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ESTIMATING ROE DEER AND WILD BOAR ABUNDANCE AND HABITAT USE IN A HETEROGENEOUS LANDSCAPE OF NORTHERN PORTUGAL

Dissertação no âmbito do Mestrado em Ecologia, orientada pelo Professor Doutor José Paulo Sousa e pela Doutora Joana Silva Alves (Universidade de Coimbra) e apresentada ao Departamento de Ciências da Vida da Faculdade de Ciências e Tecnologias da Universidade de Coimbra.

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Estimating roe deer and wild boar abundance and habitat use in a heterogeneous landscape of Northern Portugal

Dissertation in MSc in Ecology supervised by Prof. Dr. José Paulo Sousa (Department Life Sciences of the University of Coimbra and Centre for Functional Ecology - Science for People & the Planet) and Dra. Joana Silva Alves (Department of Life Sciences of the University of Coimbra and Centre for Functional Ecology - Science for People & the Planet) and presented to the Department of Life Sciences, Faculty of Sciences and Technology of the University of Coimbra

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- *Capreolus capreolus*;
- *Sus scrofa*;
- Ungulates;
- Abundance;
- Habitat use;
- Activity patterns.

Resumo

O habitat é definido pelos recursos disponíveis a um determinado organismo, os quais influenciam o número de indivíduos existentes e a capacidade de suporte do meio. A avaliação destes parâmetros é particularmente relevante em paisagens heterogéneas assim como para espécies com importância socioecológica e que são fortemente influenciadas por habitats com grande intervenção humana, como é o caso dos ungulados silvestres.

Os principais objetivos deste estudo são: i) avaliar as características ambientais e humanas que influenciam a ocorrência e o uso do habitat pelo corço e javali; ii) determinar a atividade circadiana do corço e javali; e iii) realizar uma abordagem multi-metodológica para estimar a abundância de corço. O estudo foi realizado na Paisagem Protegida de Corno de Bico (NO Portugal), através de armadilhagem fotográfica, transetos lineares com amostragem de distâncias, plots de contagem de excrementos com remoção e análise genética não-invasiva para identificação individual de amostras fecais de corço.

Os resultados obtidos demostraram que o corço e o javali estão amplamente distribuídos na área de estudo, ocupando 87% e 93% da área, respetivamente. O corço, e em menor medida o javali, apresentaram preferência por áreas de floresta, evitando zonas agrícolas. Para ambas as espécies, verificou-se uma influência de fatores antropogénicos (p.e. espécies pecuárias) na seleção do habitat e atividade circadiana. Para o corço, foi obtida uma estimativa de abundância de 13 indivíduos, com recurso aos transetos lineares e análise genética não-invasiva, e de 15 indivíduos através da armadilha fotográfica.

Com base nos resultados obtidos, são discutidas várias medidas para a conservação e gestão de ungulados silvestres em paisagens heterogéneas com elevada pressão antropogénica, e em particular nesta área protegida, assim como a necessidade de estudos futuros com base na abordagem metodológica aqui utilizada, de forma a assegurar a monitorização regular de ungulados silvestres, face às suas implicações sociais, económicas e ecológicas.

Palavras-chave: *Capreolus capreolus*; *Sus scrofa*; Abundância; Atividade circadiana; Uso de habitat.

Abstract

Habitat is defined as the resources available to a given organism, which influence the number of individuals and the ecological carrying capacity. Assessing these parameters is particularly relevant in heterogeneous landscapes as well as for species with socioecological importance that are strongly influenced by areas with high human intervention, such as wild ungulates.

The main objectives of this study are: i) to evaluate environmental and human-related parameters that influence the occurrence and habitat use by roe deer and wild boar; ii) to assess the activity patterns of roe deer and wild boar; and iii) to conduct a multi-method approach to estimate the abundance of roe deer. This study was conducted in Corno de Bico Protected Landscape (NW Portugal), by using camera-trapping, linear transects with distance sampling, clearance plots and non-invasive genetic analyses for individual identification of fecal samples from roe deer.

Results showed that roe deer and wild boar are widely distributed in the study area, occupying 87% and 93% of the area, respectively. Roe deer, and at lesser extend also wild boar, showed a preference for forest areas, avoiding agricultural lands. For both species, there was an influence of anthropogenic activities (e.g. livestock) on habitat selection and activity patterns. For the roe deer, was obtained an abundance estimate of 13 individuals, based on linear transects with distance sampling and non-invasive genetic analysis, and of 15 individuals through camera-trapping.

Based on obtained results, we discuss several actions for conservation and management of wild ungulates in heterogeneous landscapes with high anthropogenic pressure, and particularly in this protected area, as well as the need for further studies resorting to the same methodological approach used here, in order to assure a regular monitoring of wild ungulate populations, given the social, economic and ecological implications of these wildlife species.

Keywords: *Capreolus capreolus*; *Sus scrofa*; Abundance; Circadian activity; Habitat use.

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1. Introduction

1.1. Habitat use and animal abundance in heterogeneous landscapes

Habitat is defined as the resources available in an area occupied by a given organism, relating the presence of the organism to the structural and biological characteristics of its surrounding, not only through the vegetation cover, but also the sum of the resources necessary for the survival of the individual (Hall *et al.*, 1997). However, the term habitat has an ambiguous meaning and this confusion increased when there was the addition of some terminologies, such as habitat *use* (Block and Brennan, 1993). So, in this work, habitat use is considered the resources used by an animal in a certain habitat (Hall *et al.*, 1997), which can be influenced by season (Baber and Coblentz, 1986; Honda, 2009), topographic characteristics of the landscape, physiological needs (Baber and Coblentz, 1986) or human presence (Marie *et al.*, 2018) and can vary at both spatial (Burger and Gochfeld, 1998; Lone *et al.*, 2015; Kays *et al.*, 2017) and temporal scales (Oberosler *et al.*, 2017; Gaynor *et al.*, 2018).

The carrying capacity of the habitat, translated in the number of individuals that a certain area can support, is an important trait in order to manage and preserve an animal population (Mandujano and Gallina, 1995). Habitat carrying capacity is mostly influenced by the availability of food resources and refuge conditions, which are influenced by environmental or human-related factors (Aldila *et al.*, 2015), which allow organisms to reproduce and survive in a given area. In order to assess habitat carrying capacity as a tool for wildlife conservation and management, is important not only to estimate species abundances (*i.e.* number of individuals in a given area) (e.g. Marques *et al.*, 2001; Calambokidis and Barlow, 2004; Rivero *et al.*, 2004; Zerbini *et al.*, 2007) but also the landscape features where the species occur and which may influence resource use, productivity and survival (e.g. Cimino and Lovari, 2003; Coulon *et al.*, 2008; Harper *et al.*, 2008).

Landscape is defined as a geographical area where structure, function and changes are themselves scale-dependent (Turner, 1989). In this context, heterogeneous landscapes are characterized by a combination of different patches of habitat with distinct species composition and ecological conditions (e.g. levels of resource availability or human intervention), which makes them highly biodiverse and important in terms of biological conservation (Verberk, 2008). Therefore, in order to estimate habitat use and animal abundance by considering the heterogeneity of the landscapes, it is important to survey several habitat types, including areas with high human-related disturbance due to human presence or livestock grazing (Verberk, 2008; Li *et al.*, 2017; Pudyatmoko, 2017). Numerous wildlife studies have been carried out in heterogeneous landscapes (e.g. Lovett *et al.*, 2005; Pereira *et al.*, 2012; Katayama *et al.*, 2014) aiming to understand how heterogeneity influences the use of space by wild animals as well as how it promotes their abundance and survival. This is particularly important for species, either habitat generalist or specialist, which have an important socio-ecological role and are strongly influenced by the characteristics of each habitat type, including agricultural lands and other human-modified areas (Turner *et al.*, 1997; Bokdam and Gleichman, 2000; de Leeuw *et al.*, 2001), as it is the case of wild ungulates.

