrig L (2012) Do species life history traits explain population responses to roads? A meta-analysis. Biol Conserv 147:87–98.

Sabino-Marques H, Mira A (2011) Living on the verge: Are roads a more suitable refuge for small mammals than streams in Mediterranean pastureland? Ecol Res 26:277–287.

Santos SM, da Luz Mathias M, Mira AP (2010) Local coexistence and niche differences between the Lusitanian and Mediterranean pine voles (*Microtus lusitanicus* and *M. duodecimcostatus*). Ecol Res 25:1019–1031.

Santos SM, Do Rosário IT, Mathias ML (2005) Microhabitat preference of the Cabrera vole in a Mediterranean cork oak woodland of southern Portugal. Vie Milieu 55:53–59

Santos SM, Mathias MDL, Mira A, Simões MP (2007) Vegetation structure and composition of road verge and meadow sites colonized by Cabrera vole (*Microtus cabrerae* Thomas). Polish J Ecol 55:481–493

Seiler A (2001) Ecological effects of roads: a review. Introd. Res. essay 9:1–40

Shepard DB, Kuhns AR, Dreslik MJ, Phillips CA (2008) Roads as barriers to animal movement in fragmented landscapes. Anim Conserv 11:288–296.

Sokal R, Rohlf FJ (1997) Biometry, 3rd editio. San Francisco

Stumpf, W. A., & Mohr, C. O. (1962). Linearity of Home Ranges of California Mice and Other Animals. Journal of Wildlife Management, 26(2), 149–154.

Taitt MJ (1981) The Effect of Extra Food on Small Rodent Populations: I. Deermice (*Peromyscus Maniculatus*). J Anim Ecol 50:111–124

Taitt MJ, Krebs CJ (1981) The Effect of Extra Food on Small Rodent Populations: II. Voles

(*Microtus townsendii*). Source J Anim Ecol J Anim Ecol 50:125–137.

Taylor PPD, Fahrig L, With KK a (2006) Landscape connectivity: a return to the basics. Connect Conserv 29–43.

Trombulak SC, Frissell CA (2000) Review of ecological effects of roads on terrestrial and aquatic communities. Conserv Biol 14:18–30.

Van Dyke FG, Brocke RH, Shaw HG (1986) Use of road track counts as indices. J Wildl Manage 50:102–109

Ventura J, Lopez-Fuster MJ, Cabrera-Millet M (1998) The Cabrera vole, *Microtus cabrerae*, in Spain: A biological and a morphometric approach. Netherlands J Zool 48:83–100.

Way JM (1977) Roadside verges and conservation in Britain: A review. Biol Conserv 12:65–74.

White GC, Garrott RA (1990) Analysis of Wildlife Radio-Tracking Data., 1st Editio. San Diego

Wilson RF, Marsh H, Winter J (2007) Importance of canopy connectivity for home range and movements of the rainforest arboreal ringtail possum (*Hemibelideus lemuroides*). Wildl Res 34:177–184.

Wilson RR, Parrett LS, Joly K, Dau JR (2016) Effects of roads on individual caribou movements during migration. Biol Conserv 195:2–8.

Zuur AF, Ieno EN (2016) A protocol for conducting and presenting results of regression-type analyses. Methods Ecol Evol 7:636–645.

Final considerations

The present study assessed the effect of road encroachment on space use and movement patterns of Cabrera vole. Due to its restricted habitat requirements, the decline of many populations across its range, and its vulnerability to habitat fragmentation, it is important to understand the impacts an ever growing road system may have on the Cabrera vole. While other authors have already described the effects of roads on small mammals (e.g. Ascensão et al 2017; Grilo et al 2018; Galantinho et al 2017), no authors have studied the effect of roads on the Cabrera vole despite its conservation value.

The present study supports that road encroachment has a negative effect on Cabrera vole space use as well as movement patterns. However it is imperative that the mechanisms behind this effect be further explored and understood, if effective conservation measures are to be taken.

Bibliography

Ascensão F, Lucas PS, Costa A, Bager A (2017) The effect of roads on edge permeability and movement patterns for small mammals: a case study with Montane Akodont. Landsc Ecol 32:781–790.

Galantinho A, Eufrázio S, Silva C, et al (2017) Road effects on demographic traits of small mammal populations. Eur J Wildl Res 63:22.

Grilo C, Molina-Vacas G, Fernández-Aguilar X, et al (2018) Species-specific movement traits and specialization determine the spatial responses of small mammals towards roads. Landsc Urban Plan 169:199–207.

Annex

Table 2: List and description of explanatory variables used in movement pattern analyses (Name: designation of variable; Description: brief explanation of variable meaning with units and defined classes; Step: indication of whether the variable was obtained for each movement step; Path: indication of whether the variable was obtained or summarized for each movement path).

