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**Resposta espaço-temporal de corço a
novos ambientes**

**Roe deer's spatio-temporal response to
novel environments**

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(Maria Beatriz Alves Da Fonseca Samor De Almeida)



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ambientes**

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia Aplicada, realizada sob a orientação científica da Doutora Rita Maria Tinoco da Silva Torres, investigadora de pós-doutoramento no Departamento de Biologia da Universidade de Aveiro e sob co-orientação científica da Doutora Francesca Cagnacci, investigadora no Movement and Conservation Ecology Research Line Research and Innovation Centre da Fondazione Edmund Mach e do Doutor Carlos Manuel Martins Santos Fonseca, Professor Associado com Agregação do Departamento de Biologia da Universidade de Aveiro.

A todos os que,
pelo seu exemplo e capacidade de partilha, me têm ajudado a crescer.

o júri

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«Todo o trabalho criativo é, na essência, solitário, porque a disciplina e o método assim o exigem, contudo, o resultado final, é fruto de uma interioridade conjunta que motiva, une e congrega o esforço, o trabalho, a intuição e o talento de múltiplas pessoas que conosco aceitam assumir tão grande desafio. Nenhum acto criativo é por isso fruto de um Homem só.» (Abrunhosa, 1994)¹

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¹Abrunhosa, Pedro (1994). Agradecimentos. In Pedro Abrunhosa e Bandemónio, Viagens [CD áudio]. Porto. Polygram Discos, S. A.

palavras-chave

Capreolus capreolus, Reintrodução, Portugal, Área vital, Incêndios, Novos ambientes, Padrões de movimento.

resumo

O corço (*Capreolus capreolus*) é uma espécie de ungulados relativamente bem estudada na Europa central e uma das mais numerosas. Contudo, esta informação não é tão extensa no que concerne à distribuição da espécie no Sul, pelo que os fatores que condicionam a distribuição do corço e o seu comportamento, num habitat Mediterrâneo, são relativamente desconhecidos. Desde 2013, quatro eventos de reintrodução de corço tiveram lugar no centro de Portugal com o objetivo de criar um conjunto de presas naturais para o ameaçado lobo ibérico (*Canis lupus signatus*) com vista à diminuição dos seus ataques ao gado doméstico. Usando os dados do GPS dos animais rastreados no projeto de reintrodução, o objetivo deste estudo foi 1) analisar o estabelecimento das áreas vitais das populações reintroduzidas entre 2013 e 2017; 2) examinar as respostas espaço-temporais dessas populações aos novos ambientes resultantes da perturbação causada pelos incêndios ocorridos durante o referido período.

Sobre o primeiro objetivo, a análise mostrou que, aquando da sua libertação os animais tendem a afastar-se do ponto de libertação, estabilizando, posteriormente, os seus movimentos. A dimensão das áreas vitais mantidas pelos animais não variou quando se comparou o momento de libertação com os meses que se sucederam, o que, provavelmente, resultou de uma combinação entre a disponibilidade de recursos e a existência de barreiras sociais (tais como a territorialidade). No tocante ao segundo objetivo, no período imediatamente a seguir à ocorrência de um incêndio, os indivíduos demonstraram uma clara redução a nível de movimento, o que se refletiu na diminuição das áreas vitais mantidas pelos animais, espelhando uma rápida resposta à perturbação causada pelo fogo.

Este estudo para além de fornecer uma avaliação preliminar do sucesso da reintrodução do corço no centro de Portugal, também contribuirá para ajudar na gestão de esforços, atuais e futuros, relativamente à reintrodução de espécies. Além disso, representa uma das primeiras avaliações, dispondo de tecnologia GPS, no que concerne aos efeitos de elementos perturbadores, como o fogo, nos movimentos de um grande herbívoro.

keywords

Capreolus capreolus, Reintroduction, Portugal, Home-range, Fire, Naïve environments, Movement rates.

abstract

The European roe deer (*Capreolus capreolus*) is a relatively well studied ungulate species in central Europe and one of the most numerous. However, this information is not as extensive for the southern limit of the species distribution, which means that the factors that shape the roe deer distribution, as well as its ranging behaviour in a Mediterranean habitat, are relatively unknown. Since 2013, four roe deer reintroduction events have taken place in central Portugal with the aim to create a pool of wild natural prey for the endangered Iberian wolf (*Canis lupus signatus*) aiming to decrease wolf attacks to livestock. Using GPS data from the tracked animals in the reintroduction project, the purpose of this study was to 1) analyse the home range establishment of the reintroduced populations of roe deer from 2013 until 2017; 2) examine the spatio-temporal responses of these populations to novel environments under fire disturbances occurring in the same four years period. Concerning the first objective the analysis showed that at release, animals tended to move away from the release point to then stabilize their movement rates. These animals maintained home ranges of a rather constant size, possibly due to availability of resources, or social fences. Regarding the second objective, animals in proximity to a fire event showed a sharp decrease in the distance between subsequent weekly home ranges, which mirror a fast response to the disturbance caused by fire, as well as shrinkage of their home range size.

This thesis provides a valuable preliminary evaluation of the success of the reintroduction in central Portugal. Therefore, this study will help guide current and future management efforts regarding species reintroductions. Moreover, it represents an assessment, among the first of this kind with GPS telemetry, regarding the effect of disturbance effect on movement behaviour of a large herbivore, including fire events.

parole chiave

Capreolus capreolus, Reintroduzioni, Portogallo, Area vitale, Incendi, Nuovi ambienti, Tasso di movimento.

riassunto

Il capriolo europeo (*Capreolus capreolus*) è una specie ben studiata in Europa Centrale e Settentrionale, nonché una delle più numerose. Tuttavia, lo stesso non si può dire per la parte più meridionale del suo areale di distribuzione, il che significa che i determinati ecologici dell'uso dello spazio del capriolo in habitat mediterraneo, sono, ad oggi, relativamente poco conosciuti.

A partire dal 2013, sono stati effettuati quattro eventi di reintroduzione della specie nel Portogallo centrale, con lo scopo di creare un pool di prede naturali per il lupo cantabrico (*Canis lupus signatus*), in ultima analisi per ridurre il livello di danni inflitti da quest'ultimo al patrimonio zootecnico. Utilizzando dati GPS raccolti da animali rilasciati e dotati di radio-collare, gli scopi del presente studio sono stati quelli di 1) analizzare il meccanismo di formazione dell'home range in una popolazione di caprioli reintrodotti dal 2013 al 2017; 2) esaminare la risposta spazio-temporale di queste popolazioni ad una perturbazione del sistema come quella causata dai vasti incendi occorsi in questi ultimi anni.

Per quanto concerne il primo obiettivo, le analisi hanno mostrato che al rilascio gli animali tendono a muoversi rapidamente dal sito di rilascio, per poi stabilizzarsi nel tempo ad una certa distanza dal sito di rilascio stesso. La dimensione degli home range non cambia dal momento del rilascio per i mesi a venire, presumibilmente per una combinazione di disponibilità di risorse e/o barriere sociali (esempio: territorialità). Per quanto riguarda il secondo obiettivo, nell'immediato periodo successivo allo sviluppo di un incendio i caprioli mostrano una chiara riduzione del livello di movimento, nonché una riduzione delle dimensioni dell'home range.

Questa tesi rappresenta una prima valutazione preliminare del successo del progetto di reintroduzione nel Portogallo Centrale. Si auspica che tale studio possa aiutare e guidare le attuali e future scelte gestionali nell'ambito della reintroduzione di questa specie. Infine, tale studio rappresenta uno dei primi casi di analisi dell'effetto degli incendi sui movimenti animali misurati mediante GPS.

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Chapter I

Introduction

General introduction

The European roe deer (*Capreolus capreolus*) is a well-studied small ungulate, with adults having a length ranging from 90 to 130 cm, a height (until the shoulder) from 55 to 77 cm, and weight around 20 to 30 kg. Roe deer experience two yearly moults, with the summer pelage being orange-brown and the winter coat being grey, with exceptions of throat, ventral parts, and perianal region (anal mirror), which are white all year round. Fawns are spotted with white to favour mimicry with undergrowth (Andersen *et al.*, 2000). The degree of sexual dimorphism is relatively low, with males being on average slightly larger than females. The utter morphological distinction is the presence of antlers in males, which grow in winter, and shed after the reproductive season around October – November (Andersen *et al.*, 2000).

Roe deer are one of the most numerous ungulate species (Apollonio *et al.*, 2010) living in central and northern Europe. Roe deer is regarded as a typical forest species, which exhibits a high level of ecological and behavioural plasticity, that enables it to successfully colonise a wide variety of habitats (Linnell *et al.*, 1998). This results in the species being present in almost every European landscape (Jepsen & Topping, 2004), from deciduous, coniferous, and Mediterranean forests, to shrublands, moorlands, and marshes (Tellería & Virgós, 1997; Holand *et al.*, 1998; Torres *et al.*, 2011). Due to its ecological plasticity, roe deer can also be found in anthropized habitats, such as managed forests, farmland mosaics, and agricultural fields (Tufto *et al.*, 1996; Hewison *et al.*, 1998; Hewison *et al.*, 2001). The presence of roe deer within its distributional range is dependent upon a variety of factors, there including food availability (Virgós & Tellería, 1998), vegetation cover (Mysterud & Østbye, 1999), human disturbances (Torres *et al.*, 2011), terrain characteristics (Mysterud & Østbye, 1999), climatic factors (Brewka & Kossak, 1994), and predation (Melis *et al.*, 2009). However, the two crucial habitat requirements for roe deer are the presence of food resources (Duncan *et al.*, 1998), and cover (Cederlund *et al.*, 1998). Food resources are particularly relevant because, despite being a generalist herbivore, which feed on

a wide selection of plant species (Duncan *et al.*, 1998), roe deer is concentrate selector, relying on high-quality food (Illius & Gordon, 1992; Van Soest, 1994). The selection for high quality nutrients depends on the fact that roe deer are income breeders, close to the end of the continuum of energy allocation tactic. Roe deer are unable to store fat reserves, and as such they need to continuously forage to survive (Andersen *et al.*, 2000). As for the use of cover, this is an important aspect because it is pivotal as a hiding strategy for both predation and adverse climatic conditions (Mysterud & Østbye, 1999). Indeed, the presence of a canopy is crucial for thermoregulation both during summer heat waves and in the core of the winter, when it can also limit the thickness of the snow layer on ground.

The southwestern limit of roe deer distribution is in the Iberian Peninsula. In Portugal, in particular, it has always been present in few patches north of Douro river (Vingada *et al.*, 2010). In the 1990's, a series of reintroductions took place in Portuguese territory with different purposes. In central Portugal the reintroductions arose from the necessity of creating a pool of wielding prey for Iberian wolf, *Canis lupus signatus* (Carvalho *et al.*, 2008). In the south, the main objective of the reintroduction project was to provide hunting grounds for recreational hunting purposes (Torres *et al.*, 2015).