1.2. Importance of wild ungulates in Portugal

Wild ungulates have a high economic, social and ecological importance in Portugal, a southern European country dominated by heterogeneous landscapes. In Portugal, currently there are five species of wild ungulates, namely, Iberian wild goat (*Capra pyrenaica*), fallow deer (*Dama dama*), red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), and wild boar (*Sus scrofa*) (Vingada *et al.*, 2010). The most common and widespread native species of wild ungulates occurring in Portugal are the wild boar and the roe deer (Vingada *et al.*, 2010). Both species are considered to have an important economic impact, either positive due to game hunting or negative due to damages in agricultural and forestry (Verberk, 2008; Li *et al.*, 2017; Pudyatmoko, 2017). Regarding game hunting, if well managed, is an activity that may provide important economic benefits as well as a role in preserving ecosystems, conserving wildlife and controlling wild populations (Robinson and Bennett, 2004). Since both wild boar and roe deer are legally considered game species in Portugal (*Portaria* 105/2018, of April 18), they represent an important economic revenue. Although roe deer game hunting is currently a minor activity in northwestern Portugal, wild boar is an important game species, with a sharp increase in the number of harvested individuals from 423 in 1989/1990 hunting season to 8,000 in 2000/2001 (Vingada *et al.*, 2010). This increasing number of harvested wild boar is followed by the increasing number of Large Game Licenses in Portugal, which were 23,692 in 1999/2000 and 37,756 in 2004/2005 hunting season (Vingada *et al.*, 2010).

The economic impact of roe deer in terms of agricultural damages is not considered significant, despite being one of the most abundant deer in Europe (Bleier *et al.*, 2012). However, in Portugal, the impacts of wild ungulates in agriculture and forest plantations are considered relevant, especially those associated with wild boar. In fact, wild boar is considered a major threat, since they frequently feed in agricultural areas as well as seek refuge in forest plantations (Schley and Roper, 2003; Keuling and Stier, 2010). According to different studies, the extent of wildlife damages increases within shorter distances from forest edges (Naughton-Treves, 1998; DeVault *et al.*, 2007), and this boundary effect has already been demonstrated for wild boar, particularly in heterogeneous landscapes dominated by agricultural fields (Thurfjell *et al.*, 2009).

In Portugal, the exact damage caused to farmers by the wild boar is unknown, but in most cases occur in small-scale farms, which means agriculture for personal/local subsistence (Vingada *et al.*, 2010). However, several farmers complain of losses of hundreds to thousands of euros annually (Pers. Commun.). Roe deer' damages in Portugal are neither reported nor calculated, representing virtually insignificant value. However, during the pre-rut season, they can cause damage to young trees, causing their death, due to the antlers rubbing (Nielsen *et al.*, 1982).

To prevent the damages caused by wild ungulates, fences approximately 2 meters high can be placed, thus preventing their entry, however they are expensive and can have a fragmentation effect on the landscape (Rosa, 2006). Another damage prevention method that proved to be effective was the placement of individual protections on young trees, thus protecting grazing of shoots and leaves as well as rubbing for territorial marking (Hodge and Pepper, 1998; Mayle, 1999; Côté *et al.*, 2004; Rosa, 2006).

Finally, another important aspect on the socio-economic impact of wild ungulates is the involvement of these species in traffic accidents, often leading to human losses and huge economic costs in Europe (Groot Bruinderink and Hazebroek, 1996). As an example, in Portugal during 2017 and 2018, there were more than 700 accidents involving wild animals (Riso, 2018). However, based on a report on the fauna mortality of Infrastructures of Portugal, in 2019 wild ungulates killed in traffic collisions represents only 3.5% of the total of terrestrial mammals and only 1.9% of the of wild animals (Garcia, 2019).

From an ecological point of view, wild ungulate species represent an important food resource for carnivores, and particularly for Iberian wolves (*Canis lupus signatus*, Cabrera, 1907). In Portugal, the Iberian wolf is protected by law (No. 90/88 of 13 August), and considered a threatened species according to the Portuguese Red List of Vertebrates (Cabral *et al.*, 2005), with an estimated population of approximately 300 individuals and less than 60 breeding packs occurring mostly at north of Douro river (Pimenta *et al.*, 2005). The Iberian wolf in Portugal shows a high regional variation in its diet composition, with an overall high trophic dependence on domestic prey under an extensive husbandry system, such as goats, sheep, cattle and free-ranging horses, and a low consumption of wild ungulates due to their limited species diversity, range extent and population abundance (Álvares *et al.*, 2015). In fact, in Portugal the only widespread and abundant wild prey for wolves is the wild boar, while the remaining species of ungulates, such as roe deer, red deer and Spanish Ibex show a limited range and abundance in northern Portugal, mostly due to low habitat availability and intensive poaching (Vingada *et al.*, 2010; Bencatel *et al.*, 2019). However, and according to the theory of trophic strategies (Glasser, 1982), an increase in wild prey abundance and richness should cause predators to increase the consumption of such wild species. This can be found in Portugal, where wolves feed mostly on livestock due to generalized scarcity of wild prey while in Montesinho Natural Park, the only Portuguese area within wolf range with higher availability of wild prey, both in abundance and number of species, wolf diet is currently comprised in almost 80% by wild ungulates, including roe deer and wild boar (Álvares *et al.*, 2015) (Figure 1). Therefore, promoting a higher abundance and diversity of wild prey within wolf range, becomes crucial to reduce wolf predation on domestic prey and is considered one of the most important measures for wolf conservation in Portugal (Álvares *et al.*, 2015). However, the limited available knowledge on population estimates and ecological traits of wild ungulates in Portugal constrains the development of efficient management practices to promote their occurrence as a stable food resource for wolves (Vingada *et al.*, 2010; Álvares *et al.*, 2015).

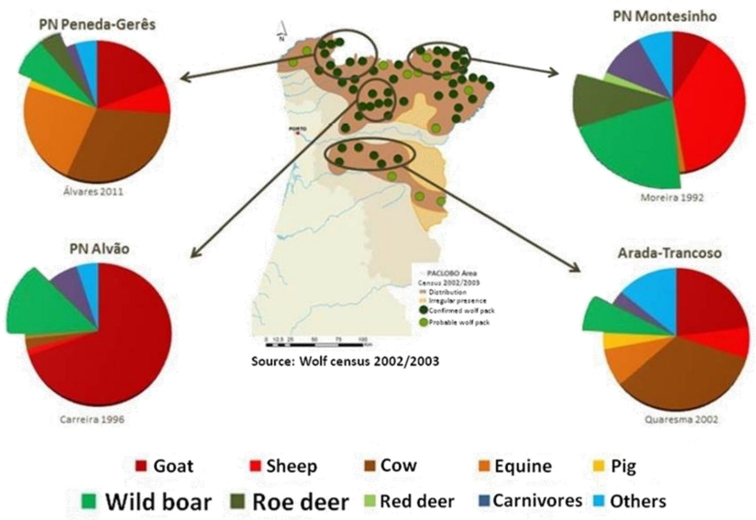

Figure 1 – Wolf diet composition in several areas of Portugal, highlighting the relevance of wild boar (light green) and the roe deer (dark green) as the two study ungulates (Source: Álvares et al., 2015).

1.3. Study species

1.3.1. Wild boar: ecology and behavior

The wild boar belongs to the family Suidae and to order Cetartiodactyla, according to the classification adopted by IUCN (Oliver and Leus, 2008). It is morphologically characterized by dark-brown hair, short neck, small and raised ears and physic robustness (Figure 2). The legs are small and end in four hoof-protected fingers. In European populations, males have an average weight of 100 kg (Rosell *et al.*, 2001), although in some southern populations, such as Iberian Peninsula, they can barely exceed 70 kg (Garzón-Heydt, 1991), while in Carpathian Mountains, adult males can reach more than 300 kg (Rosell *et al.*, 2001). Adult males have developed lower canines, projected upwards and outwards, that grow over ages of 10 years old. These canines are an important defense in the fight with other males for access to females in the reproductive season, and also helps in the territory marking (Rosell *et al.*, 2001). The sense of smell is quite developed in this species, allowing the exploration of the environment (for example, searching for food) as well as the intra and inter-specific communication, since many dangers are detected through scent (Rosell *et al.*, 2001).

