Instituto de Investigação e Formação Avançada

Persistence and Coexistence of Spatially Structured Populations in Heterogeneous Environments:

The case of Cabrera and water voles in Mediterranean farmland

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Declaração do autor

Em conformidade com o Regulamento do Ciclo de Estudos conducente ao Grau de Doutor pela Universidade de Évora (Ordem de Serviço N.º1/2010), esta tese integra um conjunto de quatro trabalhos de investigação, dos quais dois estão já publicados e dois estão submetidos para publicação (todos em revistas com *peer-review RiR*(ISI)). O autor esclarece que os trabalhos referidos foram realizados em colaboração, e que em todos eles liderou e participou activamente na sua concepção, recolha, análise e discussão de resultados, bem como na escrita dos artigos. Algumas das normas relativas ao padrão de formatação de cada revista foram retidas nesta tese.

Sines, 15 de Dezembro de 2010

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Abstract

This thesis investigates how animal populations respond to environmental heterogeneity, by analysing its importance for the persistence of Cabrera voles in highly fragmented landscapes, and for the coexistence of this species with a potentially dominant competitor (the water vole). The occurrence of Cabrera voles was partly explained by the patch size and isolation paradigms of metapopulation theory, though patch persistence and the composition of the intervening matrix also appeared to be critical for species persistence. Coexistence of Cabrera and water voles could be reasonably explained by spatial segregation within patches, probably reflecting microhabitat differentiation across multiple spatial scales. There was also some time partitioning between vole species, but this was probably a secondary mechanism favouring their coexistence. Overall, results suggest that measurements on heterogeneity at multiple spatial and temporal scales may be essential to properly assess the persistence and coexistence of ecologically similar species.

Word count: 144

Resumo

Esta tese avalia o modo como as populações animais respondem à heterogeneidade ambiental analisando a sua importância para a persistência do rato de Cabrera (RC) em paisagens fragmentadas, e para a coexistência desta espécie com o potencial competidor dominante rato-de-água (RA). A ocorrência do RC foi parcialmente explicada pelo tamanho e isolamento das parcelas de habitat, de acordo com a teoria das metapopulações, embora a persistência dos fragmentos e a composição da matriz sejam tambem críticos para a persistência da espécie. A coexistência de RC e RA pode ser explicada pela segregação espacial dentro das parcelas de habitat, provavelmente devido à diferenciação do microhabitat a multiplas escalas. A partição temporal a escalas finas poderá igualmente favorecer a coexistencia, embora provavelmente como um mecanismo secundário. Globalmente, este estudo sugere que a análise da heterogeneidade a múltiplas escalas será essencial para avaliar a persistência e coexistência de espécies ecologicamente semelhantes.

Número de palavras: 150

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CHAPTER 1

General Introduction

1. General Introduction

1.1 Responses of wildlife populations to environmental heterogeneity

1.1.1 Evolutionary mechanisms and sources of heterogeneity

Ecologists have long recognised that ecosystems are distributed heterogeneously across landscapes and that resources vary temporally within and among ecosystems (Rhodes and Odum 1996). Understanding how heterogeneity affects ecological systems thus requires an understanding of how organisms respond to the structural and functional discontinuities in space and time (Reynolds and Wu 1999; Gómez et al. 2004). In the ecological context, spatial heterogeneity may be broadly defined as the variations in composition and configuration of habitat patches across space, while temporal heterogeneity refers to the different values taken by a variable in a single point of space as a function of time (Lévêque 2003). Although the spatial and temporal dimensions of heterogeneity have generally been considered separately (Wiens 2000), there is a close relationship between them because, for instance, temporal variations in disturbance events may create patterns of heterogeneity in space (Rhodes and Odum 1996).

Both spatial and temporal heterogeneity of ecosystems imply that wildlife populations must deal with environmental uncertainty and that individual species should have evolved dynamic means of population organization to respond to the variations in their environment throughout their evolutionary trajectories (Rhodes and Odum 1996; Wiens 2000). Evolutionary mechanisms or strategies designed to deal with environmental heterogeneity may involve physiological, morphological, behavioural and demographical adaptations to survive in spatially partitioned resources at different points in time (Rhodes and Odum 1996). Adaptations providing strong selective base over many generations may alter key life-history and ecological traits of species, including spatial organization of populations, habitat selection, dispersal movements, breeding strategies and activity patterns (Wiens 2000). Since life-history adaptations are a result of evolution, it is implicit that populations may be unable to respond to accelerated rates of environmental change (Rhodes and Odum 1996). In this context, the combined effects of spatial and temporal uncertainty upon organisms and ecological processes, has received increased attention in population biology and conservation management (Rhodes and Odum 1996; Wiens 2000).

1.1.2 Scaling issues

One critical aspect to understand the myriad of species adaptations refers to the range of spatial and temporal scales at which individuals and populations respond to environmental heterogeneity (Boyce 2006; Meyer and Thuiller 2006; Meyer 2007). In order to understand how organisms will respond to heterogeneity, and thus to predict the ecological consequences of heterogeneity, we must consider the behavioural mechanism that mediate organisms responses, including movement, patch choice and perceptual scale (Wiens 2000). It is now widely accepted that the scales at which heterogeneity is perceived by organisms are multiple, depending on the species, on the organisation level of interest and on the particular ecological processes under study (Stewart et al. 2000). Environmental heterogeneity thus operates at nested spatial scales, each dictating particular spatial patterns of the species, from individual fine-scale habitat use to population geographical distribution ranges (Stewart et al. 2000). Similarly, responses to temporal heterogeneity depend on the time scale over which environmental changes operate (Stewart et al. 2000), determining the amplitudes to which animals can expect to experience a range of temporal conditions within a single foraging bout, within an individual's life-time, or among generations of the population (Brown 2000).

The primary scaling factors affecting measurements of heterogeneity are associated to the concepts of grain and extent (Li and Reynolds 1995). While grain is the finest resolution of data (e.g. minimum mapping unit in polygon vector-based data, or minimum time step for time series data), extent refers to the area or duration encompassed by a study (Li and Reynolds 1995; Meyer and Thuiller 2006). Quantification of heterogeneity depends on the sampling scheme used and is largely dictated by the nature of the behavioural process under study (Li and Reynolds 1995). Large-scale studies (e.g. species distribution ranges or long-term population trends) are usually carried-out over large extents and low resolutions, while small-scale studies (e.g.

microhabitat selection or circadian changes in behaviour) are typically conducted at small extents and high resolutions (Wiens 1989).

1.1.3 Heterogeneity-biodiversity relationships

The importance of environmental heterogeneity in controlling biodiversity is widely recognized in ecological research (Harris et al. 1996). Many theoretical studies suggest that environmental heterogeneity generates diversity (e.g. Ricklefs 1977; Kotler and Brown 1988; Cardiinale et al. 2000; Keller et al 2009) by allowing coexistence of close related and potentially competing species through resource partitioning (Stewart et al. 2000; Tylianakis et al. 2008). A mechanism of coexistence requires an axis of environmental heterogeneity and an appropriate trade-off among the species exploring or tolerating the axis. The trade-off requires that each species has some region along the axis such that it is better than the other species at exploiting or tolerating the region (Brown 2000). This implies that a more heterogeneous environment provides more ecological opportunities for more species than a less heterogeneous environment (Dornelas et al. 2009). Environmental heterogeneity across space and time is thus critical to the evolution and maintenance of biodiversity (Tylianakis et al. 2008; Griffin et al. 2009).

While many species may have evolved mechanisms for dealing with natural heterogeneity, human-induced heterogeneity at the landscape and local levels has often severe consequences for the long term persistence and coexistence of many species, particularly for those showing high habitat specialization and limited dispersal abilities (Stewart et al. 2000). Anthropogenic disturbances associated with resource extraction and land use development are increasingly the dominant forms of landscape disturbance. Rather than contributing to spatial heterogeneity, however, these activities often tend to homogenize landscape patterns (Wiens 2000), thereby reducing the likelihood of species coexistence and hence overall biodiversity patterns (Brock et al 2010). On the other hand, habitat loss and fragmentation in human-dominated landscapes may reduce the chances of species persistence if the scales of habitat fragmentation are larger than the scales of heterogeneity to which organisms are able to respond (Stewart et al. 2000). There are many examples in the literature showing that when the rates of habitat change due to human activities are higher than the rates of evolution by a species, the likelihood of populations' persistence may decrease severely (e.g. Donald et al. 2001; Lutolf et al. 2009; Féon et al. 2010).

Given the accelerated rates of habitat destruction at a global scale, there is an urgent need for conservation biologists to provide insights on how such large scale anthropogenic heterogeneity will affect wildlife populations (Stewart et al. 2000).

1.2 Heterogeneity in multi-patch farmland landscapes

1.2.1 Effects on species persistence and coexistence

Farmland landscapes with managed fields separated by semi-natural field margins may comprise highly heterogeneous mosaics of habitat types both on space and time. Indeed, conventional agricultural management practices impose periodic, and often severe, perturbations upon the spatial structure of resources in the form of ploughing, mowing or grazing by livestock, and often involving application of nutrients, herbicides and pesticides (Stewart et al. 2000). Therefore, farmland landscapes and species living therein provide suitable study systems to examine the effects of environmental heterogeneity in ecological space and time (Stewart et al. 2000). In addition, because recent developments in agriculture have considerably modified the patterns of spatial and temporal heterogeneity within landscapes and habitats worldwide (Wiens 2000), farmland areas may provide opportunities for testing the effects of habitat fragmentation and homogenisation processes on species persistence and coexistence.

Most important processes of environmental change in farmland landscapes are probably related with the expansion and intensification of agricultural production, reducing the quality and quantity of favourable habitat patches (Benton et al. 2003). As a consequence, most wildlife populations inhabiting heterogeneous agricultural landscapes are spatially structured, occurring within discrete marginal habitat patches amid a matrix of grazed and cultivated land (Gilpin 1996). Recognition of spatial structure leads to differentiation between landscape (among patches) and local (within-patch) processes reflecting different behavioural mechanisms, such as patch choice and patch use (Mouquet et al. 2005). It is the interplay between local and landscape population processes that determines the persistence ability by species in farmland areas. In addition, fragmentation and loss of habitat-patches due to agriculture intensification may reduce the likelihood of species persistence both at the local and landscape scales (Fahrig 2003; 2007).

Besides, because contemporary agricultural development is often associated with homogenization processes, at least at fine to intermediate scales (Wiens 2000), coexistence of farmland species at local and landscape scales is less likely in intensively managed areas. Indeed, by reducing the range of spatial variation available for species to differentiate, farmland homogenization reduces the chances of resource partitioning and niche segregation, which may contribute to explain the loss of biodiversity observed in many farmland areas (Benton et al. 2003).

1.2.2 Theoretical approaches to assess persistence and coexistence of spatially structured populations

1.2.2.1 Metapopulation theory and landscape ecology

Most studies aiming to describe the responses of spatially structured populations to habitat loss and fragmentation have emphasised the under-utilisation by many species of available high quality resources (e.g. Hokit et al. 2001) as a result from species inability to find those resources or to fully exploit them (Danielson and Anderson 1999). In this context, the metapopulation theory has brought an important contribution to the conservation of many species living in human dominated landscapes, mainly for its ability to act as a conceptual tool, even for patchy populations that deviate from the classical metapopulation models (e.g. Telfer et al. 2001). Metapopulation theory has its foundation in Levins's (1969) thinking of a metapopulation as a set of unstable local populations inhabiting discrete habitat patches, in the same sense in which a local population is a population consisting of individuals (Hanski 1999). Levins's model enclosed the essence of metapopulation-level persistence through the balance between local extinctions and the establishment of new populations in unoccupied sites, i.e. (re)colonisations (Hanski 1998). A key feature of classical metapopulation current thinking is that local populations interact via dispersing individuals among local populations, and that not all suitable habitats are necessarily occupied simultaneously because for instance small populations are more prone to extinction and isolated habitat patches have low colonization probabilities (Hanski 1999). It is the balance of local extinctions and colonisations that enhances the long term persistence of a species at the landscape level (Hanski 1999)

Although the metapopulation concept may be quite valuable to reduce or summarize the complexity of many ecological systems, classic metapopulation models, such as incidence function models (Hanski 1999) overemphasise the importance of size and isolation of habitat-patches on the rates of local occupancy (e.g. Hanski 1994; Moilanen 1999; Ovaskainen and Hanski 2004). In addition, metapopulation studies typically assume an environment consisting of permanent patches of suitable habitat surrounded by uniformly unsuitable habitat (the matrix) in which animals cannot survive (McCullough 1996; Hanski 1999). In contrast, landscape ecology studies consider the spatio-temporal heterogeneity of the matrix, which is viewed as a complex and dynamic mosaic of physical structures (McCullough 1996; Hanski 1999). While in classical metapopulation models the only cost involved in travelling through the matrix between patches is associated with interpatch distance, landscape ecology has shown that the structure and composition of the matrix might as well have a major influence on the viability of local sub-populations, by ruling the dispersal ability of species and hence the rates of colonisations and extinctions of habitat patches. Recognition of the importance of landscape heterogeneity in metapopulation persistence has highlighted the need to extend classical metapopulation models in order to incorporate environmental heterogeneity (e.g. Verheyen et al. 2004). Therefore, for spatially structured populations, either acting as classical metapopulations or not, the assessment of their regional persistence ability should proceed from predictive models accounting for the importance of both the patch features (local scale or within-patch level) and the landscape context and dynamics (regional scale or multi-patch level) (e.g. Sjogren-Gulve and Ray, 1996). This is probably even more relevant for agricultural landscapes, where cumulative agricultural changes are expected to severely interfere with both the availability and the accessibility of habitat-patches for species.

1.2.2.2 Ecological niche theory and trade-off mediated coexistence

Since the concept of ecological niche first appeared (Hutchinson 1957), the niche theory and the competition theory have been closely associated. According to the competitive exclusion principle (Hardin 1960), potentially competing species can only coexist if they occupy different realized niches. In the context of spatially structured populations, coexistence of ecologically similar species has been mostly explained by some form of spatial niche partitioning involving interspecific differences in competitive and colonisation abilities (e.g. Mouquet et al. 2005; Cadotte et al. 2006). The competition-colonization trade-off hypothesis states that superior competitors are dispersal-limited, while inferior competitors have higher colonization rates (Amarasekare 2003). This hypothesis has received great attention within the metacommunity framework, particularly for patch occupancy models developed under the 'patch-dynamics' perspective, i.e. based on classical metapopulation thinking (Mouquet et al. 2005). However, it assumes that when local competitive dynamics occur at higher rates than regional processes, local coexistence is impossible, a condition that may not hold in dynamic landscapes where for instance patch appearance-disapearance are likely to modulate the limiting similarity among potentially competing species (Mena-Lorca et al. 2006). Under these circumstances, the successional niche hypothesis (Amarasakere 2003) may provide an alternative explanation for local coexistence, by assuming that superior competitors may lack the ability to exploit early successional disturbed habitats, while inferior competitors may be able to exploit these habitats before superior competitors arrive and slowly displace inferior competitors (Amarasakere 2003). Thus, the mosaic of successional stages driven by the interplay between succession and disturbance in dynamic landscapes suggests that environmental gradients within patches may allow local coexistence This hypothesis agrees with the 'species sorting' and 'mass effects' perspectives, which consider that the assemble of local patches is heterogeneous in some local factors and thus the outcome of local population dynamics (both individual responses and species interactions) may change, eventually allowing local coexistence (Mouquet et al. 2005). Although both paradigms assume that patches differ in their local conditions, the 'mass effect' perspective assigns a much stronger role for dispersal than the 'species sorting' perspective (e.g. Lŏbel et al 2006; Guelat et al. 2008). The 'species sorting' perspective has thus much in common with traditional theory on niche differentiation and coexistence, by assuming that within patch resource niche partitioning may play a key role in species coexistence, even when competition-colonisation trade-offs may still interfere with the process (Jenkins 2006).

Apart from these spatially-related mechanisms potentially influencing local coexistence, a further biological trade-off related to temporal niche partitioning may as well be at play (Loreau 1992; Kronfeld-Schor and Dayan 2003). Indeed although species segregation along time has been much less explored in the context of spatially structured populations, it is likely that, at least at fine temporal scales, species may partitioning time and hence share the same habitat patches, thus allowing local coexistence (e.g. Gutman and Dayan 2005; Castro-Arellano and Lacher 2009; Di Bitetti et al. 2009).

1.3 Rationale and general purpose of this research

In this thesis I take two major theoretical threads in ecology to consider the consequences of environmental heterogeneity on spatially structured populations living in dynamic farmland landscapes: the metapopulation theory and the ecological niche theory. The main aim is to evaluate the importance of considering environmental variation in predicting species persistence ability and in interpreting coexistence in heterogeneous farmlands.

Within the metapopulation framework I explore how measurements on farmland heterogeneity may improve predictions on local and regional persistence ability by a single focal species. Although theoretical work regarding this question is still largely lacking (Wiens 1998), many empirical studies have shown that patch size and isolation are good predictors of patch occupancy status only when the matrix is homogeneous (Fahrig 2007), which is highly unlikely in complex and spatially patterned farmland landscapes. In addition, temporal patterning in farmlands may be also important because duration of habitat patches can affect species persistence abilities (Wiens 2000). Therefore, in agreement with other studies (Fahrig 1992; Kaymer et al. 2000) I consider both spatial and temporal heterogeneity typical of agricultural landscapes. Spatial heterogeneity (habitat patchiness) is determined by factors such as the number and spatial pattern of available habitat patches, as well as the composition and structure of the surrounding matrix. Temporal heterogeneity refers to the variability in the extent of habitats over time (habitat life span or patch persistence).

Under the niche theory I analyse how environmental heterogeneity (in space and time) may produce a variety of mechanisms allowing the coexistence of closely related, though different sized species, sharing similar habitat types. I focus particularly on local scale mechanisms because farmland landscapes may be highly dynamic and species living therein are likely to respond to local conditions of habitat patches (Stewart et al. 2000). Thus, assuming that farmland heterogeneity may allow local coexistence through niche differentiation at small spatial and temporal scales (Gordon 2000), it is likely that within-patch niche differentiation may provide a convincing explanation for species' regional coexistence, irrespective to eventual interspecific differences found in competition-colonization abilities (Rockwood 2006). In addition, because body-size divergence often facilitate resource partitioning among species (Basset 1995; Basset and Angelis 2007), the 'patch-dynamics' or the 'neutral' perspectives might be less relevant for explaining coexistence in heterogeneous farmland (Mouquet et al. 2005). Therefore, independently of the eventual differences in dispersal ranges between different sized species (Jenkins et al. 2007), the classical theory of resource partitioning at fine scales of heterogeneity (considered under the 'species sorting' perspective) seems the obvious starting point to describe species coexistence in heterogeneous environments (Kneitel and Chase 2004; Cottenie and Meester 2005).

1.4 Proposed model system

1.4.1 Target species

Among the vertebrates, small mammals are considered a particularly suitable taxonomic group as models organisms for addressing questions regarding the effects of environment heterogeneity at both the landscape and local scales, because they live in relatively small spatial areas, have short generation times, typically disperse from their natural areas and frequently exhibit behavioural responses to temporal (e.g. seasonal or circadian) variation (Barrett and Peles 1999).

In this research I focus on two Arvicoline species, the Cabrera vole (*Microtus cabrerae* Thomas, 1906) and the southern water vole (*Arvicola sapidus* Miller, 1908) living in Mediterranean agricultural landscapes. I consider these species particularly interesting to analyse the effects of farmland heterogeneity on population persistence and coexistence at the landscape and local scale mainly for the following reasons:

i. They are both considered habitat specialists (e.g. Fedriani et al. 2002; Fernández-Salvador 2005a) and, within their reduced geographical ranges (Figure 1, a and b), populations are patchily distributed (e.g. Fedriani et al. 2002; Fernández-Salvador 2005a). Therefore, the

Cabrera and the southern water vole are both good candidates for conducting studies rooted in metapopulation theory, because animals are largely restricted to marginal humid tall herb mosaics, which are discontinuously distributed across the landscape and may be either occupied or unoccupied (Fedriani et al. 2002; Fernández-Salvador et al. 2005b; Román 2007).

ii. There is a considerable overlapping in their distribution ranges (Figure 1 a and b), resulting in many areas where both species may occur sympatrically. Interspecific interaction between species has been referred to affect habitat use by animals, with Cabrera voles presumably avoiding sites occupied by southern water voles (Fernández-Salvador 1998). However, Cabrera and water voles may be found within the same habitat patch (Pita et al. 2006), suggesting that other mechanisms, beside eventual spatial segregation, may explain species coexistence. Sympatric Cabrera and southern water voles may therefore provide a good opportunity to test hypotheses regarding niche theory and coexistence of close-related and ecologically similar species deferring in their body sizes.

iii. In addition, both species are currently facing serious population declines largely due to fragmentation and destruction of suitable habitats (e.g. Landete-Castillejos et al. 2000; Rigaux et al. 2007). There is thus a need to design conservation and management plans for these voles, especially in farmland areas subjected to high environmental variation (Fernandes et al 2008; Rigaux et al 2008). Despite this, information regarding the biology and natural histories of Cabrera and southern water voles is still largely lacking, especially at the population and community levels of organisation.

To assess the contribution of both classical metapopulation assumptions and landscape heterogeneity in predicting the regional persistence ability by a single species, I consider the Cabrera vole as model species because, contrary to water voles (e.g. Fedriani et al. 2002; Román 2007; Centeno-Cuadros 2009), the metapopulation approach has never been applied to understand this species' spatial population structure. Evaluation of the possible mechanisms allowing local coexistence of close-related interacting metapopulations in heterogeneous environments was then carried out considering the two target species.

1.4.1.1 Cabrera vole

The Cabrera vole is one of the heaviest species of the genus Microtus, with an average body mass between 40-68g (Palomo and Gisbert 2002; Fernández-Salvador 2005b). It is an endemic species from the Iberian Peninsula with origin in the middle Pleistocene (Fernández-Salvador 1998) but yet, one of the most poorly known Iberian rodents (Fernández-Salvador et al. 2001). This species can only be found in isolated areas within the Mediterranean bioclimatic region (Figure 1a) with populations being largely restricted to humid habitats such as temporary ponds covered by rush beds, small riverine sedge/rush areas, and field margins and road verges densely covered with tall humid perennial and annual grasses (Landete-Castillejos et al. 2000, Fernández-Salvador et al. 2005a, Santos et al. 2005; 2006; Pita et al 2006).

Although the life-history and ecology of Cabrera voles are still poorly known, studies on breeding patterns and demography have suggested that this species may have a monogamous mating system and a *K*-strategy of reproduction, with neonates growing slowly and remaining in close contact with their parents (Fernández-Salvador et al. 2001 and 2005a). Individuals apparently have relatively long residence times within habitat patches, where they feed mainly on monocotyledons (mainly grasses, sedges and rushes), avoiding the consumption of plants with secondary compounds known to inhibit digestion and reproduction (Soriguer and Amat 1988; Rosário et al. 2008). Breeding patterns of Cabrera voles may thus be strongly dependent on seasonal variation in food quality, with voles often ceasing reproduction during severe summer droughts (Ventura et al. 1998), when annual herbs become unavailable (Fernández-Salvador et al. 2005a). Physiological adaptations to Mediterranean summer droughts include the ability for energy and water economy (Santos et al, 2004), as well as for lowering metabolic costs associated with thermoregulation at high ambient temperatures (Mathias et al. 2003). These traits, together with the high specialization of animals in relation to humid herb habitats, suggest that Cabrera voles may be particularly sensitive to environmental changes (Fernández-Salvador et al 2001; 2005b). In particular, empirical evidences suggest that voles' population persistence in many regions is probably affected by habitat loss, either as a result of climatic fluctuations (Fernández-Salvador et al. 2005b) or due to human activities such as agriculture, cattle overgrazing, and road construction (Landete-Castillejos et al. 2000; Fernández-Salvador et al.

2001; 2005a; Pita et al. 2006). For these reasons, the Cabrera vole is globally classified as *Near-threatened* (Fernandes et al 2008), and is listed in Appendices II and IV of the Habitats Directive (92/43/EEC), and in the Bern Convention (82/72/CEE).

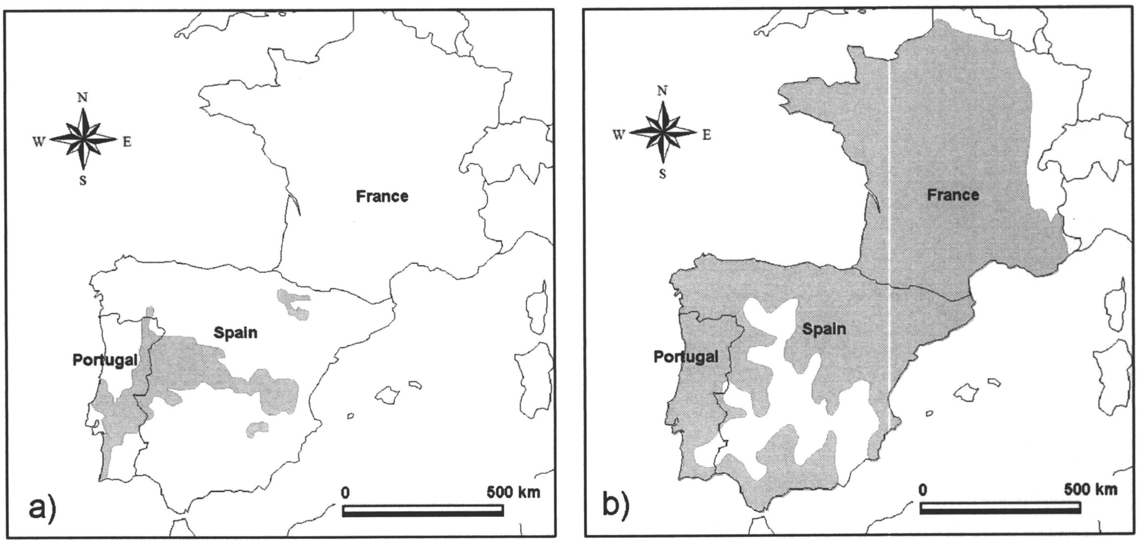

Figure 1 – Distribution ranges of the Cabrera vole (a) and the water vole (b) acording the IUCN (adapted from IUCN, 2010)

1.4.1.2 Water vole

The southern water vole is a medium sized Arvicoline, weighing about 150-310g (Palomo and Gisbert 2002) with origin in the Mindel glacial period (Pleistocene), when populations from the Iberian Peninsula became reproductively and genetically isolated from remaining European populations (Centeno-Cuadros et al. 2009), known as *Arvicola terrestris*. Present distribution of southern water voles (hereafter, water voles) is thus limited to the Iberian Peninsula and south, centre and north-west France (Rigaux et al. 2008; Fig. 1b). Water voles are referred to live closely tied to water bodies, occurring almost exclusively near streams and ponds with abundant herb vegetation (Palomo and Gisbert 2002). However, the presence of water is not a main requisite for the species, as voles are adapted to live in habitat patches with no surface water during most of the annual cycle (Fedriani et al. 2002; Román 2007). In fact, water voles may avoid permanently inundated marshes from riverbanks invaded by the more aggressive and semiaquatic brown rat, *Rattus norvegicus* (Fedriani et al. 2002).

Research on this species has contributed to understand many of its life-history traits in different regions, including the northeast (Ventura et al. 1989; Ventura and Gosálbez 1990) and south of the Iberian Peninsula (Román 2007). Studies focusing on reproductive and demographic cycles indicated that water voles have promiscuous mating system (Román 2007) and may be reproductively active throughout the year (Garde and Escala 1996; Ventura and Gosálbez 1990). However, reproductive activity is higher during the rainy periods and, similarly to Cabrera voles, may be interrupted during the hottest periods of the year, again because food quality is presumably reduced (Román 2007). During the coldest months, when extreme cold temperatures interfere with plants vegetative growth, there might be also a decrease in reproductive activity, though a complete interruption is unlikely (Garde and Escala 1996; Ventura and Gosálbez 1990; Román 2007). Preferred food items are broadly similar to those described for Cabrera voles, with animals feeding mainly on grasses, sedges and rushes (Román 2007). However, in areas permanently inundated and densely covered by reed beds, other plants, such as Typhaceae species may also be highly consumed (Ventura et al. 1989; Román 2007).

Contrary to Cabrera voles, there is already convincing indications from both ecological and genetic approaches that water vole populations respond to environmental heterogeneity and often exhibit a moderate metapopulation-like structure (Fedriani et al. 2002; Román 2007; Centeno-Cuadros 2009). These studies have shown that landscape variables may influence the likelihood of habitat patches to be (re)colonized (Fedriani et al. 2002), suggesting that dispersal should be affected by landscape matrix. Because water voles have reduced dispersal rates and mean average distances are inferior to 1 km (Róman 2007; Centeno-Cuadros 2009), it is likely that population genetic structure may be greatly influenced by the effects imposed by the inhospitable landscape. This hypothesis was not supported by a recent study (Centeno-Cuadros 2009) showing that water vole metapopulations living in naturally heterogeneous landscapes from Doñana (Spain) are structured much according to an isolation by distance pattern, with eventual physical barriers or demographic factors being of little importance in separating local populations (Centeno-Cuadros 2009). However, in heterogeneous farmland landscapes, isolation of local populations through expansion of cultivated fields is expectedly higher, and thus agriculture intensification has been often referred to negatively affect regional water vole persistence ability (Rigaux et al. 2007). Because current population trends indicate a rapid decrease in water vole numbers, particularly in agricultural areas, the species is globally classified as a *Vulnerable* (Rigaux et al. 2008), thus requiring conservation of favourable habitats.

1.4.2 Study area

The study area comprised the coastline farmland landscapes of south-west Portugal, where Cabrera and water voles are known to occur sympatrically (Pita et al. 2006). This region is included in the thermo-Mediterranean bioclimatic zone (Rivas-Martinez 1981), with mean temperature of about 16ºC and mean annual rainfall around 650 mm, of which over 80% falls between October and April (SNIRH, National System of Water Resources Information database, http://snirg.inag.pt). The arable landscape in this region is mainly devoted to irrigated agriculture and livestock production. Woody cover within the agricultural landscape is restricted to some planted woodlots and hedges with eucalyptus and pines delimiting fields and protecting crops from maritime winds. Shrubby hedges are most frequent around small fields close to agricultural warehouses and residential areas, whereas tree lines appear mostly around larger fields generally devoted to irrigated agriculture. There are also more natural areas surrounding and interspersing the arable landscape, including coastal dunes, open cork oak (*Quercus suber*) woodlands, and Mediterranean woodlands and scrublands covering the slopes of entrenched rivers and streams crossing the coastal plateau (Pita et al. 2009).