Roe deer populations have been increasing in Portugal, but densities remained low, especially when compared to those existing in Central and Northern Europe (Vingada *et al.*, 2010; Valente *et al.*, 2014; Torres *et al.*, 2015). This is probably due to the fact that the Iberian Peninsula appears to be especially susceptible to global changes, which are affecting the distribution, abundance and demography of animals across the world (Parmesan, 2006), and of wild ungulates in this area in particular (Acevedo *et al.*, 2011). Being at the southern edges of roe deer distribution, the species may especially suffer by scarcity of water in summer periods due to extended droughts, as well as, the evermore frequent wildfires (Torres *et al.*, 2015). Fires have always been present in the Mediterranean ecosystem, playing their part as catalysts of the renewal of ecosystems (Pausas & Vallejo, 1999), nonetheless over the last decades they became larger and more intense (Silva & Rego, 2007), to ultimately take catastrophic proportions (Noss *et*

al., 2006). Indeed, according to the 2017 Joint Research Centre (JRC) report, Portugal is the European country with the largest annual percentage of burned area. The number and average size of large fires in 2017 was greater than the last decade's average, which resulted in unprecedented levels of damage (San-Miguel-Ayanz *et al.*, 2018). In general, wildfires have a considerable social, economic, and environmental impact, and in Portugal they have caused an ecological disaster which has affected a large part of the county (Moreira & Pe'er, 2018).

As the trend towards larger fires is increasing due to a scenario of intensification of extreme climate events (Rajczak *et al.*, 2013), understanding how wildfires reshape ecosystems and affect individual movements is utterly needed to implement effective mitigation measures. This is further important in light of the relevant efforts which have been performed to reintroduce roe deer across Portugal. Indeed, understanding roe deer movement in a naïve environment, where they can potentially be exposed to system perturbations provided by fires is crucial to optimize reintroduction/restocking projects, e.g. by identifying the best release periods and areas, where the risk of short-term death of the released animals is minimised. In spite of the magnitude of research on roe deer ecology all over Europe, in the Mediterranean area there is remarkably little investigation on the ecology of this species (but see Focardi, 2005; Acevedo *et al.*, 2010), and Portugal is not an exception (Torres *et al.*, 2011; Torres *et al.*, 2015; Valente *et al.*, 2016). As a matter of fact, in this country the investigation of roe deer ecology has only recently taken the first steps (Torres *et al.*, 2012; Valente *et al.*, 2014; Carvalho *et al.*, 2018). The majority of the studies focused on the density estimate and space use analysis of populations ranging in the north-eastern part of Portugal (e.g. Paiva, 2004; Torres *et al.*, 2011; Torres *et al.*, 2012; Valente *et al.*, 2014; Torres *et al.*, 2015; Valente *et al.*, 2016; Carvalho *et al.*, 2018), while only a study has, thus far, been performed on a reintroduced population in Central Portugal (Carvalho, 2008). However, this population was not monitored after release, so the success of the reintroduction project was not evaluated. Moreover, to the best of my knowledge, there is an almost complete lack of investigation on the effects of

fire perturbation on roe deer space use patterns, in Portugal as well as in other countries where the species range.

My research work falls in this context, providing a contribution to fill some of the existing gaps. In particular, I focused on the analysis of roe deer movement patterns in a reintroduced population in Central Portugal, where some individuals have been provided with GPS radio-collars to track their movements, from 2013 to 2017. First, I explored the movement patterns of naïve individuals, to assess the home range emergence (**chapter II**). Then, I investigated roe deer spatio-temporal responses to disturbances caused by the wildfire occurrence in the release area (**chapter III**).

Hypotheses and predictions

Based on the premises above presented, I performed two separate analyses to assess (i) roe deer movement patterns in a naïve environment over time and (ii) roe deer immediate movement responses to a perturbation event such as wildfires.

With respect to the first topic, I addressed it by evaluating the variation of three movement metrics over time, i.e. the weekly distance from the release site, the weekly distance between subsequent relocations, and the weekly home range size, for the first year of monitoring from release event. The weekly temporal scale was selected as a compromise between catching the fast roe deer movement responses on one side, and getting enough data to compute home ranges on the other (see Methods in chapter II for details). With respect to this analysis, my general hypothesis was that, after a first unstable phase of exploration, roe deer would establish a stable home range (home range emergence hypothesis) (Owen-Smith, 2003; Calenge *et al.*, 2005; Bell, 2014). In particular, I expected that:

- Roe deer weekly distance from release site increases in the first period after release as a consequence of a first exploratory phase, to then stabilize over time (**Prediction 1.1**).

- In consequence of the post-release exploratory behaviour, roe deer weekly distance between subsequent relocations tends to decrease over time after release event, and to stabilize (**Prediction 1.2**). However, given the well-known seasonality of roe deer movement patterns that is determined by physiological and behavioural constraints (e.g. food availability or mating opportunities; Gurarie *et al.*, 2017; Peters *et al.*, 2017), I expected to observe a seasonal alternation of peaks and inflections of distances between subsequent relocations, as a consequence of home range-shift (**Prediction 1.3**).
- As a consequence of roe deer tendency to stabilize, the weekly space use area decreases over time, to then stabilise and emerge as proper home range (**Prediction 1.4**).

My second set of analysis focused on the spatio-temporal movement response of roe deer to fire disturbance. In general, the occurrence of a fire generates a perturbation that should affect individual movement, although likely at various extent between individuals (Dalerum *et al.*, 2007). In this context, my general hypothesis was that the presence of fire affects roe deer movements in the immediate phase post-fire event. Specifically, I predicted that both the distance between subsequent weekly home ranges centroids (**Prediction 2.1**) and the weekly home range (**Prediction 2.2**) decrease steadily in the immediate period after the fire event, as a consequence of the individual reaction to the stress induced by fire and of the animal aversion to explore new areas when they are exposed to several risks, there including wolf predation (Lima *et al.*, 1999).

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Chapter II

Roe deer's spatio-temporal response to novel environments

Roe deer's spatio-temporal response to novel environments

Abstract

Even if the European roe deer (*Capreolus capreolus*) is a very well-studied animal, there is a certain lack of information at the southern limit of the species distribution. The ecological factors shaping the roe deer distribution, as well as its ranging behaviour in a Mediterranean habitat, are relatively unknown. Since 2013, four roe deer reintroduction events have taken place in Central Portugal, in the context of a reintroduction project intending to create a pool of wild natural prey for the endangered Iberian wolf (*Canis lupus signatus*) in order to decrease wolf attacks to livestock. Here, I analysed the space use patterns of reintroduced roe deer in the first year after release, making use of the GPS locations derived from the collars fitted to half ($n = 35$) of the released animals. Using Generalized Additive Models, the results showed that animals tended to move away from the release point during the first year (indication of an exploratory phase), although with a bimodal increase constituted by two steps intermixed by a relative stable phase, as individuals are likely responding to other constrictions/physiological responses, as parturition for females, or the occurrence of fires. Roe deer also revealed a rather constant home range size, probably individual movements, at the weekly scale, around their home ranges barycentre remained substantially constant, while the distance between subsequent home ranges decreased in the first months to then increase again, possibly due to the need for food, combined with an increase of movements that follow a decrease of the summer temperatures. These results provide, for the first time, valuable spatial information regarding roe deer space use in Portugal, and among the first for a re-introduced population of the species in an area where it was mostly absent in recent times. The tendency of roe deer to explore a naïve environment to then settle and form a home range is showed. Further research should take into consideration other biotic components, and in particular the effect of the presence of wolf on roe deer movements after release.

Key words: *Capreolus capreolus*; Home-range emergence; Reintroduction; Naïve environments; Central Portugal; Movement patterns.

Introduction

European roe deer (*Capreolus capreolus*) populations are widely distributed and studied in central and northern Europe (Apollonio *et al.*, 2010). Currently, the species distribution range spreads from Mediterranean scrublands of Portugal, on the south-west side of its distributional range, towards the boreal forests of central Norway, on the north-west side of the species distributional range (Apollonio *et al.*, 2010), see Appendix A. In Portugal, in spite of being native, wild populations have persisted only in a few patches on the north of the Douro River, mostly restricted to forested mountain areas (Vingada *et al.*, 2010) (see Appendix A for more details). South of Douro River, existing populations are the result of several reintroduction efforts (Mattioli *et al.*, 1995; Torres *et al.*, 2012), which in the end turned in an expansion of the species over the last decades (Torres *et al.*, 2015), as observed also in other populations across Europe. Despite the population increase, roe deer densities in Portugal have remained low, particularly when compared to central and northern European populations (Valente *et al.*, 2014 & 2016).

The survival of individuals, and the establishment of a viable population after a reintroduction project, can be affected by a variety of factors affecting the intrinsic structure of the population, such as the initial size of the population released (Berger, 1990; Pedrotti *et al.*, 2007; Torres *et al.*, 2016), the age, and the sex composition of the released individuals (Komers & Curman, 2000; Apollonio *et al.*, 2003). Moreover, the habitat where the individuals are released also influences the reintroduction successes, since factors like the connectivity between populations (Festa-Bianchet, 2002), and habitat suitability (Owen-Smith, 2003) are of extreme importance (Torres *et al.*, 2016). Indeed, a release into a naïve environment unavoidably affects individuals ranging behaviour, since the individuals have to get accustomed to the new area (Jones & Witham, 1990; Dolev *et al.*, 2002), and they need to locate the new resources needed for their survival

(Owen-Smith, 2003). However, this explorative behaviour can result in an increased risk of mortality which will reduce the individual's fitness during the critical post-release period (Stamps & Swaisgood, 2007), especially when disturbance events (e.g. wildfires, which typically occur in Portugal) or survival threats (e.g. predators) are present.

In this scenario, studying the spatial ecology of the reintroduced populations of roe deer in Portugal may provide benefits to individuate the factors shaping the roe deer distribution in the mosaic of Mediterranean landscapes that typically characterize Portugal (Torres *et al.*, 2011). Even though some studies have been conducted to investigate the factors influencing fine scale distribution of roe deer populations in North-eastern Portugal (Torres *et al.*, 2011; Carvalho *et al.*, 2017), there is still a consistent gap of knowledge on the spatial behaviour of the reintroduced roe deer populations, with one exception in a central area of Portugal where home range size and altitudinal seasonal variation of five released roe deer have been analysed (Carvalho *et al.*, 2008). Notwithstanding the scarce knowledge about reintroduced populations of roe deer in Portugal, these have indisputable importance for conservation, as well as a great ecological value due to the species occupation of a Mediterranean ecosystem (Carvalho *et al.*, 2008). Furthermore, roe deer constitute one of the main wild prey for the endangered Iberian wolf, *Canis lupus signatus* (Vos, 2000). Focusing on these premises, and with the aim to reducing livestock attacks and human-wolf conflicts (Torres *et al.*, 2015), a roe deer reintroduction project was set in motion (Torres *et al.*, 2012), with the reintroduced animals coming from Spanish source populations (Torres, Brotas & Fonseca, 2018). Along this project a large group of roe deer has been released between 2013 – 2016, in previously selected suitable areas (Cruz *et al.*, 2014; Torres, Brotas & Fonseca, 2018), with particular care of maintaining a balanced sex ratio and age class structure of the reintroduced population. Moreover, half of the released individuals were equipped with GPS (Global Positioning System) collars to facilitate the monitoring of the spatial process of recolonization of the release area.