Wild boar forms herds of varying size (6 to 20 individuals) according to geographical location and season (Rosell *et al.*, 2001). However, groups with more than 100 individuals have already been registered (Oliver and Leus, 2008). Young males reach puberty at 10 months old, although they cannot compete for adult females until they reach 2 years old, while females can start reproducing at 8-10 months old under conditions of good food availability (Rosell *et al.*, 2001).

Figure 2 – Juvenile of wild boar photographed in the study area by camera-trapping (Photo: Jorge Costa).

The wild boar is a generalist species with high adaptability to the surrounding environment, being present across all Europe (Figure 3) as well as in North Africa and wide areas of Asia (Bencatel *et al.*, 2019). In Portugal, although wild boars had a limited range in the beginning of XXth century, they have shown during the last decades a sharp increase in distribution and abundance, being currently the most widespread wild ungulate, occurring throughout all continental territory, except major cities, such as Porto and Lisbon (Apollonio *et al.*, 2010; Bencatel *et al.*, 2019). Due to its adaptability and environmental plasticity, it can be found in all types of habitats, from high mountains to deserts, including human-dominated landscapes and urban areas (Rosell *et al.*, 2001).

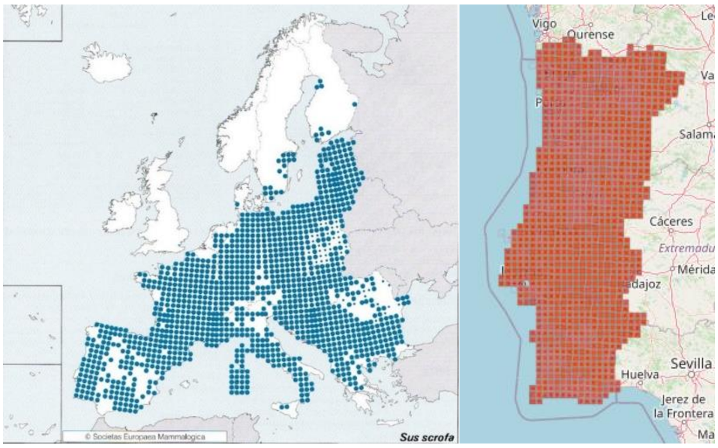

Figure 3 – Wild boar distribution in Europe (left) and in Portugal (right) (Source: Apollonio et al., 2010; Bencatel et al., 2019).

Habitat selection by wild boar can vary across regions, with the presence of water being very important for foraging areas (Abaigar *et al.*, 1994). Wild boar does not have a specific preferred habitat worldwide, which means that each population may select different environmental settings as far as food and cover is available, such as European beech-hornbeam forest (Fonseca, 2008), scrublands with pastures (Barrett, 1982), oak and mixed coniferous forest (Abaigar *et al.*, 1994; Thurfjell *et al.*, 2009), deciduous forests and open areas (Thurfjell *et al.*, 2009) or even marshes and wetlands (Dardaillon, 1986). In this context, habitat selection by wild boar can be different across the year, with the related variation being explained mostly by the seasonal availability of resources (Baber and Coblentz, 1986; Abaigar *et al.*, 1994; Thurfjell *et al.*, 2009; Fernanda Cuevas *et al.*, 2013). This species is also found in places where the soil vegetation has enough density and height to allow a safe refuge from potential predators (Baber and Coblentz, 1986; Abaigar *et al.*, 1994).

Home ranges of wild boars varies from 33.2ha in 24hours (Russo *et al.*, 1997) to 136ha in one month (Massei *et al.*, 1997), reaching up to 350ha in a mast year (defined as years when oak acorns production is high) or even 1070ha in a mast failure year (Singer *et al.*, 1981). Wild boars have a high ecological plasticity, showing a generalist and omnivorous diet that varies according to food availability (Genov, 1981; Massei *et al.*, 1996; Rosell *et al.*, 2001; Schley and Roper, 2003). Wild boar diet usually consists of energy-rich food items, such as acorns, olives and pine-seeds, and when not available, are replaced by Gramineae (Massei *et al.*, 1996) as well as other non-animal food items, which can comprise an average of 98% of frequency of occurrence in the annual diet (Massei *et al.*, 1996). In Central Portugal, a study conducted based on stomach contents from more than 200 individuals, showed that 91% of wild boar diet was composed by plant species, from Gramineae to Bryophyta, and only 5% of their diet was composed by animal species, including Insecta, Gastropoda and even Vertebrate specimens (Silva, 2009).

1.3.2. Roe deer: ecology and behavior

The roe deer belongs to the family Cervidae and to order Cetartiodactyla, according to the classification adopted by IUCN (Lovari *et al.*, 2016). It is morphologically characterized by the large ears (12 - 14 cm, Sempéré *et al.*, 1996), long neck and white anal shield, less pronounced in summer than in winter (Sempéré *et al.*, 1996), which is kidney-shaped in males and heart-shaped in females (Freire, 2012). Roe deer is the smallest deer in Iberian Peninsula (Freire, 2012), with a total length of 107 – 126 cm and shoulder height of 66 – 83 cm (Sempéré *et al.*, 1996). European roe deer have a body mass of 22 – 32 kg, and the offspring born with 1 – 1,7 kg and with light spots that fade over time (Figure 4).

Figure 4 – Young male of roe deer photographed in the study area by camera-trapping (Photo: Jorge Costa).

Breeding activity of roe deer initiates in females when they are 14 months old, and in males when they reach 3 years old, but spermatogenesis occur when males reach 7 months old (Sempéré *et al.*, 1996). Reproduction begins in late July, early August (Aitken, 1974), with gestation period lasting between 9 and 11 months (264 – 318 days). An interesting trait in this deer is that, after the ovum being fertilized and penetrates the uterus, blastocyst starts to slow down the development and having minimal mitotic activity, maintaining this state, called diapause, for 4 - 5 months (Sempéré *et al.*, 1996).

The roe deer is considered to be the most wide ranging and abundant cervid in Europe (Apollonio *et al.*, 2010). In fact, this ungulate can be found all over Europe (Figure 5), except in Ireland, Cyprus, Corsica, Sardinia and most of the smaller Mediterranean islands (Lovari *et al.*, 2016), being also present in western Russia, northern Turkey, Caucasus and Iran (Lovari *et al.*, 2016). In Portugal, the roe deer had also a limited range in the beginning of XXth century, but have shown during the last decades an increase in distribution, as also documented in most western Europe and particularly in Iberian Peninsula (Lovari *et al.*, 2016). Currently, native populations of roe deer are present across all northern half of Portugal (except coastal areas), namely in the districts of Viana do Castelo, Braga, Vila Real, Bragança, Guarda, Viseu and Castelo Branco (Figure 5) (Bencatel *et al.*, 2019). This species is also locally present in more southern areas of the country, either as the result of reintroduction programs (e.g. Serra da Freita/Arada and Serra da Lousã) or due to introductions in fenced areas for hunting purposes (Vingada *et al.*, 2010; Bencatel *et al.*, 2019).