Name	**Description**	**Step**	**Path**
patch	Binary classification of vole patch habitat (Meadow or Verge)	X	X
day period	Classification of period of the day (1: 05-09h; 2: 09-13h; 3: 13-17h; 4: 17-21h; 5: 21-01h; 6: 01-05h)	X	X
sex	Sex of each radio-tracked individual (Female or Male)	X	X
microhabitat	Classification of vegetation structure at each position fix (herb: herbaceous strata only; arb: shrub strata only; arb_herb: shrub and herbaceous strata; arv_arb: tree and shrub strata; arv_arb_herb: all three strata present)	X	
herb.cover	Classification of herbaceous cover at each position fix (1: < 50%; 2: 50-90%; 3: >90%)	X	
topo_dev	Deviation from the lowest elevation value in the habitat patch (m) (calculated from a MDT obtained for the studied patches)	X	
temp	Hourly mean air temperature (ºC)	X	
humidity	Hourly mean relative air humidity (%)	X	
rain	Hourly total rainfall (mm)	X	
herb	Proportion of steps in a path classified in herbaceous cover		X
arb	Proportion of steps in a path classified in shrub cover		X
arb_herb	Proportion of steps in a path classified in mixed shrub and herbaceous cover		X
arv_arb	Proportion of steps in a path classified in mixed tree		X

	and shrub cover		
arv_arb_herb	Proportion of steps in a path classified in mixed tree, shrub and herbaceous cover		X
mmicro	Mode of microhabitat class registered for each path		X
cherb1	Proportion of steps in a path classified as <50% herbaceous cover		X
cherb2	Proportion of steps in a path classified as 50-90% herbaceous cover		X
cherb3	Proportion of steps in a path classified as >90% herbaceous cover		X
mcobh	Mode of herbaceous cover class registered for each path		X
mtopo_dev	Average deviation from the lowest elevation value for each path (m)		X
mtemp	Average air temperature (ºC) registered during a path		X
mhum	Average relative air humidity (%) registered during a path		X
mrain	Average rainfall (mm) registered during a path		X

Table 3: Averaged coefficients, confidence intervals, P value and Importance of each variable in the group of five models (ΔAICc<2) for Path length.

	Coefficient	2.5% CI	97.5% CI	Z value	P value	Importance
intercept	3.508	2.602	4.413	7.593	0.000	-
patch	0.627	-0.007	1.261	1.937	0.053	1
mtemp	-0.056	-0.091	-0.020	3.068	0.002	0.98
day period (2)	1.378	0.152	2.604	2.203	0.028	1
day period (3)	1.213	0.125	2.302	2.186	0.029	
day period (4)	2.660	1.448	3.871	4.302	0.000	
day period (5)	-0.768	-1.936	0.399	1.289	0.197	
day period (6)	-0.280	-1.827	1.266	0.355	0.722	
patch : day period (2)	-1.269	-2.080	-0.458	3.066	0.002	1
patch : day period (3)	-1.049	-1.771	-0.326	2.846	0.004	
patch : day period (4)	-1.980	-2.713	-1.246	5.288	0.000	
patch : day period (5)	0.120	-0.606	0.846	0.324	0.746	
patch : day period (6)	-0.402754	-1.362	0.557	0.823	0.411	
Sex	0.111	-0.195	0.416	0.710	0.478	0.34

Herb	-0.128	-0.538	0.282	0.612	0.540	0.31
cherb3	0.032	-0.308	0.373	0.185	0.853	0.27
mtopo_dev	-0.009	-0.179	0.161	0.102	0.918	0.40

Table 4: Averaged coefficients, confidence intervals, P value and Importance of each variable in the group of seven models (ΔAICc<2) for Step Direction.

	Coefficient	2.5% CI	97.5% CI	Z value	P value	Importance
intercept	-1.406	-2.708	-0.104	2.117	0.034	-
Patch	0.612	-0.164	1.389	1.545	0.122	0.77
day period (2)	-0.015	-1.825	1.795	0.016	0.987	0.79
day period (3)	1.728	0.062	3.393	2.033	0.042	
day period (4)	1.416	-0.180	3.012	1.739	0.082	
day period (5)	2.180	0.227	4.133	2.188	0.029	
day period (6)	-0.759	-3.168	1.649	0.618	0.537	
patch : day period (2)	0.189	-1.098	1.475	0.288	0.774	0.59
patch : day period (3)	-1.449	-2.665	-0.232	2.335	0.02	
patch : day period (4)	-0.809	-1.848	0.229	1.528	0.127	
patch : day period (5)	-1.042	-2.161	0.076	1.827	0.068	
patch : day period (6)	1.062	-0.476	2.599	1.353	0.176	
Temp	0.034	-0.016	0.085	1.336	0.182	0.41
microhabitat (2)	-0.052	-0.519	0.416	0.217	0.828	0.39
microhabitat (3)	0.405	-0.168	0.978	1.385	0.166	
topo_dev	0.131	-0.146	0.407	0.926	0.354	0.42
herb.cover	-0.018	-0.349	0.313	0.107	0.914	0.30