I took advantage of the availability of the spatial data collected within this project to analyse post-release spatial behaviour of roe deer, aiming at exploring some aspects of the spatial ecology of reintroduced individuals in a naïve environment. Beyond presenting the first data on home range sizes of a considerable roe deer population ($n = 35$), which was monitored for several consecutive years (2013-2017), my major goal was to investigate space use behaviour after release. Usually, reintroduced individuals make extensive exploratory movements in the weeks after release, which end up in a subsequent stabilisation of the space used, to finally emerge as proper home ranges (Owen-Smith, 2003; Calenge *et al.*, 2005; Bell, 2014). Based on these premises, I hypothesized that roe deer movements vary after release, and that space use stabilizes and emerges after a time lag from release. I analysed the reintroduction pattern by measuring movements and space use with three different metrics (weekly distance from release, weekly distance between successive locations; weekly home range size; see Methods for details), and I predicted that:

- Roe deer weekly distance from release site increases in the first period after release as a consequence of a first exploratory phase, to then stabilize over time (**Prediction 1.1**)
- In consequence of the post-release exploratory behaviour, roe deer weekly distance between subsequent relocations tends to decrease over time after release event, and to stabilise (**Prediction 1.2**). However, given the well-known seasonality of roe deer movement patterns that is determined by physiological and behavioural constraints (e.g. food availability or mating opportunities; Gurarie *et al.*, 2017; Peters *et al.*, 2017), I expected to observe a seasonal alternation of peaks and inflections of distances between subsequent relocations, as a consequence of home range-shift (**Prediction 1.3**).
- As a consequence of the roe deer tendency to stabilize, weekly space use area size accordingly decreases over time, to then stabilise and emerge as proper home range (**Prediction 1.4**).

Material and Methods

Study area

The study was carried out in central Portugal, comprising both Arada and Freita hills from 2013 to 2015, and Montemuro hill starting in 2016, all of which belong to the Natura 2000 network, correspondingly PTCON0047 and PTCON0025 (Figure 2.1).

In general, the study area is in a mountainous region, where altitude ranges from 200m to 1.381m, and steep slopes are present. Forest (46%) occupies a great portion of the study site, followed by scrubland (26%), agricultural land (20%), and urban areas (8%). Forests are mainly composed of English oak *Quercus robur*, Pyrenean oak *Quercus pyrenaica*, Sweet chestnut *Castanea sativa*, Maritime pine *Pinus pinaster* that can be found in pure stands or mixed with Eucalyptus *Eucalyptus globulus*. Scrubland consists mostly of Scotch broom *Cytisus scoparius*, *Cytisus grandifloras*, various types of Gorse *Ulex* spp., a variety of Heather species *Erica* spp., *Pterospartum tridentatum*, and *Genista triacanthos*. Within the study area the human population still largely subsists on agriculture and/or pastoralism. Thus, agricultural fields and scattered pastures are easily found, and herds of cattle normally roam free around the area, escorted by a shepherd and/or shepherd-dogs. Human population is dispersed through the various valleys, in small villages with a population density of about 43 inhabitants/km².

Arada/Freita has a total area of 28.659ha, and it is characterized by extremely steep slopes, with a minimum altitude of 200m and a maximum of 1.100m. As it is precisely located in the transition area between the Atlantic and Mediterranean biogeographic regions, this part of the study area is influenced by the oceanic climate, which results in high levels of precipitation. Concerning land occupation, Arada/Freita is covered by forest (66%), scrubland (13%), agricultural area (11%) and urban area (10%).

Montemuro has a total area of 38.763ha, mostly included in a mountain massif, with a minimum altitude of 1.200m and a maximum of 1.381m. As this part of the study area is fully enclosed in the Mediterranean biogeographic region, the climate is characterised by hot, dry summers, and humid, cool winters. Concerning land occupation, Montemuro is covered by forest (26%), scrubland (39%), agricultural area (28%) and urban area (7%).

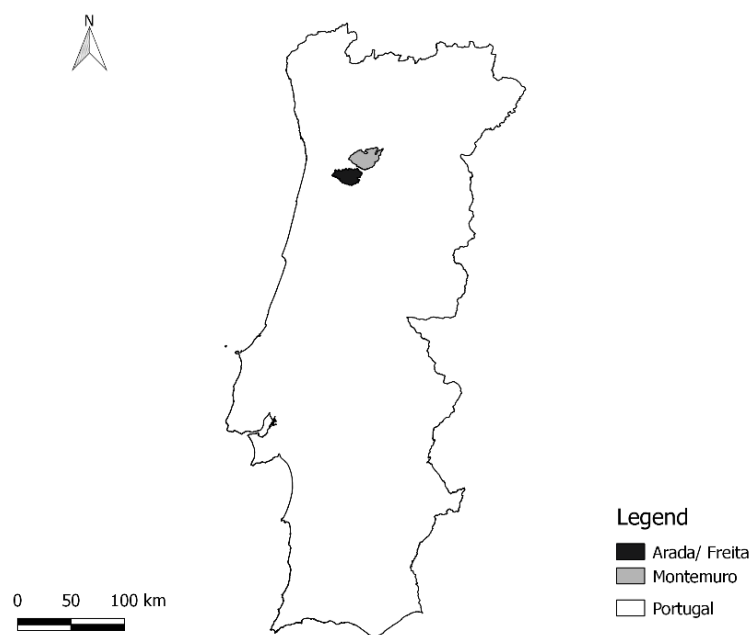


Figure 2.1 – Location of the study areas in continental Portugal.

Data collection and database management

The GPS data used for this analysis were collected from 18 males, and 17 females of roe deer, always released in November, monitored between the years of 2013 and 2017 (Table 2.1) and equipped with GPS collar (Vectronics, GPS PLUS-1 model), for a total of 82.779 GPS location with periodicity of three hours (see Appendix B). The battery lifetime of the collars spans two years, after which

the collar drops off, unless there has been a mortality event prior to the battery expiration.

Table 2.1 – Contingency of the reintroduced roe deer individuals, divided by year of reintroduction and sex.

Year	Male	Female
I (2013)	3	3
II (2014)	6	6
III (2015)	6	5
IV (2016)	3	3

Each collar was therefore associated to one animal for the time of deployment on that individual. Ancillary information on the individual (sex, age at capture) were collected to be then used for the analyses.

At the end of the monitoring of a given individual, the collar was rescued and refurbished for further deployments on other individuals released in the next reintroduction event. For this reason, the ID of a collar does not match exclusively with a given individual (e.g. the collar 13907 could be associated with an individual in the year 2013, and with another individual in the year 2015). Thus, in order to create a unique identification criterion for a given animal, an animal*year code has been created, comprising the information on the reintroduction year and the ID of the collar with which that animal had been equipped. Specifically, the code comprised the last two numbers of the GPS collar ID and the year of release, so that for instance the animal equipped with the collar 13907 in 2013 was coded as animal ID 07*2013.

The GPS dataset, and all the ancillary information regarding the animals, were added to the EUROUNGULATES PostgreSQL database (www.euroungulates.org), a large spatial data repository which is the fruitful outcome of the Euroungulates collaborative initiative carrying on large-scale

analysis on roe deer, red deer and wild boar space use patterns across Europe. The maintenance of the data in this repository facilitates the management of the data prior to the analysis stage.

The raw data has been used to compute the spatial metrics that have been used to investigate roe deer post-release spatial behaviour. In particular, weekly areas of space use, and their centroids, were computed with the Minimum Convex Polygon at 90%. The three metrics to evaluate space use by reintroduced animals were computed as follows, for each animal*year separately:

- Distance from release point of all successive weekly centroids;
- Distance between successive centroids;
- Home range size using the Kernel Density Estimator (90% isopleth), with href as the smoothing parameter (Worton, 1989).

To these weekly data, it was also associated the age class (fawn, subadult, adult), and sex of the individuals, as well as additional control information concerning each animal's exposure to fire during its monitoring period (a dummy variable 0/1 at the individual level, with 1 denoting an individual which was exposed to fire during its monitoring, and 0 denoting individuals not experiencing fires during their monitoring). This latter information was retrieved from a spatiotemporal association between the animal monitoring and the fires occurrence (see Chapter 3 for details).

The animal*year whose monitoring period was shorter than six months were not included in the analysis (marked with a diamond (◇) in Appendix A), because they could not provide valuable information on the yearly post release spatial behaviour, which was the aim of this investigation. Additionally, animal 50*2014 was also excluded, as based on visual inspection of the data it resulted clear that its movements would have strongly affected the average output, standing as outliers. For the remaining animal*year combinations, there was a further removal of almost 200 weekly records due to the lack of enough data in some weeks that did not allow to correctly compute the spatial metrics. Ultimately, for each

animal*year also the weekly records beyond the end of the first year from the beginning of the monitoring (i.e. after 365 days from release day) were excluded from the analysis. As such, the dataset for the analysis eventually contained 1042 weekly records (and their associate spatial metrics) from 19 animal*year combinations.

Statistical Analyses

The statistical analysis of the post-release space use patterns as measured by the three metrics was divided in two steps. First, a pairwise comparison between the metrics distribution of the early (1st – 2nd month), intermediate (5th – 6th months) and late period (11th – 12th months) from release was carried out. To do this, the distribution of the three metrics was firstly checked for normality, by means of visual inspection and Shapiro normality test. Since none of the three metrics followed a normal distribution, a pairwise comparison for non-normal distributions was performed, by applying the non-parametric Wilcoxon test.

For the second stage of analysis, only those metrics whose comparative analysis had shown a significant difference between early and intermediate periods, as well as between early and late periods, were taken in account. These metrics were modelled to evaluate the continuous temporal pattern of variation along the first year since release. Only the metric 'Distance from release' evidenced significant differences between early, intermediate and late period since release (see section Results for details), and as such was considered for this analysis. First, a visual exploration of the response variable was performed, which evidenced a clear non-normal distribution of the data (Figure 2.2). The best distribution family was identified as the Gamma (Figure 2.3), and as such fitted to inform the model on the metric distribution.

Histogram of Distance from release

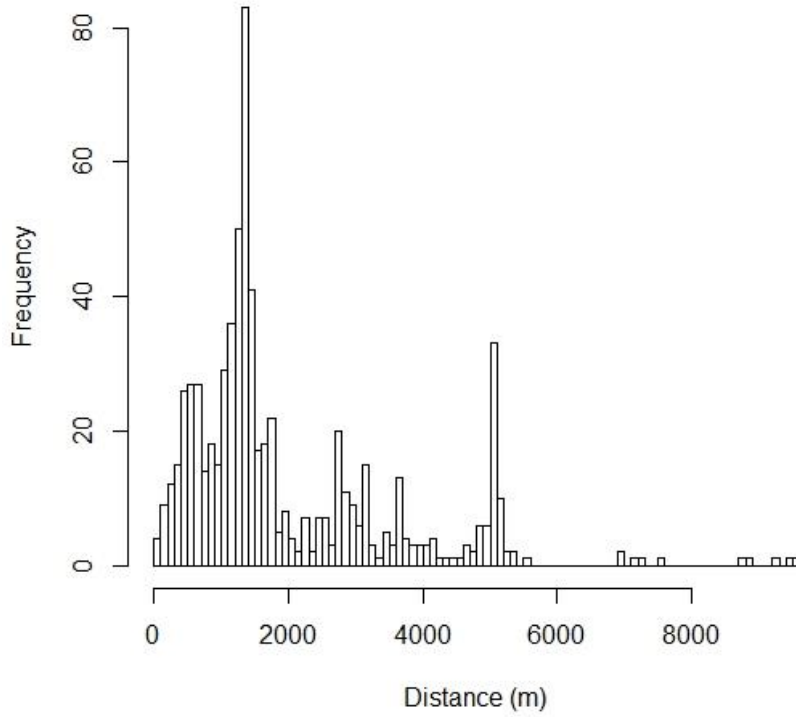


Figure 2.2 – Histogram of the Distance from release distribution.