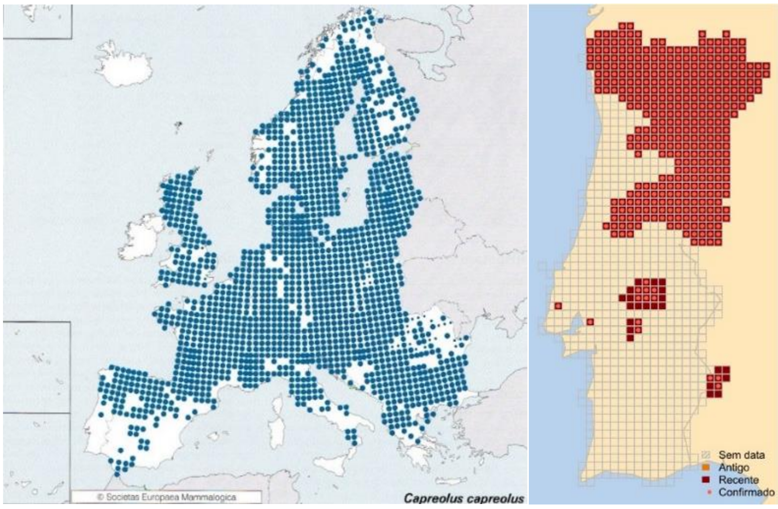

Figure 5 – Roe deer distribution in Europe (left) and in Portugal (right) (Source: Apollonio et al., 2010; Bencatel et al., 2019).

Habitat selection by roe deer, as in most ungulates, is strongly influenced by food availability and quality (Barrett, 1982; Duncan, 1983; Fernanda Cuevas *et al.*, 2013). The roe deer is found in various types of habitats, from forests to agricultural fields (Putman, 1986; San José *et al.*, 1997). However, according to several authors (Putman, 1988; Lovari and San José, 1997; Morellet *et al.*, 2011), this species is considered forest-dependent, showing some degree of habitat specialization. In fact, according to Morellet *et al.* (2011), a higher availability of forest is strongly correlated with a greater use of this habitat type by the roe deer, which suggests that forest areas should be preserved so roe deer does not need to select another type of habitats, such as agricultural areas where refuge conditions tend to be lower. A study by Gill *et al.* (1996) concluded that roe deer density decreases with increasing canopy cover, as this increase is inversely related to ground vegetation cover, which reduces the roe deer preferred habitat, that usually is related to forests with scrublands providing high refuge conditions. On the other hand, another study conducted in northern Europe during winter, where snow cover is extensive, concluded that roe deer used areas with high canopy cover and high ground cover, namely for resting and feeding areas, respectively (Ratikainen *et al.*, 2007). Based on this available knowledge, it seems that the roe deer uses mostly forests, although selecting the habitat that is most favorable for its survival, according to the need and availability at that moment.

The home ranges of roe deer are very variable, with reported values of 3ha (Tufto *et al.*, 1996), 22ha (Cederlund, 1983; Saïd *et al.*, 2005), 60ha (Kjellander *et al.*, 2004), 70ha for females and 152ha for males (Dupke *et al.*, 2017) and up to 187ha (Morellet *et al.*, 2011), being this wide variation explained by food availability (Tufto *et al.*, 1996) or habitat composition (Dupke *et al.*, 2017). Roe deer is an herbivorous species, and its diet varies according to food availability, location of the population (Barancekova *et al.*, 2010) and season (Ribeiro, 2018). In a study in Czech Republic, it was revealed the presence of various parts of forbs, shrubs, tree species, ferns and mosses (Barancekova *et al.*, 2010), with forbs (e.g. herbaceous flowering plants) being the most consumed. In another study conducted in England, *Rubus fruticosus* (shrub)*, Urtica dioica* (herb)*, Agrostis capillaris* (grass) *and Festuca ovina* (grass) were the plant species with more frequency of occurrence on the diet of this ungulate (Hearney and Jennings, 1983). In a study conducted in Portugal, in almost all seasons, tree species was the most consumed group (19-54%) followed by shrubs (27-43%), except in winter when monocotyledons (herbaceous species) were the most consumed vegetation type (Ribeiro, 2018).

1.4. Goals and hypothesis

Given the socio-ecological relevance of both wild boars and roe deer, as well as the limited available knowledge regarding the Portuguese populations, this work has three main objectives i) to identify environmental factors that influence the distribution and habitat use of roe deer and wild boar in a heterogeneous landscape of northern Portugal; ii) to asses patterns of circadian activity in roe deer and wild boar; and iii) to estimate the abundance of roe deer, using different methods, such as camera-trapping, line transects with distance sampling of fecal counts, clearance plot counts, and in addition, DNA extraction from fecal samples to determine the number of different individuals. Furthermore, the occurrence of four other species (wolf, horses, cattle and humans) will be also evaluated to assess the effect of a predator (wolf), potential competitors (livestock) and sources of human disturbance (dogs and humans) on the occurrence of the two study ungulates. For this, the activity patterns of the target ungulates, wolf and domestic animals/humans will be analyzed to assess the effect of predators, competitors and human activity on wild ungulates activity patterns, while in the analysis of habitat use, the presence of humans, horses and cattle will also be evaluated as explanatory factors, to evaluate how habitat use of the study species are affected by their presence.

Based on available knowledge, we hypothesized that roe deer will show different abundances according to the season (H1); Roe deer and wild boar will use different habitats according to refuge and food availability (H2). Regarding the first hypothesis, we predict that roe deer would show a higher abundance in summer due to births but a lower abundance in spring due to increasing dispersal (Debeffe *et al.*, 2013) to new territories out of the study area and higher mortality rates between autumn and spring (Jean-Michel *et al.*, 1993). For the second hypothesis, we predicted that roe deer would use more forest areas, since it is a forest-dependent species (Putman, 1988; Lovari and San José, 1997; Morellet *et al.*, 2011), while wild boar would use more agricultural fields, since it frequently feeds on agricultural lands (Vingada *et al.*, 2010).

2. Material and Methods

2.1. Study area

This study was conducted in Corno de Bico Protected Landscape, located in the municipality of Paredes de Coura, which is included in the Alto Minho region of northwestern Portugal. The study area covers approximately 3000 ha, comprising a sampling grid of 30 squares with 1x1km (Figure 6). The study area has a maximum altitude of 883m asl, and is characterized by an Atlantic climate, with summers having a moderate temperature, while winters have a high precipitation and cold to very cold temperatures, with occasional snow (ICNF, 2020). In fact, the average annual precipitation is between 2000 and 2500mm, with more than 100 days of rainfall per year, while the annual average temperature is 14ºC to 15ºC (Beja *et al.*, 2008). The proximity to the Atlantic Ocean, allows penetration of sea air masses into this region, leading to high levels of humidity, with an average relative humidity of 85% (Beja *et al.*, 2008).

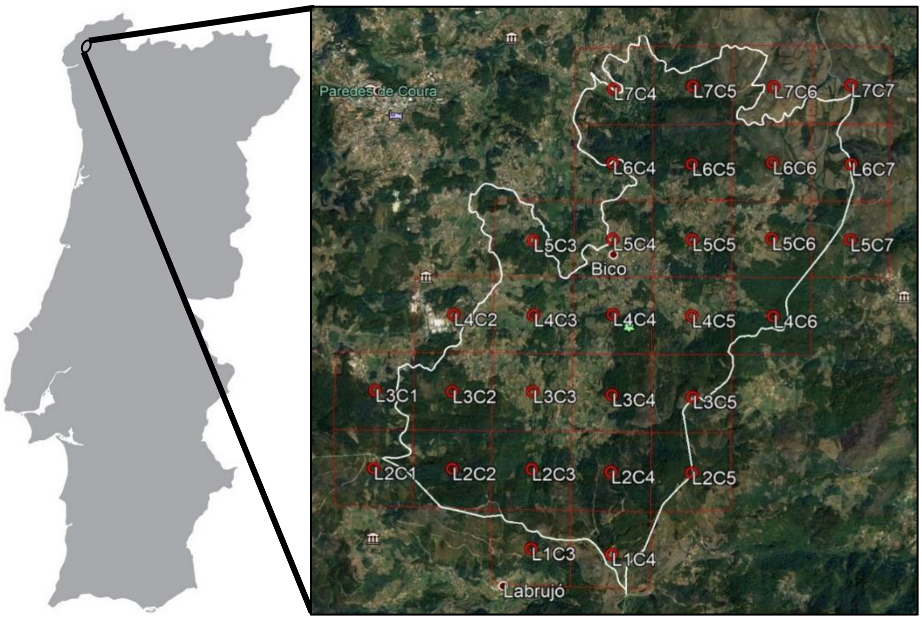

Figure 6 – Location of the study area in northwestern Portugal, including the limits of Corno de Bico Protected Landscape (white line) and the 1x1km sampling grid (red lines). Red circles indicate the center of each square and white numbers indicate the square ID.