Although the study region is included in the Natural Park of *Sudoeste Alentejano e Costa Vicentina* and within a Site of Community Importance classified under the European Directive 92/43/CEE, agriculture is becoming ever more intensive since the early 1990s (Beja and Alcazar 2003; Pita et al. 2009). Intensification processes include: (i) increases in cattle stock densities and the replacement of extensive pastureland by improved pastures; (ii) increases in the area occupied by vegetable crops for international markets, often grown in greenhouses; (iii) the loss of semi-natural habitats such as temporary ponds and scrubland; (iv) increases in the size of irrigated fields, which are mainly used for fodder crops such as corn and sorghum; and (v) the development of paved road networks (Beja and Alcazar, 2003; Pita et al., 2009). These changes have shown measurable negative impacts on amphibians (Beja and Alcazar, 2003), birds (Alcazar, 2003), and mammals (Pita et al., 2009). Despite the overall trend for agricultural intensification, some areas have been abandoned or maintain extensive agricultural land uses, due for instance to the lack of irrigation infrastructures or legal constraints (Pita et al. 2009). However it is likely that overall trends in agricultural development in south-west Portugal have detrimental impacts regarding the suitable habitats for both Cabrera and water voles, because typically these habitats are highly productive, when converted to agricultural land (e.g. Landete-Catillejos 2000; Fernández-Salvador 1998; Rigaux et al. 2007)

1.5 Specific goals and thesis outline

In order to provide general insights regarding the effects of environmental heterogeneity at multiple scales on species regional persistence and local coexistence, the following research objectives were identified:

1. To evaluate the utility of the metapopulation approach in describing the spatial structure of Cabrera voles' populations in agricultural areas;
2. To investigate the relative role of patch and matrix effects on the Cabrera vole persistence ability;
3. To assess the spatial structuring among Cabrera and water voles within habitat patches and evaluate the role of interspecific spatial segregation in explaining local coexistence;
4. To assess differential habitat selection between Cabrera and water voles at local level considering multi-scaled measures of habitat heterogeneity and to show the importance of this approach for explaining coexisting patterns of ecologically similar species;
5. To investigate the circadian activity rhythms of Cabrera and water voles and evaluate if temporal partitioning between species may provide a further mechanism for coexistence at fine temporal scales.

Overall, I expect that by linking landscape heterogeneity in space and time with classical metapopulation thinking, a more complete understanding of the factors influencing the likelihood of regional persistence by Cabrera voles may be achieved (Wiens 1997). On the other hand, I expect that environmental heterogeneity at small spatial and temporal scales may provide opportunities for interspecific resource partition favouring local coexistence of Cabrera and water voles metapopulations in Mediterranean farmland, as expected from the differences in species body sizes (Basset and Angelis 2007).

The thesis is organized in 6 Chapters. **Chapter 1** provides the motivation, scope and background information of the research subjects. Chapters 2 to 5 comprise four scientific papers published (Chapters 2 and 3) or submitted to publication (Chapters 4 and 5) in peer-reviewed journals.

In **Chapter 2**, the spatial population structure of the Cabrera vole in Mediterranean farmland is analysed and the relative effects of habitat patch attributes (e.g. size, isolation, connectivity and temporal persistence) and of matrix composition and structure are evaluated. To achieve this, a variation partitioning approach adapted to logistic regression is used and a spatially realistic patch-occupancy model (*sensu* Fahrig 2007) is developed from a data set consisting on occupancy-status recordings made along almost three years within a typical farmland of south-west Portugal.

The next chapters (3 to 5) focus on the possible mechanisms that allow coexistence of Cabrera and water voles within shared habitats. In **Chapter 3** eventual spatial segregation between Cabrera and water voles is investigated from radio-telemetry data, using static interaction analysis. This study also provides information on important aspects related with the spatial ecology of both species (e.g. site-fidelity, home-ranges and core areas sizes, and intraspecific range overlap), allowing elucidation on many life-history traits described for both species (e.g. social organisation, breeding strategies, mating systems).

Chapter 4 presents a multi-scaled hierarchical approach to analyse seasonal microhabitat selection and differentiation between Cabrera and water voles. In particular this chapter explores the utility of considering the effects of spatial extent and resolution of habitat mapping in detecting resource partitioning and niche overlap between these close-related, though different-sized species. It is proposed that multi-scaled differential habitat selection may be essential to understand the spatial dimensions at which niche partitioning between species occurs.

In **Chapter 5**, the circadian activity rhythms of Cabrera and water voles are analysed using sophisticated, thought largely underused rhythmometry techniques developed to describe complex biological rhythms. Voles´ activity rhythms are analysed in relation to abiotic and biotic factors, which allowed inferring on eventual temporal partitioning between Cabrera and water voles, as a further mechanism allowing species coexistence.

Chapter 6 provides an integrated overview of the most important results from the previous chapters. This final chapter summarises the main conclusions and implications of this thesis and presents possible approaches for further research programs aiming to understand the importance of environmental heterogeneity on ecological processes.

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CHAPTER 2

Spatial population structure of the Cabrera vole in Mediterranean farmland: The relative role of patch and matrix effects

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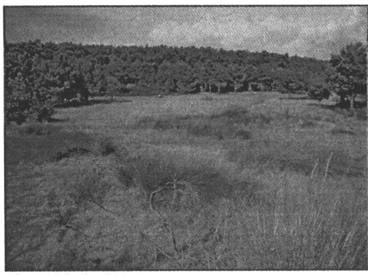
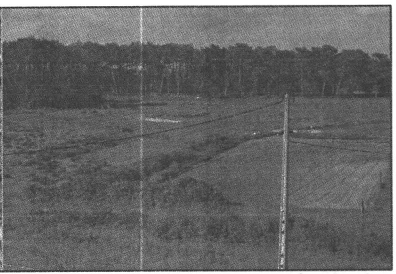


Suitable habitat

Cabrera voles' droppings



Suitable habitat in heterogeneous farmland

2. Spatial population structure of the Cabrera vole in Mediterranean farmland: The relative role of patch and matrix effects

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Abstract

There is mounting evidence that both patch networks and the intervening matrix influence species persistence in fragmented landscapes, though the relative importance of each of these factors in determining spatial population structure remains poorly understood. This study examined this issue using a three-year data set on the distribution of Cabrera voles (*Microtus cabrerae*) in Mediterranean farmland. The spatial pattern appeared consistent with a metapopulation structure, as voles occupied discrete tall herb patches scattered across the agricultural landscape, where local extinctions and colonisations induced temporal changes in occupancy patterns. Patch dynamics determined deviations from classical metapopulation assumptions, with over half the extinctions resulting from agricultural disturbance or vegetation succession, and recolonisations often occurring after the recovery of suitable habitat conditions sometime after disturbance. Occupancy in undisturbed patches was more stable, with vole occurrence in one year strongly reflecting that in the previous year. Overall, occupancy increased with both patch size and connectivity, but the unique contribution of patch variables to explain variation in vole occurrence was far smaller than that of matrix attributes. Voles occurred more often in patches surrounded by natural pastures, while prevalence declined with increasing cover by shrubland, pine plantations, improved pastures and grazed cropland. It is hypothesised that unfavourable land uses may increase the effective isolation of habitat patches through increased predation risk of dispersing voles. Conservation of the Cabrera vole in Mediterranean farmland should thus strive to maintain lightly grazed fields surrounding well-connected networks of suitable habitat patches.

Keywords: Agri-environment scheme, Mediterranean farmland, Metapopulation, Patch dynamics, Effective isolation, *Microtus cabrerae*

2.1 Introduction

In agricultural landscapes, many species persist in natural and semi-natural habitat patches amid a matrix of grazed and cultivated land, and so they are highly vulnerable to land use changes reducing the amount and quality of favourable patches and increasing their isolation (Halley and Lawton, 1996; Swihart and Moore, 2004). Wildlife conservation in these human-dominated landscapes is challenging, frequently involving lengthy and costly negotiations with private landowners to preserve critical habitats for target species. In Europe, for instance, many such agreements are made under the EU agri-environment schemes, whereby farmers are compensated for maintaining practices compatible with biodiversity conservation (Stoate et al., 2001; Kleijn and Sutherland, 2003).

Designing effective conservation prescriptions for a given target species in fragmented landscapes requires understanding on the amount and spatial arrangement of habitats needed to guarantee its survival (Swihart and Moore, 2004). Current approaches to this problem are strongly embedded in the metapopulation paradigm (e.g., Breininger et al., 2002), often assuming that the number, size and isolation of habitat patches are particularly critical factors driving species persistence (Harrison, 1994; Hanski and Simberloff, 1997). Taking this perspective, a great deal of effort is usually devoted to identifying and protecting an optimal network of favourable habitat patches, while largely ignoring or disregarding as little important the habitat between fragments (the 'matrix'). However, there is increasing evidence that the matrix itself strongly influences species occurrences in habitat fragments, for instance by determining the permeability to movements or buffering patches against external impacts (Haynes and Cronin, 2003; Revilla et al., 2004; Kupfer et al., 2006). The matrix may be particularly important if it provides conditions for a species to forage or live there, at least at low density or during short periods (Aldrich and Hamrick, 1998; Cook et al., 2004). Proper interpretation of fragmentation effects, and thereby the selection of the best conservation management approaches, thus requires due consideration on the roles of patch and matrix attributes determining species persistence (Vandermeer and Carvajal, 2001). This information may be particularly important for poorly known species inhabiting habitat patches in dynamic agricultural mosaics, for which adopting simple metapopulation frameworks may misdirect conservation efforts.

This may be the case of the Cabrera vole (*Microtus cabrerae*), a threatened arvicoline rodent endemic to the Iberian Peninsula (Palomo and Gisbert, 2002; Cabral et al., 2005), where its distribution is patchy and its populations seem to be decreasing rapidly (Ventura et al., 1998; Landette-Castillejos at al., 2003; Fernández-Salvador et al., 2005). This vole is considered a habitat specialist, living in discrete and easily recognisable breeding colonies associated with humid tall herb communities (San Miguel, 1992; Fernández-Salvador, 1998; Landette-Castillejos et al., 2003; Santos et al., 2005, 2006; Pita et al., 2006). In Mediterranean farmland, these are relatively rare and marginal habitats, which occur as rather isolate patches (Pita et al., 2006). It is possible that Cabrera voles inhabiting this network of habitat patches may present a metapopulation structure, making it likely that the amount, size and spatial configuration of patches can play a key role in determining its persistence. However, it is possible that the matrix itself may also affect patch occupancy, as patches are surrounded by a complex mosaic of agricultural habitats shaped by a variety of land uses.

In the present study we addressed these issues by examining the factors shaping the spatial population structure of the Cabrera vole in a complex farmland mosaic. In particular, we used a variation partitioning approach (sensu Bocard et al., 1992) to isolate the unique contributions of patch and matrix sets of variables to the explanatory power of vole distribution models across habitat fragments, thereby identifying the critical landscape elements that should be considered for the conservation management of this species. This information was then used to formulate agri-environment prescriptions favouring the persistence of the Cabrera vole in Mediterranean farmland.

2.2 Methods

2.2.1 Study area

The study was carried out on the coastal plateau of south-western Portugal. Climate is Mediterranean with oceanic influence; mean monthly temperatures range between 6ºC and 29ºC, and average annual rainfall is around 650 mm, of which > 80% falls in October-March. This is an agricultural landscape, with almost half the land devoted to irrigated annual crops. The production of beef cattle is also important, resulting in large areas occupied by pastures, fodder crops, and silage corn or sorghum. Wood cover in the agricultural landscape is restricted to arboreal windbreaks and a few woodlots. Since about 1990 there has been a strong intensification of agricultural practices, with negative consequences for farmland biodiversity (Beja and Alcazar, 2003; Pita et al., 2006).

2.2.2 Habitat and vole survey

Surveys were made in a 1600-ha square representative of the agricultural landscape of south-western Portugal (Fig. 1), where the presence of Cabrera voles had previously been recorded (Pita et al., 2006). The area was considered sufficiently large, since colonies of this vole usually are <0.2 ha (e.g., Fernández-Salvador et al., 2005; Santos et al., 2005 and 2006; Pita et al., 2006) and individuals apparently have a reduced mobility, with home ranges <0.01 ha (Fernández-Salvador, 1998).

Patch occupancy was surveyed in 12 sampling occasions at 2.7±0.03 (mean±sd) month intervals, from February 2002 to September 2004. In each occasion, all the area was walked over and patches with potential habitat conditions for this species were identified and mapped. Habitat suitability was judged from previous studies showing that these voles are restricted to areas of dense and tall herbs with high superficial groundwater table, both in the study area (Pita et al., 2006) and elsewhere (San Miguel, 1992; Fernández-Salvador, 1998; Landette-Castillejos et al., 2003; Santos et al., 2005, 2006). Potentially suitable habitat patches included all dense (about 100% cover) and tall (mean height around 30-40 cm) herbaceous communities dominated by grasses, sedges and rushes, located near small streams, temporarily flooded or waterlogged soil depressions, as well as tall grasslands in agricultural field margins, ditches and road verges (Pita et al., 2006). The actual occurrence of Cabrera voles was assessed on every sampling occasion in each potential habitat patch, from a 30-minute survey of characteristic signs such as droppings, runways made on grasses and heaps of grass clippings (e.g., San Miguel, 1992; Santos et al., 2006). If no sign was recorded in a given patch, a second 30-minute survey was carried out, so as to detect the species at low densities and to offset the variation in the abundance of field signs over the seasons (Pita et al., 2006). In every case, the presence of voles was detected during the first searching period, suggesting that the sampling effort was adequate.

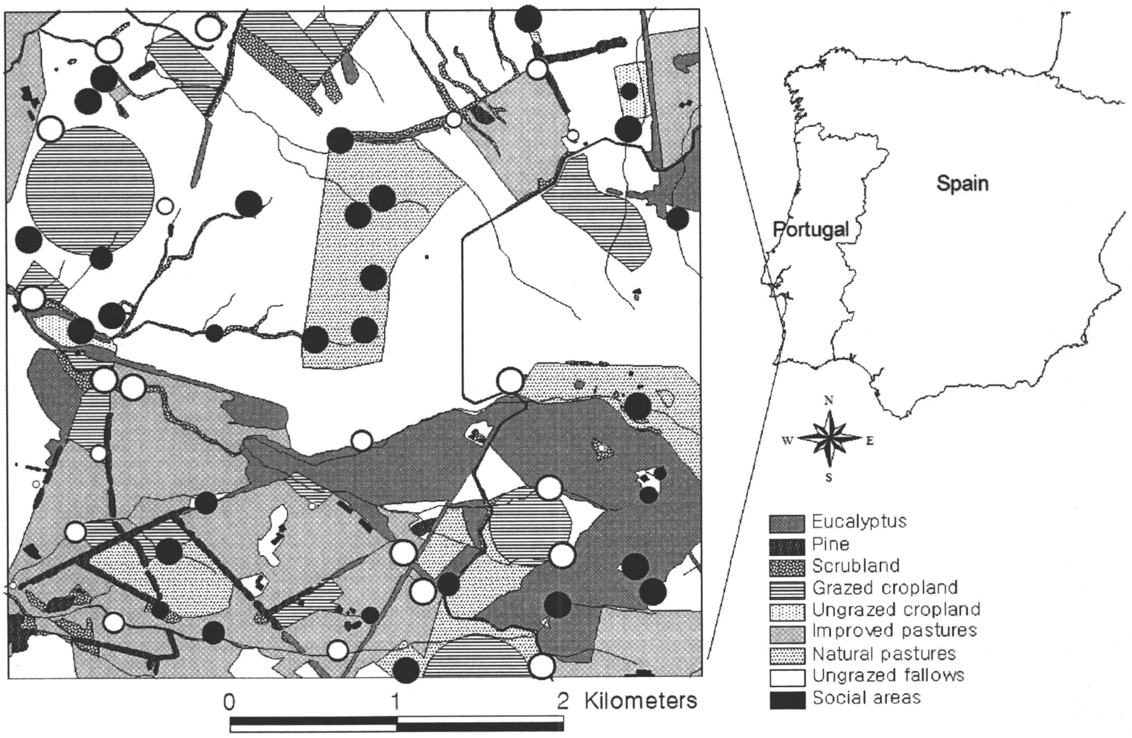

Fig. 1 - Map of the area sampled for Cabrera voles in southwestern Portugal, showing dominant land uses and location of habitat patches occupied (black circles) and unoccupied (grey circles) at some time in 2002-2004. The size of each circle is proportional to patch persistence throughout the three study years. Full thick lines are irrigation channels and thin lines are streams.

2.2.3 Patch and matrix attributes

Factors influencing patch use by the Cabrera vole were estimated considering two sets of variables reflecting patch and matrix attributes. Patches were characterized from variables describing its size, connectivity and temporal persistence. The size of patches was categorized in four size classes, thereby accounting for small-scale fluctuations due to management interference (Landette-Castillejos et al., 2003) or seasonal effects (San Miguel, 1992; Ventura et al., 1998): (1) small, <0.05 ha; (2) medium, 0.05-0.10 ha; (3) large, 0.10-0.20 ha; and (4) very large, >0.20 ha. Connectivity of a patch *i* (S_i) was estimated using the following metrics (Hanski, 1999):

$$S_i = \sum_{j \neq i} \exp\left(-d_{ij} / d'\right) p_j A_j$$

where d_{ij} is the straight-line distance between patches *i* and *j*, d' is the parameter determining the shape of the negative exponential distributions, p_j equals 1 for occupied and 0 for empty patches, and A_j is the area of patch *j*. The parameter d' is biologically related to the average effective interpopulation dispersal distance (Harrison et al., 1988), and it was estimated empirically by examining how changing its value in 50-m increments affected the fit of models relating patch occupancy to connectivity (Telfer et al., 2001). In each case, the value of d' yielding the best fitting model was used in further analysis. In common with other studies (e.g., Telfer et al., 2001), the distance to the nearest occupied patch was also used as a measure of patch isolation (the inverse of connectivity). Patch persistence was quantified considering the proportion of visits that the patch maintained favourable conditions for the Cabrera vole, as judged from the habitat requirements described above. In each case, the reason for the eventual destruction or degradation of the habitat patch was identified and recorded.

Matrix composition was estimated in 150-m radius circles from the centre of each habitat patch, corresponding to about half the mean distance between nearest-neighbouring patches in this landscape. The proportion of each of nine dominant land use classes was quantified in a Geographic Information System (GIS), from maps prepared using aerial photography and field surveys (Fig. 1): social areas - habitations and agricultural warehouses; eucalyptus (*Eucalyptus* spp.) plantations; pine (*Pinus* spp.) plantations; shrublands – areas dominated by *Cistus* spp., *Ulex* spp. and other Mediterranean shrubs; ungrazed fallows - covered by herbs and the soft-wooded shrub *Dittrichia revoluta*; natural pastures - long-term fallows irregularly grazed by cattle; improved pastures - sown and irrigated lawns permanently grazed by cattle; grazed cropland - arable land used each year for both cultivation (e.g., dry cereals, irrigated corn) and cattle grazing; and ungrazed cropland - cultivation of either dry or irrigated crops alternating each year with ungrazed short-term fallows. Distance from the centre of a patch to the nearest stream was also measured, because previous studies referred that colonies were frequently associated with stream networks (Fernández-Salvador, 1998; Santos et al., 2005; Pita et al., 2006).

2.2.4 Statistical analysis

Prior to statistical analysis, skewed variables were transformed to approach normality and to reduce the influence of a few large values, using the angular transformation for proportional data and the logarithmic transformation for other continuous variables. Principal Component Analysis (PCA) was then used to examine the interrelationships among habitat variables, describing the main gradient in patch and matrix attributes (Legendre and Legendre, 1998).

Logistic regression was used to estimate the effects of patch and matrix variables on annual patch occupancy by Cabrera voles. The analyses were based on spring surveys, because breeding takes place predominantly in winter and spring, and so this is the period when population densities are presumably close to their peaks, just before the declines occurring during the hot and dry summer season (e.g, Ventura et al., 1997; Fernández-Salvador, 1998; Fernández-Salvador et al., 2005; Pita et al., 2006). The same approach was used to discriminate between patches where voles occurred at least once during the three-years study and patches that were always empty.

For each data set, a preliminary screening of habitat variables was undertaken using univariate analysis, to detect variation between patches with and without voles. Quadratic terms were introduced into univariate models to check for unimodal responses to habitat variables. Multiple logistic models were then built separately for patch and landscape sets of variables, selecting in each case a reduced subset of explanatory variables using the all-possible-subsets approach (Rao, 1998). Only significant ($P < 0.05$) and nearly significant ($0.05 < P < 0.10$) variables in univariate analysis were considered in model building, to restrict the number of possible submodels and to avoid the incorporation of spurious effects. The best models were selected using the Aikaike Information Criteria (AIC; Burnham and Anderson, 1998). The presence of interactions between variables was tested and incorporated in the models if they significantly increased explained variation and reduced AIC. The statistical significance of each individual variable in a multivariate model was assessed using likelihood-ratio tests controlling for the effects of all the remaining variables included in the model (marginal or Type III effects).

The patch and matrix models were combined to produce a global habitat model, and an adaptation to logistic regression of the variation partitioning method of Bocard et al. (1992) was used to isolate the unique and shared contributions of each subset of variables to the explained variation in voles distribution (e.g., Reino et al., 2006): i) pure patch effects, ii) pure matrix effects; and iii) combined patch and matrix effects. The Nagelkerke coefficient of determination (R^2; Nagelkerke, 1991) provided a measure of variation explained by each logistic model. Model predictive performance was assessed by calculating the area under the curve (AUC) of a receiver operating characteristic (ROC), that is a plot of the true positive rate against the false positive rate (Pearce and Ferrier, 2000). Similar multivariate modelling and variation partitioning were carried out using simple and partial linear regression methods, to investigate patch and matrix effects on the average patch occupancy by Cabrera voles.

2.3 Results

2.3.1 Patch and matrix patterns

A total of 57 herbaceous patches appearing to provide adequate habitat conditions for the Cabrera vole were recorded, with a mean number (± sd) of 45.8±2.1 favourable patches recorded per sampling season. Just over half the patches (54.4 %) persisted through the three-year study, while 21.5% were present less than half the time. In any one sampling occasion 3.7% ± 2.3 (0-8.5%) of the patches recorded during the previous seasonal survey had been destroyed by farming operations or scrub encroachment, while 16.0% ± 10.0 (0-33.3%) of the records resulted from the recovery of previously destroyed patches. Over one year, 9.1-14.6% of the patches were destroyed, with 61.5-66.7% recovering their previous condition.

Habitat patches were generally < 0.2ha (77.2%), and they were at a mean distance of 331 m ± 126 (155 – 676) from the nearest habitat fragment. The matrix surrounding the patches was primarily composed by ungrazed fallows (30.6%), improved pastures (22.7%), natural pastures (19.7%) and eucalyptus plantations (14.2%). The PCA of habitat variables did not identify any clearly dominant environmental gradient, with no PC axis reflecting more than 20% of variation in habitat conditions and five axis showing eigenvalues > 1 (Table 1). The first axis (19.2%)

underlined a positive covariation between patch size and persistence, which was negatively related with cover by improved pastures and pinewoods. The second axis (14.0%) reflected increasing cover by ungrazed cropland, social areas and eucalyptus plantations, along with a decline in patch isolation. The third axis (12.6%) contrasted cover by ungrazed fallows and natural pastures. The fourth axis (11.1%) was related to increasing cover by grazed cropland, along with increasing patch isolation and reduced distance to the nearest stream. The fifth axis (9.3%) only reflected increasing cover by shrubland.

Table 1. Loadings of patch and matrix variables on the first five axes extracted by PCA (eigenvalues > 1), and the proportion of variance accounted for by each axis, for 57 grassland patches with potential habitat conditions for the Cabrera vole in SW Portugal. For clarity, only loadings > |0.40| were listed.

Variables	PC1	PC2	PC3	PC4	PC5
Patch variables					
Persistence	0.77				
Area	0.60				
Isolation		-0.62		-0.51	
Matrix variables					
Distance to nearest stream				0.66	
Grazed cropland				-0.71	
Ungrazed cropland		0.50			
Improved pastures	-0.74				
Natural pastures			-0.70		
Ungrazed fallows			0.81		
Shrubland					-0.87
Pine	-0.75				
Eucalyptus		0.55			
Social areas		0.70			
% Var.	19.3	14.0	12.6	11.1	9.3

2.3.2 Patch occupancy by voles

Cabrera voles were present at some time during the three years in 56% of the patches (Fig. 1). The percentage of occupancy in any one sampling occasion was relatively stable over the years and across seasons at about 45-50% (Table 2). Overall, there were 18 extinction events, of which

10 were deterministic resulting from habitat destruction or heavy degradation due to ditch clearance operations, burning, ploughing, overgrazing and scrub encroachment. In eight cases the extinctions seemed stochastic, as the species disappeared from apparently suitable habitat. Far more extinctions were recorded in 2004 than in 2003, with most stochastic extinctions occurring in winter and most deterministic extinctions occurring in summer and autumn (Table 2). Colonisations totalled 13 events, of which four were recolonisations. Colonisations were most frequent in winter and summer, but little variation was recorded between years (Table 2). The mean distance of a colonization event to the nearest patch occupied in the previous season was 453 ± 172m (176-762m). Overall annual turnover was 11.1% in 2002-2003 and 28.9% in 2003-2004, but this reduced to 7.0% and 17.0%, respectively, when considering only those patches remaining undisturbed in each pair of years.

Table 2. Annual (spring) and seasonal rates of patch occupation, extinction, and colonization for Cabrera voles occurring in a network of habitat fragments in south-western Portugal (2002-2004).

	Occupation	Deterministic Extinction	Stochastic Extinction	Colonization
Annual rates				
2002	47.7	-	-	-
2003	50.0	4.8	0.0	12.9
2004	44.7	16.7	16.7	16.7
Seasonal rates				
Winter	49.0	4.8	7.1	10.6
Spring	47.5	0.0	3.0	1.5
Summer	48.2	6.2	3.0	7.9
Autumn	45.6	6.2	1.4	1.4

2.3.3 Determinants of patch occupancy

Both patch and matrix characteristics differed between patches occupied and unoccupied by voles during the study (Table 3). The probability of voles occurring in one patch at some time during the study and the average patch occupancy increased with increasing connectivity and decreasing distance to the nearest occupied patch (Table 4). The strongest connectivity effect was recorded for *d'* = 50m, suggesting that the availability of colonizers to a patch declined abruptly with increasing distance to its neighbours. The distance to the nearest occupied patch was a strongest predictor of patch occupancy than the connectivity metrics. Nearly significant relationships with average patch occupancy were recorded for both patch area and persistence. Area was the only patch variable significantly affecting patch occupancy in 2002 and 2003, while no patch effect was detected in 2004 (Table 4). Cover by grazed cropland and improved pastures showed the strongest negative matrix effects on patch occupancy, while the strongest positive effect was that of natural pastures (Table 4). Shrublands and pinewoods were also negative correlates of patch occupancy, though their effect was weaker and more variable among years. There was no evidence for unimodal responses to either patch or matrix variables.

Table 3. Summary statistics (Mean ± standard errors) of patch and matrix variables in habitat patches occupied (n = 32) and unoccupied (n = 25) by Cabrera voles at some time during the three-years study. For each variable we report the estimated effect of each variable on patch occupancy as assessed from univariate logistic regressions, indicating significance levels (* - $P < 0.10$; ** - $P < 0.05$; *** - $P < 0.01$; n.s. – not significant), and directions of association (+, positive; -, negative).

Environmental variables	**Absences mean±sd**	**Presences mean±sd**	**Univariate Logistic regression**
Patch variables			
Persistence (score; PERSIS)	0.7 ± 0.3	0.8 ± 0.2	n.s.
Area (score; SIZE)	2.4 ± 1.0	2.4 ± 1.2	n.s.
Isolation (m; ISOL)	471.0 ± 218.2	332.1 ± 162.9	(-)***
Connectivity (CONNECT)[a]	0.008 ± 0.021	0.027 ± 0.038	(+)**
Matrix variables			
Distance to nearest stream (m; DSTREAM)	89.0 ± 95.7	92.5 ± 106.8	n.s.
Grazed cropland (%; GRCROP)	11.3 ± 15.8	4.2 ± 11.0	(-)*
Ungrazed cropland (%; UNCROP)	0.2 ± 1.0	5.3 ± 15.8	n.s.
Improved pastures (%; IMPAST)	35.9 ± 34.6	12.3 ± 27.5	(-)***
Natural pastures(%; NATPAST)	2.8 ± 7.6	33.0 ± 39.5	(+)***
Ungrazed fallows(%; FALLOW)	29.6 ± 1.2	32.8 ± 1.0	n.s.
Shrubland (%; SHRUB)	3.5 ± 6.8	1.2 ± 4.0	(-)*
Pine (%; PINE)	2.5 ± 5.4	0.6 ± 2.3	(-)**
Eucalyptus (%; EUCAL)	12.8 ± 21.8	15.3 ± 26.9	n.s.
Social areas(%; SOCIAL)	0.2 ± 0.4	0.4 ± 1.0	n.s.

[a] Estimated considering *d′*= 50m (see text for details).

Table 4. Summary of relationships between patch occupancy by the Cabrera vole and explanatory variables estimated in a Mediterranean farmland landscape in southwestern Portugal, as assessed from logistic regression (annual and overall patch occupancy) and linear regression (average patch occupancy). Significance levels and directions of association, negative (–) or positive (+), are given for patch and matrix variables showing significant ($P < 0.05$) or nearly significant ($P < 0.10$) relationships with the predictor variables. Variables in bold are those incorporating the best multivariate models while other variables showing significant univariate relationships are in italics. The amount of explained variation (R^2) is given for each of the best patch and matrix models. See Table 3 for definition of variables.

	Patch models					**Matrix models**					
	R^2	**AREA**	**ISOL**	**CONNECT**	**PERSIS**	R^2	**GRCROP**	**IMPAST**	**NATPAST**	**SHRUB**	**PINE**
Annual surveys											
Spring 2002	12.0	**0.041 (+)**				31.4	**0.032 (-)**	**0.007 (-)**	*0.040 (+)*		*0.055 (-)*
Spring 2003	16.8	**0.011 (+)**				35.9	**0.020 (-)**	**0.009 (-)**	*0.017 (+)*	**0.049 (-)**	*0.026 (-)*
Spring 2004	-					32.3	*0.090 (-)*	**0.001 (-)**			
Overall Study											
Occupation	15.4		**0.007 (-)**	*0.014 (+)*		54.0	**0.039 (-)**	**0.003 (-)**	**<0.001 (+)**	**0.056 (-)**	*0.025 (-)*
Average occupancy	11.6	**0.094 (+)**	**0.047 (-)**	*0.061 (+)*	*0.064 (+)*	25.6	*0.084 (-)*	**0.003 (-)**	**0.002 (+)**	*0.095 (-)*	*0.038 (-)*

In multivariate modelling, all patch models explained less variation in patch occupancy than matrix models (Table 4), and they showed a much poorer predictive power (Table 5). Area was the only predictor included in the best AIC patch models for 2002 and 2003, while distance to the nearest occupied patch was the only patch predictor of overall occupancy. Both area and isolation were influential in the average occupancy model. Patch models always explained less than 20% of variation in the occurrence of voles (Table 4), though they showed some discrimination ability between sites with and without voles (Table 5). Matrix models consistently incorporated the negative effects of cover by improved pastures, with all but the 2004 and the average occupancy model also including the negative effect of grazed cropland. The positive effects of natural pastures were included in both the overall and the average occupancy models, while the model for patch occupancy in 2003 incorporated the negative shrubland effects. Matrix models always explained over 25% of variation in patch occupancy (Table 4), reaching over 50% in the overall occupancy model, and they showed a good predictive power (Table 5).