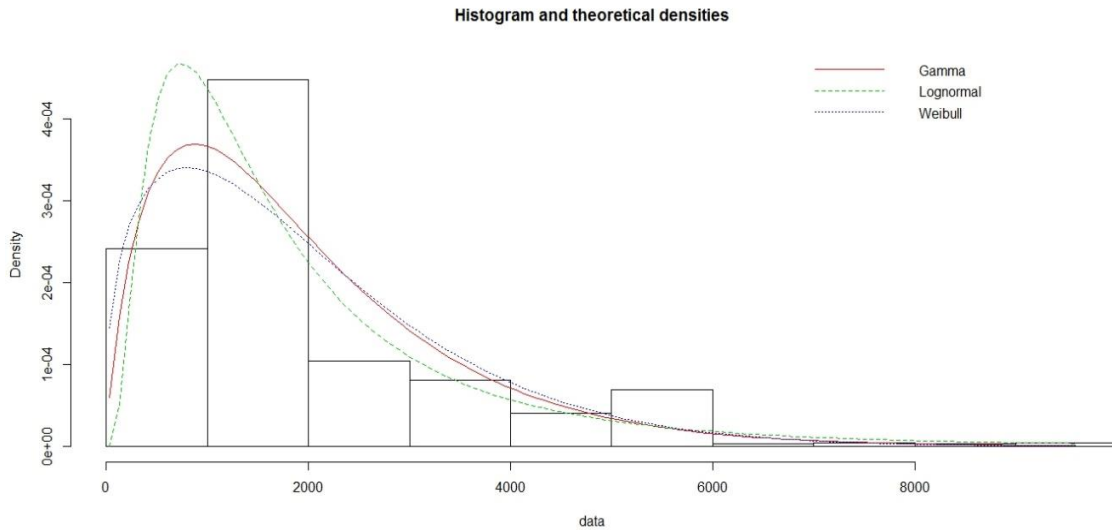


Figure 2.3 – Histogram of the Distance from release distribution, with the fit of the three best theoretical distribution families.

Specifically, the Distance from release was fitted either within the framework of Generalized Linear Models with Mixed effect (GLMMs) or, alternatively, within the framework of Generalized Additive Models with Mixed effect (GAMMs). In both the models, the mixed effect allows to account for the presence of a random variable, in this case the animal*year ID, e.g., the individual auto-correlation of spatial data. While GAMMs allow to take in account temporal non-linear patterns by fitting splines of any covariate with an expected temporal component, this is not the case for GLMMs, where the same covariates are fitted as fixed linear effects. Both the full models had therefore a similar structure:

- GLMM: $DR \sim \text{Day} + \text{EF} + \text{Sex} + \text{Age} + \text{RE}(\text{animal*year})$
- GAMM: $DR \sim s(\text{Day}) + \text{EF} + \text{Sex} + \text{Age} + \text{RE}(\text{animal*year})$

Where:

- DR = Distance from Release site, i.e. the response variable
- Day = Day from Release, ranging from 0 to 365
- EF = Exposure to Fire, dummy covariate
- Sex = sex of the individual, dummy covariate
- Age = age class of the individual (categorical covariate, three levels)
- RE = random effect of the animal*year

These two full models were compared based on Akaike Informative Criterion (AIC) scores (Burnham & Anderson, 2002). Then, the best of the two models was further screened and simplified by means of several ANOVA based on deviance procedure (Statski *et al.*, 1993), based on the principle of parsimony. The simplified best model was eventually used to determine a plot of the predicted variation of Distance from release site in function of time from release.

Results

The non-parametric comparison between early and intermediate periods from release showed a significant decrease for the Distance from previous centroid ($p = 0.03$) and a significant increase for the Distance from release site ($p = 0.001$), while no significant difference emerged for the home range size (Table

2.3). As for the comparison between early and late periods, a significant increase for the Distance from release site was found ($p = 0.0008$), while there were no significant differences for the other two metrics analysed (Table 2.2).

Table 2.2 – Summary statistics for the pairwise comparisons between early, intermediate and late periods from reintroduction event, for the three metrics analysed. Significant p values are denoted with stars ($p < 0.001 = ***$; $0.001 < p < 0.01 = **$; $0.01 < p < 0.05 = *$).

	Dist. from Previous	Dist. from Release	HR Size
Early vs Intermed.	0.03*	0.001*	> 0.05
Early vs Late	> 0.05	0.0008*	> 0.05

The best model accounting for the temporal variation of the Distance from release in function of time was Generalized Additive Model where the random effect of the animal*year and the spline of the day from release were retained as significant covariates, while none of the other fitted covariates was retained (Table 2.3).

Table 2.3 – Summary of the best model accounting for the distance from release. Significant p values are denoted with stars ($p < 0.001 = ***$; $0.001 < p < 0.01 = **$; $0.01 < p < 0.05 = *$).

Distance_From_Release ~ s(Time_In_Day_Round) + s(Animal_Id, bs = "re")				
Parametric coefficients:				
	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	0.0008172	0.0003376	2.42	0.0158 *
Approximate significance of smooth terms:				
	edf	Ref.df	F	p-value
s(Time_day_round)	6.703	7.818	7.178	6.64e-09 ***
s(Animal_Id)	16.921	17.000	42.531	< 2e-16 ***
R-sq.(adj) = 0.611		Deviance explained = 63.6%		
GCV = 0.24968	Scale est. = 0.22332			n = 639

The best model, which had a quite high R^2 value (0.611), thus confirmed a strong temporal pattern in the variation of the Distance from release over time, as well as, a significant inter-individual difference, accounted for by the random effect of the animal*year. The predicted plot (Figure 2.4) confirmed the observed trend, with a sharp increase of the distance from release site in the first months after release, followed by a tendency to the stabilization.

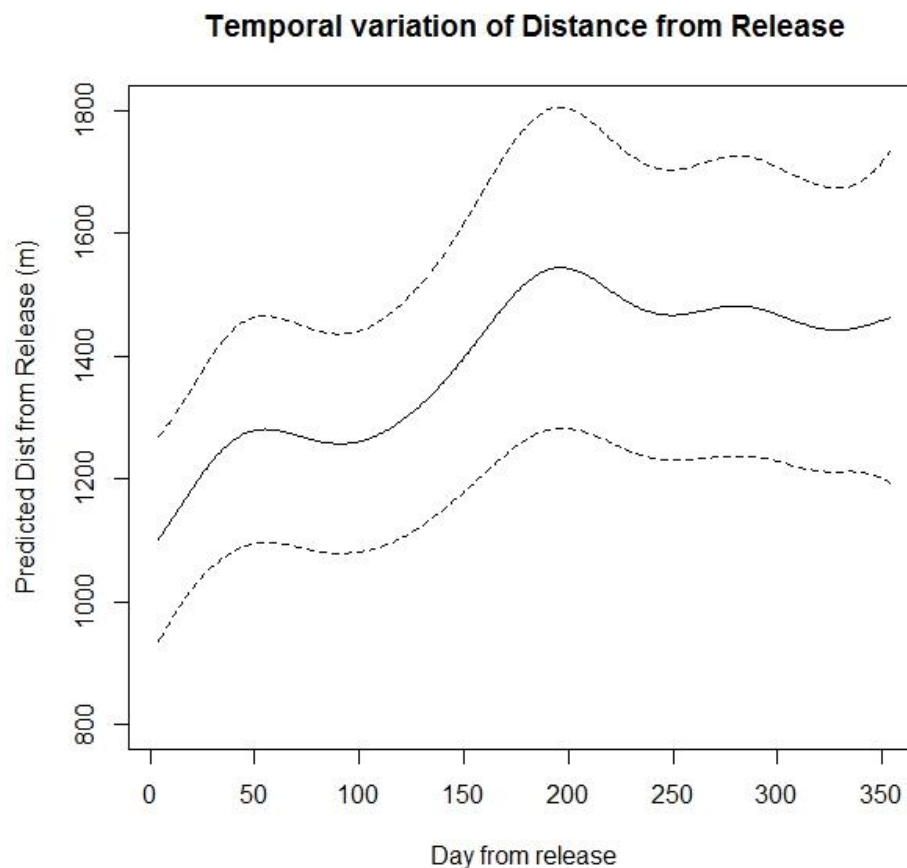


Figure 2.4 – Predictive plot of the variation of the distance from release in function of the day of the year.

Discussion

To the best of my knowledge, this work represents the first spatial analysis on a reintroduced population of roe deer in Portugal, and a rare example in

general of post-release of the species in a naïve environment with no or few conspecifics. Overall, the presented results fit with the general ecological knowledge on behaviours expected in reintroduced individuals in a naïve environment, although some of my predictions were not, or only partially, respected.

Whenever a reintroduction event takes place, the reintroduced animals undergo a unique process in which they are transported from their natal habitat, and placed in an entirely unfamiliar landscape (Bell, 2014). When released in these novel environments, they need to gain information about their surroundings (Frair *et al.*, 2007), while paying attention to the costs deriving from this exploration phase (e.g. increased predation risk and enhanced allocation of energy in long displacements). Thus, animals face a trade-off between their need to learn about the new environment, and the necessity to minimize risks associated with this exploration (Eliassen *et al.*, 2007; Berger-Tal *et al.*, 2014). This trade-off is likely dependent upon an individual phenotypic condition, and as such it varies within a pool of individuals. In accordance with this general theoretical framework, and in compliance with my first prediction (**P1.1**), the distance from release site increased over months, hence showing the tendency to perform exploratory movements aimed at constructing a spatial representation of the new environment in order to maximize their survival probability, e.g. by identifying best resources patches, or hiding sites to escape from the existing predator, the Iberian wolf predators (Burns, 2005; Russel *et al.*, 2010; Berger-Tal & Avgar, 2012). Remarkably, not all individuals behaved exactly the same way, probably as a consequence of differential age at release and phenotypic condition, as caught by the highly significant random effect fitted in the model. Interestingly, the model predicting the temporal variation of the Distance from release showed a rising trend in the first months from release, followed by an apparent smoothing period, which ends with a new increase in distance to the release site. The two peaks of these 'steps' seem to correspond with January and May. Possibly, after 2-3 months after release (i.e. in January), the first exploratory phase is over, and the animals tend to settle for a while. However, in May, when the second peak is observed, individuals are likely responding to other constrictions/physiological responses,

such as those of territorial males which establish territory in that period. Certainly, if this was the case, we should have observed a relevant effect of sex in explaining the pattern, which was not the case. Though, we cannot exclude that other elements contributed to delineate the observed pattern, such as parturition for females, or the occurrence of fires (see chapter 3 for details). Interestingly, the reintroduced group of animals exhibits a smaller dispersion scale than those of other areas of Europe (e.g., Aspromonte; Cagnacci F., pers. comm.). Possibly, this might be due to the closeness of the reintroduction sites to roads, which even if back roads, could still represent barriers preventing animals from expand their movements, eventually reducing their dispersing capability. The presence of small villages at the base of the hills, regardless of being shown not to constrain roe deer movements elsewhere (Torres *et al.*,2011), may be another constraining factor. This is certainly an issue to be taken in account, because the presence of barriers may importantly alter the success of the reintroduction operations.