Corno de Bico is dominated by a heterogeneous landscape, consisting in 56% of agricultural area (pastures: 19%, arable: 21%, arboreal: 16%), 19% of forested area dominated by oak trees, 17% of natural pastures and scrublands, while 8% of urban and industrial areas or areas without vegetation cover (ICNF, 2018). For this study, we considered only 3 main types of habitat (Oak forest, Shrubland and Agricultural Area) which were attributed to each sampling square based on the most dominant habitat (Figure 7). The study area includes several small urban settlements, and according to the 2011 population census, there were 1935 inhabitants inside the protected landscape, most of them working in the primary sector related to agriculture and livestock production of extensively grazed sheep, cattle and free-ranging horses (Beja *et al.*, 2008; INE, 2012).

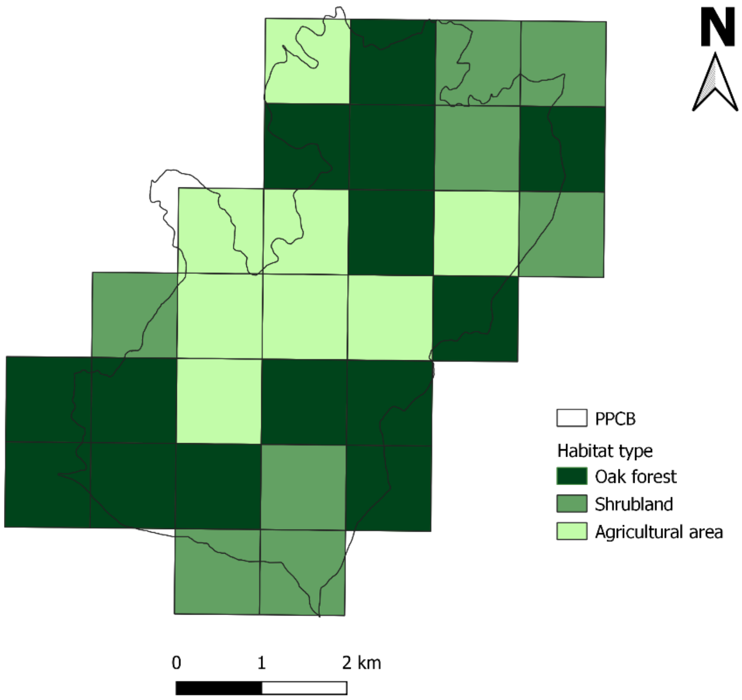

Figure 7 – Habitat distribution in the study area, based on the most dominant habitat type in each sampling 1x1km square.

The study area harbors a high biological richness, with the Protected Landscape of Corno do Bico presenting 25 species considered as conservation priority, including 3 fishes, 4 amphibians, 5 reptiles, 5 birds and 8 mammals (Beja *et al.*, 2008), which justified the classification of this area in the Natura 2000 Network, as site of community importance in the Atlantic biogeographic region (ICNF, 2018). The vegetation cover is predominantly European Oak (*Quercus robur*), with high abundance of European Blueberry (*Vaccinium myrtillus*), Iberian White Birch (*Betula celtiberica*) and Holly tree (*Ilex aquifolium*) (ICNF, 2018). Forest cover is also represented by non-native trees, such as forests of Cedars (*Cupressus lusitanica*) without understory vegetation. The study area has a vast community of colonizing bryophytes such as mosses as well as various lichens (Leiras-do-Carvalhal *et al.*, 2017). Corno de Bico stands out by the presence of several rare plant species, with limited occurrence at national level, such as *Narcissus cyclamineus*, *Bruchia vogesiaca* and *Bryoerythrophyllum campylocarpum* (ICNF, 2018).

Regarding animal species, the study area is important for birds, reptiles and amphibians, as well as for several medium to large sized mammals, including the two study ungulates, wild boar and roe deer, the latter species with a regular occurrence only in the last few decades (ICNF, 2018). This area has excellent habitat conditions also for the Iberian wolf, comprising the main territory of a breeding pack and being important to ensure the connectivity between the wolf population of Peneda-Gerês National Park and the most marginal western populations of this species, such as Serra d'Arga (ICNF, 2018). The local pack, named “Alcateia da Cruz Vermelha”, has an average pack size of 4 adults + 3 pups and after a period of local extinction during late 1990s - early 2000s, it recovered due to the natural incorporation of dispersing individuals and has been reproducing since 2010 (Álvares *et al.*, 2019). The diet of this wolf pack is based essentially on livestock (46% horse, 31% goat and 15% cattle) as the most common available prey, with only 5% corresponding to wild ungulates (3% roe deer and 2% wild boar) (Álvares *et al.*, 2019).

2.2. Data collection

2.2.1. Camera trapping

A 1x1km grid was overlaid in the study area, and in each of the 30 quadrats a camera-trap was placed as much as possible in the center of each square, although in some cases this was not possible due to inaccessibility (see Figure 6 in Study Area description). A total of 30 camera-traps were deployed continuously from mid-August 2019 to mid-April 2020. From the 30 cameras, 20 cameras were brandless (trigger speed: 0,2sec; photo resolution: 8MP; wide view angle: 120º; infrared range: 20m) and 10 were Moultrie M-40i Cam (trigger speed: 0,3sec; photo resolution: 16MP; wide view angle: 80º; infrared range: 24m). During the eight months of sampling, a total of 76,504 files (photographs or videos) were obtained and an average of 210 night-traps per camera was sampled, comprising a total effort of 6302 night-traps. Cameras were put at approximately 40-80cm above the ground without bait (Figure 8) and programmed to take one to three pictures and one video whenever the motion sensor was activated, with a 10sec interval between consecutive events. Each camera was visited 2 times per month to download files and check batteries.. The total number of contacts for each detected species per month in the study area is represented in Appendix 1.

Figure 8 – Example of one camera trap (black arrow) deployed in the study area (Photo: Marta Oliveira).

2.2.2. Linear transects with distance sampling

In each of the 30 quadrats described previously, a line transect with distance sampling was performed to count fecal pellets of roe deer (Figure 9), once per each season with biological relevance for the study species: birth season (spring: may), reproduction (summer: August-September), post-reproduction (autumn: November) and winter dispersal (winter: March). The line transects were performed following Buckland *et al.* (2001) and consisted in counting pellets and measuring its perpendicular distance (cm) to the line transect, recording species, distance from the starting point (m), perpendicular distance (cm), among other factors that we considered important, such as age of the pellet group and number of individual pellets. A group of pellets was defined as a dung group of seven or more pellets of similar shape and age, attributed to a single animal. The line transects were randomly distributed in each quadrat (starting point and direction were defined at random), with a length of 200 meters and maximum width of 5 m to each side. The total distance traveled was 6,000m and were covered 60,000m^2, comprising all main types of habitat (agricultural fields, forest areas and shrubland).

Figure 9 – Example of a linear transect across a shrubland in the study area (Photo: Marta Oliveira).

2.2.3. Fecal collection for DNA extraction

During the performance of the linear transects in the summer sampling season, 68 fecal samples of 5 droppings of roe deer were collected and stored in silica-gel for genetic analysis as a way of determining species and individual identification and, consequently, the abundance of individuals present in the study area. Fecal samples were subsequently sent to CIBIO/InBIO (Research Centre in Biodiversity and Genetic Resources) for genetic analysis carried out by researcher João Queirós.