Table 5. Discrimination ability estimated by the area under the curve (AUC) of a receiver operating characteristic, for logistic regression models of patch occupancy by Cabrera voles in southwestern Portugal. The asymptotic significance level under the null hypothesis AUC = 0.5 is provided in each case.

	Patch model		Matrix model		Global model	
	AUC	(*P*)	AUC	(*P*)	AUC	(*P*)
Annual surveys						
Spring 2002	0.737	(0.003)	0.772	(0.001)	0.825	(< 0.001)
Spring 2003	0.704	(0.015)	0.793	(0.001)	0.840	(< 0.001)
Spring 2004	-		0.757	(0.003)	0.757	(0.003)
Overall Study						
Occupation	0.676	(0.023)	0.871	(< 0.001)	0.908	(< 0.001)

Combining patch and matrix variables increased the predictive power of patch occupancy models (Table 5), though the unique contributions of matrix effects to explained variation were always much higher than that of patch effects (Table 6). No significant unique patch components were apparent for 2002, 2004 and the average occupancy model, while the proportion of unique explained variation for occupancy in 2003 and over the study was rather small (Table 6).

Conversely, there was a significant proportion of unique variation explained by matrix effects in all occupancy models, which was three to seven times that of patch models (Table 6).

Table 6. Partitioning of variation in patch occupancy by Cabrera voles in southwestern Portugal explained by habitat models incorporating patch and matrix effects. Significance levels are given in brackets for pure components.

	Pure components		**Shared components**	**Unexplained**
	Patch	**Matrix**	**Patch/matrix**	**variation**
Annual surveys				
Spring 2002	3.0 (0.251)	22.4 (0.011)	9.0	65.6
Spring 2003	8.8 (0.033)	27.9 (0.004)	8.0	55.3
Spring 2004	-	28.0 (<0.001)	-	67.8
Overall Study				
Occupation	11.0 (0.004)	49.1 (<0.001)	4.9	35.0
Average occupancy	6.2 (0.104)	20.2 (0.001)	5.4	68.2

Besides the patch and matrix variables, patch occupancy in one year was strongly influenced by that in the previous year. Indeed, logistic regression models incorporating occupancy status in 2002 and 2003, showed a very good performance to predict patch occupancy in 2003 ($R^2 = 0.82$, $P < 0.001$; AUC = 0.935, $P < 0.001$) and 2004 ($R^2 = 0.50$, $P < 0.001$; AUC = 0.830, $P < 0.001$), respectively. When the occupancy status in the previous year was forced into logistic habitat models, all patch and matrix variables lost significance.

2.4 Discussion

2.4.1 Spatial population structure

Like other small mammals inhabiting fragmented landscapes (Lambin et al., 2004), Cabrera voles in Mediterranean farmland seemed to meet at least some conditions for a spatially structured population to be considered a metapopulation (Hanski and Kuussaari, 1995; Hanski, 1999). They lived in spatially distinct habitat patches, occupying discrete fragments of dense (> 80 % cover) and tall (> 30 cm) Mediterranean humid herb communities (Pita et al., 2006), with no evidence for its presence in the surrounding woodlots, shrublands and cultivated and grazed fields. There also appeared to be no 'mainland' population, with all local populations presumably showing some risk of extinction. Indeed, all the colonies occupied rather small habitat patches and there was no evidence for the presence of one or a few larger colonies that might act as a permanent source of colonists for neighbouring patches. The importance of dispersal processes for the regional dynamics was supported by the influence of patch isolation on occupancy status, with voles being much more likely to be recorded at some time during the study in patches closer to other occupied patches than when they were farther apart. In the absence of direct information on the dispersal capacity of Cabrera voles, the very low value estimated for the average effective interpopulation dispersal distance (50m) also suggests that dispersal was strongly limited by distance, though care should be taken when interpreting estimates of this metric (see below). The level of spatial correlation in population processes could not be assessed from the present data, though the uneven spatial and temporal occurrence of extinctions and colonisations suggests that local dynamics were unlikely to be completely synchronous. Studies from other small mammals suggest that metapopulation persistence may occur despite some level of spatial correlation (Lambin et al., 2004).

Although Cabrera voles appeared to present a metapopulation structure in the agricultural landscape of SW Portugal, the assumption of a static landscape implicit in classic metapopulation theory was not met. Traditional metapopulation models assume that patches are neither created nor destroyed (e.g., Hanski, 1999), whereas the patches inhabited by Cabrera voles were often destroyed through cultivation or grazing, eventually recovering sometime after anthropogenic disturbance ceased. Loss of habitat patches was also associated with vegetation succession resulting from the absence of grazing or other agricultural disturbance for extended periods, with scrub encroachment turning the habitat unsuitable to these voles (Pita et al., 2006). As a consequence, over half the extinctions recorded in this dynamic landscape were deterministic, with only a few reflecting apparently the stochastic processes that are generally assumed in metapopulation models. Also, new populations often resulted from the (re)colonization of patches that became available due to habitat recovery. Agricultural disturbance apparently added much variability to the spatial occupancy patterns, as in undisturbed patches the turnover was lower and occupancy patterns were strongly related to that in the preceding year. Recent modelling studies showed that in these circumstances metapopulation persistence is conditional on both classical metapopulation parameters, such as colonization and extinction rates, but also on the rates of patch destruction and creation (Keymer at al., 2000; Feng and DeWoody, 2004). However, even these rather complex models still have limited utility in estimating the conditions for metapopulation persistence in real landscapes, as they fail to explicitly incorporate many realistic complexities such as for instance the strong matrix influences on patch occupancy detected in this study.

2.4.2 Patch and matrix effects

Cabrera voles were influenced by both patch and matrix attributes, with the latter assuming a particularly relevant role in determining patch occupancy. Although the importance of the matrix in fragmented landscapes has been increasingly recognized (Vandemeer and Carvajal, 2001; Cook et al., 2004; Kupfer et al., 2006), few studies have attempted to empirically estimate the relative contributions of patch and matrix effects to metapopulation patterns and processes (but see Haynes and Cronin, 2003). The variation partitioning approach adopted here statistically isolated such unique contributions, offsetting the potential confounding effects of intercorrelated patch and matrix characteristics (Haynes and Cronin, 2003), thereby demonstrating that in at least some cases matrix effects may actually be greater than the size and isolation of patches commonly considered in metapopulation models. This result adds to the growing evidence that the matrix should no longer be treated as an equally inhospitable, structurally uniform and ecologically irrelevant component of the landscape, because it may influence strongly population persistence in fragmented landscapes (Vandermeer and Carvajal, 2001; Kupfer et al., 2006). Matrix effects may be particularly important in complex and dynamic landscapes such as the Mediterranean farmland inhabited by Cabrera voles, where habitat patches are surrounded by a heterogeneous mosaic of agricultural habitats differing in vegetation structure and composition, which are shaped by a range of contrasting land uses. In this context, understanding species persistence across fragmented landscapes requires consideration on the processes operating at the scale of individual patches, networks of patches and the intervening matrix.

Cabrera voles were more likely to be found in the largest habitat patches in two out of three spring surveys, though over the study they eventually occurred in all but the most isolated patches irrespective of their size. As a consequence, average occupancy was primarily influenced by patch isolation and only marginally by patch area. Lack of relationships between area and occupancy were also reported elsewhere for Cabrera voles (Fernández-Salvador et al., 2005). These patterns contrast with theoretical predictions and empirical observations of strong positive effects of area on occupancy, which are usually assumed to reflect a lower probability of stochastic extinction associated with larger populations (e.g., Hanski, 1999). This relationship was not clear in this study probably because over half the extinctions were deterministic, resulting from the destruction of habitat patches independently of their size. This may explain why area affected occupancy in 2003 but not in 2004, as the rate of deterministic extinction was four times as high in the later. In a less dynamic landscape, it might probably be expected far greater effects of patch area, as vole densities tend to be higher and the reproductive output better in larger patches (Fernández-Salvador et al., 2005), which may therefore be less prone to stochastic extinction.

The negative effects of isolation on occupancy suggested that colonization of empty patches by dispersing voles also drives the regional dynamics of this population. Dispersal may be particularly important for species persistence given the relatively high rate of patch destruction and subsequent recovery, thereby continuously creating empty habitat patches. Colonization was apparently limited by the small dispersal capacity of the Cabrera vole, as the average effective dispersal distance (d') was estimated at only 50m. However, this very small d' may be an artefact of the estimation method, as it was smaller than the nearest distance between occupied patches (147m). The estimate of d' was the value maximizing the fit of a logistic regression model relating patch occupancy to the connectivity metrics. The connectivity of a patch *i* was simply a weighted sum of neighbouring population sizes, with weights given by a negative exponential of interpatch distances d_{ij} scaled by d'. When d' is small relative to the minimum of d_{ij} the weights decline very sharply, suggesting that only the nearest patches contribute effectively to patch recolonization. This view was supported by the higher predictive power of the distance to the nearest patch than the connectivity metrics. This results supports the view that estimates of d' based solely on distribution data may underestimate the importance of dispersal for the regional dynamics (Telfer et al., 2001).

The strong matrix effects on path occupancy were associated with the increasing prevalence of Cabrera voles in patches surrounded by lightly grazed fields and their negative response to the amount of both heavily grazed and ungrazed land. Contrary to other small mammals (e.g., Cook et al., 2004), these results were unlikely to be determined by voles using the surrounding matrix, as no signs of their activity were ever found outside the tall herb humid habitat patches. A more important factor was probably the impact on habitat patches of cattle using the surrounding matrix. Occasional cattle grazing presumably contributed for maintaining suitable habitat conditions within patches by delaying scrub encroachment, while high cattle densities probably destroy the tall herbaceous vegetation required by voles (Fernández-Salvador, 1998; Pita et al., 2006). Another important aspect may be the resistance offered by different agricultural land uses to vole dispersal. Heavily grazed land may be little permeable, as predation risk is probably high due to the low and homogeneous sward. On the contrary, the taller and more heterogeneous herbaceous layer of lightly grazed fields is expected to provide better cover to dispersing voles. Predation risk may also justify the negative role of shrublands, as these are preferential habitats for a range of carnivore predators in Mediterranean ecosystems (e.g., Fedriani et al., 1999). Although actual data on movements by Cabrera voles is lacking to support these views, reduced mobility of other vole species under increased predation risk was reported elsewhere (Norrdahl and Korpimaki, 1998). The surrounding land uses may thus determine an "effective isolation" of the habitat patches that may be higher or lower than simple straight-line distances would imply (Ricketts, 2001).

2.4.3 Conservation

Results from this study suggested that conservation of Cabrera voles in Mediterranean farmland requires a network of tall herb humid habitat patches, surrounded by lightly grazed pastureland. Each patch should be as large as possible (0.2-0.5 ha) and separated from its nearest neighbour by no more than about 300-400 m, corresponding to 1.6-2.8 patches.km^{-1}. In some circumstances these patches might eventually be fenced off to prevent overgrazing, though this would imply managing the land to prevent the loss of habitat conditions through successional scrub encroachment. Although the conservation of these small and scattered habitat patches may be relatively simple and inexpensive to implement under an agri-environment program, keeping the low livestock densities that appear to be required in the surrounding matrix may be far more complicated and eventually unaffordable over large areas. In these circumstances, further information is necessary on factors affecting the dispersal of Cabrera voles among habitat patches, to understand in greater detail the mechanisms through which the matrix composition affects the regional population dynamics. This would allow the development and testing of conservation management alternatives, such as for instance the creation of grassy corridors along field margins or road verges to increase the effective connectivity among habitat patches (e.g., Polla and Barret, 1993; Aars and Ims, 1999). In general, this study suggests that conservation of the Cabrera vole, like that of many species inhabiting habitat fragments in human-dominated landscapes, should shift from a patch-oriented strategy to a landscape mosaic perspective recognizing the importance of both the patch and the matrix components for species persistence (Lindenmayer and Franklin, 2002; Kupfer et al., 2006).

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CHAPTER 3

Spatial segregation of two vole species (*Arvicola sapidus* and *Microtus cabrerae*) within habitat patches in a highly fragmented farmland landscape

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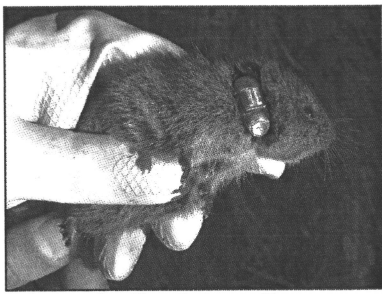

Cabrera vole with radio-transmitter.
(Photo: António Mira)

TRX - 1000S Receiver

Water vole with radio-transmiter

3. Spatial segregation of two vole species (*Arvicola sapidus* and *Microtus cabrerae*) within habitat patches in a highly fragmented farmland landscape

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Abstract:

Spatial segregation is one of the common mechanisms allowing the coexistence of similar interacting species in heterogeneous environments. Analysing spatial segregation requires information on individual home-range sizes and their degree of spatial overlap. In this study, we used radio-tracking to report for the first time the home-range and core-area sizes of sympatric Cabrera and water voles, and to analyse intra- and inter-specific space sharing within habitat patches in a highly fragmented landscape. Results indicated that both species exhibited strong fine-scale site-fidelity and reduced variation in range size across sexes and seasons. Monogamous mating system seemed to prevail for both species, although water voles may also exhibit poligynous breeding strategies. Mean home-range and core-area sizes of water voles (946.3 m^2 and 156.6 m^2) were about twice that of Cabrera voles (418.2 m^2 and 55.1 m^2). Within habitat patches, individuals of both species often overlapped their home-ranges, particularly during the dry season (May – September), though intra-specific home-range overlap was generally higher than inter-specific overlap. Inter-specific space sharing was restricted to areas outside the centre of activity of animals, as no core-area overlap was ever recorded between Cabrera and water voles. Taken together, results support the view that coexistence of Cabrera and water voles in Mediterranean patchy habitats may in part result from spatial segregation among individuals, which may reflect competitive displacement or small-scale habitat partitioning. Results highlight the need to account for species interactions when designing conservation management strategies for sympatric Cabrera and water voles in fragmented landscapes.

Keywords: Competition, Fragmentation, Spatial ecology, Species coexistence, Mediterranean farmland

3.1 Introduction

Understanding the mechanisms determining the coexistence of closely related species and their organizing dynamics in spatially structured environments has received increasing attention in recent years (Amarasekare 2003; Dammhahn and Kappeler 2008). In particular, coexistence of similar interacting species in patchy habitats has often challenged researchers to meet the predictions from the competitive exclusion principle, especially when niche dimensions at which species differentiate are still to be identified, or life-history trade-offs remain unclear (Hoopes et al. 2005). In this context, spatial segregation is often referred to as an important mechanism facilitating the coexistence of ecologically similar species, because competition for resources is reduced when they occupy different parts of the same habitat patches (Urayma 1996; Amarasakere 2003).

Analysing spatial segregation between interacting species requires primarily information on their spatial ecology and individual life-history traits (Eccard and Ylonen 2003; Brunjes et al. 2009). Because animals tend to defend a certain territory for breeding, food gathering or breeding at least during particular periods of their life, information on site-fidelity, home-range sizes and their degree of overlap is fundamental to assess eventual segregation mechanisms at the individual level (e.g. Rosenzweig 1991; Borowski 2003; Hillen et al. 2009), which in turn will determine species distribution patterns at the population level (South 1999; Eccard and Ylonen 2003; DeAngelis and Mooij 2005). Studies aiming to describe species ranging behaviour, socio-spatial organization and inter-specific relations might also have a major practical importance when interpreting habitat-size required for keeping viable populations of threatened species, on which decisions about the design and management of protected areas should be based (Simcharoen et al. 2008; Hillen et al. 2009). In particular, the spatial aggregation or segregation within and between closely related, ecologically similar, and sympatric species that are threatened by the same processes, should be highly informative to determine the appropriate scale for conservation planning (Brunjes et al. 2009; Oro et al. 2009).

The Cabrera vole (*Microtus cabrerae*) and the water vole (*Arvicola sapidus*) are two Arvicoline rodents with reduced and overlapping geographical ranges, occurring exclusively in the Iberian

Peninsula in the case of the Cabrera vole, and in the Iberian Peninsula and parts of France in the case of the water vole (Palomo et al. 2007). Both species are currently facing serious population declines and in need for conservation and management, mainly due to fragmentation and destruction of the habitats on which both species have specialized (IUCN 2009). Typically, these habitats include little disturbed and often seasonally flooded tall wet meadows and some adjacent shrubby vegetation able to provide food and protection from predators (e.g. Fedriani et al. 2002; Pita et al. 2006; Santos et al. 2006; Luque-Larena and López 2007; Pita et al. 2007; Román 2007). Overall, agricultural development, intensive herbivory by livestock and severe drought episodes are apparently the most important threats across the species' distribution ranges (Fedriani et al. 2002; Fernández-Salvador et al. 2005; Pita et al. 2007; Rigaux and Charruau 2007; Román 2007).

Although the habitat characteristics required for Cabrera and water voles are relatively well known, information on their spatial ecology and hence on some of their basic life-history traits is still scarce, limiting the effectiveness of conservation efforts recommended for these species (Pita et al. 2006, 2007; Rigaux and Charruau 2007; Román 2007). Moreover, because of their ecological similarities, it has been hypothesised that space use by Cabrera and water voles in sympatric areas might be influenced by competitive interactions, and that coexistence might in part result from spatial segregation between the two species (Pita et al. 2006). Because of this, the potential for competitive interactions should be duly considered when designing habitat conservation management strategies to protect these species (Pita et al. 2006).

In this study we addressed these issues by analysing home-range and core-area size and overlap by Cabrera and water voles within habitat patches in highly fragmented landscapes. Firstly, we assessed the foraging site fidelity of Cabrera and water voles in farmland habitats of south-western Portugal and quantified their home-range and core-area sizes using radio-tracking techniques. Secondly, we analysed how conspecifics partition their individual home-ranges and core-areas, and assessed the degree of spatial overlap between the two species. This information was then used to test predictions on the spatial ecology of these two species, derived from general ecological theory and previous empirical observations on their life-histories and ecological requirements. Specifically, we predicted that (i) home-ranges of water voles should be larger than those of Cabrera voles, due to the much larger body size of the former species (Lindstedt et al. 1986; Swihart et al. 1988); (ii) home-range sizes should change across seasons, because both the Cabrera and the water voles often cease reproduction during the driest months, when the availability of food resources is presumably much reduced (Ventura et al. 1998; Fedriani et al. 2002; Pita et al. 2007; Román 2007); (iii) intra-specific variation in home range sizes and overlap should be influenced by gender in promiscuous water voles (Román 2007), but not as much in monogamic Cabrera voles (Fernández-Salvador, 2005), reflecting differences in mating systems between species (Wollf 2007); (iv) there should be inter-specific spatial segregation of home ranges, because this is usually considered essential to enable the coexistence of similar species that apparently show no obvious partitioning of resources (Amarasekare 2003; Brunjes et al. 2009). Results of this study were then used to discuss the role of spatial processes at the individual level in explaining the coexistence of this two species in highly fragmented landscapes, and to derive management prescriptions favouring the conservation of sympatric Cabrera and water voles in Mediterranean farmland.

3.2 Material and Methods

3.2.1 Study area and species

The study was carried out on the coastal plateau of south-western Portugal (37°57' – 37°35' N, 08°51' – 08°48' W) which is included in the thermo-Mediterranean bioclimatic zone (Rivas-Martinez 1981). Mean temperature is about 16 °C and mean annual rainfall around 650 mm, of which over 80% falls between October and April (SNIRH, National System of Water Resources Information database, http://snirh.inag.pt). The landscape is predominantly flat and devoted to irrigated agriculture and livestock production, with natural and semi-natural habitats occurring marginally in dunes, entrenched stream valleys and cork oak woodlands surrounding the farmed area. Surface waters in this landscape are mostly associated with temporary ponds which flood during the rainy season and dry out in summer, whereas permanent water bodies are scarce and mostly associated with irrigation infra-structures such as concrete channels and reservoirs. Over the past two decades the landscape has changed considerably as a result of social and land management transformations, with an overall intensification of agriculture, along with the abandonment of some marginal and less productive areas (Beja and Alcazar 2003; Pita et al. 2007, 2009).

As in other regions of the Iberian Peninsula, both the Cabrera vole and the water vole have a highly discontinuous distribution within this agricultural landscape, where they are largely restricted to patches of little disturbed tall grass communities typical of temporary ponds, and to narrow herbaceous strips along the margins of small intermittent streams, field boundaries and road verges (Pita et al. 2006, 2007). Although water voles are generally reported to be dependent on permanent and stable water bodies (e.g. Garde and Escala 1993; Ventura 2004), in our study area they often occupy seasonally flooded or moist habitats (R. Pita, Unpublished Data), as it seems to be the case in other dry Mediterranean areas (Fedriani et al. 2002; Román 2007). Cabrera voles are also associated with these habitat types, and so the two species often occur within the same patches, which tend to be separated from other patches by a largely inhospitable agricultural matrix (Pita et al. 2006, 2007).

Within habitat-patches, Cabrera voles are usually organised in monogamic breeding pairs exhibiting relatively high residence times of over 10 months (Fernández-Salvador 2005). Water voles generally mate promiscuously and rarely survive beyond 12 months (Román 2007). Both species tend to exhibit diurnal peaks of activity (Ventura 2004; Fernández-Salvador 2005) and to feed mainly on leaves, stems and seeds of grasses, sedges and rushes (Soriger and Amat 1988; Román 2007; Rosário et al. 2008). Reeds may also be an important food item for water voles nearly permanently inundated habitats, such as river banks (e.g. Ventura at al. 1989). Because of the severe Mediterranean summer droughts, seasonality in food availability is regarded as a key factor influencing population dynamics and breeding patterns of both species in south-western Iberia (Pita et al. 2007; Román 2007). In particular, food quality is considerably reduced during the summer, when annual plants are scarce and voles increase their consumption on perennial plants with lower protein content and higher concentrations of secondary compounds inhibiting digestion and reproduction (Soriguer and Amat 1988; Román 2007; Rosário et al. 2008).

3.2.2 Study design

The spatial ecology of Cabrera and water voles was analysed with radio-tracking, from individuals captured within 18 discrete habitat patches distributed across two farmland areas in the Portuguese south-west coast, where agreement with landowners to capture and radio-track voles could be obtained (Figure 1). Mean (± se) nearest neighbour distance between sampling sites was 1.2 ± 0.3 km (0.12 m – 4.0 km). Sampling sites that were relatively close to each other were sill treated as distinct units in the context of this study, because they were separated by inhospitable matrix (e.g. heavily grazed pastureland or ploughed land) and it was very unlikely that voles crossed such areas within their routine movements. All sampling sites consisted of a mosaic of mixed grasses and forbs, as well as tall wet and riparian meadows, shrubs and trees, embedded in a predominantly agricultural matrix. No site was associated with a water body, although all flooded partly during rainy periods, eventually resulting in small, scattered and shallow surfaces of water, lying beneath the vegetation.

The presence of Cabrera and water voles within each habitat patch was initially confirmed from surveys based on systematic searches for the typical presence signs of each species, mainly droppings and the characteristic pathways on ground vegetation (e.g. Fedriani et al. 2002; Santos et al. 2006; Pita et al. 2007), both of which are about two times larger for water voles (Román 2003; Pita et al. 2006). These preliminary surveys indicated that seven out of 18 sampling sites showed signs of both species, while seven were apparently used by Cabrera voles only and four by water voles only (Figure 1). Because the number of voles inhabiting each habitat patch was generally very small and we wanted to keep disturbance to a minimum, we restricted sampling to a small number of individuals from each habitat patch. Also, we avoided repeated disturbance to small populations by sampling each patch in a single occasion between April-2006 and April-2008. To account for potential seasonal differences in range use, 10 patches were surveyed during the wet season (October – April) and 8 during the dry season (May – September), with the later corresponding to the period with high temperature and very low rainfall.

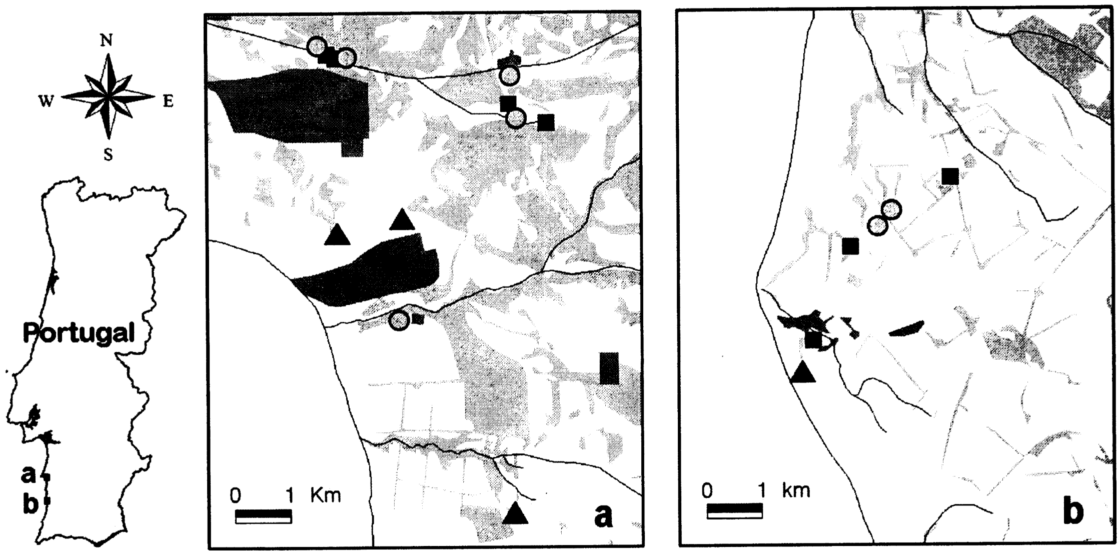

Figure 1 – Location of 18 sites sampled in two farmland areas of south-western Portugal. Squares and triangles indicate the presence of Cabrera voles and water voles, respectively, while circles represent sites where species co-occurred, as revealed from sign surveys. Built up areas and forestry habitats are shown in dark and light grey, respectively, while white areas represent the agricultural matrix. Lines represent the main streams crossing the study area.

3.2.3 Captures and radio-tracking

Voles were captured using Sherman live-traps (7 x 23 x 9 cm^3 for Cabrera voles and 10 x 37 x 11 cm^3 for water voles) baited with apple and supplied with hay and hydrophobic cotton for bedding. Traps were placed at likely capture sites, which were assessed by checking eaten apple trials left in the area during the previous 1-3 days. After setting the traps, these were checked every 8 hours (around 08:00, 16:00 and 00:00). A total of 804 traps were used during 108 days of trapping distributed through the study period. The sampling effort varied among sites depending on the evidence for the presence of voles signs, patch size, capture-recapture success, and whether radio-tracking was in progress. Mean (± se) sampling effort to capture Cabrera voles was 186.3 ± 63.8 trap-nights per sampling site during the dry season (n = 6; range: 50 – 450), and 195.7 ± 41.3 trap-nights per sampling site during the wet season (n = 8; range: 45 – 315). Sampling effort for water voles averaged 207.8 ± 68.8 trap-nights during the dry season (n=6; range: 42 – 420), and 244.2 ± 84.3 trap-nights per sampling site during the wet season (n=5; range: 72 – 540).

All Cabrera and water voles captured were weighed and sexed, and the reproductive status (active or non active) of non-juveniles (> 28 g for Cabrera voles, Fernández-Salvador et al. 2005; > 94 g for water voles, Román 2007), was assessed based on the testis position (scrotal or abdominal) for males and on vulva perforation and nipples size (small or large) for females. Animals of non target species were immediately released at the point of capture. Individual Cabrera and water voles were fitted with collar radio-transmitters (Wildlife Materials, Inc., Illinois USA) adding no more than 5% of the animals weigh, so as to ensure no significant additional energetic costs for voles (Gannon et al., 2007). Pregnant females were identified by abdominal palpation and they were not collared to reduce potential negative effects on vole populations (Mendonça, 1999). All animals were lightly sedate with a subcutaneous injection of Dormitor® (0.2mg/kg) to reduce handling stress. After transmitter attachment, voles were induced out of anaesthesia using an equivalent dose of Antisedam®, a reversing agent to Dormitor®. Before release, collared animals were kept under observation for at least 2 hours to ensure that they were suffering no ill-effects or loss of mobility. During this short observational period, uncovered wire cages supplied with hay and hydrophobic cotton were used and apple and water were provided *ad libitum*. Radio-tracking started at least 4 hours after trap removal and the release of animals at their point of capture (e.g. Gray et. al. 1998).

Six different 4-hour radio-tracking intervals covering a complete 24-hours cycle (06:15 – 10:00; 10:15 – 14:00; 14:15 – 18:00, 18:15 – 22:00; 22:00 – 02:00; 02:15 – 06:00) were alternately surveyed for each animal. Each tracking session started at least eight hours after the previous session and consisted in recording the location of each individual at 15-minute intervals, totalling 16 fixes recorded per animal in each session. Whenever possible, tracking was carried out until at least a minimum of 96 locations was reached for each individual, corresponding to the number of locations needed to obtain a complete 24-hour cycle. Voles were located using a TRX-1000S receiver and an external 3-element yagi directional antenna (Wildlife Materials, Inc., Illinois USA). Locations were made by homing and by multiple triangulations when the tracker was close to the animals. At each radio-location a positioning measurement was recorded using a Garmin eTrex® handheld GPS, except when the animal remained in the same location in successive fixes, for which the coordinates of the previous fix was assigned to minimize GPS measurement error. GPS-positioning typically yielded an error of ± 4m and was considered sufficiently accurate, as the range used by of voles may cover hundreds of square-meters (see Results). At each radio-location we recorded whether the animal was active or inactive, as judged by fluctuating vs. stationary radio-signal. After radio-tracking, each sampling site was re-trapped, so as to remove collars from tracked voles.