In partial accordance with my second prediction (**P1.2**), the intrinsic movements of animals, measured here as distance from previous centroid, exhibited a decrease from the early to the intermediate period, while no pattern emerged when comparing the first two months after release and the 11th – 12th months. The lack of a signal of decrease in this latter case is relatively surprising, although it may be explained by the insurgence of ecological drivers which lead to an increase of the movements in the late autumn period (the animals were all released in November, and as such the months 11th – 12th since release refer to the autumn). Arguably, autumn is the season when individuals can recover after the most expensive energetic phase of their biological cycle, i.e. the rut. Males are no longer territorial, and need to gain the energy lost in territorial defence and mating attempts, while females are getting out of the most expensive phase of parental care, as well as of the rutting period. Possibly, the need for food, which usually lacks in the core of the summer in arid Mediterranean ecosystems, in combination with an increase of movements that follow a decrease of the hot summer temperatures, lead to an overall increment of the Distance between subsequent home ranges, with individuals tracking or shifting resources (Gurarie *et al.*, 2017; Peters *et al.*, 2017). This may explain the lack of signal from months

1st – 2nd after release, and months 11th – 12th, which is the outcome of two very different biological processes, in partial compliance with my prediction **P1.3**. Alternatively, it is important to consider that Wilcoxon test measures differences in the median values of ranked distributions, and therefore it is possible that when a pattern is not evident enough, this is not portrayed accurately by the test.

As for the home range size variation, the lack of significance of differences over time was quite surprising, and contrary to my prediction **P1.4** that weekly space use area size decreases over time, to then stabilise and emerge as proper home range. Indeed, because of decreasing exploratory movements, home ranges should also decrease (Michallet & Toïgo, 2000; Dolev *et al.*, 2002). Nonetheless, in our case, the weekly area used proved to be relatively constant. Possibly this is due to the fact that distance from release, and distance between subsequent home ranges, measure intrinsic properties of individual movement, while home range size is more related to spatial patterns of use. In other words, it is possible that individuals moved more, or less, at the weekly scale from one site to another, but that their weekly movements around their home ranges barycentre remained substantially constant.

This work provides some first indications on roe deer space use patterns and movements in a released area in Portugal. Which is particularly relevant since the behaviour of this species at the southern end of its distribution is poorly known, i.e. where the climatic conditions contrast with respect to northern or continental climates, with milder winters and dryer and hotter summers. Clearly, several other aspects should be taken in account for a more comprehensive analysis, such as e.g. the presence of the wolf in the area. The inclusion of this and other biotic and abiotic covariates in the analysis goes beyond the scope of the present work, but definitely represents a crucial step to better understand the drivers of roe deer movements in Portugal – with the final aim to favour their persistence and management, thus valorising the reintroduction efforts.

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Chapter III

Light my fire! A story of roe deer, fire, and escapes

Light my fire! A story of roe deer, fire, and escapes

Abstract

In Portugal, wildfires occurrence and destructive impact are increasing rapidly, in consequence of climate change. Among other impacts, a better understanding of wildfires influence on wildlife populations is necessary. This is particularly relevant in roe deer (*Capreolus capreolus*), where some populations have been recently reintroduced, in order to understand the reaction of individuals to this disturbance and optimize reintroduction efforts in future projects. Relying on the GPS data of 12 radio-tracked roe deer which have been recently released in Central Portugal and which have been exposed to fire occurrence during their monitoring, I examined individual immediate response to fire occurrence. I did this by using Generalized Linear Mixed Models to predict the impact of the occurrence of a fire on individuals' movements and space use patterns, measured respectively as distance between subsequent weekly home ranges and home ranges size. I found that roe deer rapidly respond to the occurrence of fire events, by decreasing their movements and by shrinking their home range size. Though resilient to human disturbances, roe deer thus demonstrate that they quickly respond to fire events, minimizing their movements probably as a strategy to minimize the exposure in unknown areas, or because small unburned patches remained in the closeness of their previous burned home ranges. Although preliminary, these results indicate the need to investigate the spatio-temporal response of roe deer to fire occurrence, also to better direct reintroduction efforts in areas which are subject to fire occurrence.

Key-words: Roe-deer; Portugal; Wildfires; Movement Ecology; Home-range disturbance.

Introduction

Climate change is recognized as a global problem with vast local repercussions, amongst which the increase of wildfires occurrence (Parmesan & Yohe, 2003), with large impacts especially on Mediterranean countries, such as Portugal. Indeed, this country is the one facing the largest annual percentage of burned area, in consequence of increasing number of fires and expanding average size, which resulted in unprecedented levels of damage (San-Miguel-Ayanz *et al.*, 2018).

From an ecological perspective, wildfires are an important element of the Mediterranean ecosystem because of their role as catalysts of the vegetation community renewal (Pausas & Vallejo, 1999; Noss *et al.*, 2006). Bond and Keeley (2005) considered wildfires a recurrent disturbance that applies pervasive effects on many terrestrial systems' structure and function. Certainly, one of these effects is the alteration of climax vegetation, where nutritious forage for several species such as large herbivores is provided by easing the establishment of early seral stages (Fryxell, 1991). Energy and protein availability for herbivores after a fire often increase, peaking after some years from fire, and then slowly declining as succession progresses (Long *et al.*, 2008). The alteration of the nutritional landscape, which typically comes in the form of a mosaic where some areas have just been burned, while others have been untouched by fire for some years, turns in a modification of animal's distribution in space and time, affecting several levels of the trophic chain (Pulliam, 2000), up to the apex predators such as wolf. Despite the post-fire nutritional benefits, there are also reasons why animals may avoid recently burned areas, such as energetic expenditure and predation risk (Lima *et al.*, 1999).

The increase of wildfires vastness and intensity over the last decades (Silva & Rego, 2007) reduced their important natural role in the ecosystems (Pereira *et al.*, 2006), leading to the occurrence of wildfires of catastrophic proportions (Gomes, 2006). The occurrence of extended wildfires brings several threats to the maintenance of local ecosystems. First, it promotes the spread of invasive

species, which in turn enhance the spread of wildfires (Brooks *et al.*, 2004). A good example of this is the expansion of eucalyptus on Portuguese forest areas (Marchante *et al.*, 2010; Silva, 2016). The occurrence of wildfires and the abandonment of forest land after a fire incident lead to the development of eucalyptus areas, which have been reshaping the Portuguese forest landscapes over the last decades (Silva, 2016). The combination of increasing forest fires and eucalyptus resilience will likely continue to affect and alter the Portuguese forest ecosystem (Silva, 2016). Second, the occurrence of vast wildfires can seriously threaten wildlife, affecting individual movement both directly and indirectly, at different spatiotemporal scales. Where, initially, fires can cause an immediate displacement of animals towards not burned areas, with potential consequences on individual survival due to stress increase and individual exposition in unknown areas. And, at a longer temporal scale, fires can cause a temporary abandonment or avoidance of burned areas as consequence of lack of natural resources which are of vital importance for species (see e.g. Dalerum *et al.*, 2007), such as food resources and cover, thus eventually affecting population distribution and locally altering ecosystemic trophic chains.

As a consequence of the increment of the impact of fires, a new discipline, namely fire ecology, developed over the past century (Pechony *et al.*, 2010), especially in North America where large fires occur more frequently than in Europe. As such, numerous papers have described relations between ungulates, and their habitats in North America after large fires (e.g. Vogl & Beck, 1970; Peek, 1974; Klinger *et al.*, 1989; Pearson *et al.*, 1995; Cherry *et al.*, 2018; Spitz *et al.*, 2018), while so far, in Europe, multi-scale responses of herbivores to burned areas have rarely been documented (but see Borkowski, 2004), leaving a critical gap in our ability to anticipate the strength, direction, and duration of the effect of fire history (i.e., post-fire successional changes on forage availability and vertical structure) on animal distributions.

Amongst the species that in Mediterranean ecosystems are more exposed to the occurrence and to the effects of wildfires, there is the European roe deer (*Capreolus capreolus*), given the species wide distribution range (see Appendix A

for more details). Roe deer can be considered as a model species to evaluate the impact of wildfires on animals. Indeed, taking advantage of its high ecological plasticity (Linnell *et al.*, 1998), it is likely that roe deer are capable to efficiently track post-fire regeneration taking advantage of new available food items on which they selectively browse. However, both the immediate and the long-term response of roe deer to wildfires occurrence have received so far little attention, probably because fire ecology has not been investigated in this species whose majority of spatial range covers areas which – so far – have not been particularly affected by wildfires. In a comparison study between red deer and roe deer habitat use following the occurrence of a large fire in Poland, roe deer was found to be less dependent from cover than red deer, which entailed an earlier use of the burned area by roe deer (Borkowski, 2004), suggesting a higher plasticity of this small ungulate and recolonization capability. However, these results cannot blindly be applied to roe deer populations living in completely different ecosystems as the Mediterranean one in Portugal.

Starting from these premises, in this chapter I provide a contribution to understand the immediate movement response of a pool of individuals in a Mediterranean ecosystem. To do this, I analysed the GPS movement data collected between 2013 and 2017 in a group of reintroduced roe deer in Central Portugal, where a reintroduction project has been set up to create a pool of wielding natural prey for the wolf, as a management measure to decrease predation on livestock (Torres *et al.*, 2014). Specifically, I compared movement and space use metrics pre and post fire events of individuals which have been exposed to the occurrence of a fire along their monitoring. Relying on the principle that fire events should affect animal spatial behaviour, I hypothesized that roe deer movements and space use patterns, measured respectively by the distance between subsequent weekly home ranges and by the weekly home range size (see Methods for details), change in the immediate period (two months) after the occurrence of a fire to which they have been exposed. In particular, I predicted that the distance between subsequent weekly home range centroids and the home range size will decrease after the fire event, in an attempt to minimize movements

in a perturbed area and to reduce explorative movements in a naïve area out of their known home range.

Material and Methods

Study area

The study was carried out in central Portugal, in two different study sites, the first comprising both Arada and Freita hills, from 2013 to 2015, and the second one, in the Montemuro hill, in 2016. Both study areas are part of the Natura 2000 network, PTCO0047 and PTCO0025 respectively (Figure 3.1).

The study area is encompassed in a mountainous region, where altitude ranges from 200m to 1.381m, and steep slopes are present. Forest (46%) occupies a great portion of the study site, followed by scrubland (26%), agricultural land (20%), and urban areas (8%). Forests are mainly composed of English oak *Quercus robur*, Pyrenean oak *Quercus pyrenaica*, Sweet chestnut *Castanea sativa*, Maritime pine *Pinus pinaster*, that can be found in pure stands or mixed with Eucalyptus *Eucalyptus globulus*. Scrubland consists mostly of Scotch broom *Cytisus scoparius*, *Cytisus grandifloras*, various types of Gorse *Ulex* spp., a variety of Heather species *Erica* spp., *Pterospartum tridentatum*, and *Genista triacanthos*. Within the study area the human population still largely subsists on agriculture and/or pastoralism. Thus, agricultural fields and scattered pastures are easily found, and herds of cattle normally roam free around the area, escorted by a shepherd and/or shepherd-dogs. Human population is dispersed through the various valleys, in small villages with a population density of about 43 inhabitants/km².