Briefly, DNA was extracted from 68 faecal samples using the EasySpin DNA Kit (Citomed, Portugal) and E.Z.N.A Tissue DNA Kit (Omega, BIO-TEK GA, United States), respectively, and following the manufacture's recommendations. Sex was determined using the ZFX and SRY primers pairs and following the PCR conditions reported by Matosiuk *et al.* (2014). To identify the species of non-invasive samples a fragment of mitochondrial DNA cytochrome-b gene was amplified using the primer pair GLUDG-L and CB2-R, and following the PCR conditions reported by Palumbi (1991). Successful amplifications were purified using the enzymes exonuclease I and shrimp alkaline phosphatase, and then sequenced with BigDye chemistry (Applied Biosystems), using the GLUDG-L primer and following the BigDye Terminator v3.1 cycle sequencing protocol (Applied Biosystems). Electropherograms were checked and aligned using SEQSCAPE 2.5 (Applied Biosystems).

From the 53 microsatellites that have been used in roe deer studies (Poetsch *et al.*, 2001; Lorenzini *et al.*, 2003; Vial *et al.*, 2003; Randi *et al.*, 2004; Lorenzini and Lovari, 2006; Royo *et al.*, 2007; Mucci *et al.*, 2012; Baker and Rus Hoelzel, 2013; Olano-Marin *et al.*, 2014; Biosa *et al.*, 2015; Horcajada *et al.*, 2018), a total of 16 microsatellites (NVRT16, NVHRT21, NVHRT24, NVHRT48, NVHRT71 (Røed and Midthjell, 1998), Roe01, Roe03, Roe5, Roe6, Roe08 (Fickel and Reinsch, 2000), BM1818, INRA006, (Álvarez *et al.*, 2004; Álvarez *et al.*, 2005), CSSM66 (Røed, 1998), MAF70 (Crawford *et al.*, 1995), ETH225 (Vial *et al.*, 2003), and RT1 (Wilson *et al.*, 1997)) were selected for individual molecular identification based on high polymorphism content and allele size range observed in several European populations.

2.2.4. Clearance plots

Clearance plots were performed in order to evaluate if this sampling method could be applied to this population and to compare results from different methods. Clearance plots were performed following Buckland *et al.* (1993) and located in the surroundings of the linear transects, on a total of 72 plots with a radius of 5m (5,890.5m^2 covered), randomly placed in the 30 quadrats with 1x1km. Plots were permanently marked, according to the area available at each site to be carried out and the cleaning occurred 120 days prior to sampling. Pellets found within a radius of 5 meters were counted and the distance to the central point was recorded.

2.3. Data analysis

2.3.1. Occurrence maps

Occurrence maps were created using the number of independent contacts (minimum of 10 min interval between contacts of the same species) from camera-trap survey (see Appendix 1) and represented at the 1x1km grid previously described. The number of contacts were divided into classes, which were converted into a color gradient with the lightest color representing the smallest-class value, while the darkest color represents the highest-class value. Occurrence maps were produced for each study ungulate (wild boar and roe deer), as well as for wolf, cattle, horses and humans, by using the QGIS (version 3.14). Based on the distribution maps it was possible to analyze the distribution of each species and compare patterns between species. Finally, for roe deer was also recorded opportunistic data regarding visual contacts and known mortalities, by recording the date, sex and geographical coordinates.

2.3.2. Activity patterns

Data obtained from camera-trapping observations was analyzed to estimate the activity patterns of both wild ungulates, wolf, livestock (including goat, cattle and horses), domestic dogs and humans, using the kernel density analysis, which is a non-parametric method (Worton, 1989; Ridout and Linkie, 2009). The overlap of the activity patterns of the different species was calculated by fitting kernel density functions to the temporal activity pattern of the different species (Ridout and Linkie, 2009). The degree of overlap between the two kernel density functions was calculated and corresponds to the area between the two kernel density curves. The overlap value ranges from 0 (no overlap) to 1 (complete overlap). The confidence intervals of the overlap were estimated using bootstrap. The statistical analyses were performed using R 3.5.0 (Team, 2013) with the package "overlap" (Meredith and Ridout, 2014).

2.3.3. Abundance

The software DISTANCE 7.3 (Thomas *et al.*, 2010) was used to analyze the pellet counts from linear transects. This method assumes that all groups of pellets located on the line (perpendicular distance = 0 cm) are detected with 100% certainty and calculates the detection probability as the distance to the central line increases. To remove overdispersion and increase model fitting, measurements were truncated at 4m. In the sampling with linear transects, an observer counts the number of objects (fecal samples) while crossing a line with a random start and direction of predetermined length *L* (Buckland *et al.,* 1993). The perpendicular distance of each fecal sample from the linear transect is recorded. When performed the stipulated distance, the abundance of objects in the sampled area (*D* in objects per square meter) is estimated according to Buckland *et al.* (1993) as: $\widehat{D} = \frac{n \times \hat{f}(0)}{2L}$.

The software package considered six models when generating separate $f(0)$ estimates for each site: uniform-cosine, uniform-simple polynomial, half normal-hermite, half normal-cosine, hazard rate-cosine and hazard rate-simple polynomial (Buckland *et al.*, 1993). To choose the model that best fits our data, we rely on the combination of low Akaike's information criterion (AIC), a low variance and nonsignificant goodness-of-fit value (Marques *et al.*, 2001). The density of animals per km^2 was estimated by dividing the density of pellet groups by the decay and defecation rates (Marques *et al.*, 2001). Since there are no data on the defecation rate of this population and on the decay rate in this study area, we used as defecation rate 23.4 ± 6.5 (Mitchell *et al.*, 1985) and decay rate of 164.5 ± 15.5 (Tsaparis *et al.*, 2009). ANOVAs were used to test the effects of season on animal abundance and the effects of season on abundance averages (calculated using DISTANCE). Using the DISTANCE software, we calculated the coefficient of variation (Marques *et al.*, 2001).

2.3.4. Habitat use

Generalized linear models with a Poisson distribution and a log link function were fitted to camera-trapping contacts to access the influence of environmental variables on the number of animals. The environmental variables considered were distance to agricultural fields, to forest and to road, elevation, slope, northness and eastness, and also the presence of domestic ungulates and humans. Although initially considered as environmental variables, distance to urban areas was not included due to collinearity with distance to agricultural areas, while the distance to water was not included due to the lack of accurate information on the hydrological network that fits the scale of the study area, as only major rivers were available. The statistical analyses were performed using IBM.SPSS, version 23. The results were expressed as mean ± standard error (SE), unless otherwise stated. *P* values lower than 0.05 were considered significant.

3. Results

3.1. Species occurrence

In total, we obtained 866 contacts of roe deer and 1677 contacts of wild boar by camera-trapping (see Appendix 1 and 2 for more information). Based on camera-trapping contacts, the results show that roe deer and wild boar are widely distributed, being present, respectively, in 26 (87%) and 28 (93%) of the 1x1km quadrats comprising all study area (Figure 10). For roe deer and wild boar, there are two quadrats that had zero contacts for both species, which are in fact the quadrats where a higher presence of horses (Figure 11B) and humans (Figure 12B) was recorded.