3.2.4 Data analysis

The first aspect investigated concerning space use by voles was a test for random movement at fine-scale (site fidelity analysis). This analysis estimated whether voles moved through space at random, or whether the animals made directional choices for particular areas within habitat patches (Shanahan et al. 2007). One thousand random walks were generated for each tracked vole using the Animal Movement Analysis Extension (AMAE, Hooge and Eichenlaub 2000) for ArcView GIS 3.2 (ESRI, Redlands, CA, USA). Each distance travelled between locations was equal to that observed in true vole tracks, and random turning angles substituted all actual angles taken (Shanahan et al. 2007). The parameters used to compare real and random walks were the average straight-line distance each consecutive location moved away from the start point of the track (R^2); and the total linear distance between the start and end points of an animal's path, divided by the total length of the path (*LI*) (Spencer et al. 1990). For each individual, observed R^2 and *LI* values were compared with the range of values given by the random track. If R^2 and *LI* of an observed animal track was in the lower 5% of the range of values for the random walk tracks, then the observed track was considered significantly more constrained than would be expected by chance, indicating that the animal exhibited site fidelity (Hooge and Eichenlaub 2000), which in turn reveals the existence of a measurable home-range (Spencer et al. 1990).

Individual home ranges were estimated using both the minimum convex polygon (MCP, Mohr 1947) and the fixed Kernel method (FK, Worton 1989), because a combination of polygon and contouring methods is often recommended to account for the potential limitations and shortcomings of each of these techniques (e.g. Kernohan et al. 2001, Boyle et al. 2009). Home-ranges based on MCPs were estimated for each animal by connecting the outermost points considering all locations made (MCP100), while checking for home-range asymptotes using the area-observation plots produced in AMAE. Using kernel analysis we calculated home-ranges based on 95% utilization contour (FK95, the area where animals spend 95% of its time) and defined the core-area as the area enclosed by the 50% isopleths (FK50, the area where voles spend 50% of its time) (Millspaugh et al. 2006). Smoothing parameters were determined by Least Squares Cross-Validation (LSCV), which results in less-biased home range estimates than other methods (Seaman and Powell 1996), and grid size was selected automatically by the AMAE. In order to ensure that home-ranges and core-areas better represented the active selection of any particular area, only active fixes were considered in range use estimates (Herr et al. 2009), thereby reducing potential problems of LSCV non-convergence due to identical or spatially closed fixes (Hemson et al. 2005). This also reduced autocorrelation between locations, although time to independence between locations (Swihart and Slade 1985) was not a main concern in this study, as some autocorrelation might be desirable to add biological meaning in range use analysis, in particular for that of small mammals (Rooney et al. 1998; De Solla et al. 1999; Fieberg 2007). All FK95 and FK50 estimates were based on > 30 locations, the minimum number required when using Kernel estimates with LSCV for bandwidth selection (Seaman et al. 1999). For each species, the MCP100 and FK95 home-ranges were compared using Mann-Whitney U tests (M-W, Siegel and Castellan 1988) in order to determine whether estimates were affected by the analytical technique. Differences in used ranges according to gender and season were investigated likewise for each species, with data pooled across years. Bonferroni corrections for two repeated tests on each species data set were used at the level of 5%, i.e. $p<0.025$ (Sokal and Rohlf 1995). Analysis were based on data combined for the two farmland areas considered in this study, because sample sizes were too small for carrying out separate analysis, particularly in the southern area ($n < 10$ for each species, see Results). Differences between species were compared by M-W after pooling gender, season and year data.

Static intra- and inter-specific interactions were examined using range overlap analyses for those animals radio-tracked within the same habitat patch during the same period. In this analysis two-dimensional home-ranges (HR_i and HR_j) were superimposed and the overlapped area ($O_{i,j}$) was estimated using the 'clip' tool in ArcView GIS project. The measure of space sharing was computed as the proportion of overlap of HR_i on HR_j ($HR_{i,j}$) and HR_j on HR_i ($HR_{j,i}$) for any dyad of ranges (Mizutani and Jewell 1998; Kernohan et al. 2001), i.e.:

$$HR_{i,j} = O_{i,j} / HR_i \quad \text{and} \quad HR_{j,i} = O_{i,j} / HR_j$$

A mean overlap value was calculated by using all overlap percentages, with a sample size of 2k where k is the number of dyads. These procedures were also employed for core-area overlap analysis. Intra and inter-specific overlaps of home-range and core-area were compared considering overall data and data from each season separately, using M-W tests (Siegel and Castellan 1988). Inter and intra-sexual overlaps were compared likewise, whenever the number of dyads within groups allowed statistical testing (i.e. ≥ 5 dyads per group, Siegel and Castellan 1988). Throughout the paper, mean values are presented together with the corresponding standard errors and range of variation.

3.3 Results

Altogether, 34 Cabrera voles were captured 53 times in 13 habitat patches, and 43 water voles were captured 78 times in 10 habitat patches, from a total of 239 captures made in the 18 patches surveyed. Although sign surveys suggested the occurrence of both species in seven of the surveyed areas, capture data only confirmed simultaneous coexistence in five of these. Mean (± se) number of Cabrera and water voles captured per sampling site was 2.6 ± 0.5 individuals (1 – 6) and 4.3 ± 1.2 (1 – 14), respectively. The Algerian mouse (*Mus spretus*) was also captured frequently (32.6% of the captures), occurring together with Cabrera voles in four sites, together with water voles in three sites, and together with both species in four sites. Rats (*Rattus* sp) were captured less often (9.6% of the captures), occurring in two sites with Cabrera voles, four sites with water voles, and three sites with both species. The greater white-toothed shrew (*Crocidura russula*) was rarely captured (2.5% of captures), occurring in two sites with Cabrera voles, one site with water voles, and in one site with both species. Finally, a single weasel (*Mustela nivalis*) was captured in a site occupied by water voles only (0.4% of captures).

A total of 31 Cabrera voles and 29 water voles were fitted with collar radio-transmitters. Mean (± se) weight of collared Cabrera voles was 48.4 ± 1.5 g (27 – 62 g), with no variation between sexes (U = 73, df = 1, p = 0.13), while that of water voles was 175.7 ± 7.9 g (92 – 261 g), also with no variation between sexes (U = 67, df = 1, p = 0.11). From the 60 animals collared, three water voles and one Cabrera vole were juveniles at the time of collaring, though they were sub-adults by the end of radio-tracking. The mean (± se) number of Cabrera and water voles radio-tracked per sampling site was 2.4 ± 0.4 (1 – 5) and 2.9 ± 0.8 (1 – 8), respectively. The percentage of animals radio-tracked during the dry season was 48% for Cabrera voles and 45% for water voles. Females represented 64% of the Cabrera voles radio-tracked and 55% of water voles radio-tracked. Overall, about 87% of the Cabrera voles tracked were reproductively active, with no significant variation between the wet and the dry season ($U = 89.0$, $df = 1$, $p = 0.717$), while the proportion of reproductively active water voles was 83%, also with no variation between seasons ($U = 78.5$, $df = 1$, $p = 0.268$). The number of Cabrera ($n = 9$) and water voles ($n = 6$) collared in the southern farmland area was relatively small.

Altogether, 9664 locations were obtained during the study, with a mean (± se) number of locations of 148.5 ± 11.9 (48 – 368) per Cabrera vole and 174.4 ± 18.1 (96 – 512) per water vole. Loss of signal due to predation, dispersion or discharge of transmitter batteries, prevented us to complete a full 24-hour cycle for two of the tracked Cabrera voles, although the number of locations for these animals was still greater than 30. Each individual vole was radio-tracked during a single season. The mean (± se) number of tracking days per Cabrera vole was 10.3 ± 1.1 (5 – 20 days) during the dry season and 8.8 ± 1.3 (4 – 23 days) during the wet season. The mean number of tracking days per water vole was 12.3 ± 1.7 (5 – 27 days) in the dry season and 8.3 ± 0.8 (5 – 10 days) in the wet season. Recovery of radio-transmitters was possible for 42% of the Cabrera voles and 65.5% of the water voles tracked. Signs of predation were evident for 16.1% and 3.4% of the Cabrera and water voles tracked respectively.

3.3.1 Site-fidelity

Results from radio-telemetry indicated that for most of animals the majority of fixes was densely clustered within a particular area. Fine-scale site-fidelity analysis showed that both R and LI values for 90.3% ($n = 31$) of tracked Cabrera voles and for all the 29 tracked water voles, were significantly lower ($p < 0.05$) than those corresponding to random walks. There was thus evidence that generally the movements by Cabrera and water voles were more constrained than would be expected from random tracks, and that individual ranges were sufficiently well defined for their boundaries to be determined with a high degree of confidence. It should be noted, however, that one Cabrera vole male exhibited fine-scale site-fidelity only after dispersive movement of 448 m, travelled during one night to a different habitat patch. Therefore, one further habitat patch was considered in the study, totalling 19 patches surveyed for radio-tracking (see Electronic supplementary material).

3.3.2 Range size

Area-observation curves of individual MCP100 home-range estimates approached satisfactorily an asymptote for all animals showing site fidelity. MCP100 home-ranges varied between 57.5 and 987.5 m^2 for Cabrera voles (mean ± se = 375.1 ± 45.7) and between 230.5 and 2,858.5 m^2 for water voles (mean ± se = 828.0 ± 120.2). Although slightly smaller, these estimates did not vary significantly from those produced by 95% kernels (Cabrera voles: U = 370, df = 1, p = 0.72; water voles: U = 366, df = 1 p = 0.40). Therefore, subsequent analyses were based on FK95 home range estimates (Figure 3 and Electronic supplementary material), as the kernel techniques are expected to provide a better representation of the internal structure of individuals range use (Harris et al. 1990; Marker et al. 2008). FK95 home-ranges of Cabrera voles ranged between 39.3 and 1,075.6 m^2 (mean ± se = 418.2 ± 56.3 m^2), while for water voles it ranged between 198.3 and 2,600.2 m^2 (mean ± se = 946.3 ± 126.3 m^2). Core-area sizes (FK50) of Cabrera voles ranged between 1.9 and 182.4 m^2 (mean ± se = 55.1 ± 9.3 m^2) whereas estimates for water voles ranged between 21.1 and 562.4 m^2 (mean ± se = 156.6 ± 28.2 m^2). The M-W tests revealed that water voles had significantly larger home ranges (U = 174, df = 1, $p < 0.001$) and core areas (U = 164, df = 1, $p < 0.001$) than Cabrera voles. Within each species, there were no sexual or seasonal differences either in home-range or core-area estimates (M-W: $p > 0.05$ for all tests, after Bonferronni corrections).

3.3.3 Spatial overlap

Overlap of home ranges was always higher than overlap of core-areas for intra-specific space sharing analysis (M-W: $p < 0.05$ for all tests), considering either overall data or data from each season separately (Figure 2). Inter-specific overlap of home-ranges was significantly lower than that observed among conspecifics (Figure 3 and Electronic supplementary material), considering both the overall data (U = 1431, df = 1, $p < 0.001$) and data from the wet season (U = 398, df = 1, $p < 0.001$) (Figure 2). During the dry season, home-range sharing by Cabrera and water voles was apparently higher than that observed during the wet season (Figures 2, 3 and Electronic supplementary material), reaching up to 60.4%. However, rank-based comparison did not show significant differences between seasonal inter-specific home-range overlap (U = 87, df = 1, p = 0.588). Despite this, inter-specific home-range overlap during the dry season was high enough to be considered similar to that observed within conspecifics (U = 308, df = 1, p = 0.230). There was no overlap between core-areas of Cabrera voles and water voles, in either the dry or the wet season (Figure 2).

Considering the inter-individual static interactions among conspecifics, results suggest a reduced home-range overlap among water vole males (Figure 2), with significant differences from the overlap measured between males and females (U = 387, df =1, p = 0.002) and nearly significant differences in relation to female overlap (U = 239, df = 1, p = 0.061). Maximum home-range overlap observed among water voles was high, reaching over 86% in all groups compared. Water vole males never shared core-areas (Figure 2), while females did and in a greater extent than the core-area sharing between sexes (U = 169, df = 1, $p < 0.001$). Despite this, maximum inter-sexual core-area overlap observed for water voles reached 100%, while among females it was 66.2%. In the case of Cabrera voles, only two males were followed simultaneously in the same sampling site, showing no spatial overlap. Because of this, only female and inter-gender overlaps were compared for Cabrera voles, with no differences observed both in the case of home ranges (U = 293, df = 1, p = 0.890) and core areas (U = 280, df = 1, p = 0.617). Maximum home-range and core-area overlap observed for Cabrera voles reached over 87% and 100% respectively for both groups considered (between sexes and among females).

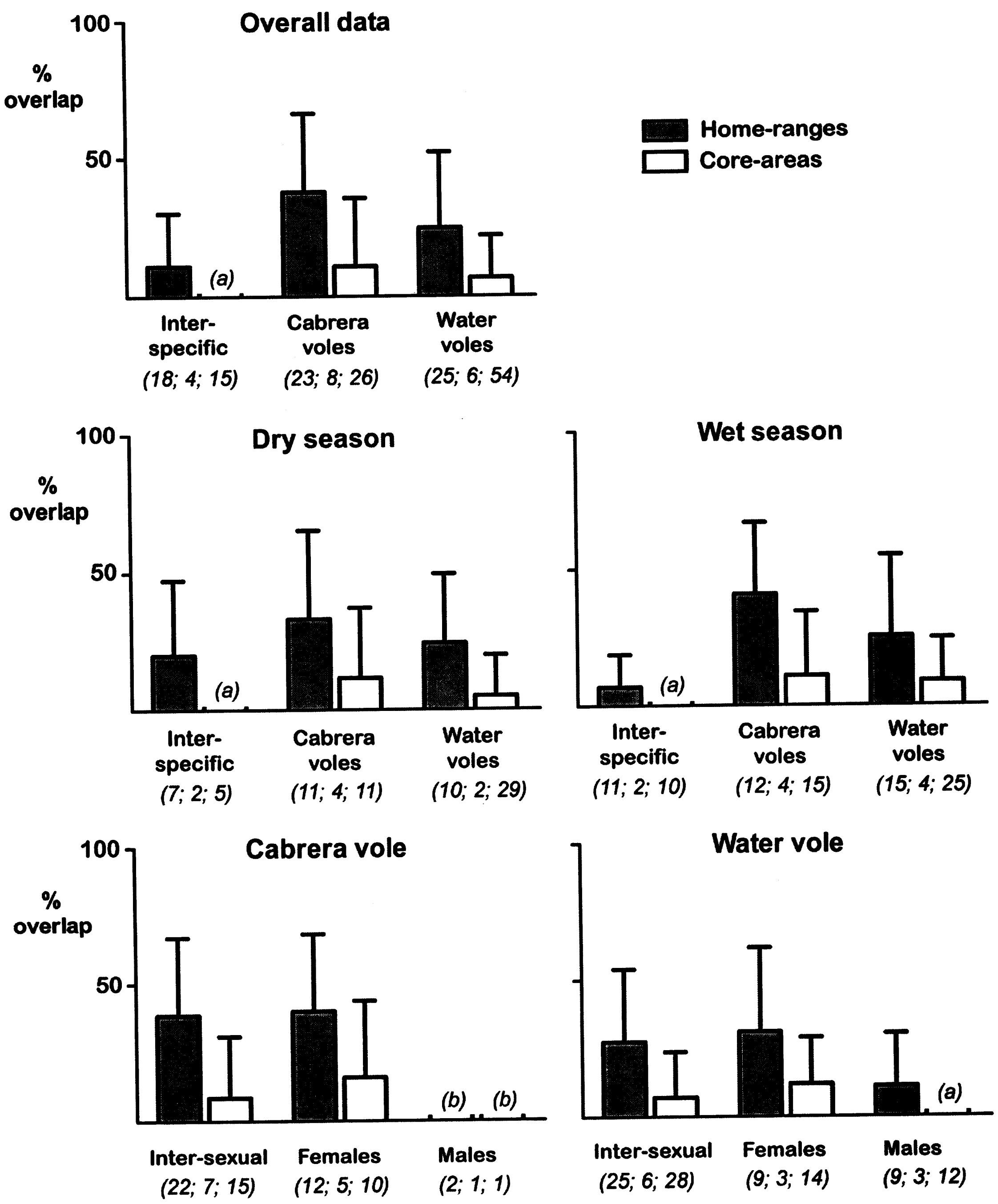

Figure 2 – Static interactions showing the mean percentage of spatial overlap between individual radio-tracked during the same period at the same habitat patch. Error bars show 95% confidence intervals. Sample sizes are given as *(number of voles, number of sampling sites, number of dyads)*. *(a)* no spatial overlap; *(b)* insufficient data.

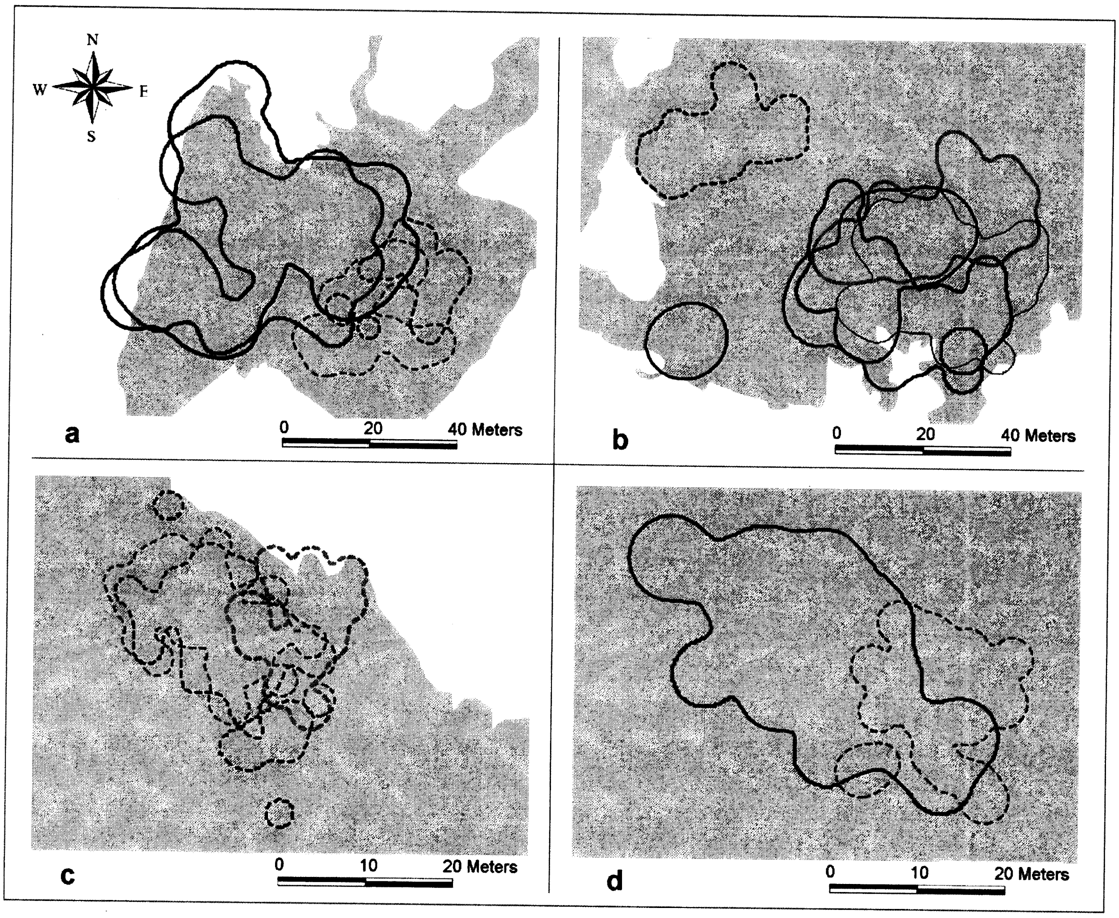

Figure 3 – Examples of individual home-ranges and spatial overlap of Cabrera (dashed lines) and water voles (solid lines), based on 95% fixed kernel (FK95), during the wet (A and B) and dry (C and D) seasons (see also Electronic supplementary material). Sampling site A is located at the southern farmland area, while B, C, and D are located in the northern farmland area. For both species, males are represented by black lines and females are represented by lines with different scales of greys. Suitable habitat-patches are shown in light grey.

3.4 Discussion

To the best of our knowledge, this is the first study describing from radio-tracking the use of space by wild ranging Cabrera and water voles, and to document the spatial interactions among individuals of the two species. The study provided important novel information regarding the spatial ecology of each species, allowing inferences on several relevant and still understudied traits of their life-histories. Also, our results evidenced that the distribution of Cabrera and water voles may be driven, at least partially, by spatial processes reducing inter-specific encounters, which in turn may reflect eventual competitive displacement or niche differentiation at small-scales of habitat heterogeneity (Mouquet at al. 2005). These findings have important implications for the conservation management of habitats of the two species in areas of sympatry.

3.4.1 Range use and spatial organisation within species

This study revealed that both Cabrera and water voles tended to limit their movements to a circumscribed home-range, at least during tracking periods of up to about 25 days. Such strong fine-scale site-fidelity probably reflected the relatively high seasonal association of individual voles to a particular habitat patch, as reported for both species based on long-term capture-recapture studies (Fernández-Salvador et al. 2005; Román 2007). Faithful behaviour of Cabrera and water voles to an area may be related with the very demanding habitat characteristics required by animals from both species to fulfil at least some their basic life-history traits (e.g. foraging, resting, thermal regulation, mating). In addition, because habitat patches may be crossed either by other potential competitors, such as the more aggressive *Rattus* sp. (Fernández-Salvador 1998; Fedriani et al. 2002; Ventura 2004; Román 2007), or by predators specialised on voles, such as the weasel (Brandt and Lambin 2007), fine-scale site-fidelity might be particularly important for voles by providing knowledge of escape routes to secure sites within the home-range.

In general, home-ranges and core-areas sizes estimated for both species agree with those expected according to the allometric relationship between body size and home-range size in mammals (Swihart et al. 1988), with water voles moving over ranges about twice that of Cabrera voles. Despite this, home-ranges estimated for Cabrera voles were larger than those inferred for the species from capture-recapture data (between 80 – 100 m^2, Fernández-Salvador 1998). Similar comparisons concerning water voles were not possible, because no other studies reported quantitatively on their home range sizes. However, our results suggests that apparently, home-range sizes of water voles in our study area might be slightly larger than those usually referred for its congener *Arvicola terrestris* along narrow (1- to 2-m) linear habitats of northern Europe (length ranges < 300 m, e.g. Moorhouse and Macdonald 2005). Results on home-range and core-area sizes of Cabrera and water voles also revealed that variation between seasons was reduced, which could be related with the fact that most of the collared voles were reproductively active at the time of tracking, irrespective of the seasonality trends in reproductive cycles referred for both species (Fernández-Salvador et al. 2005; Román 2007)

Home-range and core-area sizes of Cabrera and water voles showed also little variation among sexes and considerable inter-sexual overlap, reaching over 85% for home-ranges and up to 100% for core-areas in both species. Low inter-sexual variation in range sizes together with high inter-sexual overlap and reduced sexual dimorphism in body size are typical traits of monogamous species (Gaulin and FiztGerald 1988; Roberts et al. 1998; Wolf 2007). Although this could not be fully confirmed from our data, monogamous mating system was suggested for the Cabrera vole (Fernández-Salvador 1998; Fernández-Salvador at al. 2001). Monogamy in water voles was referred to occur only in small habitat patches with reduced chances of being occupied by more than one couple of reproductive animals, otherwise prevailing the promiscuous mating system (Román 2007). It is also worth noting however that space sharing among the water voles studied here was relatively high between females, while males apparently avoided overlapping their ranges with other males, particularly at the core-area scale. Thus, our data concerning water voles could also fit the resource-defence polygyny models, in which males defend one or more females or a critical resource that will give them access to the females using that resource, as opposed to promiscuous tactics (Wolff 2007). There are thus reasons to hypothesise that water voles may exhibit some plasticity in the mating system according to local variations in quality and abundance of habitat patches. The high spatial segregation among water vole males tracked in this study further suggests that this species may have a tendency for a male-biased dispersal in our study area, as also suggested for the species in other regions (Román 2007).

3.4.2 Spatial interactions between species

This study confirmed that, at least in habitat patches with no permanent surface waters, Cabrera and water voles might not only co-exist nearby, but also share the same areas within patches. In addition, the strong fine-scale site-fidelity of co-existing Cabrera and water voles suggests that none of the species appeared to be actively driving the other out of the area, supporting the view that spatial coexistence was stable. However, overall spatial overlap between species was relatively low at the home-range scale and there were even evidences for a considerable spatial segregation between species at the core-area scale across all seasons. Therefore, space sharing among species was in practice limited to foraging areas outside the centre of activity of individuals (exploitative competition) and species may thus co-exist via spatial segregation. Because core-area overlap indicates a greater potential for competition than home-range overlap (Brunjes et al. 2009), non overlapping core areas between species suggests that inter-specific competition influenced the spatial distribution of voles more than intra-specific competition. However during the dry season, when the availability of food resources was presumably lowest (Ventura at al. 1998; Román 2007; Rosário et al. 2008), the potential for exploitative competition was highest, as indicated by the increased inter-specific home-range overlap. This suggests that the strength of competition concerning space sharing between Cabrera and water voles may vary locally according to the quality and abundance of habitat patches (Amarasekare et al. 2004), which in turn suggests that other mechanisms of coexistence besides spatial segregation might occur. These may include spatial partitioning on a temporal scale or habitat partitioning at finer spatial scales than can be detected by our home-range overlap analysis (Amarasekare 2003; Brunjes et al. 2009). Spatial segregation between Cabrera and water voles may indeed result from differences in the way species utilise their habitat at small scales rather than direct competition between species. The discrepant spatial range sizes between Cabrera and water voles, suggests that differences in the scale of resource perception might in part explain the coexistence of species (Christopher and Barret 2006; Cromsigt and Olff 2006) and should be accounted in habitat selection studies aiming to investigate habitat differentiation between species.

The higher body size of water voles suggests that this species may be a superior competitor, which in the context of source-sink metapopulation dynamics, might indicate that Cabrera voles are superior at colonizing empty patches (Amarasekare and Nisbet 2001), irrespective to dispersal-range abilities of species. Although this could not be checked from our data, results on fine-scale site-fidelity analysis revealed that the only individuals showing no site fidelity (n=3) or dispersive movements (n=1) were Cabrera voles, which could be indicative of their higher propensity to move away from a habitat patch than water voles. Eventual differences in trade-offs between competitive and dispersal abilities might thus contribute as a further mechanism allowing Cabrera and water voles coexistence in fragmented farmland. Therefore, although our study suggests an effective spatial segregation among Cabrera and water voles, it also reinforces that further research on specific life-history traits and niche differentiation is needed in order to fully understand coexistence of these species within patches in Mediterranean farmland.

3.4.3 Management implications

The southern water vole and the Cabrera vole are globally vulnerable and near-threatened species (IUCN, 2009), respectively, and so securing their populations within farmland landscapes may be considered a conservation priority (Pita et al. 2007; Rigaux and Charruau 2007). Previous studies suggested that achieving such goal requires a network of little grazed and well connected tall herb humid habitat patches (Pita et al., 2007; Fedriani et al. 2002), which might be protected even in intensively managed landscapes through agri-environment and cross-compliance schemes promoting the retention of grass margins and other interstitial habitats (Stoate et al., 2009). This view is supported by the present study, which showed that individuals of both species circumscribe their routine movements to small areas of suitable habitat, where they likely find adequate foraging and breeding conditions. Based on estimates of home range size and maximum inter-sexual overlap, the study suggests that areas of about 500 m^2 and 1,100 m^2 might be sufficient to support one Cabrera and one water vole breeding pair, respectively, which underlines the importance of even relatively small habitat patches. However, larger areas should be considered to increase the chances of local population persistence, because the likelihood of extinction is generally higher when population units are small (Legendre et al. 2008). In the case of Cabrera voles, Pita et al. (2007) showed that population persistence was indeed higher in larger patches, recommending that conservation programs in fragmented landscapes should strive to maintain patches of about 2,000-5,000m^2. The area requirements estimated in this study suggests that about 4-10 breeding pairs may inhabit patches of this size, corresponding to very small populations with a presumably high risk of stochastic extinction (Hanski 1999; Legendre et al. 2008). This strengthens the need to maintain the connectivity among habitat patches, thereby increasing the chances of metapopulation persistence, despite the frequent extinction of local populations (Fernández-Salvador et al. 2005; Pita et al. 2007)

Results of this study also support the view that species interactions may need to be accounted for when designing conservation management strategies for Cabrera and water voles in fragmented landscapes (Pita et al. 2006), given the significant levels of inter-specific spatial segregation observed. Although both species appeared to coexist within the same habitat patch, it is possible that local spatial segregation resulted from larger water voles displacing Cabrera voles from potentially favourable habitats, as it has been found elsewhere for species of different sizes exploring the same or similar resources (e.g., Oro et al., 2009 and references therein). Therefore, the presence of water voles may effectively reduce habitat availability and thus Cabrera vole population size at local habitat patches, thereby increasing the probability of metapopulation extinction at the landscape scale (Pita et al., 2007). This possibility requires further investigation, for the conservation of asymmetric competitors in fragmented landscapes is a challenging task, which should be based on a detailed understanding of the spatial and temporal mechanisms of resource partitioning that allow species coexistence in areas of sympatry (e.g., Oro et al., 2009).

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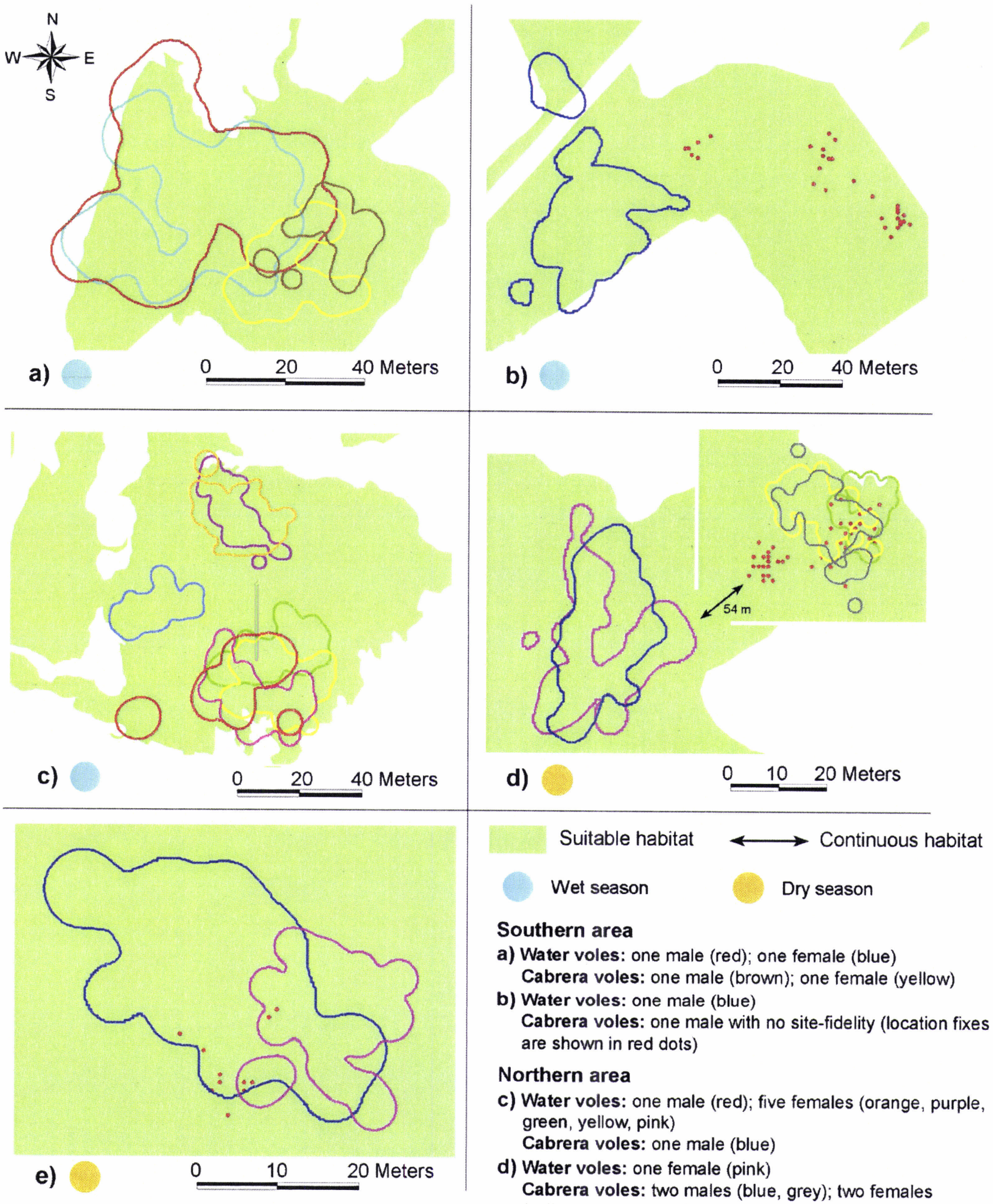
Supplementary Material

Home-ranges estimated for 28 Cabrera voles and 29 water voles radio-tracked whithin 18 + 1 habitat patches in Mediterranean farmland of south-western Portugal: **1.** Habitat patches with both species; **2.** Habitat patches with water voles only; **3.** Habitat patches with Cabrera voles only.