Arada/Freita has a total area of 28 659ha, a minimum altitude of 200m and a maximum of 1100m, nonetheless its average altitude is 500m. This area is featured by steep slopes. As it is precisely located in the transition area between the Atlantic and Mediterranean biogeographic regions, this part of the study area is influenced by the oceanic climate, which results in high levels of precipitation.

Concerning land occupation, Arada/Freita is covered by forest (66%), scrubland (13%), agricultural area (11%) and urban area (10%).

Montemuro has a total area of 38 763ha, with most of this area in a massif, with a minimum altitude of 1200m and a maximum of 1381m, containing extremely steep slopes. As this part of the study area is fully enclosed in the Mediterranean biogeographic region, the climate here is characterised by hot, dry summers, and humid, cool winters. Concerning land occupation, Montemuro is basically covered by scrubland (39%), forest (26%), agricultural area (28%), with a small urban area (7%).

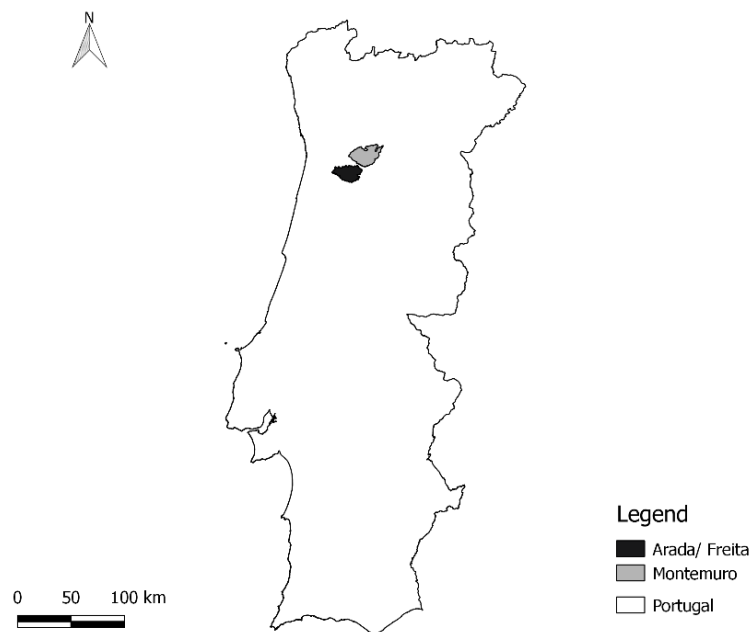


Figure 3.1 – Location of the study areas in continental Portugal.

Data collection and database management

The GPS data used for this analysis were collected from 6 males and 6 females of roe deer monitored between the years of 2013 and 2017 (Table 3.1) and equipped with GPS collars (Vectronics, GPS PLUS-1 model). These individuals represent a subsample of the 35 monitored individuals along the reintroduction project in Central Portugal, selected because they are the animals which have been exposed to the fire along their monitoring (see below for details).

Since at the end of the monitoring of a given individual the collar was rescued and refurbished for a further deployment on another individual, the ID of the collar does not match with a given individual exclusively. For this reason, a unique identification criterion was established for each animal, rising from the combination between the year of release and the collar ID (animal*year code). For example, the animal code 07*2014 refers to the animal 07 released in 2014, where 07 represent the last two digits of the collar ID (in this case 13907). Each animal*year was associated with information on the individual (sex, age at capture).

Table 3.1 – Contingency table of males and females exposed to fires during their monitoring, for each year separately

<i>Year</i>	<i>Male</i>	<i>Female</i>
II (2014)	1	3
III (2015)	5	3

The collection of the information on the fires included several steps. First, it was necessary to get information regarding the fires that took place in the different years of the study. This information came in shapefile format from the Portuguese government (ICNF - Instituto da Conservação da Natureza e das Florestas). Though these files were precise in their spatial component (accurate information on fire location, and its limitation), the same did not happen with the temporal component (meaning that, for most of the fire polygons there was no information regarding beginning and/or end date and/or time). Given the obvious constraints

that the lack of temporal information meant for the analysis, and the study itself, a new source of information had to be found. In order to fill this gap, NASA (MODIS C6, Moderate Resolution Imaging Spectroradiometer, and VIIRS, Visible Infrared Imaging Radiometer Suite) satellites fire data were additionally used. In order to match the time window of the study, I used the NASA data comprised between the 1st of January of 2013 and the 31st of December of 2017. Since NASA dataset was gathered from three different satellites (Table 3.2), with different passes time, some temporal mismatching of the fire periods as recorded by the three satellites data occurred. It was decided to account for this by considering different fire dates as contemporary (i.e. related to the same fire) when the interval between the dates was less than nine days. Furthermore, only NASA's fire data overlapping with ICNF's spatial polygons were considered valid and maintained for the study. Overall, 543 fire polygons from 2013 to 2017 were available for the analysis. Appendixes C to G sum up all the information, per year, gathered from the three sources for all the fires within the study area.

Table 3.2 – Informative table on the NASA satellites

Satellite	Data acquisition/ Passages over Equator	Spatial resolution
MODIS Terra	Passes from north to south across the equator in the morning	1000m
MODIS Aqua	Passes south to north over the equator in the afternoon	1000m
VIIRS	Crosses the equator twice, every day, at approximately 13:30 PM (ascending node) and 1:30 AM (descending node)	375m

Prior to modelling, several database management operations had to be undertaken. First, there was the need to associate the individuals with the fires, both in space and time. As for the spatial association, for each animal*year only the fires whose border was closer than 300 meters to at least one GPS location of that given animal*year were considered. Then, animals*year and wildfires were associated in time, i.e. only the combination animal*year - wildfire which temporally overlapped were considered. This way, it was possible to evaluate, for

each combination individual-wildfire, the response of that animal to the fire event. The application of this double filter led to the identification of 15 associations animal*year*overlapping fire, with two animals*year associated with more than one overlapping fire (44*2014 associated with two fires; and 52*2015 associated with three fires). Beyond this general procedure, it was also necessary to remove an outlier animal (50_2014) and its associated fires, because of the extreme large range covered, that would drive all further analysis in a relatively small sample. For the retained combinations animal*year, weekly areas of space use were computed using the Minimum Convex Polygon at 90%. Then, the distance between successive centroids of weekly MCP home ranges was computed. In parallel, the home range size was computed with the Kernel Density Estimate method (KDE) at 90% (Worton, 1989), with href as the smoothing parameter. Eventually, the Delta time of each weekly record from the associated fire event (ranging from '-x' to '+x', where 0 corresponds to the day of the fire) was computed; the data were then filtered to retain only the range between - 60 days and + 60 days from fire event to catch the immediate response of an animal to the fire event. The choice of this temporal window for this specific analysis was taken to avoid any misinterpretation of the patterns caused by long-scale movement patterns due to other biological constraints of the species, for example seasonal or physiological responses. Lastly, based on the Delta time, another temporal metric was computed, i.e. the preceding (pre)/posterior (post) fire period, in order to contrast the spatial behaviour pre or post the occurrence of a given fire. Overall the dataset was made of 204 data (each of which referring to a temporal window of a week, for which home range and distance from the centroid of the previous week have been computed), 109 pre-fire records, and 95 post-fire records.

Statistical analyses

First, the distribution of the response variable was visually explored, highlighting a non-normal distribution of the data, which were fitted in the model with a Gamma family distribution of residuals. For both metrics, the modelling procedure was analogous. Specifically, the pattern was modelled within the context of Generalized Linear Models with Mixed effect (GLMMs). First, it was evaluated the importance of the random effect of the animal*year to explain the variance of the fitted data, by comparing two analogous models, with or without the random effect (AIC scores, Burnham & Anderson, 2002):

- GLMM: $\text{Metric} \sim \text{PP} + \text{Sex} + \text{RE}(\text{animal}*\text{year})$
- GLM: $\text{Metric} \sim \text{PP} + \text{Sex}$

Where:

- Metric = Distance between subsequent home ranges or Home Range size, i.e. the response variables
- PP = Pre/Post fire occurrence, dummy covariate
- Sex = sex of the individual, dummy covariate
- RE = random effect of the animal*year

Then, the best of the two models was further screened and simplified by means of several ANOVA based on deviance procedures (Skalski *et al.*, 1993), based on the principle of parsimony. The simplified best model was eventually used to determine a barplot of the predicted variation of the metric in function of the covariate Pre/Post fire events.

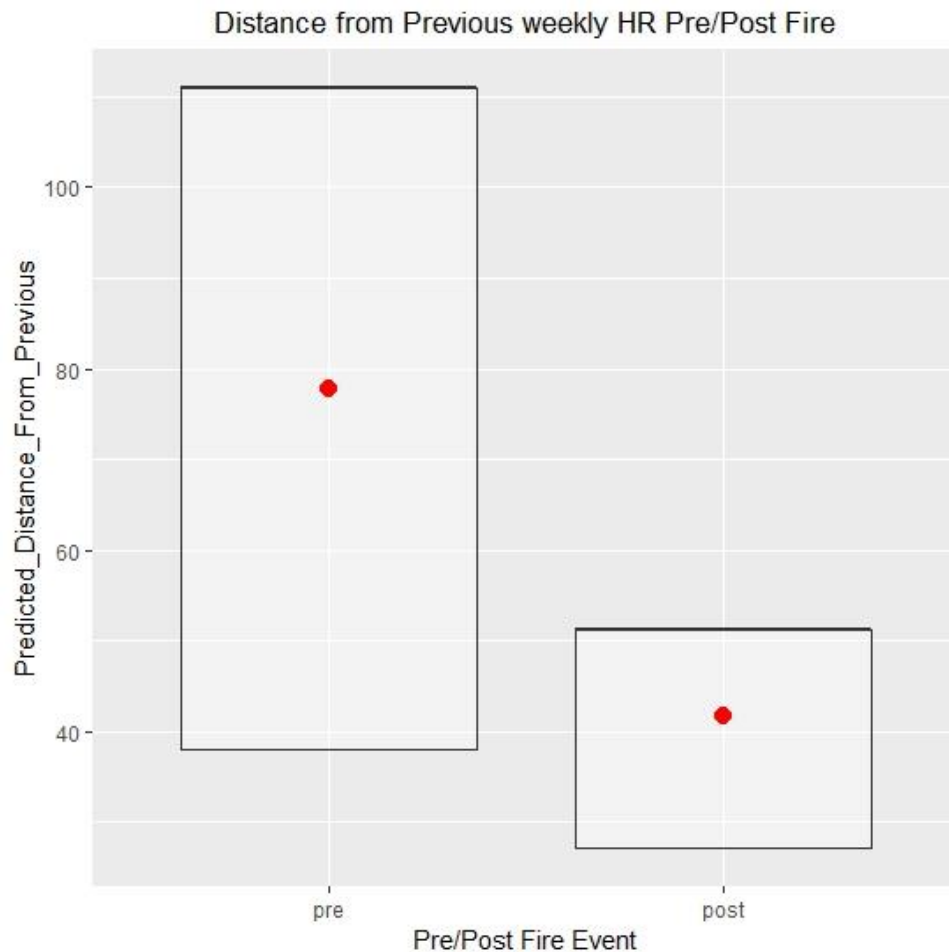
Results

The analysis of the distance between subsequent home ranges in function of the occurrence of the fire showed, as expected, a significant decrease of the metric after the fire event with respect to the pre-fire period (Table 3.2 and Figure 3.2). The pattern was more evident in males than females, and rather homogeneous among the individuals, since the random effect was not retained in the best model. It is important to note that the link function of the gamma distribution is the reciprocal. As such, getting negative beta coefficients on the Gamma distribution scale (Table 3.3) means that the 'actual' beta coefficient is positive with respect to the reference category (in this case, reference categories are, respectively, 'post fire event' and 'sex = female').