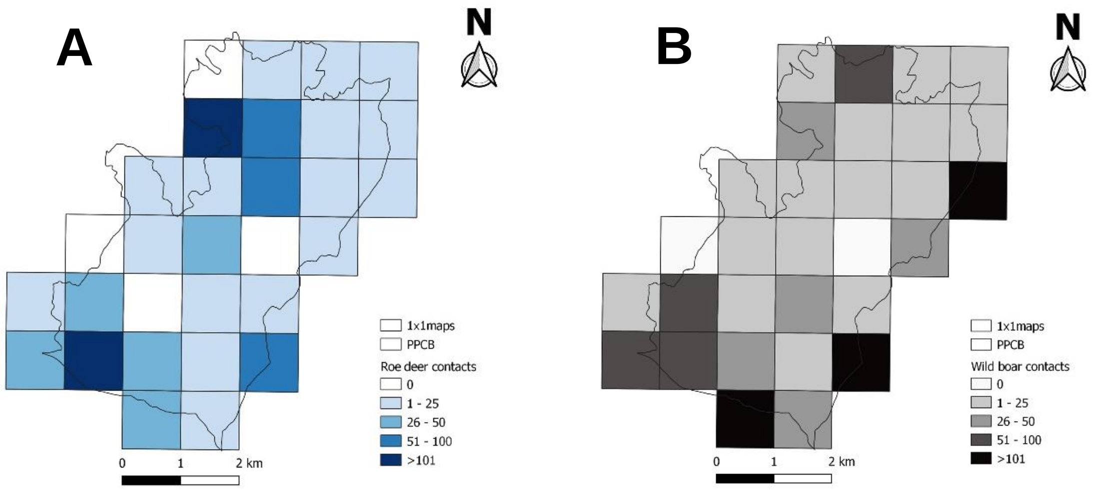

Figure 10 – Detected presence of roe deer (A) and wild boar (B) in the study area based on camera-trapping contacts per 1x1km square.

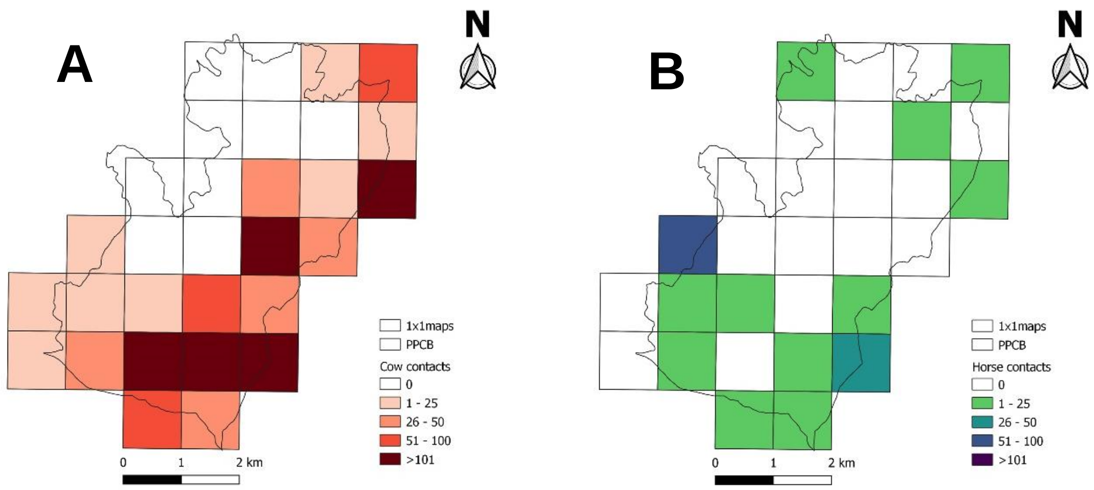

Figure 11 – Detected presence of cattle (A) and horses (B), as potential competitors, in the study area based on camera-trapping contacts per 1x1km square.

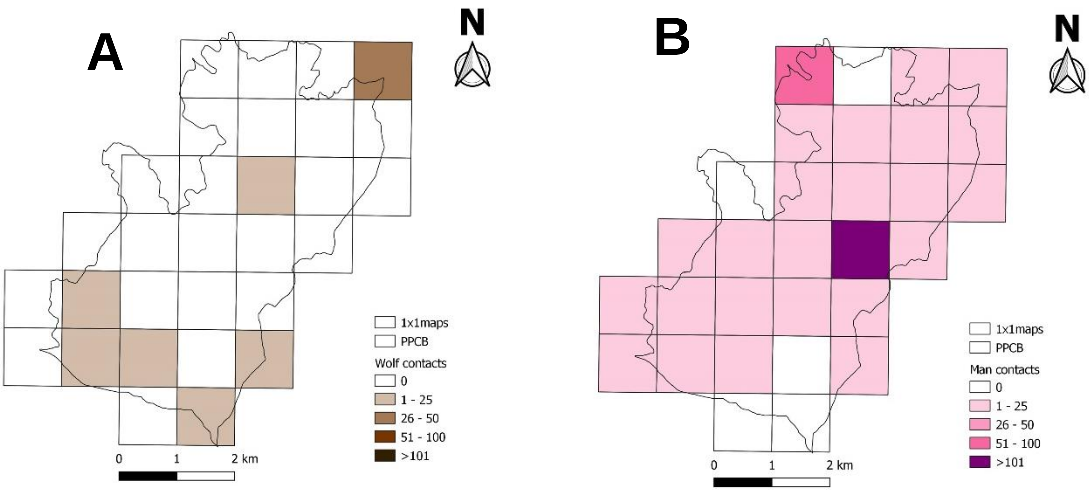

Figure 12 – Detected presence of wolves (A) and humans (B), as potential predators, in the study area based on camera-trapping contacts per 1x1km square.

In terms of species occurrence per habitat type by comparing the distribution maps with the habitat map (see Figure 7 in Study Area description), the results from camera-trapping surveys showed that quadrats dominated by oak forest, which represents around 47% of the study area, is the habitat with more contacts by roe deer (79.6%) and cattle (53%). Shrubland was the habitat type with more contacts of wild boar (50.6%), horses (67.5%) and wolf (67.4%). In agricultural areas, despite representing 26.7% of the study area, only a total of 7.5% contacts of roe deer and 2.6% contacts of wild boar were detected (Figure 13) and 87.9% of the human contacts were in this habitat type.

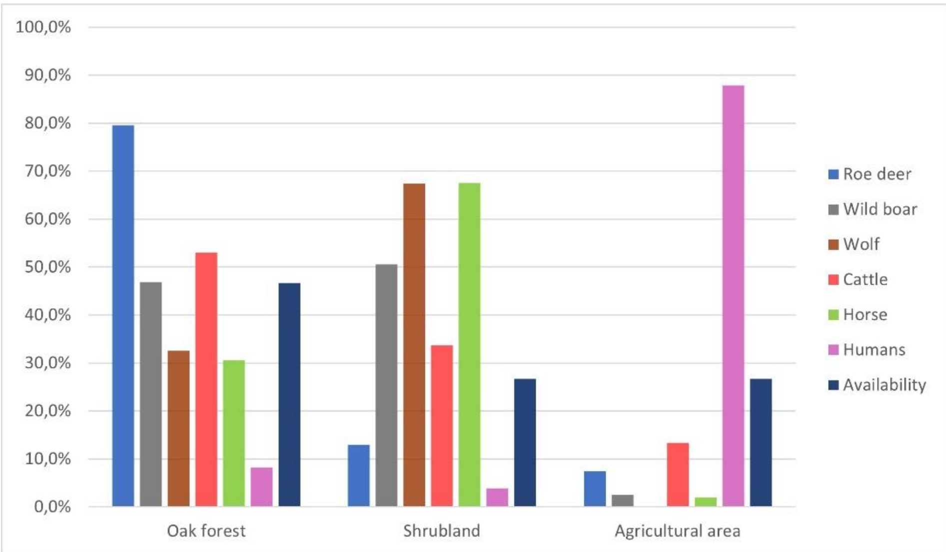

Figure 13 – Percentage of contacts for each species per habitat type considering 1x1km grid in the study area.

3.2. Habitat use

Regarding the habitat use by roe deer, the results show that this species selects areas near or inside oak forests (β=-0.783) and avoids the proximity to agricultural areas (β=1.231). This species also showed avoidance for the proximity to roads (β=0.387), high elevations (β=-0.958) and high slopes (β=-0.065). Roe deer also showed a higher use of areas facing south (β=-0.060) and east (β=0.064) (Table 1).

Table 1 – Generalized linear model (GLM), with a Poisson distribution, of the effect of environmental variables on the habitat used by roe deer, based on camera-trapping contacts (number of animals as response variable). SE is the standard error of the model coefficient and β is the coefficient.