1. Habitat patches with both species



Southern area

a) Water voles: one male (red); one female (blue)
Cabrera voles: one male (brown); one female (yellow)

b) Water voles: one male (blue)
Cabrera voles: one male with no site-fidelity (location fixes are shown in red dots)

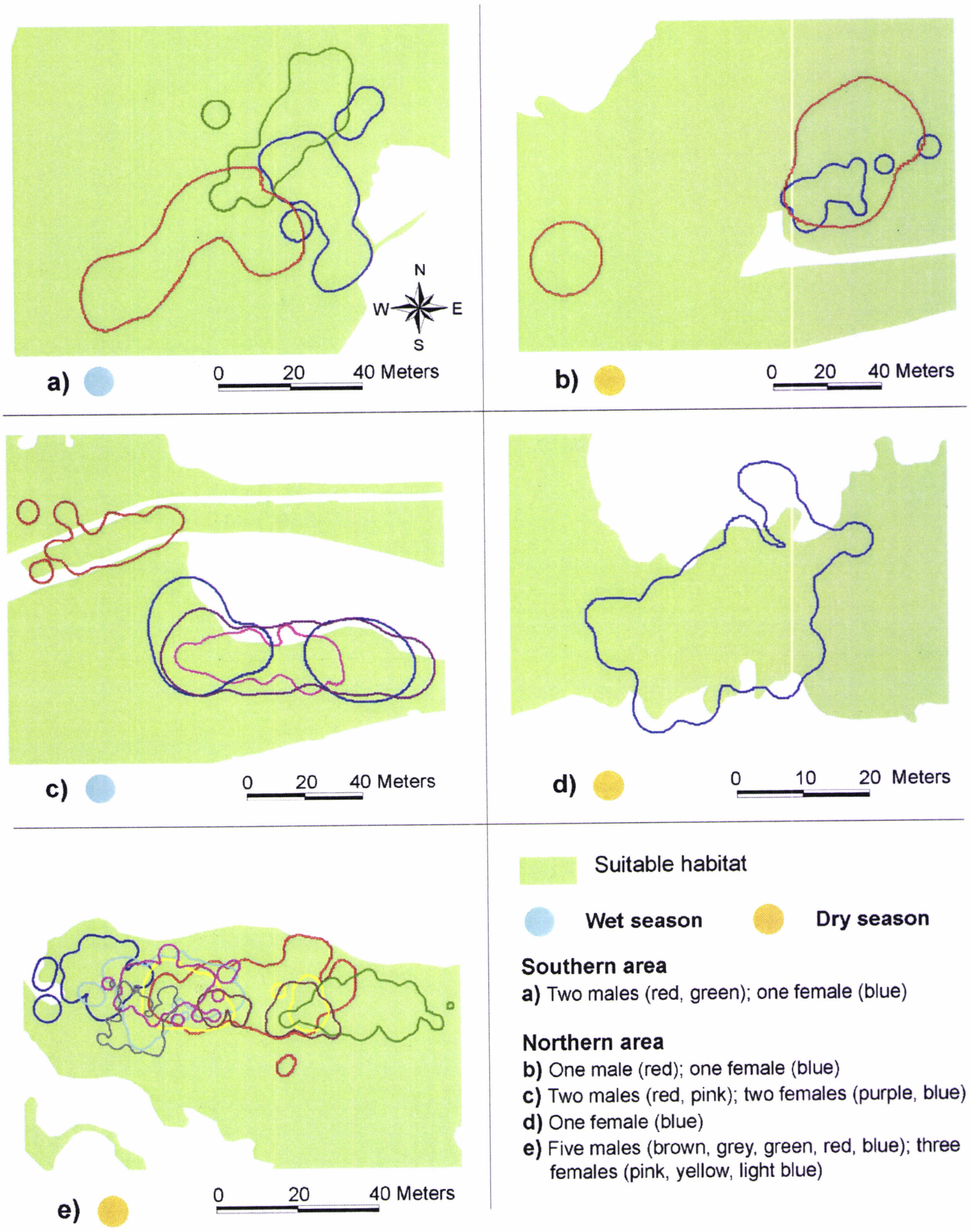
Northern area

c) Water voles: one male (red); five females (orange, purple, green, yellow, pink)
Cabrera voles: one male (blue)

d) Water voles: one female (pink)
Cabrera voles: two males (blue, grey); two females (yellow, green); one female with no site-fidelity (location fixes are shown in red dots)

e) Water voles: one female (blue)
Cabrera voles: one female (pink); one female with no site-fidelity (location points are shown in red dots)

2. Habitat patches with water voles



🟩 Suitable habitat

● **Wet season** ● **Dry season**

Southern area

a) Two males (red, green); one female (blue)

Northern area

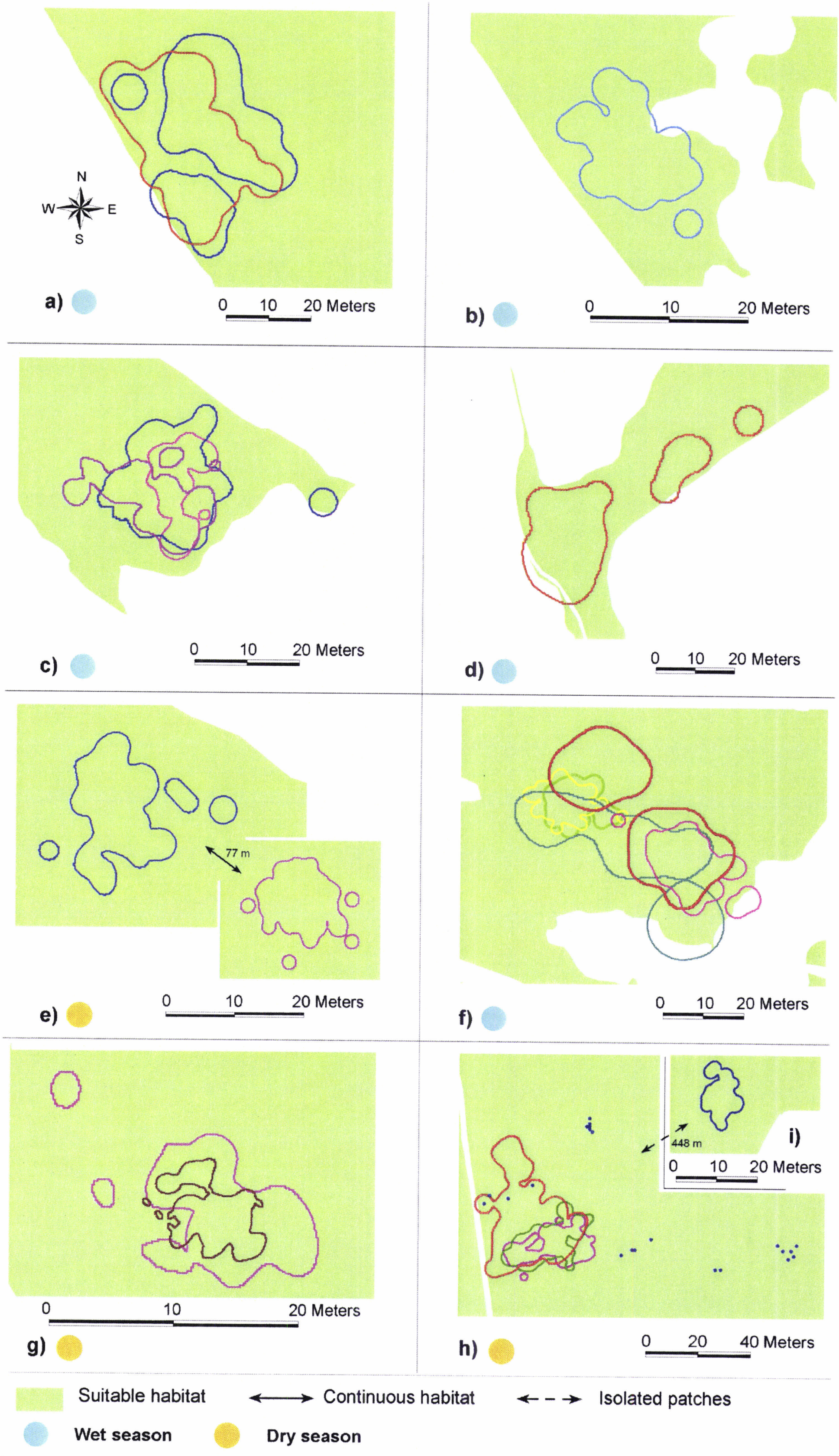
b) One male (red); one female (blue)

c) Two males (red, pink); two females (purple, blue)

d) One female (blue)

e) Five males (brown, grey, green, red, blue); three females (pink, yellow, light blue)

3. Habitat patches with Cabrera voles



Southern area:
a) One male (red); one female (blue) **b)** One female (blue) **c)** One male (blue); two females (purple, pink)

Northern area:
d) One female (red) **e)** One male (blue); one females (pink) **f)** One male (red); four females (blue, pink, yellow, green)
g) Two females (red, blue) **h)** One male (red); two females (pink, green); one male with no site-fidelity (location fixes are shown in blue dots) and the same male in **i)** after dipersal from *h)*

CHAPTER 4

Assessing microhabitat differentiation between coexisting species: The role of spatial scale

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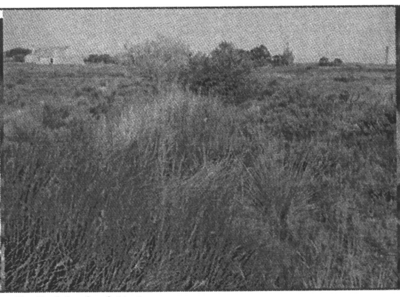
Water vole



Suitable habitat

Cabrera vole

4. Assessing microhabitat differentiation between coexisting species: The role of spatial scale

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Abstract:

Although the importance of spatial scale for understanding habitat selection patterns and processes has long been recognized, little is known about its impact on the identification of habitat differentiation between sympatric species, despite its likely utility in assessing niche partitioning and thus explaining species coexistence. Here we used radio-telemetry data to examine seasonal microhabitat selection and differentiation by Cabrera (n=28) and water voles (n=29) within habitat patches in a highly fragmented landscape, across spatial extents and resolutions. Microhabitat selection was found for both species at the home-range and core-area scales, tending to be strongest for water and Cabrera voles at coarse and fine spatial resolutions, respectively. Water voles showed higher preference for humid sedge/rush (SR) and reed (RE) microhabitats across seasons and spatial scales. Cabrera voles consistently selected tall grass (TG) and shrub microhabitats during the wet season, whereas dry season preference was higher for SR and TG at fine and coarse spatial resolutions, respectively. Niche overlap was highest during the dry season, lowest at the core-area scale, and increased with spatial resolution. These patterns likely reflected the fine scale, seasonal habitat preferences of the Cabrera vole, which during the dry season increased the use of small SR patches embedded in larger TG meadows, thereby bringing it in closer contact to the humid microhabitats selected at coarse spatial resolutions by the water vole throughout the year. Overall, this study suggests that spatial scale may critically influence the perception of habitat differentiation between coexisting species.

Keywords: Cabrera vole, Habitat selection, Mediterranean farmland, Niche overlap, Spatial scale, Species coexistence, water vole

4.1 Introduction

Analysing the range of spatial scales at which habitat selection by animals operates has important implications for understanding how species perceive and respond to their environment (Mayor et al., 2007; Schaefer and Mayor, 2008) and how they discriminate between habitats of different quality (Chalfoun and Martin, 2007, Bellier et al., 2010). Multi-scaled habitat selection studies provide more detailed characterization of species habitat profiles (Boyce, 2006; Mayor et al., 2009) by including multiple hierarchical orders of selection (e.g. Rettie and Messier, 2000; Beasley et al., 2007) and multiple spatial resolutions of habitat resources across varying domains or extents of analysis (e.g. Thompson and McGarigal, 2002; Wheatley, 2010). As a consequence, an increasing number of habitat selection studies are routinely conducted at multiple spatial scales (Mayor et al., 2009).

Although the utility of multi-scaled habitat selection studies is now widely recognized, this approach has rarely been used to assess differentiation among interacting species sharing the same habitat types, despite its presumably high potential to understand niche segregation and species coexistence (Boyer and Rivault, 2006; Gilbert et al., 2008, Indermaur et al., 2009). Nevertheless, there is already some evidence that it may be inappropriate to analyse habitat differentiation at a single spatial scale, because species often differ in the way they perceive and react to their habitat (Zollner, 2000; Girvetz and Greco, 2007). Even within guilds, there is no single best scale for habitat differentiation studies (Holland et al., 2005; Moura et al., 2005), because for instance larger species tend to have larger home-ranges and are expected to perceive their habitat at coarser scales, while smaller species should move and identify habitat patches within their home-ranges at finer spatial scales (Mech and Zollner, 2002; Holland et al., 2005; Cromsigt and Olff, 2006). Therefore, in order to properly understand differential habitat selection as a potential mechanism for coexistence, studies on resource partitioning between sympatric species should evaluate how differentiation changes across spatial scales (Holland et al., 2005; Cromsigt and Olff, 2006; Indermaur et al., 2009). In addition, because multi-scaled habitat selection by a species might change across seasons (e.g. Beasley et al., 2007), habitat differentiation between species is also expected to change accordingly. Information on how habitat differentiation among interacting species changes across space and time may be particularly important to understand coexistence of close-related sympatric species (Barrows et al., 2005; Boyd et al., 2008), particularly by providing information on the quantitative effects of patchiness on the success with which species move and identify preferred habitats.

This study evaluated the impact of spatial extent and resolution of analysis on the perception of microhabitat differentiation between two closely related species, and how this changed across seasons with contrasting environmental conditions. Specifically, we used radio-telemetry data to explore within-patch, multi-scaled habitat differentiation between the Cabrera vole (*Microtus cabrerae*) and the water vole (*Arvicola sapidus*), living sympatrically in a highly fragmented Mediterranean farmland landscape (Pita et al., 2007; in press). These species were considered particularly adequate, because they are both habitat specialists, which seem to require similar sedge/rush vegetation (e.g. Fedriani et al., 2002; Santos et al., 2006; Pita et al., 2007; Román, 2007) and often coexist within the same habitat patch (Pita et al., 2006; in press). Preliminary studies showed considerable spatial avoidance between sympatric Cabrera and water voles inhabiting the same patch (Pita et al., in press), suggesting that fine-scale habitat differentiation might be a primary form of inter-specific ecological partitioning. We firstly assessed how each species respond to spatial scaling of their habitat across hierarchical orders and seasons, and then investigated how spatial scaling determines the type and magnitude of habitat differentiation between species. We predicted that a) seasonal habitat selection should change according to the spatial scale considered in terms of hierarchical orders, domain of analysis, and spatial resolution of habitat mapping, reflecting the scale of the behaviour processes under study and the response by animals to the spatial heterogeneity and level of fragmentation of their habitats (Mayor et al., 2009); b) seasonal niche overlap may as well change according to the spatial scale of the analysis, reflecting the varying levels of habitat segregation across species utilization distributions (Pita et al., in press) and the differential response to the scale of habitat patchiness perceived by animals (Indermaur et al., 2009); c) because of their larger body size and home-ranges (Pita et al., in press) water voles should perceive preferred habitats at coarser spatial scales than Cabrera voles (Zollner, 2000; Holland et al.. 2005). Results were then used to discuss the importance of multi-scaled habitat differentiation selection when interpreting species coexistence in heterogeneous environments.

4.2 Materials and methods

4.2.1 Study area

The study was conducted in the coastal plateau of south-western Portugal, which is included in the thermo-Mediterranean bioclimatic zone (Rivas-Martinez, 1981). Mean annual temperature is about 16 °C and mean annual rain fall around 650 mm, of which < 15% falls during the hot and dry season. This region is largely devoted to agriculture and livestock production, with arable land and pastures covering about over 65% of the landscape (Pita et al., 2009). Wood cover is restricted to a few woodlots and hedges with planted pines and eucalyptus delimiting irrigated fields, while natural and semi-natural habitats (cork oak woodlands, shrubby and marshy vegetation) are most frequent in the surroundings of extensive agricultural fields (Pita et al., 2009). Agricultural practices have strongly intensified during the last two decades, with increases in the area devoted to irrigated agriculture and in cattle stock densities, and detrimental impacts on biodiversity (Beja and Alcazar, 2003; Pita et al., 2006; 2007; 2009).

4.2.2 Study design

The study was based on radio-telemetry data from 28 Cabrera voles and 29 water voles tracked between April-2006 and April-2008, at 19 habitat patches (details in Pita et al., in press; Table 1). These data provided information on the use of space and habitats by each vole, and to estimate its home-range and core-area from the 95% and 50% fixed Kernel (FK) method (Worton, 1989) using the Animal Movement Analysis Extension (AMAE, Hooge and Eichenlaub, 2000) for ArcView 3.2 (ESRI, Redlands, CA, USA). In these estimates, the Least Square Cross Validation (LSCV) was used to calculate smoothing parameters and the locations where the animals remained inactive were discarded, so as to ensure that home-ranges and core-areas represented the active foraging selection of any particular habitat (Pita et al., in press). Habitat selection was then investigated hierarchically based on the use-availability approach (e.g. Aebischer et al., 1993; Thomas and Taylor, 2006), considering two orders of selection reflecting three different spatial extents defined by voles behaviour: second-order home-range and core-area selection, and third-order core-area selection (Johnson, 1980; Beasley et al., 2007). Second-order selection was estimated by comparing habitat composition within home-ranges and core-areas, with habitat composition within a surrounding area that was assumed to be available to voles. To reduce subjectivity in defining habitat availability, points generated by random walks tests (Pita et al., in press) were used to estimate the 95% fixed Kernel_{LSCV} probability range area for each animal, using AMAE (Hooge and Eichenlaub, 2000). This surface limits the 95% probability of finding each vole in the case of random habitat use and accounts for the individual ranging behaviour during tracking, thereby reflecting the spatial scale at which animals make habitat-use decisions (Heithaus et al., 2006). This approach alleviated many problems related with arbitrary definition of available area boundaries (Arthur et al., 1996), as it makes no assumptions about the structure of the study area, or the directionality and distances of steps, and does not assume that starting points are random with respect to habitat (Heithaus et al., 2006). Third-order selection was estimated by comparing habitat composition between core-areas and home-ranges (Beasley et al., 2007).

Table 1 – Summary of radio-telemetry data used to analyse habitat differentiation by Cabrera and water voles. Core-area and home-range estimates were based respectively on 50% and 95% fixed Kernel (FK) techniques using active fixes and Least Square Cross Validation (LSCV).

		Cabrera vole	Water vole
Number of animals (females/males ratio)	Overall	28 (1.8)	29 (1.2)
	Dry season	13 (1.6)	13 (1.2)
	Wet season	15 (2.00)	16 (1.3)
Mean number of position records per animal	[range]	154.9 [64-368]	174.4 [96-512]
Mean 50% FK core-area [range] (m^2)		55.1 [1.9-182.4]	152.6 [21.1-562.4]
Mean 95% FK home-range [range] (m^2)		418.2 [39.3-1075.6]	946.3 [198.3-2600.2]

4.2.3 Habitat mapping

Habitats were quantified in a Geographic Information System, from maps prepared using recent (2005) high resolution (0.5 m/pixel) aerial photographs and field surveys. Delimitation and classification of habitat coverages were made at the scale of 1:300, considering 10 habitat classes reflecting the main land-use practices and farmland management options in each area, including the type of vegetation within uncultivated arable land (Table 2). To reduce eventual subjectivity in polygon delineation, areas with less than $3m^2$ were dissolved into adjacent polygons. For the analysis of habitat selection in relation to grain size, we further generated four additional cover maps considering Minimum Mapping Units (MMU) of $10m^2$, $30m^2$, $100m^2$ and $300m^2$.

Table 2 – Classes considered in habitat mapping to study habitat selection and differentiation among Cabrera and water voles in south-western Portugal.

Habitat class	Description
Roads	Dirt and paved roads
Houses	Human buildings and social areas
Agricultural	Ploughed and cultivated land.
Open Water	Ponds, streams and irrigation channels
Short Herbs	Short (~ < 30cm) herbaceous vegetation resulting from heavy grazing by cattle or mowing
Tall Grasses	Tall (~ ≥ 30cm) mesic grasses and forbs with reduced grazing and often with scattered soft-wooded shrubs (e.g. *Dittrichia* sp, *Carpobrotus* sp.), small shrubs or planted trees.
Sedges/Rushes	Tall wet vegetation mainly composed by the families Juncaceae (e.g. *Juncus* sp, *Luzula* sp.) and Cyperaceae (e.g. *Carex* sp., *Scirpus* sp., etc)
Reeds	Tall riparian herbaceous vegetation mainly composed by *Phragmites* sp. and/or *Thypha* sp.
Shrubs	Shrubs (e.g. *Rubus* sp, *Ulex* sp., *Acacia* sp., brushy *Quercus* sp., *Salix* sp, etc.)
Planted trees	Upper canopy trees of *Eucalyptus* sp and *Pinus* sp.

4.2.4 Statistical analysis

Compositional analysis (CA, Aebischer et al., 1993) was used to determine whether habitat use differed from random, by comparing the matrices of log-ratio-transformed use and availability distributions with a log-likelihood ratio statistic (Wilks' lambda, λ). By treating individuals as the experimental unit, this analysis avoids auto-correlation and pseudo-replication problems encountered when telemetry locations are used as sampling units (Aebischer et al., 1993), allowing population-level inference of habitat use by each species (Millspaugh et al., 2006; Sawyer et al., 2007). The logarithmic transformations underpinning compositional analysis require that all habitat types are available to and used by each animal (Aebischer et al., 1993), which was not fulfilled in our data, in particular for the largest grains of analysis. Therefore, for each particular hierarchical scale of habitat selection, grain was analysed by starting from the smallest MMU to the largest MMU not exceeding 30% of the average used-range being analysed (Chow et al., 2005), thereby keeping enough resolution to accurately identify the details of forage selection by animals (Fortin et al., 2005). As a further attempt to reduce missing values, the habitat types with low prevalence (bare ground, farm houses and open water) and those seldom used by voles (agricultural, short grazed herbs and tree plantations) were excluded from analysis. Therefore, before CA we subtracted their areas (when non-zero) from the areas being compared. Even so, there were still some null proportions in both available and used areas. When zero values were found in the matrix of available habitats, we computed a mean λ by weighting each dominator-dependent value of λ by the number of non-missing values involved in its calculation and determining the level of significance by randomization (Aebischer et al., 1993). Missing values in the matrix of used habitats were replaced by 0.01%, which is about an order of magnitude smaller than the smallest non-zero value (Britschgi et al., 2006) and avoided inflating type I error rates when very small values (0.0001-0.001) are used (Bingham and Brennan, 2004). Misclassification rate of habitat selection was also minimized by using randomization procedures (1000 replicates) for both the habitat ranking and habitat selection tests (Bingham and Brennan, 2004; Thomas and Taylor, 2006). If use was significantly non-random, habitat types were ranked from the most to the least selected, using a matrix of mean and standard deviation of log ratio differences for all habitat types. Habitat selection was analysed separately for the dry (May – September) and the wet (October – April) seasons, with data pooled across years. All CA's were implemented in the package adehabitat (Calenge, 2006) for R 2.10.10 software (R Development Core Team 2009).

Inter-specific overlap in habitat use between Cabrera and water voles was assessed for each season and at each spatial scale (grain and extent), using Piankas's niche-overlap index (Pianka, 1974). This is a symmetrical measure that ranges between 0 when no habitats are used in common, to 1 when there is complete overlap. Overlap indexes were computed in the pgirmess R package (Giraudoux, 2010) and the 95% Confidence Intervals (CI) of estimates were assessed using 1000 permutations. We further explored habitat differentiation for each season and at different spatial scales by comparing the log-ratios produced in CAs. One thousand random pairwise differences between Cabrera and water voles log-ratios of used habitats were estimated for each particular habitat type at each spatial scale, with positive values indicating higher use by Cabrera voles, and negative values indicating higher use by water voles. Because there were missing values in the matrices describing the available habitat, log-ratios obtained considering each reference habitat were averaged and used for differentiation analysis. This allowed the evaluation of the selection strength relative to species, which is not measurable by using CA only (e.g. Gosselink et al., 2003; Gehrt and Prange, 2007).

4.3. Results

4.3.1 Habitat selection

Cabrera voles showed large differences in habitat composition between available and used home-ranges and core-areas (Supplementary material, Tables S1 and S2), reflecting significant second-order habitat selection (Table 3 and 4). At the home-range scale, habitat selection appeared stronger at fine spatial resolutions during the dry season, with voles showing preference for sedges and rushes (SR) and, to a much lesser extent, for tall grasses (TG) (Table 3). Habitat selection during the dry season was weaker at coarser resolutions and preferences appeared to changed, with TG ranking first, followed by SR. Reeds (RE) and shrubs (SH) consistently ranked third and fourth across spatial resolutions (Table 3). Second-order habitat selection at the core-area scale during the dry season also revealed a strong preference of Cabrera voles for SR, followed by TG, although at this spatial extent RE ranked lastly (Table 4). Cabrera voles also tended to show third-order habitat selection at the highest spatial resolution during the dry season (λ=0.01; p=0.06), with habitats ranked as SH>TG>SR>RE. During the wet season, second-order habitat selection at both the home-range and core-area scales was significant across all spatial grains analysed, with TG and SH ranking first and second, respectively, followed by SR and RE (Table 3). However, according to Wilks' lambda scores, second order selection by Cabrera voles at the home-range scale was particularly strong at fine spatial resolutions (Table 3). No third-order selection by Cabrera voles was detected during the wet season.

Water voles also showed strong second-order selection (Table 3 and 4), with large differences in habitat composition between available and used areas (Supplementary material, Tables S1 and S2). At the home-range scale, SR was consistently the most preferred habitat across seasons and spatial resolutions. RE tended to be the second most preferred habitat across seasons, except at coarser spatial resolutions, for which it was among the least selected habitats, with an increased importance of SH and TG during the dry and wet seasons, respectively (Table 3). Analysis at the core-area scale yielded much similar results, underlining a consistent preference for SR and a strong avoidance of TG, particularly during the dry season, when SH was more selected (Table 4). According to Wilks' lambda scores, second order habitat selection by water voles at the home-range scale tended to be stronger at coarser resolutions (Table 3). Third-order selection was only apparent during the wet season and at the finest spatial resolutions ($\lambda=0.61$, $p=0.04$ for the $3m^2$ MMU, and $\lambda=0.52$; $p=0.03$ for $10m^2$ MMU), with habitats ranked as SR>RE>TG>SH.

Table 3 - Compositional analysis results of second-order home-range scale habitat selection by Cabrera voles and water voles in south-west Portugal across spatial resolutions of habitat mapping. *ns* - non-significant; * $p<0.1$; ** $p<0.05$; *** $p < 0.01$; >> significant difference in selection between two habitat types; TG- Tall Grasses; SR – Sedges/Rushes; RE – Reeds; SH – Shrubs.

	$3m^2$		$10m^2$		$30m^2$		$100m^2$		$300m^2$	
	Λ	**Habitat ranking**	**λ**	**Habitat ranking**	**λ**	**Habitat ranking**	**λ**	**Habitat ranking**	**λ**	**Habitat ranking**
Cabrera vole										
Dry season	0.20 ***	SR>>TG>>RE>SH	0.24 *	SR>TG>RE>SH	0.17 *	TG>SR>RE>SH	0.19 *ns*	-	-	-
Wet season	0.25 ***	TG>SH>>SR>RE	0.25 ***	TG>SH>SR>RE	0.09 ***	TG>SH>SR>RE	0.03 ***	TG>>SH>SR>RE	-	-
Water vole										
Dry season	0.07 ***	SR>>RE>SH>>TG	0.09 ***	SR>>RE>SH>>TG	0.12 ***	SR>>RE>SH>>TG	0.41 **	SR>SH>RE>TG	0.28 ***	SR>SH>>TG>RE
Wet season	0.12 ***	SR>>RE>>SH>TG	0.10 ***	SR>>RE>>SH>TG	0.09 ***	SR>>RE>>SH>TG	0.20 ***	SR>>TG>RE>SH	0.34 **	SR>TG>RE>SH

Table 4 - Compositional analysis results of second-order, core-area scale habitat selection by Cabrera voles and water voles in south-west Portugal, across spatial resolutions of habitat mapping. ns - non-significant; * p<0.1; ** p<0.05; p<0.01; >> - significant difference in selection between two habitat types; TG- Tall Grasses; SR – Sedges/Rushes; RE – Reeds; SH – Shrubs.