Table 3.3 – Summary of the best model accounting for the distance from previous home range. Significant p values are denoted with stars ($p < 0.001 = ***$; $0.001 < p < 0.01 = **$; $0.01 < p < 0.05 = *$).

Distance_From_Prev ~ Fire_Active + Sex				
# Deviance Residuals:				
Min.	1Q	Median	3Q.	Max.
-6.1729	-2.2070	-0.7945	0.3581	3.0076
#Coefficients:				
	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	0.036916	0.005016	7.360	4.61e-12 ***
Fire_Activepre	-0.010550	0.003567	-2.958	0.003469 **
Sexm	-0.017358	0.004545	-3.819	0.000178 ***
Null deviance: 1203.2 on 203 degrees of freedom				
Residual deviance: 1146.4 on 201 degrees of freedom				
AIC: 1557.1	Explained deviance: $1 - (1146.4/1203.2) = 4,7\%$			

Figure 3.2 – Predictive barplot of the distance from previous weekly home range in function of the occurrence of fire event.



Regarding the analysis of the home range size metric in function of the occurrence of the fire event, a significant difference between pre-fire and post-fire records emerged (Table 3.4 and Figure 3.3). In particular, home range size was significantly smaller after the fire occurrence than before, while no sex effect was detected. The random effect of the animal*year was retained in the best model, and it explained a relevant proportion of the overall variance.

Table 3.4 – Summary of the best model accounting for the home range size. Significant p values are denoted with stars ($p < 0.001 = ***$; $0.001 < p < 0.01 = **$; $0.01 < p < 0.05 = *$).

Hr_Size ~ Fire_Active + (1 Animal_Id)				
# Random effects:				
Groups	Name	Variance	Std.Dev.	
Animal_Id	(Intercept)	543.0076	23.303	
Residual		0.8612	0.928	
#Number of obs: 204, groups: Animal_Id, 12				
#Fixed effects:				
	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	11.450026	7.790662	1.470	0.142
Fire_Activepre	-0.029679	0.006529	-4.546	5.47e-06 ***

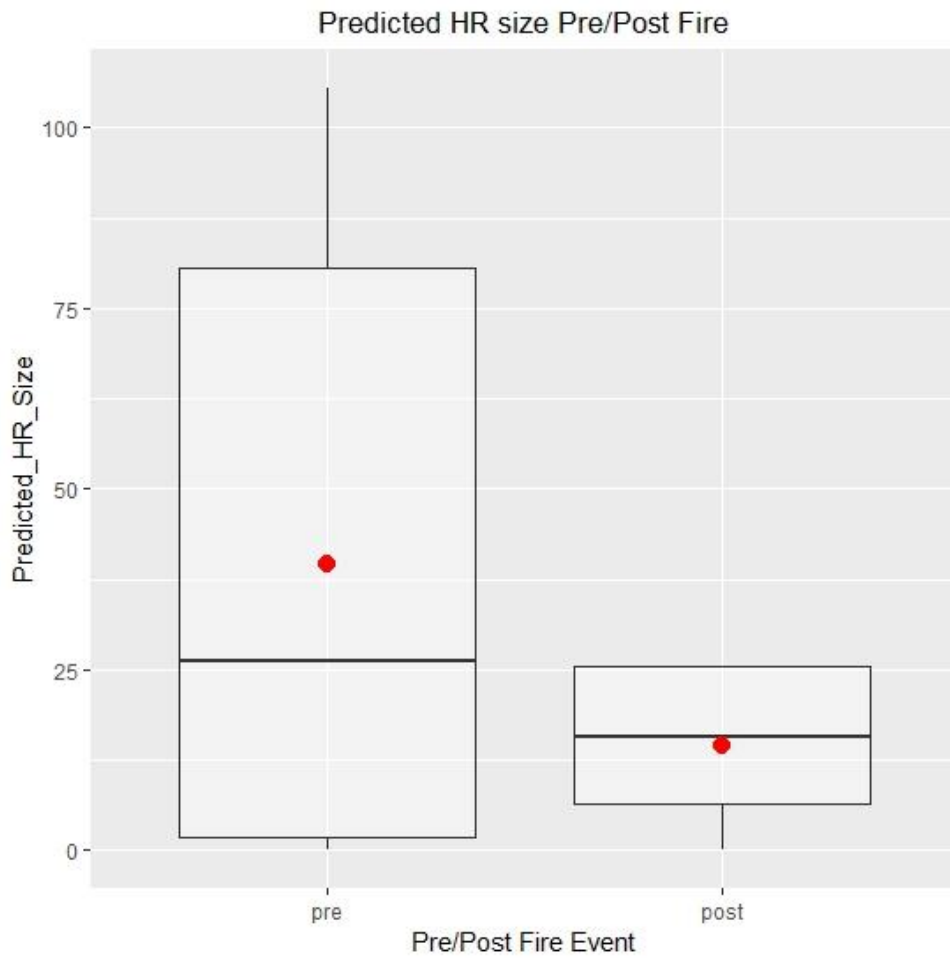


Figure 3.3 – Predictive barplot of the weekly home range size in function of the occurrence of fire event.

Discussion

The results presented in this work highlight the relevance of fire occurrence on the movements and space use pattern of roe deer in Portugal. Clearly, these results are far from being fully informative, and they actually open a series of exciting questions on the effects of fires on roe deer spatial behaviour that will need further consideration.

One aspect which is clearly evident both from the analysis of the pattern of Distance between subsequent home ranges, as well as from the analysis on the home range size, is the strong response of roe deer to the fire occurrence. Both these metrics decreased after the occurrence of the fire, which means that individuals tended to reduce their movements and shrink their space use, although at a different extent, confirming the high plasticity of this species. In principle, one could also have expected a different behavioural response to the fires, with an increment of post-fire occurrence movements as resultant of an escape reaction and exploration of new unburned areas. However, this was not the case. Seemingly, roe deer shrunk their movements because they found themselves in small patches of terrain within the home range not affected by the burning event, as, on a small scale, some patches of good habitat (e.g. high resource availability and cover) were left among the burnt areas. Furthermore, since recently burn areas provide a new source of easily assessable high-quality nutrients (Hobbs *et al.*, 1991; Eby *et al.*, 2014; Silva *et al.*, 2014), animals might stay nearby the burned patch rather than move away (Cherry *et al.*, 2018) to then restart the exploration process once again. Additionally, due to their small size roe deer are one of the first deer species to occupy a burned patch, as the emerging plants will offer cover them sooner than any of the other larger deer species (Borkowski, 2004). Also, within a burned area new forage items become available for roe deer which is a selective browser. It is possible therefore that roe deer tend to minimize movements in the surroundings of burned areas, ready to exploit the new resources as soon as they become available. In order to evaluate these aspects, longer-term analysis is needed, focusing on the pattern of recolonization of burned areas by roe deer.

Another factor that should be taken into account is that most of the fires happen in the summer months, when roe deer mating season occurs. The difference in the Distance between subsequent home ranges that was found for females and males, with males moving more than females, may provide a signal of this potentially confounding effect, and it could be related with the need for males to establish and defend a territory for the mating season. Indeed, it is possible that males, pushed by their biological need to maximise their fitness opportunity, tend to move more than females in order to find a new territory to defend, notwithstanding the modified post-fire conditions. On the other hand, it is possible that at least some females had fawns with them (although no information was available in this sense), and that this limited their movements, although roe deer hide, and are not closely followed, by their fawns.

The knowledge on the response of this species to fire events is so poor that comparisons with previous knowledge are substantially impossible. The understanding of fire ecology has only increased in the last period due to climate change, fire suppression, and proliferating invasive plants (Pechony *et al.*, 2010). However, the lack, in Europe, of multi-scale responses of herbivores to burned areas poses a criticality in our ability to understand the short- and long-term effects of fires on animal movements (i.e., post-fire successional changes on forage availability), which affect the individual fate, the population dynamics and the species distribution (Nathan *et al.*, 2008). Given the increase of fires all over Europe, and not only in the Mediterranean countries, it is desirable that the studies on this (and other) species response to fire events will receive more attention in the coming future. Indeed, although roe deer are notorious for their resilience to human-made environments, and for being able to return to their home ranges after disturbance has ended (Reimoser, 2012), in the case of fire events the appearance of a burned area could lead - also in the medium-term - to the avoidance of that area with consequent forcing movements towards unknown areas where the risks for animal survival are certainly more relevant (e.g. predation by wolf). Therefore, understanding the processes regulating animal movements in response to fire events is crucial, especially in a context- as the Portuguese one – where important reintroduction efforts have been performed with

the aim to maintain a self-growing population. Indeed, reintroduction release sites should be carefully considered, e.g. by checking the historical fire tradition of that area. Certainly, larger scale analyses (in time, and, possibly, in space with comparison with other populations) are needed to shed light on this interesting, and critically relevant topic.

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Chapter IV

Final considerations

Final considerations

Reintroduction of animals following their extinction from the wild is a central part of ecosystem restoration (Polak & Saltz, 2011; Seddon *et al.*, 2012). Nowadays, with increasing success rates of reintroduction programs, and more efficient post-release monitoring, gathering information on these new populations to study their ecology became more accessible.

Thus far, there was only another study, conducted in central Portugal, featuring a reintroduced population of roe deer, aiming at understanding how translocated roe deer establish their home-range (Carvalho *et al.*, 2008). However, that study had a limited data sample (5 animals), it was carried out in a different and smaller study area, with different monitoring methods (VHF instead of GPS collars), and one year after release the animals were no longer followed, therefore the success of the reintroduction, the space use, and home range establishment were not fully evaluated. Using roe deer data gathered from an ongoing reintroduction project in Central Portugal, the present study attempted to analyse roe deer home range emergence after release in a naïve environment (chapter II), in addition to examine spatio-temporal response of this population after their environment is disrupted by fire incidents (chapter III).

Throughout the thesis, all the major aims were achieved, making it possible to better understand the movement of roe deer in Portugal, as this is one of the last frontiers in the species' European distribution. Firstly, in chapter II, I showed, for the first time in Portugal, how animals tended to move away from the release point, displaying a two phased dispersing movement characterized by a first phase of exploration, an apparent stabilization, and a further movement away from release site matching with the reproductive season. I also found that the intrinsic movements of these animals first exhibited a decreasing tendency in the distance between different centroids, i.e. a decrement of movement rate, to then stabilize over time, while no difference in home range size was detected throughout the first year of monitoring after release. These novel and exciting results will merit further

investigation, especially for what concerns the presence of wolves and the spatio-temporal variation of food resources.