	3m^2		10m^2		30m^2	
	λ	**Habitat ranking**	**λ**	**Habitat ranking**	**λ**	**Habitat ranking**
Cabrera vole						
Dry season	0.25 **	SR>TG>SH>RE	0.30 *	SR>TG>SH>RE		
Wet season	0.11 ***	TG>SH>SR>RE	0.12 ***	TG>SH>SR>RE		
Water vole						
Dry season	0.31 **	SR>>RE>SH>TG	0.32 **	SR>>RE>SH>TG	0.53 *	SR>RE>SH>TG
Wet season	0.23 ***	SR>RE>TG>SH	0.23 ***	SR>RE>>TG>SH	0.23 ***	SR>RE>>TG>SH

4.3.2 Habitat differentiation

Interspecific overlap in habitat use increased with spatial resolution of analysis (Table 5). Habitat overlap among Cabrera and water voles was also consistently lower at the core-area scale and higher during the dry season (Table 5). The analysis of habitat differentiation between vole species revealed that segregation resulted to a large extent from the much higher use of TG by Cabrera voles during the dry season (Fig. 1). Interspecific differentiation in the use of SR was also high, with water voles generally using these habitats more than Cabrera voles, except at the home-range scale during the dry season, when the use of SR by the two species seemed much similar, particularly at the finest spatial grains (Fig. 1). In general, the differentiation of TG and SR was higher at the core-area scale and increased at coarser spatial resolutions in home-range scale analysis. This pattern was also likely at the core-area scale, although only two spatial grains could be analysed for habitat differentiation (Fig. 1). The use of RE was also consistently higher for water voles, particularly at the core-area scale during the wet season. However, contrarily to TG and SR, differential use of RE decreased at coarser spatial resolutions (Fig. 1). Differentiation in the use of SH was less evident and apparently interchangeable across seasons, with Cabrera voles using these habitats more than water voles during the wet season, whereas the opposite was found during the dry season (Fig. 1).

Table 5 – Pianka's niche overlap indexes between Cabrera and water voles (means and 95% CI's) after 1000 permutations (see also Electronic supplementary material, Tables S1 e S2).

Season	Grain (m^2)	Extent	
		Home-range	Core-area
Dry	3	0.832 (0.583-0.979)	0.781 (0.480-0.963)
	10	0.812 (0.573-0.975)	0.691 (0.388-0.935)
	30	0.765 (0.497-0.949)	
	100	0.735 (0.453-0.937)	
Wet	3	0.762 (0.526-0.937)	0.622 (0.368-0.865)
	10	0.753 (0.504-0.936)	0.578 (0.316-0.832)
	30	0.704 (0.470-0.903)	
	100	0.682 (0.406-0.907)	

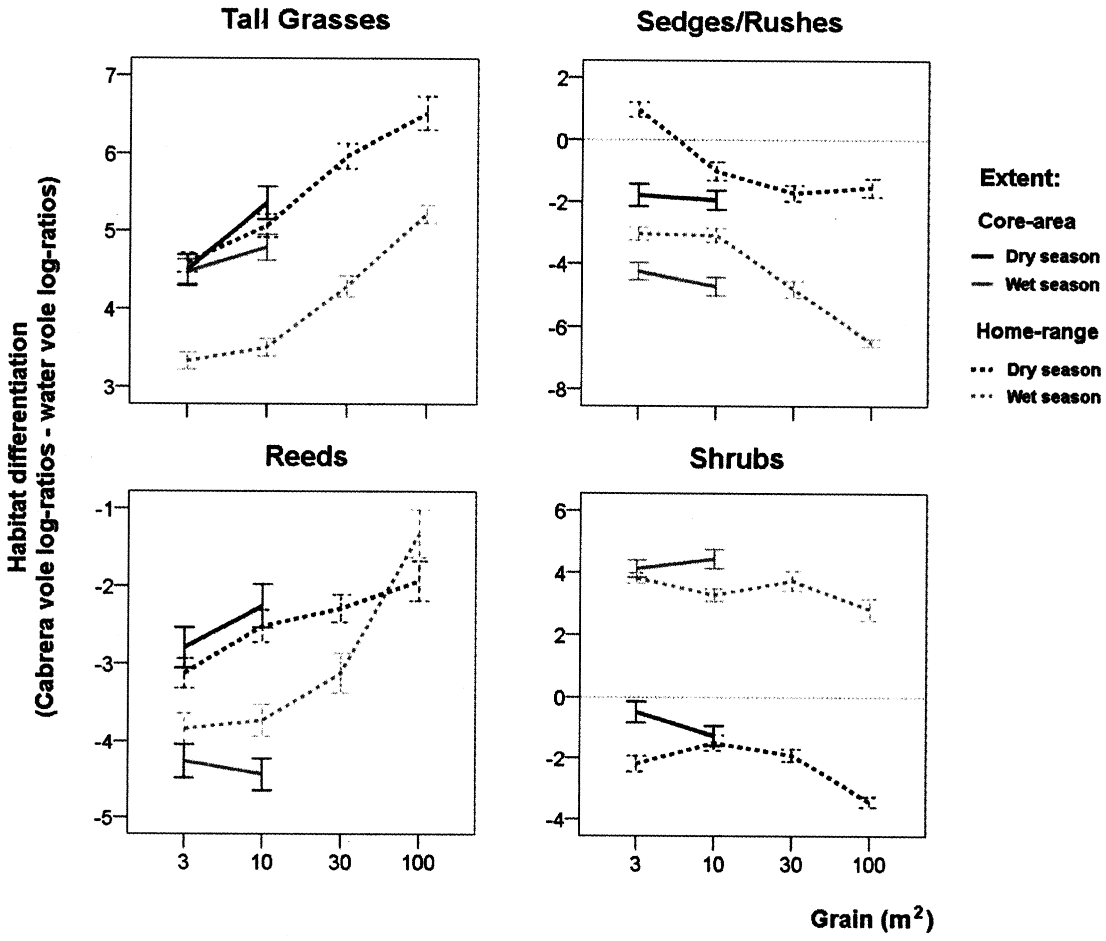

Fig. 1 – Habitat differentiation between Cabrera and water voles across different spatial scales (grain and extent) for the wet and dry seasons, given by 1000 random pairwaise differences between log-ratios of used habitats (see Supplementary material, Tables S3 and S4). Positive values indicate higher use by Cabrera voles, while negative values indicate higher use by water voles. Bars indicate 95% CI.

4.4 Discussion

This study evidenced that spatial scale strongly influenced the perception of habitat selection patterns by Cabrera and water voles. In addition, our multi-scale approach on the seasonal habitat overlap between species suggested that differentiation may as well operate at multiple spatial scales, both in terms of resolution and spatial extent of analysis. These results support the view that studies of habitat differentiation between species should incorporate multiple-resolution and multiple-order hierarchical designs, across different periods of the annual cycle (Gilbert et al., 2008; Indermaur et al., 2009).

4.4.1 Habitat selection

Results emphasized the high habitat specialisation exhibited by Cabrera and water voles (e.g. Fedriani et al., 2002; Pita et al., 2007), underlining a strong association with ungrazed patches of herbaceous vegetation composed by tall grasses, sedges, rushes, reeds and shrubs, and a strong avoidance of short herb pasturelands, agricultural fields, forest plantations, and human infra-structures. Water voles showed a clear preference for sedge/rush and reed microhabitats, suggesting that this species was dependent on the wettest parts of the humidity gradient found within herbaceous patches. Although water voles may be able to cope with the severe summer droughts typical of Mediterranean areas (Fedriani et al., 2002; Pita et al., in press), preference for wet vegetation was consistent across seasons. However, eventual dryness within water voles' core-areas during the dry season may indeed occur, as long as the foraging areas within home-ranges afforded enough high-quality patches of sedge/rush and reed habitats. Despite this strong preference for wet vegetation, selection of reeds by water voles was reduced at coarser grains of habitat mapping, particularly during the summer, suggesting that animals responded to these habitats at multiple-spatial scales. This may be related with the way individuals perceived their environment, particularly concerning the likelihood of predation risk (Román 2007), which in the study region is presumably high along large or continuous riparian corridors where many mammalian carnivores often concentrate their foraging activity (Matos et al., 2009, Pita et al., 2009).

Cabrera voles also showed strong preference for sedges and rushes during the dry season, particularly considering second-order selection at fine resolutions of habitat mapping. However, contrary to water voles, they seemed to avoid reeds at all spatial scales, particularly during the wet season, probably because these habitats are much more prone to flooding (Fernández-Salvador et al., 2005). In addition, the importance of sedges and rushes for Cabrera voles during the summer was apparently reduced when less spatial detail on resource distribution was used, suggesting that this species perceives and uses those habitats at finer spatial scales, probably reflecting the empirical relation between body size, home-range size and the scales of habitat perception (Mech and Zollner, 2002; Gehring et al., 2003). Indeed, at coarser resolutions of habitat mapping Cabrera voles tended to prefer tall grasses, indicating that grain size effectively average out the spatial heterogeneity occurring at finer resolutions (Thompson and McGarigal, 2002).

Cabrera voles exhibited also multi-scaled responses to shrubby habitats, as during the summer these tended to be the most selected habitat at the highest resolutions of third-order selection, being among the least preferred habitats across all grains of second-order selection. This suggests that within the predominantly wet herbaceous vegetation used by Cabrera voles during the dry season, small patches of shrubs mixed with tall grasses may provide an important refuge at the core-area scale, with voles probably avoiding locating their nests near those sites that are more likely to become inundated, as also suggested for other vole species living in humid herbaceous habitats (e.g. Starck, 1963). On the contrary, third-order selection by Cabrera voles was never detected during the wet season, while second-order selection indicated a preference for tall grasses at all spatial resolutions. During the wet season, sedges, rushes and reeds were among the least preferred habitats by Cabrera voles, suggesting that the dependence of this species on wet vegetation may be relaxed when other grassland habitats that usually dry out in summer may retain suitable humidity. This probably also explained the higher preference for shrubs by Cabrera voles at the second-order scale during the wet season, suggesting that multi-scaled responses by voles to particular habitats may change according to seasonal changes in resource availability, as referred for other mammal species (Bond et al., 2002; Beasley et al., 2007).

4.4.2 Habitat differentiation

Results on habitat differentiation between Cabrera and water voles suggested that the observed species-specific multi-scaled responses to habitat may have created opportunities for spatial resource partitioning among heterospecifics. Indeed, seasonal habitat differentiation between Cabrera and water voles apparently did not simply happen at one spatial scale but actually species used differentially complex habitat mosaics where a hierarchical set of factors determined the spatial heterogeneity of resource quality and quantity at different spatial scales (Cromsigt and Olff, 2006; Roshier et al., 2008). Multi-scaled differential use within shared habitat types has been similarly found among other close-related mammal species (Gabor et al., 2001; Laca et al., 2010), as well as within other taxa, including amphibians (Indermaur et al., 2009), and insects (Gilbert et al., 2008).

According to the seasonal habitat selection profiles exhibited by voles, the degree of interspecifc overlap in habitat use was higher during the summer, particularly considering the likelihood of fine-scale sedge/rush habitat selection within home-ranges. Interspecific differentiation regarding the relative use of reeds and shrubs was also lower during the dry season, although water voles generally used these habitats more than Cabrera voles. Summer habitat segregation was however strong when considering the likelihood of using tall grasses, which was much higher for Cabrera voles, highlighting the overall stronger association of this species with relatively drier habitats comparing to those preferred by water voles.

Interspecific niche overlap changed according to the spatial extent considered, being lowest at the core-area scale. This was probably due to the strong differential use of tall grasses and sedge/rush habitats by Cabrera and water voles within their core-areas across seasons, reflecting a strong spatial segregation between the two species at this spatial extent (Pita et al., in press). Furthermore, habitat overlap increased when more spatial resolution was allowed in habitat mapping at least within home-ranges, probably reflecting the high differentiation in species' relative use of tall grasses and sedge/rush habitats at coarser scales of habitat mapping. Indeed, even considering the contrasting effects of grain on the differential use of reeds, and the corresponding weak effects regarding the use of shrubs within seasons, habitat differentiation was apparently greatly influenced by the scale of patchiness measured for tall grass and sedge/rush habitats, as suggested from the multi-scaled habitat selection analysis. Our study thus provided empirical evidence that niche differentiation between Cabrera and water voles may have occurred along fine-scale spatial gradients of habitat use, thus acting as an additional dimension over which animals partitioned resources. Specifically, results suggest that, within a size hierarchy, Cabrera voles seemed to use sedges and rushes mostly in patches that may be too small for water voles and probably a threshold area of sedge/rush habitats below ca. $100m^2$ may not constitute high-quality patch for water voles and will do for Cabrera voles. This may be a key mechanism facilitating species co-existence at least during the dry season, when the distribution of humid habitats is presumably reduced (Román, 2007; Ventura et al., 1998), forcing individuals to share preferred resources in a greater degree, as also suggested by the increased space sharing among voles in summer (Pita et al., in press).

Taken together, results suggested that interspecific differences in the scales of habitat perception may operate as a plausible mechanism allowing the coexistence of Cabrera and water voles within habitat patches, because the scales of resolution and extent of analysis were likely to form axes along which species partitioned preferred habitats. Eventual interspecific competition between sympatric Cabrera and water voles may be particularly influential during the dry season at fine-scale habitat selection within home-range areas. It should be noted however that we did not experimentally test the seasonal effects of interspecific competition across the range of spatial scales considered. Although such a test was beyond the scope of this study, we suggest that future research should focus on the strength of inter-specific competition between the species and on how eventual competitive asymmetries change across space and time.

4.4.3 Multi-scaled differential habitat selection as a mechanism for coexistence

Quantifying differential habitat selection is probably the most common approach used by ecologists to understand ecological segregation between close-related interacting species (e.g. Morris, 2003; Nicholls and Racey, 2006). Despite the growing evidence that habitat selection is a scale dependent process (Mayor et al., 2009), scaling issues have frequently been ignored in habitat differentiation studies (WallisDeVries et al., 1999, Laca et al., 2010), which in many cases might have prevented a complete characterization of the dimensions at which habitat segregation may facilitate coexistence. In common with a few recent studies analysing the effects of spatial scale on habitat segregation between species (Indermaur et al., 2009; Laca et al., 2010), our study provided support for the idea that multi-scaled differential habitat selection may create opportunities to define spatial niches that are not measurable using single scale approaches. Scaling processes may thus be involved in niche segregation between sympatric species, by adding a source of complexity that reflects the spatial scales at which animals respond to their habitat regarding a particular behavioural process (Cromsigt and Olff, 2006; Laca et al., 2010). This may have deep implications for future studies aiming to evaluate habitat differentiation as a possible mechanism favouring species coexistence in spatially heterogeneous environments. By increasing the likelihood of detecting niche segregation between species, multi-scaled differential habitat selection studies may be particularly useful to understand the functional scales of habitat fragmentation from the animals' point of view (Girvetz and Greco, 2007; Mayor et al., 2009), thereby providing information on critical thresholds regarding area requirements and spatial distribution of resources for target species (Thompson and McGarigal, 2002).

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Supplementary material

Table S1 - Mean (± se) percent cover by each habitat type considered within available areas, home-ranges and core-areas of voles radio-tracked during the dry season, according different grains of habitat mapping, used to study hierarchical habitat selection and differentiation among Cabrera and water voles.

Species	Extent	Grain	Tall Grasses	Sedges/Rushes	Reeds	Shrubs
Cabrera vole	Available	3m^2	85.95 ± 3.13	4.09 ± 1.18	1.74 ± 0.71	8.23 ± 2.43
		10m^2	86.62 ± 3.18	3.56 ± .15	1.74 ± 0.71	8.08 ± 2.40
		30m^2	88.62 ± 3.07	2.35 ± 0.77	1.76 ± 0.72	7.28 ± 2.36
		100m^2	90.21 ± .87	1.74 ± 0.65	1.67 ± 0.69	6.38 ± 2.18
	Home-range	3m^2	69.99 ± 4.97	21.70 ± 5.62	0.43 ± 0.37	7.88 ± 3.63
		10m^2	75.54 ± 4.72	16.94 ± 4.92	0.50 ± 0.38	7.02 ± 3.15
		30m^2	82.11 ± 4.86	11.73 ± 4.36	0.51 ± 0.38	5.65 ± 2.98
		100m^2	84.96 ± 4.75	11.73 ± 4.36	0.88 ± 0.88	2.43 ± 1.46
	Core-area	3m^2	54.07 ± 9.01	31.88 ± 10.21	0.20 ± 0.20	13.85 ± 6.01
		10m^2	51.26 ± 10.11	38.20 ± 11.17	0.20 ± 0,20	10.33 ± 5.62
Water vole	Available	3m^2	78.26 ± 2.28	6.75 ± 0.75	3.61 ± 0.79	11.38 ± 1.67
		10m^2	78.49 ± 2.28	6.48 ± 0.70	3.62 ± 0.80	11.41 ± 1.65
		30m^2	78-90 ± 2.29	5.98 ± 0.65	3.91 ± 0.83	11.22 ± 1.61
		100m^2	79.31 ± 2.20	5.94 ± 0.71	3.97 ± 0.87	10.78 ± 1.48
		300m^2	79.77 ± 2.39	5.02 ± 0.74	4.12 ± 0.94	11.09 ± 1.42
	Home-range	3m^2	36.45 ± 4.03	36.00 ± 3.99	9.09 ± 1.80	18.45 ± 2.88
		10m^2	37.47 ± 4.13	34.45 ± 4.20	9.14 ± 1.79	18.93 ± 2.94
		30m^2	37.77 ± 4.40	32.55 ± 4.90	9.96 ± 1.79	19.73 ± 2.82
		100m^2	35.80 ± 5.28	34.78 ± 6.31	9.41 ± 2.09	20.02 ± 3.29
		300m^2	36.81 ± 5.71	31.76 ± 7.17	3.90 ± 1.95	27.54 ± 4.02
	Core-area	3m^2	23.24 ± 5.49	40.89 ± 7.16	15.66 ± 5.62	20.22 ± 6.20
		10m^2	23.97 ± 6.04	38.83 ± 7.31	15.12 ± 5.66	22.08 ± 6.41
		30m^2	23.31 ± 6.24	38.77 ± 8.44	14.70 ± 4.91	23.21 ± 7.49

Table S2 - Mean (± se) percent cover by each habitat type considered within available areas, home-ranges and core-areas of voles radio-tracked during the wet season, according different grains of habitat mapping, used to study hierarchical habitat selection and differentiation among Cabrera and water voles.

Species	Extent	Grain	Tall Grasses	Sedges/Rushes	Reeds	Shrubs
Cabrera vole	Available	3m^2	71.82 ± 1.77	4.95 ± 0.66	4.13 ± 1.01	19.10 ± 1.59
		10m^2	72.62 ± 1.76	4.84 ± 0.64	4.13 ± 1.01	18.41 ± 1.63
		30m^2	73.99 ± 1.83	4.43 ± 0.72	4.30 ± 0.72	17.28 ± 1.65
		100m^2	77.28 ± 1.88	4.18 ± 0.87	3.53 ± 0.91	15.01 ± 1.81
	Home-range	3m^2	69.59 ± 1.93	6.21 ± 1.04	1.94 ± 0.56	22.27 ± 1.98
		10m^2	72.49 ± 1.86	5.61 ± 0.93	1.94 ± 0.56	19.96 ± 2.00
		30m^2	74.40 ± 1.85	4.41 ± 1.17	0.18 ± 0.09	21.01 ± 2.21
		100m^2	86.98 ± 2.36	2.41 ± 0.91	0.07 ± 0.04	10.54 ± 2.39
	Core-area	3m^2	64.92 ± 3.62	9.15 ± 2.57	0.53 ± 0.27	25.40 ± 3.30
		10m^2	67.66 ± 3.60	8.09 ± 2.53	0.53 ± 0.27	23.72 ± 3.26
Water vole	Available	3m^2	68.50 ± 3.76	12.79 ± 1.48	4.02 ± 0.42	14.68 ± 3.16
		10m^2	69.65 ± 3.64	12.76 ± 1.53	4.12 ± 0.43	13.47 ± 2.79
		30m^2	70.75 ± 3.63	12.83 ± 1.53	4.62 ± 0.46	11.80 ± 2.46
		100m^2	71.48 ± 3.81	13.96 ± 1.85	4.30 ± 0.46	10.26 ± 2.09
		300m^2	71.11 ± 4.15	13.86 ± 2.41	3.16 ± 0.63	11.87 ± 2.23
	Home-range	3m^2	41.38 ± 4.21	37.60 ± 4.22	8.63 ± 1.48	12.39 ± 2.55
		10m^2	40.63 ± 4.58	38.24 ± 4.50	8.96 ±1.42	12.18 ±2.68
		30m^2	39.37 ±4.94	39.41 ± 4.69	10.16 ± 1.75	11.06 ± 2.83
		100m^2	41.74 ± 4.74	37.99 ± 5.14	12.18 ± 3.80	8.09 ± 2.13
		300m^2	40.61 ± 5.57	45.61 ± 8.48	8.42 ± 4.13	5.36 ±2.69
	Core-area	3m^2	28.80 ± 2.40	46.13 ± 2.90	15.12 ± 1.97	9.96 ± 1.22
		10m^2	27.36 ± 2.71	46.78 ± 3.22	16.69 ± 2.02	9.17 ± 1.08
		30m^2	25.52 ± 3.05	48.45 ± 3.29	16.48 ± 2.33	9.55 ± 1.48

Table S3 - Mean (± se) log-ratios from Compositional Analysis of each habitat type considered within available areas, home-ranges and core-areas of voles radio-tracked during the dry season, according different grains of habitat mapping, used to study hierarchical habitat selection and differentiation among Cabrera and water voles.

Species	**Extent**	**Grain**	**Tall Grasses**	**Sedges/Rushes**	**Reeds**	**Shrubs**
Cabrera vole	Available	3m^2	4.53 ± 0.51	-0.67 ± 0.39	-4.06 ± 0.69	0.20 ± 0.42
		10m^2	4.93 ± 0.64	-1.80 ± 0.63	-3.66 ± 0.63	0.52 ± 0.45
		30m^2	5.67 ± 0.79	-1.86 ± 0.54	-2.96 ± 0.61	-0.85 ± 0.78
		100m^2	6.08 ± 0.79	-2.16 ± 0.64	-2.80 ± 0.61	-1.12 ± 0.71
	Home-range	3m^2	5.59 ± 0.48	1.85 ± 1.12	-5.09 ± 0.44	-2.36 ± 1.16
		10m^2	6.24 ± 0.59	0.11 ± 1.35	-4.48 ± 0.60	-1.87 ± 1.12
		30m^2	6.92 ± 0.68	-0.96 ± 1.19	-3.89 ± 0.59	-2.07 ± 0.93
		100m^2	7.28 ± 0.63	-0.63 ± 1.18	-4.02 ± 0.69	-2.65 ± 0.70
	Core-area	3m^2	5.63 ± 0.64	0.69 ± 1.51	-4.89 ± 0.59	-1.43 ± 1.23
		10m^2	6.40 ± 0.71	0.60 ± 1.21	-4.49 ± 0.61	-2.51 ± 1.13
Water vole	Available	3m^2	2.77 ± 0.24	-0.59 ± 0.20	-2.20 ± 0.46	0.02 ± 0.18
		10m^2	2.78 ± 0.24	-0.63 ± 0.19	-2.20 ± 0.46	0.04 ± 0.17
		30m^2	2.79 ± 0.24	-0.73 ± 0.19	-2.08 ± 0.46	0.02 ± 0.18
		100m^2	2.90 ± 0.26	-0.65 ± 0.22	-2.34 ± 0.62	0.09 ± 0.25
		300m^2	2.97 ± 0.27	-0.88 ± 0.24	-2.29 ± 0.64	0.19 ± 0.25
	Home-range	3m^2	1.04 ± 0.30	1.06 ± 0.28	-2.03 ± 0.76	-0.07 ± 0.39
		10m^2	1.08 ± 0.30	0.98 ± 0.29	-2.02 ± 0.76	-0.04 ± 0.36
		30m^2	0.95 ± 0.27	0.70 ± 0.30	-1.57 ± 0.60	-0.09 ± 0.35
		100m^2	0.83 ± 0.76	0.73 ± 0.83	-2.16 ± 0.94	0.59 ± 0.42
		300m^2	1.85 ± 0.91	1.51 ± 0.79	-4.82 ± 0.99	1.47 ± 0.74
	Core-area	3m^2	0.94 ± 0.65	2.50 ± 0.73	-2.21 ± 1.10	-1.22 ± 1.05
		10m^2	0.99 ± 0.66	2.44 ± 0.74	-2.31 ± 1.09	-1.11 ± 1.07
		30m^2	0.51 ± 0.94	1.84 ± 1.10	-1.45 ± 1.01	-0.91 ± 1.12

Table S4 - Mean (± se) log-ratios from Compositional Analysis of each habitat type considered within available areas, home-ranges and core-areas of voles radio-tracked during the wet season, according different grains of habitat mapping, used to study hierarchical habitat selection and differentiation among Cabrera and water voles.

Species	Extent	Grain	Tall Grasses	Sedges/Rushes	Reeds	Shrubs
Cabrera vole	Available	3m^2	3.38 ± 0.29	-1.17 ± 0.55	-3.58 ± 0.79	1.37 ± 0.37
		10m^2	3.46 ± 0.30	-1.25 ± 0.56	-3.54 ± 0.78	1.33 ± 0.40
		30m^2	4.00 ± 0.42	-1.71 ± 0.67	-3.97 ± 0.85	1.68 ± 0.49
		100m^2	5.34 ± 0.59	-3.00 ± 0.82	-3.60 ± 0.82	1.26 ± 0.95
	Home-range	3m^2	4.51 ± 0.43	-2.01± 0.93	-4.99 ± 0.81	2.49 ± 0.47
		10m^2	4.75 ± 0.42	-1.89 ± 0.97	-4.81 ± 0.80	1.95 ± 0.72
		30m^2	5.82 ± 0.42	-3.46 ± 1.07	-3.67 ± 0.97	1.31 ± 1.03
		100m^2	7.77 ± 0.39	-3.88 ± 0.50	-4.30 ± 0.31	0.41 ± 1.07
	Core-area	3m^2	5.63 ± 0.52	-2.57 ± 1.08	-5.34 ± 0.61	2.28 ± 0.92
		10m^2	5.89 ± 0.53	-2.51 ± 1.05	-5.15 ± 0.61	1.77 ± 0.99
Water vole	Available	3m^2	2.22 ± 0.18	-0.14 ± 0.13	-1.65 ± 0.13	-0.43 ± 0.28
		10m^2	2.26 ± 0.18	-0.13 ± 0.13	-1.60 ± 0.13	-0.53 ± 0.28
		30m^2	2.40 ± 0.24	-0.01 ± 0.17	-1.33 ± 0.19	-1.06 ± 0.50
		100m^2	2.56 ± 0.28	0.22 ± 0.20	-1.65 ± 0.43	-1.13 ± 0.51
		300m^2	3.29 ± 0.32	0.80 ± 0.33	-2.82 ± 0.94	-1.26 ± 0.93
	Home-range	3m^2	1.25 ± 0.19	1.09 ± 0.20	-1.08 ± 0.32	-1.27 ± 0.50
		10m^2	1.22 ± 0.26	1.13 ± 0.15	-0.98 ± 0.33	-1.37 ± 0.55
		30m^2	1.46 ± 0.36	1.49 ± 0.20	-0.47 ± 0.42	-2.49 ± 0.85
		100m^2	2.48 ± 0.32	2.22 ± 0.37	-2.35 ± 1.15	-2.35 ± 1.06
		300m^2	3.91 ± 0.30	3.09 ± 0.80	-3.74 ± 1.21	-3.25 ± 0.99
	Core-area	3m^2	1.21 ± 0.43	1.77 ± 0.46	-1.04 ± 0.71	-1.94 ± 0.70
		10m^2	1.26 ± 0.50	2.03 ± 0.59	-0.56 ± 0.65	-2.72 ± 0.82
		30m^2	1.30 ± 0.51	2.35 ± 0.50	-0.42 ± 0.77	-3.23 ± 0.95

CHAPTER 5

Circadian activity rhythms in relation to season, sex and interspecific interactions in two Mediterranean voles

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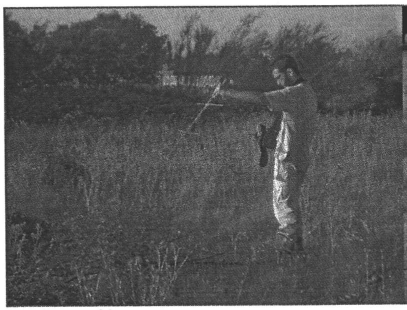
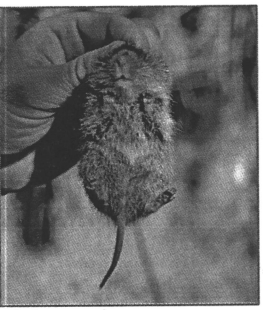


Radio-tracking
(Photo: Francisca Lima)

Suitable habitat



Cabrera vole

5. Circadian activity rhythms in relation to season, sex and interspecific interactions in two Mediterranean voles

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Abstract

Understanding the mechanisms driving flexibility in activity patterns by mammals remains a key problem in behavioural ecology. This study investigated the circadian activity rhythms by sympatric Cabrera and water voles in relation to season, sex and interspecific interactions, using activity data from 60 individuals radio-tracked for 1,032 hours in Mediterranean farmland. Results based on logit mixed-effects multicosinor regression modelling, revealed that the fundamental circadian period, together with the hemi-circadian and ultradian periods, described much of the diel variation in vole activity. Both species apparently presented episodes of activity every 6 hours that were synchronized by major activity bouts related to sunrise and sunset. Cabrera voles were markedly diurnal and exhibited higher rhythm changes, although water voles were generally more active. At fine temporal scales there was evidence for interspecific time-lagged activity bouts related to sunrise and sunset. Both species showed higher rhythm amplitudes during the dry season. Cabrera voles were globally less active during summer days, when their diurnality was considerable reduced. Water voles were more diurnal during the wet season and nocturnal during the dry season, though their bimodal crepuscular pattern was consistent across seasons. Sex and interspecific interactions showed little effect on the activity patterns of each species. Overall, results suggest that the most important environmental factors driving flexibility in circadian activity rhythms of voles seem to be related with predictable seasonal changes provided by abiotic zeitgebers (day length and air temperature), as biotic interactions showed relatively limited influence in producing variation at the population level.

Keywords: Activity patterns, Cosinor analysis, Cabrera vole, Water vole, Species coexistence, Mediterranean farmland

5.1 Introduction

In mammals, circadian rhythms seem to be controlled by an endogenous biological clock or circadian pacemaker that interacts with a number of exogenous abiotic and biotic factors (zeitgebers) related to the environmental cycles (Goldman 2001; Mistlberger & Rusak 2005). The daily light-dark cycle is the most important abiotic zeitgeber for most species, resulting in relatively stable nocturnal, diurnal or crepuscular chronotypes that are consistent with the long-term phylogenetic history of species (Fernandez-Duque 2003; Ocampo-Garcés et al. 2006; Roll et al. 2006). Other abiotic stimuli affecting temporal niches in mammals include temperature and humidity cycles, while biotic zeitgebers are usually related to food availability, metabolic needs, digestion, social behaviour, interspecific competition and predation risk (Woods & Kennedy 1997; Mistlberger & Rusak 2005; Halle 2006). Interactions between intervening zeitgebers are complex, depending on their relative timing, direction, and magnitude in producing phase shifts. The overall integration of multiple zeitgebers by multiple, formally distinct pacemakers and oscillators may thus generate high variation in circadian rhythm not only between species, but also within a species and between individuals within single breeding populations (Goldman 2001). In this context, small mammals have provided the most illustrative examples showing how circadian pacemakers are affected by multiple abiotic and biotic zeitgebers (Halle & Stenseth 2000; Refinetti et al. 2007; Kronfeld-Schor & Dayan 2008).

The seasonal variation in light entrainment is probably the most obvious environmental factor inducing changes in the circadian organization of small mammal activity (Decoursey and & Manon 1991; Halle & Stenseth 2000; Kronfeld-Schor & Dayan 2008). In particular, the annual progression of day length provides direct time-of-year signals that allow animals to anticipate important seasonal changes (Goldman 2001). Seasonal variation in circadian activity mediated by predictable changes in the light-dark cycle highlight the phenotypic plasticity in activity timing by many small mammals, reflecting species behavioural adaptations to particular photoperiods at different times of the year. Biotic interactions among individuals are also known to phase-shift, entrain or modify the period of activity rhythms (Ocampo-Garcés at al. 2006; Kronfeld-Schor & Dayan 2008; Favreau et al. 2009). For instance, strong social entrainment between males and females has been reported for many small mammals, resulting in sex differences in activity levels and length of activity periods (e.g. Jechura et al. 2000; Lightfoot 2008). Interspecific interactions may also affect the plasticity of response to ecological selective forces, as the time-niche axis may facilitate niche partitioning between co-occurring species (Kronfeld-Schor & Dayan 2003; Halle 2006; Kronfeld-Schor & Dayan 2008). Predation risk is arguably the most well documented biotic interaction driving shifts in small mammals' activity patterns (e.g. Fenn & Macdonald 1995; Harrington et al. 2009), while rhythm changes due to competition are much less reported (Jones et al. 2001; Gutman & Dayan 2005). However, competition is most often asymmetrical, including a dominant (superior or larger) and a subordinate (weaker or smaller) competitor, often involving direct aggression that may be perceived similarly to predation risk (Harrington et al. 2009). Despite this accumulating empirical evidence on the abiotic and biotic drivers of small mammal circadian rhythms (Caldelas et al. 2005), flexibility in diel activity patterns by species integrating ecological mechanisms that take place in natural environments remains poorly understood (Kronfeld-Schor & Dayan 2008), as most research has been conducted under captivity or semi-captivity conditions (e.g. Halle 1995; Demas et al. 2001; Tavernier et al. 2004).

This study addresses these issues, by analyzing flexibility in the circadian activity rhythms of Cabrera (*Microtus cabrerae*) and water voles (*Arvicola sapidus*), using time-series data collected from free-ranging animals radio-tracked under natural conditions (Pita et al. in press). We first investigated the overall population activity patterns by voles, using curve-fitting rhythmometric techniques developed to describe complex waveforms of daily activity. This modelling approach was then used to test predictions on the activity rhythms of these species, derived from general ecological theory and previous empirical observations on their life-histories and ecological requirements. Specifically, we predicted that (a) vole circadian activity should be rhythmic, with cycles corresponding to the typical rhythmic components occurring in the sub-family Arvicolinae (e.g. Halle 2006); (b) circadian rhythms should be broadly similar across species because of their close phylogenetic position (e.g. Roll et al. 2006), with activity peaks occurring mostly during the day (Fernández-Salvador et al. 2005; Ventura 2004) and probably synchronised with sunrise and sunset photic clues (Halle & Stenseth 2000); (c) despite broad similarities, circadian rhythms of both species should differ in detail, primarily due to differences in body size (Halle & Stenseth 1994) and eventual time partitioning of resource utilization (e.g. Kronfeld-Schor & Dayan 2003; Gutman & Dayan 2005); (d) adjustments of daily time allocation and duration of activity periods should occur in relation to the seasonal changes in environmental conditions (e.g. Kronfeld-Schor & Dayan 2008); (e) sex should affect circadian activity rhythms and the overall activity levels, which in vole species, contrary to most rodents, are usually greater in males (Mead et al. 1996); (f) Cabrera voles should change activity rhythms in the presence of water voles so as to reduce eventual agonistic encounters (Kronfeld-Schor & Dayan 2003; Pita et al. in press). Results were then used to discuss the relative importance of biotic and abiotic mechanisms influencing flexibility of circadian activity rhythms in small mammals.

5.2 Material and Methods

5.2.1 Study area and populations

The study was carried out in south-western Portugal (37°57' – 37°35' N, 08°51' – 08°48' W), within an agricultural landscape where Cabrera and water voles occur sympatrically (Pita et al. 2006, 2007). The region has a Mediterranean climate, with mean temperatures of about 16°C and mean annual rainfall around 650 mm, of which >85% falls between October and April. In this landscape the distribution of these species is largely restricted to marginal and relatively small-sized patches, typically composed by little disturbed and often seasonally flooded tall wet meadows and some shrubby vegetation, embedded in the predominantly agricultural matrix (Pita et al. 2006, 2007, and 2009). These species seem to show a metapopulation structure, where distinct sub-populations (breeding colonies) are connected by dispersal and local extinction-(re)colonization events are relatively common (Pita et al. 2007, unpublished data). Cabrera and water voles often coexist within the same patches, though they show to some extent within-patch habitat differentiation and spatial segregation, which may contribute for reducing interspecific competition (Pita et al. 2006, in press, unpublished data).

5.2.2 Capture and handling procedures

The circadian activity of Cabrera and water voles was analysed with radio-tracking, from individuals captured within 18 discrete habitat patches distributed across two farmland areas in the Portuguese south-west coast, where agreement with landowners to capture and radio-track voles could be obtained (Pita et al. in press). The presence of the species was initially confirmed from surveys based on systematic searches for the typical, species-specific signs of occurrence (Román 2003; Pita et al. 2006), indicating that seven patches were occupied by both species, while eight and four were only occupied by Cabrera and water voles, respectively (Pita et al. in press). Capture and handling of voles were carried out under permission of the Instituto da Conservação da Natureza e Biodiversidade (ICNB, Portugal) and conformed to the guidelines approved by the American Society of Mammalogists for the use of wild mammals in research (Gannon et al. 2007), and to the guidelines for the treatment of animals in behavioural research (ASAB 2006). Because the number of voles inhabiting each habitat patch was generally very small and we wanted to keep disturbance to a minimum, we only captured a small number of individuals from each patch. Also, we avoided repeated disturbance by sampling each patch in a single occasion between April-2006 and April-2008 (Pita et al. in press).

Voles were captured using Sherman live-traps (7 x 23 x 9 cm^3 for Cabrera voles and 10 x 37 x 11 cm^3 for water voles) baited with apple and supplied with hay and hydrophobic cotton for bedding. Traps were placed at likely capture sites, which were assessed by checking eaten apple trials left in the area during the previous 1-3 days (Pita et al. in press). A total of 804 traps were used during 108 days of trapping distributed through the study period. The sampling effort varied among surveyed areas depending on the evidence for the presence of voles, patch size, capture-recapture success, and whether radio-tracking was in progress (see details in Pita et al. in press). Traps were checked every 8 hours (around 08:00, 16:00 and 00:00). All Cabrera and water voles captured were weighed and sexed, and the reproductive status (active or non active) of non-juveniles (> 28 g for Cabrera voles, Fernández-Salvador et al. 2005; > 94 g for water voles, Román 2007), was assessed based on the testis position (scrotal or abdominal) for males and on vulva perforation and nipples size (small or large) for females. Animals of non target species were immediately released at the point of capture. Individual Cabrera and water voles were fitted with collar radio-transmitters weighing 1.2 g and 3.2 g (SOM-2018 and SOM-2070, Wildlife Materials, Inc., Illinois USA). Only those animals for which radio-transmitters added no more than 5% of the animals weigh were collared, so as to ensure no significant additional energetic costs for voles (Gannon et al., 2007). The collars were made from teflon-coated wires and were easely attached through a ratchet mechanism designed for holding cables ties together. Pregnant females were identified by abdominal palpation and they were not collared to reduce potential negative effects on vole populations (Mendonça, 1999). All animals were lightly sedate with a subcutaneous injection of Dormitor® (0.2mg/kg) to reduce handling stress. After transmitter attachment, voles were induced out of anaesthesia using an equivalent dose of Antisedam®. Before release, collared animals were kept under observation for at least 2 hours to ensure that they were suffering no ill-effects or loss of mobility. During this short observational period, uncovered wire cages supplied with hay and hydrophobic cotton were used and apple and water were provided *ad libitum*. Radio-tracking started at least 4 hours after trap removal and the release of animals at their point of capture (e.g. Gray et. al. 1998). After radio-tracking, each sampling site was re-trapped, so as to remove collars from tracked voles.

5.2.3 Study animals and data collection

Altogether, 34 Cabrera voles were captured 53 times in 13 habitat patches, and 43 water voles were captured 78 times in 10 habitat patches, from a total of 239 captures made during the study period (Pita et al. in press). Mean (± se) number of Cabrera and water voles captured per sampling site was 2.6 ± 0.5 individuals (1 – 6) and 4.3 ± 1.2 (1 – 14), respectively. A total of 31 Cabrera voles and 29 water voles were fitted with collar radio-transmitters. Mean (± se) weight of collared Cabrera voles was 48.4 ± 1.5 g (27 – 62 g) while that of water voles was 175.7 ± 7.9 g (92 – 261 g). From the 60 animals collared, three water voles and one Cabrera vole were juveniles at the time of collaring, though they were sub-adults by the end of radio-tracking. The mean (± se) number of Cabrera and water voles radio-tracked per sampling site was 2.4 ± 0.4 (1 – 5) and 2.9 ± 0.8 (1 – 8), respectively. About 42% of Cabrera voles and 41% of water voles were sampled in sites occupied by both species. Females represented respectively 64% and 55% of the Cabrera and water voles sampled. The percentage of animals radio-tracked during the dry (May-September) season (SNIRH, National System of Water Resources Information database, http://snirh.inag.pt) was 48% for Cabrera voles and 45% for water voles. Overall, about 87% of the Cabrera voles tracked were reproductively active, while the proportion of reproductively active water voles was 83%. About 90.3% of tracked Cabrera voles and all tracked water voles showed high site-fidelity (Pita et al. in press). Recovery of radio-transmitters was possible for 42% of the Cabrera voles and 65.5% of the water voles tracked. Although signs of predation were evident for

16.1% and 3.4% of the Cabrera and water voles tracked respectively, collar transmitters showed no adverse effects on animals tracked (Pita et al. in press).

Voles were located using a TRX-100S receiver and an external 3-element yagi directional antenna (Wildlife Materials, Inc., Illinois USA). Locations were made by homing and by multiple triangulations when the tracker was close to the animals. Activity was sampled from records of fluctuating vs. stationary radio-signal, taken every 15 minutes during 4-hour tracking periods surveyed alternately across the 24-hour cycle (0615-1000; 1015-1400; 1415-1800, 1815-2200; 2200-0200; and 0215-0600). Sampling thus followed a combination of longitudinal and transverse sampling (Fernández et al. 2004; Refinetti et al. 2007), which is usually preferred in chronobiological studies (Refinetti et al. 2007). Specifically, several individuals provided repeated samples at fixed time-intervals covering several 24-hour cycles, enabling rhythm characteristics to be summarized across all sampled individuals to obtain an assessment of circadian activity generalizable to the populations (Fernández et al. 2004; Refinetti et al. 2007). Radio-tracking provided 9,664 activity records, made during 1,032 hours. Mean ± s.e. fixes per animal was 148.5±11.9 (48-368) for Cabrera and 174.4±18.1 (96-512) for water voles, and mean ± s.e. tracking time was 37.16±3.05 (12-92) hours per Cabrera vole and 43.59±4.38 (48-128) hours per water vole.

5.2.4 Statistical analysis

Analysis of circadian activity rhythms was based on mixed effects multi-periodic logistic regression models, which are structurally similar to population-mean multi-cosinor (MC) harmonic regressions (Nelson et al. 1979; Bingham et al. 1982; Fernández et al. 2009), representing the periodic components of time-series by pairs of sine and cosine functions. Unlike common MC approaches, this method uses the natural log of the odds of activity occurring or not (Flury & Levri 1999). A multiple components approach was used because it improves the quality of fit over those of single-cosinor models, and it is recommended for time-series where perfect sinusoidality might be lacking (Koukkari & Sothern 2007; Fernández et al. 2009) as suggested for our data after visual inspection of time plots. Mixed effects models were used to allow specification of the tracked animal and the tracking period as random effects (RE), thereby accounting for potential lack of independence in the data (Mikulich et al. 1999; Albert & Hunsberger 2005). The fixed component of the models was specified using functions reflecting different alternative hypothesis concerning vole activity patterns, which were then evaluated using the Information Theoretical Approach (ITA; Burnham & Anderson 2002). For each set of alternative candidate hypothesis, the Akaike Information Criteria (AIC) of each model and the respective Akaike weights were computed, and evidence ratios were used to quantify model parsimony. Alkaike weights were taken as the approximate probabilities that each model is the best of those considered, while evidence ratios reflect the log of the odds of the best model over another. Where sample sizes were small in relation to the number of parameters, ITA was based on second order AIC values (AICc)

Data analysis started by fitting a global model of circadian activity for each species. Although successive addition of harmonic terms in the cosinor may improve model performance (Alonso & Fernández 2001; Albert & Hunsberger 2005; Refinetti et al. 2007), increasing the number of sinusoidal components may lead to overfitting. To avoid model over-parametrizaton in trying to model the 'noisy' component in the original circadian rhythms of activity, we considered only the main cyclic components judged to affect Arvicoline voles (Halle & Stenseth 2000; Mistlberger & Rusak 2005; Halle 2006). Besides the fundamental circadian 24-hour cycle (CD), a hemi-circadian 12-hour cycle (HCD) was chosen to approximately represent sunrise and sunset timings, which were spaced by a mean ± s.e. 12:34±00:07 (9:31-14:52 hours) during our sampling days (AOL, Astromomic Observatory of Lisbon database, http://www.oal.ul.pt/). Also, we included an ultradian UD period, chosen from a set of plausible UD rhythms for voles (2-, 3-, 4-, 5- and 6-hour cycles), as determined by ITA. This model seemed adequate to describe activity rhythms, because the summation of circadian plus the two harmonics cosine waveforms with different amplitudes and phases permits an infinite number of shapes to be fitted. In its general form, the activity model was specified as:

$$\operatorname{logit}\left(Y_{(t)}\right) = MC + RE = m_0 + \sum_{i=1}^{k}\left[b_i.\operatorname{sen}\left(\frac{2.\pi.t}{T}\right) + c_i.\cos\left(\frac{2.\pi.t}{T}\right)\right] + \mathit{random\ effects} + \varepsilon_{(t)}$$

where Y is the probability of activity at time t, k is the number of sinusoidal components considered (k=3) and T represents the time in hours of each fitted period (24, 12 and 2-6 hours).

Because the best fitting UD periods were the same for Cabrera and water voles (see Results), five alternative plausible models were fitted to the combined data set to investigate hypothesis on the effects of species identity specified as a dummy variable (SP) on circadian activity rhythms:

i) $Y_{overall} = RE$ (Null RE model - lack of circadian activity rhythms);

ii) $Y_{overall} = MC + RE$ (Null MC model - similar circadian activity rhythms between species);

iii) $Y_{overall} = SP + RE$ (Additive RE model – differences in overall activity in species with no circadian rhythms);

iv) $Y_{overall} = SP + MC + RE$ (Additive MC model – differences in overall activity in species with similar circadian rhythms);

v) $Y_{overall} = SP{*}MC + RE = SP + \mathrm{MC} + SPxMC + RE$ (Interaction MC model – differences between species in circadian activity rhythms).

Species-specific waveforms were then described from the best approximating model by estimating MC parameters: i) the Midline Estimated Statistic of Rhythm (MESOR), corresponding to the mean level around which the cosine functions oscillate; and for each fitted single harmonic component, ii) the amplitude, corresponding to the distance from the MESOR to the extremes, peak or nadir, of the oscillation; and the acrophase, corresponding to the time interval at which the fitted function has its peak value. Because models report the odds ratio of activity, MESORs were computed as

$$MESOR = \frac{\exp m_0}{1 - \exp m_0}$$

The MESOR is closely related to the 24-hour mean activity, whereas the amplitudes of each component measure the magnitude of the rhythmic change, and the acrophases describe their timings. Amplitudes were given as $\sqrt{b_i^2 + c_i^2}$, while acrophases were given by $\tan^{-1}(-c_i/b_i)$ and chosen to be in the correct quadrant, according to c_i and b_i signals (Bingham et al. 1982; Fernández et al. 2009). Acrophases were converted into hours after midnight by back-transformation. Because several animals were followed during the same tracking session and time measurements at each location might involve some error, we expressed acrophases as the 15-minutes time interval within which higher activity occurred, corresponding to the time interval between locations. We also estimated the global amplitudes and acrophases resulting from the sum of the fundamental and the harmonic components. Global amplitudes were given by one half of the difference between the peak and trough of the waveform and were computed numerically after all model parameters were found, by solving the regressions. The 15-min time intervals within which models peaked corresponded to the global acrophases of resulting waveforms (Fernández et al. 2009). Bootstrapped 95% Confidence Intervals (CI) for each MC parameter were calculated in alternative to the delta method, because unlike asymptotic normal approximations based on Taylor series, bootstrap does not assume any particular distribution function (Fernández et al. 2009). Using a leave-one-out bootstrap cross-validation scheme, models were re-run excluding one animal at the time with replacement. Amplitudes with 95%CI including 0 were considered equivocal, meaning that the respective cyclic components may be of little importance in explaining the overall rhythm of activity. Differences between species in each MC parameter were assessed by examining overlap in the corresponding 95%CIs.

A second set of analysis tested whether species-specific circadian activity was affected by season (SEA; dry *versus* wet season), sex (SEX; male *versus* female), and species interactions (SPI; presence *versus* absence of the other species). This was done by specifying alternative MC models incorporating for each explanatory variable ($factor_i$) the additive ($factor_i + MC + RE$) and interaction ($factor_i*MC + RE$) effects, which were then compared with the corresponding null MC model ($MC + RE$) using ITA. Support for the additive model was taken to indicate that the explanatory variable was associated with differences in overall activity but not in activity rhythms, whereas support for the interaction model suggested effects in the activity rhythms. For variables showing interaction effects, a model was fitted separately for each group and MC parameters were estimated to describe waveforms obtained. Bootstrapped 95% confidence intervals were also estimated to assess the importance of each cyclic component and respective variations according to SEX, SEA and SPI, and within each of these factors, according to species. All models were fitted in the R 2.10.0 software (R Development Core Team 2009) using the lmer function of the lme4 package (Bates & Maechler 2009).

5.3 Results

5.3.1 Circadian activity rhythms

The 6-hour period was the UD component best fitting water and Cabrera vole data sets (Table 1). The simultaneous fit of the three cosine functions (CD, HCD and UD) provided support for rhythmicity in circadian activity patterns by both species, though there were marked differences in species-specific overall activity patterns (Table 2). The MESOR was largest for water voles, suggesting that they were generally more active than Cabrera voles (Table 3). The amplitudes corresponding to each harmonic component of the MC also differed between species (Table 3). The fundamental CD component was the most important for Cabrera voles (Table 3) with the acrophase consistently occurring between 1200-1215 hours. For water voles, the CD amplitude was relatively low and, although the 95%CI did not include 0 (Table 3), the corresponding acrophase was equivocal because 95%CI for CD timing of activity included the whole 24-hour cycle. The most important cyclic component for water voles was the HCD period, which as for Cabrera voles, determined a first maximum between 0600-0615 hours. The UD period was the least important for Cabrera voles and the second most important for water voles, and indicated similar timings for both species, the first one occurring between 0245-0300 hours.

Table 1 – Summary results of information-theoretic model selection for multi-cosinor models of vole activity, considering five alternative ultradian (UD) periods, showing the ΔAIC and rank of each candidate model.

UD periods	**Cabrera vole**		**Water vole**	
	ΔAIC	**Rank**	**ΔAIC**	**Rank**
6-hour	0	1	0	1
5-hour	10	2	52	4
4-hour	13	3	28	2
3-hour	15	4	50	3
2-hour	19	5	55	5

Table 2 – Summary results of information-theoretic model selection for multi-cosinor models of vole activity, comparing candidate models reflecting the effects of species identity (Cabrera versus water voles) on circadian rhythms. SP = Species Effects; RE = Random Effects; MC = Multi-cosinor function;

Candidate models	Nº of parameters	ΔAICc	Akaike weights	Evidence ratio	Rank
RE	2	333.951	3.0E-73	3.4E+72	5
MC + RE	8	92.965	6.4E-21	1.6E+20	3
SP + RE	3	320.953	2.0E-70	5.1E+69	4
SP + MC + RE	9	78.969	7.0E-18	1.4E+17	2
*SP * MC + RE*	15	0	≈ 1	1	1

Table 3 – Comparison of MESORS and Amplitudes (CD, HCD, UD and global) for Cabrera and water voles, estimated from multi-cosinor models (MC) of circadian activity. Estimated parameters are provided with 95% confidence intervals

MC parameter	MC Component	Cabrera vole	Water vole
MESOR		0.336 [0.326;0.345]	0.517 [0.505;0.529]
Amplitude	CD	1.159 [1.063;1.256]	0.051 [0.019;0.091]
	HCD	0.879 [0.786;0.974]	0.802 [0.739;0.867]
	UD	0.299 [0.258;0.340]	0.406 [0.367;0.445]
	Global	0.253 [0.244;0.263]	0.211 [0.202;0.220]

Similarities between species in activity timings, at least for HCD and UD components, suggests that differences in amplitudes of each of the three cyclic components analyzed were enough to produce distinct average waveforms for each species overall activity (Fig. 1). Globally, activity peaks for the two species occurred twice across the 24-hour cycle, reflecting the relative importance of the HCD component, with timings approximately occurring just-after sunrise and sunset (Fig. 1). Cabrera voles were considerable diurnal in their circadian activity, as expected from the high importance of the CD component (Table 3). This was particularly evident during the afternoon, the only period when Cabrera voles were apparently more active than water voles (Fig. 1). Despite the general similarities between species concerning the effects of sunrise and sunset in increasing activity, there were differences in the respective timings at fine temporal scale, with sunrise-related activity bouts occurring between 0730-0830 hours (global peak) for water voles, and between 0800-0900 hours for Cabrera voles, and sunset-related activity bouts occurring between 1900-1945 hours (global peak) for Cabrera voles and between 19:30-2030 hours for water voles. The global amplitude of activity rhythms was greater for Cabrera voles (Table 3).

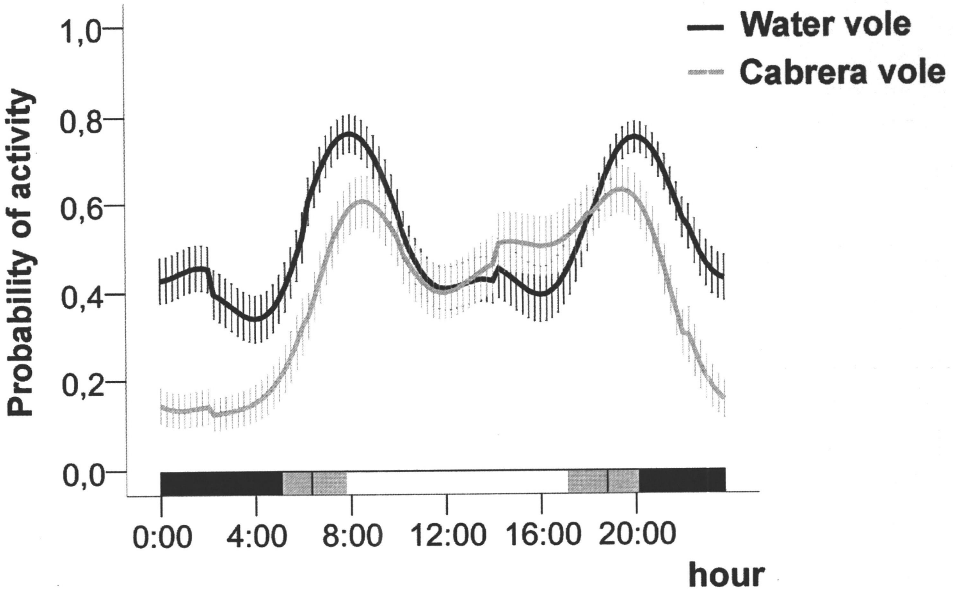

Figure 1 – Overall composite waveforms showing the circadian activity of Cabrera and water voles, derived from mixed effects multi-periodic logistic regression models. Error bars indicate 95% confidence intervals. Bars at the bottom represent night time (black), day time (white), minima and maxima timings of sunrise and sunset (grey) an respective means (vertical lines), considering overall days of activity sampling.

5.3.2 Effects of Season, Sex and Interspecific Interactions

The seasonal trend model for the circadian activity by Cabrera voles was about 2.5 times more likely than the model with no effects (Table 4), and the MESORs of the bootstrapped models were consistently higher in the wet than in the dry season (Table 5). However, the model including the effects of season on the MC rhythm parameters had the highest support (Table 4), suggesting that circadian activity rhythms by Cabrera voles changed across seasons. This model also had the highest support for water voles, although the mean circadian activity remained constant across seasons for this species (Table 4). The model corresponding to the circadian activity by Cabrera voles in the wet season had greater CD amplitudes than the model for the dry season (Table 5). This suggests that, although for both models the 95% CI of acrophases for the CD harmonic component were included within the time-interval 1200-1215 hour, the diurnality of Cabrera voles is probably more pronounced during the wet season, as shown by the averaged composite waveforms produced for each season (Fig. 2a). The CD amplitudes of the water voles seasonal models were relatively small and the respective 95%CIs overlapped (Table 5). Despite the relatively reduced importance of the CD cycle for water voles across seasons, there was a tendency for some diurnality during the wet season and some nocturnality during the dry season, as judged from the 95%CIs for acrophases (within 0000-0015 hour during the dry season, and within 1200-1215 hour during the wet season). The HCD and UD components of the MCs were more important for both species during the dry season, as judged from their higher amplitudes (Table 5). However, the HCD and UD phases remained constant across species and seasons, with 95%CIs for the first maximum occurring within 0600-0615 hour for HCD and within 0245-0300 for UD.

The distinct average waveforms produced from the seasonal models for each species (Fig. 2a and b), reflected mostly the differences in the seasonal amplitudes of the MC components. Global amplitudes were higher during the dry season for both species, with water voles having less rhythmic changes than Cabrera voles across seasons (Table 5). The differences in activity phase between species were higher during the wet season, with 95% CIs timings of sunrise- and sunset-related peaks of activity by water voles occurring respectively between 0730-0830 hours and 1915-2015 hours (global peak), and with Cabrera voles showing a unimodal-like distribution pattern peaking between 1045-1845 hours. During the dry season the global peak for each species was related to sunrise, occurring between 0745-0845 hours for Cabrera voles and between 0730-0830 hours for water voles. Sunset-related peak of activity by Cabrera voles during the dry season occurred between 1915-2000 hours, and between 1945-2030 hours for water voles.

Table 4 – Summary results of information-theoretic model selection for multi-cosinor models (MC) of vole activity, comparing alternative models reflecting the effects of sex, season and interspecific interactions on circadian rhythms of Cabrera and water voles. SEX = Sex Effects; SEA = Season Effects; SPI = Species Interaction Effects; RE = Random Effects; MC = Multi-cosinor function

Candidate models	**Nº of parameters**	**Cabrera vole**				**Water vole**			
		ΔAICc	**Akaike weights**	**Evidence ratio**	**Rank**	**ΔAICc**	**Akaike weights**	**Evidence ratio**	**Rank**
Season									
MC + RE	8	24.926	2.340E-6	2.59E+5	3	32.927	7.08E-8	1.41E+7	2
SEA + MC + RE	9	22.935	6.,335E-6	9.55E+4	2	33.934	4.278E-8	2.34E+7	3
*SEA * MC + RE*	15	0	0.605	1	1	0	≈ 1	1	1
Sex									
MC + RE	8	0	0.605	1	1	2.992	0.18	4.464	2
SEX + MC + RE	9	1.008	0.366	1.655	2	0	0.805	1	1
*SEX * MC + RE*	15	6.073	0.029	20.834	3	8.065	0.014	56.412	3
Interspecific Interactions									
MC + RE	8	0	0.423	1	1	0	0.581	1	1
SPI + MC + RE	9	0.008	0.421	1.004	2	2.008	0.213	2.728	2
*SPI * MC + RE*	15	2	0.156	2.718	3	2.073	0.206	2.820	3

Sex and interspecific interactions had little effect on the activity rhythms of Cabrera and water voles (Table 4). For water voles the MESOR changed according to sex, with a mean probability of activity of 0.585 (95%CI: 0.562-0.609) for males and 0.457 (0.434-0.480) for females, but still with no effects on rhythmic patterns (Table 4).

Table 5 - Comparison of seasonal MESORS and Amplitudes (CD, HCD, UD and global) for Cabrera and water voles, estimated from multi-cosinor models (MC) of circadian activity. Estimated parameters are provided with 95% confidence intervals.