As for the analysis on the fire effect on individual movements (chapter III), I showed that the studied animals exhibited a contraction of their mobility post-fire. Remarkably, this thesis is the first assessment combining roe deer fire ecology based on GPS data. The fire reality in Portugal is changing rapidly. According to the 2017 Joint Report Centre (JRC) report, Portugal was, beyond a doubt, the country most affected by forest fires, with 41% of the country mapped as burnt area in 2017. Fires of over 30ha were accounted every month of the year, and 2016's total (itself considered the worst for some years) was surpassed in August. The report also stated that these fires are getting so large and numerous that several merged together, creating mega-fires which resulted in problematic classification of the exact number of fire occurrences and their delimitations. Furthermore, by the end of 2017, 563.674ha of burnt area had been recorded in Portugal, i.e. more than the previous 6 years put together, and more than the total burnt area mapped for Europe, Middle East and North Africa in all but two of the last 10 years (San-Miguel-Ayanz *et al.*, 2018).

As the trend towards larger fires increases, owing to the intensification of extreme climate events, it is pivotal to analyse their effects on wild populations. My work represents a first step in this direction. Larger spatio-temporal scale analysis, likely available in the future, will bring knowledge on the long-term effects of fires on exposed populations. I also encourage researchers to move beyond local studies, comparing the observed patterns across different population to get more robust information on the effects of wildfires. As a final management remark, I retain that in view of future management plans for reintroducing roe deer in Portugal, release areas should be identified also based on the risk of fire occurrence in that area, i.e. the fire history of a given area should be considered as a crucial factor for this decision.

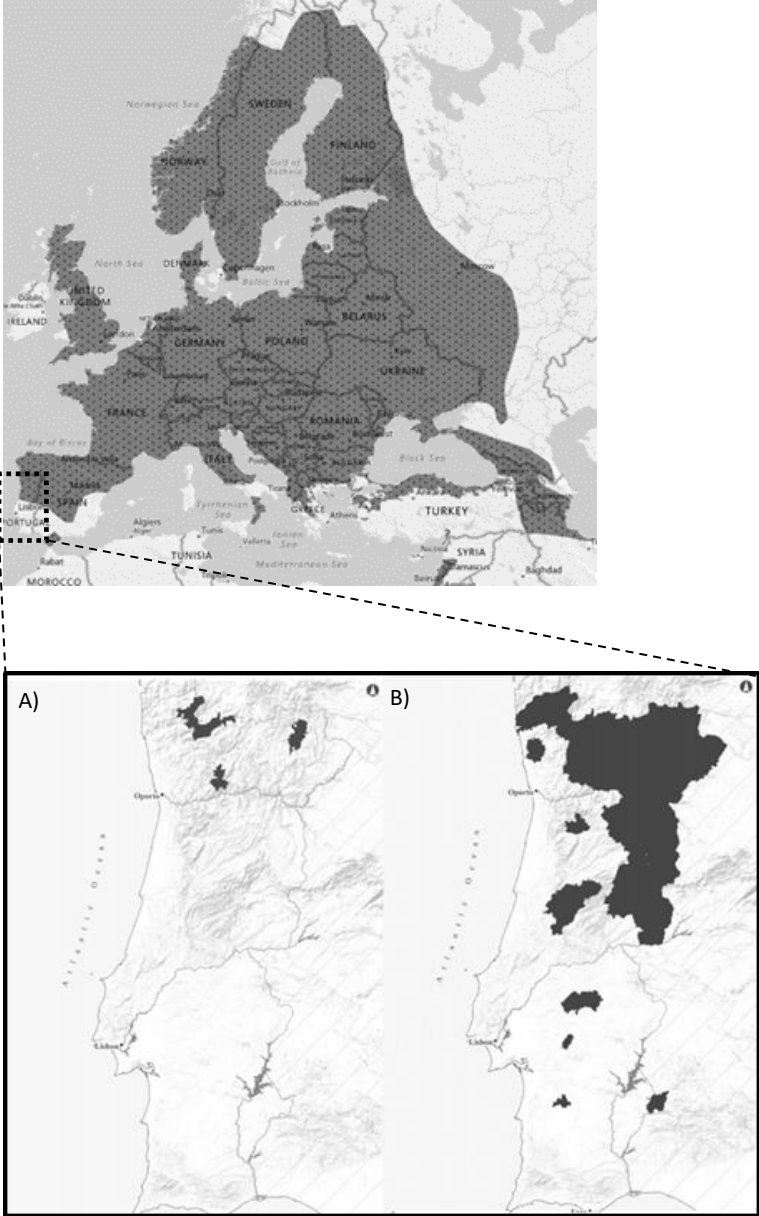
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Appendix

Appendix A – Roe deer distribution in Europe, and its evolution since 1970's (A), to 2015 (B) in Portugal.

Source: IUCNred list & Torres *et al.*, 2015



Appendix B – Track record of the collars in each year of reintroduction.

Animal ID	Collar ID	Reintroduction year	Sex	Age	Notes
6_2013	13906	I	Male	0,5	No signal #
7_2013	13907	I	Male	0,5	Died of natural causes ◇
8_2013	13908	I	Male	2,5	Pouching #
9_2013	13909	I	Female	0,5	No signal #
10_2013	13910	I	Female	0,5	Killed by wolves ◇
11_2013	13911	I	Female	6	Dead ◇
8_2014	13908	II	Male	2,5	Pouching #
10_2014	13910	II	Male	3,5	No signal #
45_2014	15745	II	Male	1,5	Drop off
48_2014	15748	II	Male	3,5	Mortality event #
50_2014	15750	II	Male	1,5	Mortality event
7_2014	13907	II	Female	7,5	Killed by wolves ◇
11_2014	13911*	II	Female	3,5	No signal
43_2014	15743	II	Female	Adult	Drop off
44_2014	15744	II	Female	1,5	Drop off
46_2014	15746	II	Female	1,5	Drop off
49_2014	15749	II	Female	1,5	Mortality event
51_2014	15751	II	Female	5,5	Pouching #
7_2015	13907	III	Male	1,6	Drop off
51_2015	15751	III	Male	1,6	Drop off
52_2015	16852	III	Male	1,6	Drop off
71_2015	16871	III	Male	1,6	Drop off
81_2015	16881	III	Male	2,6	Drop off
82_2015	16882	III	Male	1,6	Drop off ◇

8_2015	13908	III	Female	4,6	Drop off
48_2015	15748	III	Female	1,6	Drop off #
72_2015	16872	III	Female	1,6	Drop off
78_2015	16878	III	Female	4,6	Drop off ◊
79_2015	16879	III	Female	5,6	Drop off
45_2016	15745	IV	Male	3,5	Still been tracked
49_2016	15749	IV	Male	3,5	Still been tracked
50_2016	15750	IV	Male	1,5	Still been tracked
43_2016	15743	IV	Female	3,5	Still been tracked
46_2016	15746	IV	Female	2,5	Still been tracked
64_2016	22964	IV	Female	2,5	Still been tracked

Appendix C – Count matrix/contingency table, for fire information of 2013.

		GOV 2013									
		No date/time + no fire dept info	No date/time + fire dept info	Beginning date only + no fire dept info	Beginning date only + fire dept info	Beginning date/time only + no fire dept info	Beginning date/time only + fire dept info	Both beginning and end date + no fire dept info	Beginning and end date/time + no fire dept info	Beginning and end date/time + fire dept info	
NASA	No date/time	80	18	10	59	10	0	0	0	0	167
	Beginning date only	0	0	0	0	0	0	0	0	0	0
	Beginning date/time only	28	18	2	8	2	0	0	0	0	56
	Both beginning and end date	0	0	0	0	0	0	0	0	0	0
	Both beginning and end date/time	23	19	1	11	1	0	0	0	0	54
		131	55	13	78	13	0	0	0	0	TOTAL = 277

Appendix D – Count matrix/contingency table, for fire information of 2014.

		GOV 2014											
		No date/time + no fire dept info	No date/time + fire dept info	Beginning date only + no fire dept info	Beginning date only + fire dept info	Beginning date/time only + no fire dept info	Beginning date/time only + no fire dept info	Beginning date/time only + fire dept info	Both beginning and end date + no fire dept info	Both beginning and end date + fire dept info	Beginning and end date/time + no fire dept info	Beginning and end date/time + fire dept info	
NASA	No date/time	6	0	0	0	0	0	0	0	0	4	0	10
	Beginning date only	0	0	0	0	0	0	0	0	0	0	0	0
	Beginning date/time only	1	0	0	0	0	0	0	0	0	0	0	1
	Both beginning and end date	0	0	0	0	0	0	0	0	0	0	0	0
	Both beginning and end date/time	1	0	0	0	0	0	0	0	0	0	0	1
		8	0	0	0	0	0	0	0	0	4	0	TOTAL= 12

Appendix E – Count matrix/contingency table, for fire information of 2015.

		GOV 2015										
		No date/time + no fire dept info	No date/time + fire dept info	Beginning date + no fire dept info	Beginning date + fire dept info	Beginning date/time + no fire dept info	Beginning date/time + fire dept info	Beginning and end date/time + no fire dept info	Beginning and end date/time + fire dept info	Beginning and end date + no fire dept info	Beginning and end date + fire dept info	
NASA	No date/time	4	0	0	0	0	0	0	42	0	0	46
	Beginning date only	0	0	0	0	0	0	0	0	0	0	0
	Beginning date/time only	3	0	0	0	0	0	0	0	0	0	3
	Both beginning and end date	0	0	0	0	0	0	0	0	0	0	0
	Both beginning and end date/time	6	0	0	0	0	0	0	0	7	0	13
		13	0	0	0	0	0	0	42	7	0	TOTAL= 62

Appendix F – Count matrix/contingency table, for fire information of 2016.

		GOV 2016										
		No date/time + no fire dept info	No date/time + fire dept info	Beginning date + no fire dept info	Beginning date + fire dept info	Beginning date/time + no fire dept info	Beginning date/time + no fire dept info	Beginning and end date/time + no fire dept info	Beginning and end date/time + fire dept info	Beginning and end date + no fire dept info	Beginning and end date + fire dept info	
NASA	No date/time	14	0	0	0	0	0	0	85	0	0	99
	Beginning date only	0	0	0	0	0	0	0	0	0	0	0
	Beginning date/time only	10	0	0	0	0	0	0	0	1	0	11
	Both beginning and end date	0	0	0	0	0	0	0	0	0	0	0
	Both beginning and end date/time	11	0	0	0	0	0	0	0	11	0	22
		35	0	0	0	0	0	0	85	12	0	TOTAL= 132

Appendix G – Count matrix/contingency table, for fire information of 2017.

		GOV 2017										
		No date/time + no fire dept info	No date/time + fire dept info	Beginning date + no fire dept info	Beginning date + fire dept info	Beginning date/time + no fire dept info	Beginning date/time + fire dept info	Beginning and end date/time + no fire dept info	Beginning and end date/time + fire dept info	Beginning date + no fire dept info	Beginning date + fire dept info	
NASA	No date/time	0	0	0	0	0	0	0	0	19	0	19
	Beginning date only	0	0	0	0	0	0	0	0	0	0	0
	Beginning date/time only	1	0	0	0	0	0	0	0	19	0	20
	Both beginning and end date	0	0	0	0	0	0	0	0	0	0	0
	Both beginning and end date/time	1	0	0	0	0	0	0	0	20	0	21
		2	0	0	0	0	0	0	0	58	0	TOTAL= 60