MC parameter	MC Component	Cabrera vole		Water vole	
		Dry Season	Wet Season	Dry Season	Wet season
MESOR		0.304 [0.286;0.322]	0.380 [0.364;0.396]	0.512 [0.478;0.547]	0.503 [0.488;0.518]
Amplitude	CD	0.881 [0.652;1.110]	1.445 [1.264;1.626]	0.242 [0.144;0.340]	0.189 [0.103;0.274]
	HCD	1.275 [1.126;1.423]	0.512 [0.315;0.710]	1.177 [1.029;1.326]	0.432 [0.320;0.545]
	UD	0.477 [0.330;0.564]	0.169 [0.116;0.222]	0.489 [0.418;0.559]	0.345 [0.286;0.404]
	Global	0.313 [0.292;0.334]	0.226 [0.206;0.246]	0.266 [0.241;0.291]	0.167 [0.149;0.184]

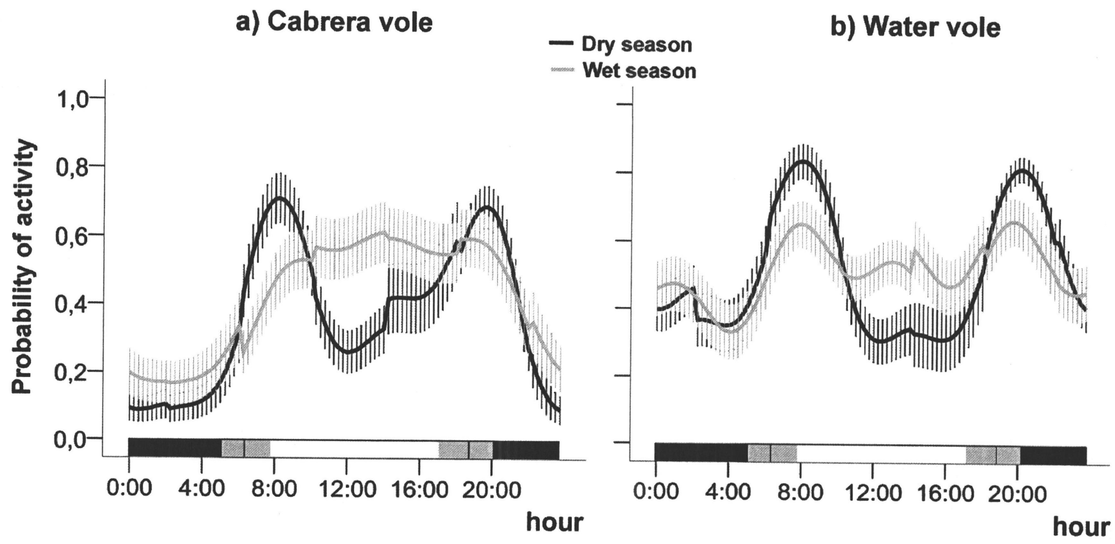

Figure 2 – Overall composite waveforms, showing the seasonal circadian activity by Cabrera (a) and water voles (b), derived from mixed effects multi-periodic logistic regression models. Error bars indicate 95% confidence intervals. Bars at the bottom represent night time (black), day time (white), minima and maxima timings of sunrise and sunset (grey) an respective means (vertical lines), considering overall days of activity sampling.

5.4 Discussion

The circadian activity of Cabrera and water voles followed a rhythmic pattern, apparently exhibiting, at least in some degree, the ultradian (UD), hemi-circadian (HCD) and circadian (CD) rhythmic components assumed to affect arvicoline rodents (Halle & Stenseth 2000; Mistlberger & Rusak 2005; Halle 2006). For both species, there was evidence for a 6-hour UD period at the population level, which was higher than expected for water voles (about 4.7-hours periods) and particularly so for Cabrera voles (about 3.2-hours periods), according to the allometric equation relating period length with body weight in vole species (Halle & Stenseth 2000). In water voles, however, the UD period was within the range estimated for its congener *Arvicola terrestris* (Halle & Stenseth 2000). Because UD rhythms are ultimately affected by metabolic and digestive needs (Halle & Stenseth 1994), the relatively long UD period of Cabrera voles may be a consequence of their relatively low basal metabolic rates (Mathias et al. 2003), and high digestive efficiency of the rich-cellulose and poor-protein content of their diet (Santos et al. 2004). Energy and water economy by Cabrera voles may be particularly important during the dry season, when ambient temperatures are higher and animals may lower their metabolic rates for thermoregulation (Mathias et al. 2003; Santos et al. 2004). This probably explained why the importance of the 6-hour UD period increased for Cabrera voles during dry season, when UD amplitudes were highest.

According to our predictions, hemicircadian profiles were largely related with the two main circadian photic clues (sunrise and sunset), during which voles considerably increased their activity. Because these daily events synchronized UD activity bouts of both species, with the first peak occurring about 6 hours after the sunset, their overall activity was relatively similar both in terms of the periods affecting activity and respective timings across the 24-hour cycle. This may result from the strong relationship between intra-family taxonomic affiliation and daily activity patterns by species (Kronfeld-Schor & Dayan 2003; Gutman & Dayan 2005). However the magnitude of rhythm change determined by each period differed between species and because Cabrera voles were more active in daytime, the overall variation in vole circadian activity was largely explained by species-specific responses to circadian changes. The Cabrera vole may be globally characterized as a diurnal species with major episodes of activity after dawn and dusk synchronizing relatively long UD activity bouts. The water vole may be either diurnal or nocturnal, although higher activity occurred always just after sunrise and sunset, and reset the short-term population activity bouts. In addition, the overall amplitude of circadian activity was higher for Cabrera voles, while the mean activity of water voles was globally higher throughout the day, with the only exception of the afternoon period.

The season was the most consistent factor influencing rhythm change by Cabrera and water voles, suggesting that they were able to respond to the predictable environmental events that recur in the form of annual cycles, as suggested for other vole species (Halle & Stenseth 2000). Seasonal time cues for voles were probably related with the differences in day length and temperature ranges, as other seasonal indicators potentially affecting activity, such as eventual changes in food availability or habitat quality, were unlikely given the lack of intra-specific differences in home-range size among tracked voles across seasons (Pita et al. in press). Cabrera voles exhibited a much stronger diurnal profile during the wet season, without systematic drifts toward sunrise or sunset, while during the dry season they exhibited a more prominent bimodal pattern associated to the light-dark transitions. Mean circadian activity by Cabrera voles was also higher during the wet season, which may be a consequence of the relatively reduced activity around noon during the dry season, when voles were probably forced to adjust their physiological and metabolic needs to the higher temperatures (Mathias et al. 2003). Water voles displayed a markedly bimodal-crepuscular pattern during the dry season, although they also showed some tendency to be more active during the night. This bimodal pattern remained relatively stable during the wet season, although sunrise and sunset photic entrainments seemed more effective in producing rhythm during the dry season. In addition, because water voles were apparently more active in daylight during the wet season, there was support for a possible seasonal phase shifts in their circadian activity. Similar seasonal phase shifts have been described for other vole species, supporting the view that some arvicolines may shift the predominance in their activity from nocturnal in summer to diurnal in winter, maintaining the strong crepuscular component throughout the year (e.g. Erkinaro 1961; Rowsemitt 1991; Gliwicz & Dabrowski 2008). Such seasonal changes in activity timings are probably facilitated by the short-term rhythms displayed (Rowsemitt 1991), which in our study were globally more pronounced in water voles. Seasonal alteration in activity timing has probably evolved to avoid extreme microclimates, such as thermal gains in summer days and losses in winter nights (Rowsemitt 1991). The global amplitudes of the circadian activity rhythms were much higher during the dry season for both species, suggesting that rhythm changes were more pronounced during the dry season.

Biotic interactions showed little influence on activity rhythms for both Cabrera and water voles. The effects of sex were only apparent for water voles, with a small tendency for males showing the highest mean circadian activity. Because sex differences in activity levels by rodents seem to reflect species-specific evolutionary mechanisms of sexual selection (Mead et al. 1996), it is likely that the pattern observed in water voles may be related to differences also found in intra-sexual home-range overlap, which are consistent with the spatial properties of polygynous systems (Pita et al. in press). Therefore, despite the likely ability by water voles to adopt alternative mating systems according to environmental conditions (Román 2007; Pita et al. in press), the higher activity by males might have probably evolved to improve the odds of finding a mate (Mead et al. 1996). Likewise, the lack of sex differences in the overall circadian activity among Cabrera voles agrees with the tendency for the species to mate monogamously (Mead et al. 1996; Fernández at al. 2001; Pita et al. in press).

Contrary to our predictions, Cabrera voles showed no rhythm changes in the presence of water voles within the same habitat patch. It is plausible that evolutionary constrains related with phylogenetic imprint could greatly diminish the ability of voles to shift their diel activity rhythms due to interspecific interactions, and hence to differentiate across the time niche-axis in the presence of potential competitors (Kronfeld-Schor & Dayan 2003; Roll et al. 2006). Indeed, even when interspecific competition might be relevant, rhythm shifts by species towards temporal resource partitioning among competitors are relatively rare and should require significant depletion of resources before it is no longer optimal to use a period frequented by competitors (Kronfeld-Schor & Dayan 2003). It may be also hypothesized that the apparent higher specialization by Cabrera voles to diurnal periods was sufficient to guarantee enough temporal partitioning between co-existing hetero-specifics, thereby adding to spatial segregation (Pita et al. in press) and habitat partitioning (Pita et al. unpublished data) in allowing species co-existence. There was also some evidence that temporal partitioning may have occurred within the preferred parts of the diel cycle at fine temporal scale, with sunrise activity bouts occurring first for water voles and sunset activity bouts occurring first for Cabrera voles.

Overall, results suggest that environmental factors associated to seasonal changes are by far more important in driving the flexibility in circadian activity rhythms than biotic interactions among individuals. Because light and temperature changes provided the major time cues of environmental seasonality for voles, it seems that, at least at the population level, the seasonal changes in abiotic factors are probably the prime environmental parameters shaping the evolution of circadian activity rhythms by voles. We thus suggest that, although biotic interactions might still serve as an important mechanism producing immediate behavioural responses at the individual level (Sharma & Chandrashekaran 2005), they might provide poor selective base in terms of the long time evolutionary forces driving the flexibility of activity patterns within vole species.

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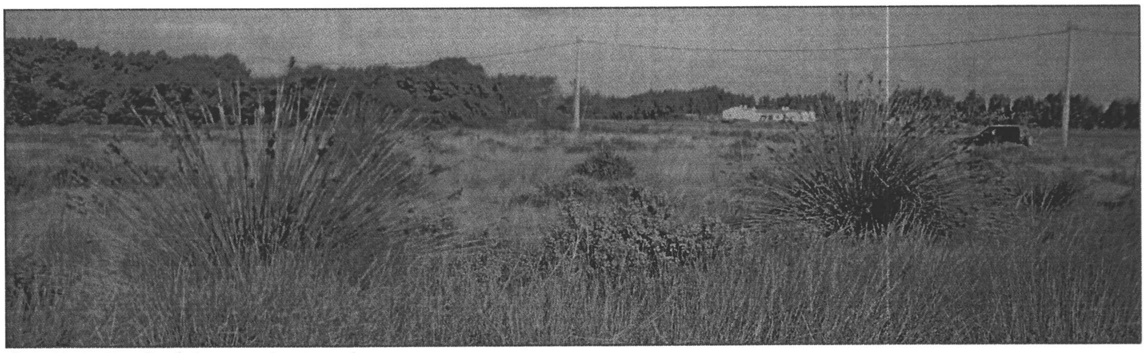
CHAPTER 6

General discussion



Suitable habitat for Cabrera and water voles

6. General discussion

6.1 Overall patterns

Understanding how spatial and temporal heterogeneity affect the dynamics of populations and communities is a fundamental issue in ecological research (Rhodes and Odum 1996; Wiens 2000). Recognition that organisms experience variation regarding both abiotic (e.g. weather, climatic changes) and biotic factors (e.g. interspecific interactions, habitat changes) at many spatial (e.g. across landscapes, within habitat patches) and temporal scales (e.g. seasonal, circadian), implies that answers to most prominent questions in ecology should involve the pervasive influence of heterogeneity (Wiens 1997). However, dealing with environmental variation in space and time is not straightforward, which has often deterred field ecologists to consider the effects of heterogeneity on ecological processes (Weins 1997). Indeed, because measurement of all the heterogeneity in every property of an ecosystem is virtually impossible and parsimony tells that ecological models should be kept as simple as possible (Lovett et al. 2005), researchers have historically tend to focus on patterns and dynamics of ecological systems within relatively homogeneous environments and based on well established ecological theories (Wiens 1997). There is therefore a need for empirical studies illustrating how environmental heterogeneity may be addressed in ecological studies, in order to contribute for the development of conceptual frameworks to analyse responses of organisms to environmental change (Wiens 2000; Lovett et al. 2005).

The case-studies outlined in the previous chapters provide a route to understand how environmental heterogeneity affects persistence and coexistence of spatially structured populations, using two arvicoline species living in Mediterranean farmland as model system. Specifically, the example presented in Chapter 2, regarding the factors ruling the spatial population structure of Cabrera voles, supported the view that considerations on an eventual metapopulation structure may be incomplete when the context of the underlying landscape mosaic is disregarded (e.g. Wu 1995). Therefore, this work showed that ideas from both metapopulation dynamics and landscape ecology may indeed provide cleared insights on species persistence abilities in heterogeneous environments (Wiens 1997). In fact, results from this thesis evidenced that the patch size and isolation paradigms of current metapopulation theory were weaker predictors of species persistence abilities than explanatory variables describing landscape heterogeneity, such as land use patterns. This result has been increasingly recognised for other spatially structured populations from many different taxa living in different systems (Pellet et al. 2007; Prugh et al 2008), suggesting that heterogeneity of the intervening matrix may be highly influential in determining spatial population structure of diverse species. Therefore, while consideration of the spatial structure of habitat patches across the landscape under the metapopulation theory may provide an important reference source for ecologists and conservationists, the developments regarding spatial pattern analysis using geographic information systems and spatial statistics under landscape ecology, have shown that the type of land cover separating habitat patches may strongly affect sensitivity of species to patch area and isolation (Prugh et al 2008), such as seems to be the case of Cabrera voles in Mediterranean farmland. On the other hand, results also provided evidence that the dynamics of patches themselves (temporal heterogeneity) may strongly affect overall population persistence, by producing changes in the spatial patterns and relationships of patches within the matrix (Wiens 1997).

While results from Chapter 2 suggested that environmental heterogeneity in space and time may interfere with ecological processes at the landscape level, such as the likelihood of species persistence, the studies presented in Chapters 3 to 5 showed that consideration of heterogeneity may be essential to understand coexistence of close-related species at the local (and thus landscape) scale. The empirical model represented by Cabrera and water voles in Mediterranean farmland provided evidences that, despite the apparent niche overlap between species, coexistence of voles within habitat patches may be enhanced by environmental heterogeneity at fine spatial (Chapters 3 and 4) and temporal scales (Chapter 5). Specifically, while in Chapter 3 it is suggested that there may be considerable spatial segregation between species within habitat patches, the multi-scaled analysis of microhabitat selection and differentiation presented in Chapter 4, showed that local coexistence is possible by means of resource partitioning at multiple small spatial scales. Likewise, the interspecific differences regarding the circadian activity of voles at fine temporal scales described in Chapter 5, suggested that temporal heterogeneity may contribute as further mechanism enhancing coexistence. Overall, the most important message from these studies is that focussing on only one source of heterogeneity and on a single spatial scale does not answer the question of how species can coexist.

As a whole, by assuming the importance of considering environmental heterogeneity in assessing persistence and coexistence of Cabrera and water voles in Mediterranean farmland, this thesis has some strong applied implications directly related with the conservation of the studied species, and indirectly related with the evaluation of the approaches used to study persistence and coexistence of spatially structured populations living in heterogeneous environments.

6.2 Summary of main findings

6.2.1 Spatial structure of Cabrera vole populations

6.2.1.1 The utility of the metapopulation approach

Information gathered in this research indicated that, in common with water voles (Román 2007; Centeno-Cuadros 2009) as well as with other small mammals living in heterogeneous environments (Lambin et al. 2005; Olivier et al. 2009), Cabrera voles apparently exhibit a metapupulation-like structure, with the overall population consisting of an assemblage of small colonies (local populations) inhabiting spatially distinct habitat patches. Support for such spatial structure is given by the fact that during the census interval, we found local population extinctions and the establishment of new colonies in previously empty habitat patches, resulting in regular population turn-over. Moreover, patch occupancy was affected by patch area and isolation, suggesting that dispersal may be a key process for population persistence (Hanski 1999). In addition, although population equilibrium (i.e. the balance between extinctions and colonisations) could not be assessed from the data, the relative stable percentage of occupied patches across seasons and years, suggests that local dynamics was sufficiently asynchronous to make simultaneous extinction of all local populations unlikely. This could reflect habitat heterogeneity, reduced dispersal rates between local populations, and variation in community processes, trophic interactions or interspecific relationships, in contrast with synchronous populations resulting from high dispersal rates or correlated changes of environmental conditions (Olivier et al. 2009). Results also indicated that there seems to be no obvious permanent 'core' population, although larger habitat patches may be occupied for relatively long periods. Overall, these attributes suggest that the metapopulation concept as defined by Hanski (1999) can be applied to the studied Cabrera vole population. However, since habitat patches may appear and disappear, many of the extinctions recorded were deterministic, i.e. resulting from habitat destruction or reduction, either due to natural causes (e.g. habitat drought, scrub encroachment) or to human management (e.g. habitat conversion, burning, overgrazing or land abandonment). Such temporal variation in habitat availability, which probably is not an exclusive particularity of our study system, thus adds a source of local and landscape variation that usually is not considered under classical metapopulation models. Despite the deviations from its classical premises, the metapopulation approach used to assess the spatial population structure of Cabrera voles facing environmental heterogeneity was found to be a useful conceptual tool to improve our understanding of species persistence in heterogeneous Mediterranean farmlands.

6.2.1.2 The prevailing role of matrix heterogeneity

The nature of the intervening land between habitat patches appeared to be crucial to improve predictions on persistence abilities of Cabrera voles, as habitat patches were more likely to be occupied when surrounding matrix was predominantly composed by natural, lightly-grazed pastures, and tended to be empty when enclosed in heavily grazed land or woody habitats. Because dispersal ability is an important determinant of population persistence (e.g. Hanski 1998 and 1999), it is possible that some habitat types of the matrix may provide higher chances of survival (including reduced predation risk) during long distance movements between habitat patches, as it seems to be the case of pastures with low-input grazing. Therefore, landscape composition and structure may determine an 'effective isolation' (*sensu* Ricketts 2001) of habitat patches, resulting from the complex interactions that are likely to occur between matrix permeability and straight-line distance between suitable habitats. This suggests that simple models assuming a homogeneous matrix ignore a potentially important aspect of patch isolation (Ricketts 2001). Since real landscapes are complex and resistance of a given matrix type is probably species-specific, attempts to generalise results towards the development of modelling frameworks to deal with landscape heterogeneity, may be somehow infertile. However, results suggest that a promising start to overcome this, may be the incorporation of certain characteristics of matrix heterogeneity that are likely to affect autecological traits of the species under study, such as the rates of interpatch movement (Ricketts 2001). This may be particularly relevant in agricultural areas subjected to homogenisation and fragmentation processes, as a result of increasing coverages of monocultures and increasing distances separating habitat patches.

6.2.2 Resource partitioning and niche overlap between Cabrera and water voles

6.2.2.1 Insights from spatial structuring

Space is one of the niche axes where ecological differences may arise between species, making possible their coexistence in a given area (e.g. Segurado and Figueiredo 2007; Amarasekare 2003). Although in Mediterranean farmland landscapes of south-western Portugal, Cabrera and water voles may occur simultaneously within the same habitat patches, results on spatial interactions among coexisting voles evidenced a considerable amount of interspecific spatial segregation, contrasting with a high level of intraspecific spatial overlap recorded for both species and for all range estimators. In particular, core foraging areas of the two species were essentially mutually exclusive, suggesting that spatial segregation occurred mostly at fine spatial scales. High intraspecific overlap may reflect specific socio-spatial organization, which was consistent with a monogamous mating system for Cabrera voles (see also Fernández-Salvador et al. 2001 and 2005), and a polygyny (resource-defence) strategy for water voles, with shifts to facultative monogamy, when habitat patches are rather small (see also Román 2007). Conversely, the reduced space sharing between species may be a consequence of either direct (behavioural) or indirect (resource partitioning) interspecific interaction. Under the direct interspecific interaction perspective, it could be hypothesized that water voles would more often displace Cabrera voles, because of the interspecific differences in body sizes and contrasting home-range area requirements. However, the strong site fidelity exhibited by Cabrera and water voles was not affected by the presence of heterospecifics, suggesting that other mechanisms beyond spatial segregation may be operating at fine scales. In addition, preliminary analysis at the landscape scale (Pita et al., unpublished data) suggests considerable spatial association between Cabrera and water vole colonies, which could imply low interspecific spatial segregation at the large spatial ranges.

Although results suggest that fine-scale spatial segregation between Cabrera and water voles may facilitate local coexistence, interspecific space sharing varied along the annual cycle, being apparently higher during the summer, i.e. when food and water availability become a strong limiting factor (e.g. Ventura et al 1998; Román 2007). Therefore, presuming that overlap in space correlates with the probability of encounter between individuals, it could be expected that for instance fine scale habitat differentiation or temporal partitioning under the classical niche theory (Hutchinson 1957), would provide critical additional mechanisms enhancing local coexistence of Cabrera and water voles in Mediterranean farmland.

6.2.2.2 Perceptual ranges and multi-scaled microhabitat differentiation

How foraging animals respond to habitat heterogeneity at different spatial scales reflects their perception of resource availability, and the amount of resource they are able to find (McClure and Shipley 2009). The characterisation of hierarchical microhabitat selection and differentiation between Cabrera and water voles across multiple spatial grains and extents, revealed that spatial scale may critically influence tacit inferences regarding species habitat profiles, and interpretation of coexistence within shared habitat patches. While second-order selection analysis revealed that sedge/rush habitats were apparently the most selected cover types by both species at small spatial grains during the summer, preferred habitats differed when coarser grains of analysis were used, and when other resources, such as food and water, became more abundant (i.e. during the wet season). Under these circumstances, Cabrera voles preferred tall mesic grasses, while water voles retained their preference for tall wet vegetation. In addition, preferred habitats also changed considering the spatial extent, as revealed for example from third-order selection by Cabrera voles, which indicated a preference for shrubs. In accordance to these results, niche overlap increased at the highest spatial resolutions and during summer periods, decreasing with spatial extent and during the wet season.

Overall, these results showed that habitat selection and partitioning between Cabrera and water voles did not simply seem to happen at one spatial scale. Moreover, selection by Cabrera voles was apparently stronger at fine spatial scales, while that of water voles strengthened when coarser scales were used, probably reflecting the interspecific differences in perceptual ranges by each species, as expected from their differences in body sizes and home-range areas. There is thus evidence that studies aiming to explain coexistence of ecologically similar, different sized species in complex habitat mosaics, should integrate the various spatial scales at which animals are likely to make habitat-use decisions (Boyce 2006; Meyer and Thuiller 2006; Meyer 2007). Although no analytical protocol currently exists for incorporating and comparing animal decisions across scale into the statistical approaches that characterize the outcomes of these decisions (McClure and Shipley 2009), results indicate that understanding the responses of species to habitat heterogeneity, may be best achieved when measurements on heterogeneity are conducted at multiple spatial scales, and considering the temporal variation in resource availability (e.g. seasonal fluctuations).

6.2.2.3 Fine-scale temporal partitioning

Apart from the differences found in microhabitat selection between Cabrera and water voles, a further mechanism related with fine-scale temporal partitioning, may contribute for species coexistence within habitat patches. The analysis of circadian activity rhythms by both species suggested general similarities between Cabrera and water voles regarding the three rhythmic components presumed to affect voles' dial activity (circadian = 24 hours, hemicircadian = 12 hours, and ultradian = 6 hours), probably reflecting their close phylogenetic relation (Roll et al. 2006). In addition, species were relatively inflexible in changing their overall circadian activity patterns in the presence of heterospecifics. Despite these general traits, the differences between species in their overall patterns of circadian rhythms, were probably enough to facilitate coexistence (Kronfeld-Schor and Dayan 2003). Indeed, the Cabrera vole showed to be a more diurnal species (particularly during the wet season), presenting major episodes of activity after dawn and dusk synchronizing relatively long ultradian activity bouts. Conversely, water voles may be either diurnal (during the wet season) or nocturnal (during the dry season), although higher activity occurred always just after sunrise and sunset, and reset the ultradian population activity bouts. In addition, there was also some evidence that temporal partitioning may have occurred within the preferred parts of the diel cycle (sunrise and sunset) at fine temporal scale.

Because interspecific differences in circadian activity rhythms were apparently higher during the wet season, it could be hypothesised that the potential for competition between Cabrera and water voles is higher during the summer, as also suggested from results regarding spatial overlap and habitat differentiation. This may have important implications for species survival when a combination of circumstances takes place. For instance, populations living in farmlands facing ongoing homogenization and fragmentation processes may difficultly resist to consecutive years of severe summer droughts limiting resource availability. Although merely prognosticative, such scenario may not be an uncommon situation, considering the current global patterns of land transformation (e.g. Jongman 2002) and global warming (Botkin et al. 2007).

6.3 Conservation implications

Conservation biology is often concerned with the persistence and coexistence of wildlife populations under different landscape scenarios, and the effects of environmental heterogeneity are of major concern in this regard (McCullough 1996, Wiens 1997). Determining the spatial structure of a population is essential to formulate conservation guidelines, and the application of metapopulation theory has shown to provide an useful tool to formulate a set of ‘rules’ for reserve design and management derived from the area and isolation paradigms (Hanski 1999). The relevance of metapopulation theory to wildlife conservation and management has been particularly recognized in the face of increasingly rapid habitat fragmentation occurring worldwide (McCullough 1996, Hanski 1998). However, if one wishes to guarantee the persistence of a metapopulation structure of a particular species in a given area, it may be necessary to manage not only the network of habitat patches that contain (or could contain) local populations, but also the landscape features that influence interpatch movement (Wiens 1997).

Results from this thesis suggest that environmental heterogeneity may be a key factor for conserving Cabrera and water voles in Mediterranean farmland. At the landscape scale, Cabrera voles conservation seems to require a network of large (ideally ~>2000m^2, minimum ~500m^2)

suitable habitat patches composed by a mixture of tall grasses, sedges, rushes, reeds, and shrubby vegetation. Preferentially these habitats should be separated by less than 300-400m, and surrounded by mosaics of little grazed pastures. Although comparable inferences regarding water voles were not assessed in this thesis, it is likely that the species could as well benefit from similar management principles. However, the critical thresholds regarding interpatch distance could probably be relaxed to around 600-800m (according Centeno-Cuadros 2009 and Román 2007, respectively), while minimum patch area should be greater (~>1000m^2). On the other hand, because spatial scales did not perform identically on seasonal microhabitat selection and differentiation between Cabrera and water voles, conservation prescriptions involving habitat management at the local scale should require attention to the quantitative effects of patchiness on the success with which voles move and identify preferred habitats. In particular, the patchiness of sedge and rush habitats was apparently the most important spatial pattern determining how the studied voles perceived and used their environment. Given the likely importance of small (~<100m^2) patches of sedge/rush habitats within tall grassy areas for Cabrera voles and the preference of water voles for larger patches of wet vegetation, maintenance of heterogeneous mosaics combining different-sized sedge/rush habitat patches, is probably a key step to guarantee species co-existence within marginal humid grasslands in Mediterranean farmland. Indeed, by providing a variety of compositions regarding the habitats used and shared by the species, the potential impacts of increased interaction between Cabrera and water voles could be reduced, thereby increasing individuals' chances to survive, particularly during the dry season. Habitat heterogeneity within vole areas may thus provide a variety of spatial niches favouring the coexistence of their metapopulations in Mediterranean farmland (Amarasekare et al. 2004; Román 2007).

6.4 Future research directions

Real metapopulations do not consist of identical and equally connected populations such as assumed in Levins model (Hansky 1998), and thus the patch size and isolation paradigms developed from the theory of Island Biogeography (MacArthur and Wilson 1967), have been routinely taken into classical metapopulation models (e.g. Hanski 1999). Besides, an increasing number of empirical examples, such as that provided in this thesis, have shown that insights from landscape ecology may further contribute to improve predictions on metapopulation dynamics, particularly in spatially and temporally heterogeneous environments (Rhodes and Odum 1996; Wiens 2000). By adding parameters that describe, for instance, the resistances of different matrix types to animal movements, researchers may improve causal inferences to be made regarding critical parameters in metapopulation dynamics, such as species dispersal rates and ranges (e.g. Hanski 1998 and 1999). Although the scale at which metapopulation processes act is largely determined by species dispersal abilities (Hanski 1998 and 1999), this proves to be one of the most difficult parameters to be measured in the field (e.g. Ims and Andreasson 2005). For Cabrera voles, results based on isolation measures taken approximately at 3 moth intervals, were concordant with the only dispersal movement successfully detected directly through radio-tracking, both suggesting that dispersal range abilities by animals may average around ca. 400m. However, at larger time intervals, Cabrera voles may move over larger ranges, as suggested in a post-fire re-colonisation study, showing that animals may travel at least 1364 m within eight months (Rosário et al. 2007). There is thus some support for the idea that stepping-stone dispersal, rather than single long dispersal movements, may occur in Cabrera voles, as suggested for water voles (Román 2007). In this context, further studies, including genetic approaches (Centeno-Cuadros 2009) are needed to deepen our understanding on overall dispersal patterns in heterogeneous environments and its implications on species persistence.

Improvements in metapopulation predictive modelling for Cabrera and water voles could also be obtained by using a more detailed approach, in which it would be possible to specify the population size on each patch. As a patch-occupancy model, the example provided for Cabrera voles used the state variables presences and absences of voles within a patch, i.e. a vector of 1s and 0s (Gilpin 1996), and habitat heterogeneity within habitat patches was not considered. Although such an inclusive modelling was beyond the scope of this thesis, these issues should be addressed in future studies, as voles are likely affected by fine scale spatial and temporal heterogeneity.

A particularly relevant issue emerging from this research regards on the possible interactions between Cabrera and water voles at the landscape scale. Although this issue was not directly addressed in this thesis, the outcomes regarding within-patch niche segregation between species raised a number of questions related with metacommunity functioning, competitive metapopulation dynamics and species coexistence. While the studies from this thesis supported initial predictions that resource partitioning under the classical niche theory may reasonably explain species coexistence, eventual competition-colonisation trade-offs between Cabrera and water voles should be also accounted for in future researches, as this is often referred to be a key mechanism enhancing coexistence in competitive metapopulations (Amarasekare 2003; Amarasekare et al. 2004; Mouquet et al 2005). Future studies should thus involve hypothesis testing, directly regarding the potential role of competitive-colonisation trade-offs in explaining species coexistence, and how the relative importance of each operating mechanism may change with variations in environmental heterogeneity at multiple scales. Such approach would also require more detailed information on species autoecological traits, particularly those reflecting their dispersal abilities in the face of spatial and temporal variation.

Usually competition-colonisation trade-offs imply that local displacement of one species by another occurs, and thus that local coexistence is unlikely (Mouquet et al. 2005). Despite the lack of evidence found for eventual displacement events, it could be hypothesised that the rates at which eventual local displacement of Cabrera voles by water voles may operate at wider temporal scales than that imposed by the radiotracking sampling scheme used here. This may imply that future research regarding competitive abilities resulting in displacement of one species by the other, may require sampling designs allowing analysis of home-range drift and size variation over longer sampling periods. In addition, because local heterogeneity may produce variation in competition asymmetries (Orrock and Watling 2010), there may be patches simultaneously occupied by both species, where the amount and patchiness of sedge/rush habitats may be limiting factors for water voles, with Cabrera voles taking advantage of such conditions and colonizing territories previously occupied by water voles.

Summarising, although results presented here provided evidence that, at least for close related species differing in their body sizes and perceptual ranges, heterogeneity may provide opportunities for resource partitioning at fine spatial and temporal scales, future research aiming to explain coexistence of Cabrera and water voles should focus on analysing whether interspecific interactions at the local scale may be significantly reflected in overall metacommunity dynamics and function or if the two metapopulations are relatively independent (Kneitel and Chase 2004).

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