	β	SE	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	2.745	0.0041	2.737	2.753	457991.596	1	<0.001
Distance to							
Agricultural fields	1.231	0.0057	1.219	1.242	46879.412	1	<0.001
Forests	-0.783	0.0093	-0.802	-0.765	7157.785	1	<0.001
Roads	0.387	0.0029	0.382	0.393	17982.784	1	<0.001
Elevation	-0.958	0.0065	-0.971	-0.945	21542.365	1	<0.001
Slope	-0.065	0.0031	-0.071	-0.059	433.852	1	<0.001
Northeness	-0.060	0.0031	-0.067	-0.054	374.894	1	<0.001
Eastness	0.064	0.0032	0.058	0.071	401.679	1	<0.001

Considering the co-occurrence of domestic ungulates and humans, the results showed that roe deer tend to avoid areas with high presence of humans (β=-0.015) and high presence of horses (β=-0.041) but use the same areas than cattle (β=0.003) (Table 2).

Table 2 – Generalized linear model (GLM), with a Poisson distribution, of the influence of domestic ungulates and humans on the habitat used by roe deer, based on camera-trapping contacts (response variable). SE is the standard error of the model coefficient and β is the coefficient.

	β	SE	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
Intercept	3.242	0.0037	3.235	3.250	749011.011	1	<0.001
Cattle	0.003	0.00003	0.003	0.003	9024.923	1	<0.001
Horse	-0.015	0.0002	-0.016	-0.015	4702.009	1	<0.001
Human	-0.041	0.0005	-0.042	-0.040	6849.812	1	<0.001

In relation to the habitat used by wild boar, the results show that this species select areas near or inside oak forests (β=-0.608), avoids the proximity to agricultural areas (β=0.430) and avoids the proximity to roads (β=0.267). This species uses more areas at higher elevations (β=0.097), higher slopes (β=0.490) and areas facing north (β=0.811) and east (β=0.424) (Table 3).

Table 3 – Generalized linear model (GLM), with a Poisson distribution, of the effect of environmental variables on the habitat used by wild boar, based on the camera-trapping contacts (response variable). SE is the standard error of the model coefficient and β is the coefficient.

	β	SE	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	3.220	0.0030	3.214	3.226	1185076.032	1	<0.001
Distance to							
Agricultural fields	0.430	0.0037	0.422	0.437	13601.989	1	<0.001
Forests	-0.608	0.0040	-0.616	-0.600	23152.304	1	<0.001
Roads	0.267	0.0023	0.262	0.271	12918.489	1	<0.001
Elevation	0.097	0.0042	0.088	0.105	528.412	1	<0.001
Slope	0.490	0.0025	0.485	0.495	38020.348	1	<0.001
Northeness	0.811	0.0028	0.805	0.816	81553.983	1	<0.001
Eastness	0.424	0.0024	0.419	0.429	32049.931	1	<0.001

The analysis of habitat use by wild boar show, as for roe deer, an effect of the presence of domestic ungulates (cattle and horses) and humans in the study area (Table 4). Wild boar tends to avoid areas with high presence of humans (β=-0.010) and horses (β=-0.038) but use areas similar to cattle (β=0.008).

Table 4 - Generalized linear model (GLM), with a Poisson distribution, of the influence of domestic ungulates and humans on the habitat used by wild boar, based on the camera-trapping contacts (response variable). SE is the standard error of the model coefficient and β is the coefficient.

	β	SE	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi-Square	df	Sig.
Intercept	3.228	0.0031	3.221	3.234	1068171.634	1	<0.001
Cattle	0.008	0.00003	0.008	0.008	82471.890	1	<0.001
Horse	-0.038	0.0002	-0.039	-0.038	38115.735	1	<0.001
Human	-0.010	0.0003	-0.011	-0.010	1319.409	1	<0.001

3.3. Activity patterns

Roe deer and wild boar show a marked activity pattern in this study area. Roe deer is a crepuscular species, with peaks of activity at sunrise and sunset, while wild boar is markedly nocturnal, where its activity starts right after the sunset until slightly before the sunrise (Figure 14).

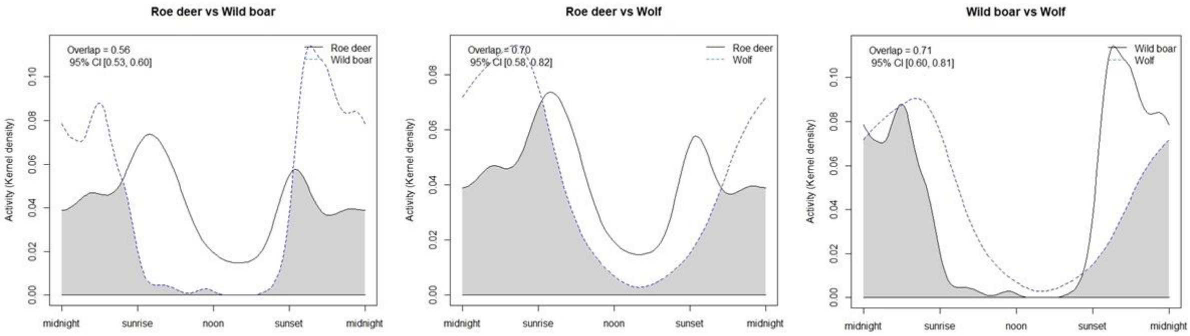

Figure 14 – Activity pattern of roe deer, wild boar and wolf, and their overlap two by two (grey area) based on camera trapping.

Iberian wolf show a peak of activity right before the sunrise and, overall, has a higher time of activity, comparatively with the wild prey species of this study. Considering the activity overlap, the level of overlap between roe deer and wild boar (56%), was lower than their overlap with the Iberian wolf (around 70% for both wild ungulates) (Figure 14).

Regarding the activity patterns of domestic animals and humans, they are markedly diurnal with most of the activity during the morning and afternoon, in opposite to wild animals (Figure 15). It is important to note that at midday both domestic animals and humans reduce their activity.

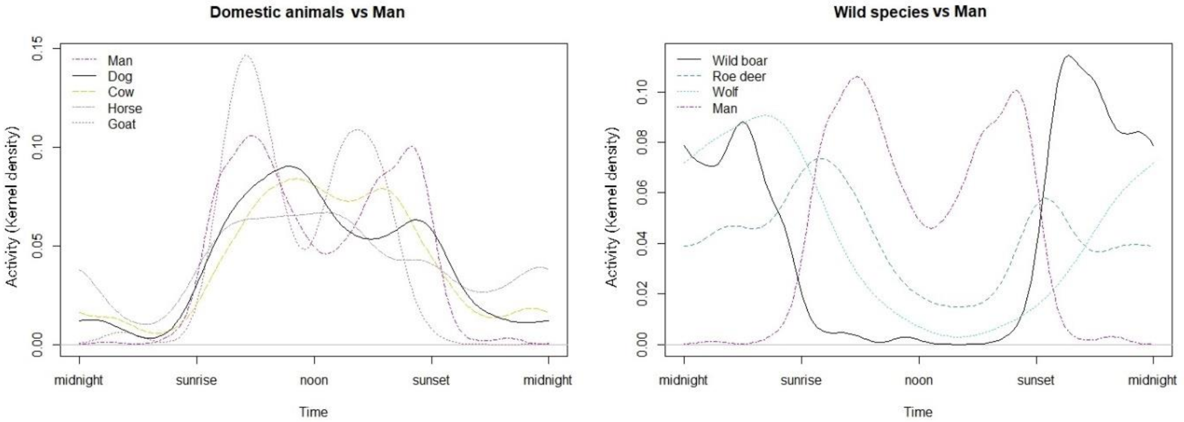

Figure 15 – Activity patterns of domestic animals and humans (on the left) and wild species and humans (on the right) based on camera trapping.

3.4. Roe deer abundance and population estimates

Based on the data collected using linear transects (see Appendix 3), and after model selection, the detection function with the best fitting was a half normal function, resulting in the histogram of distances represented in Figure 16.

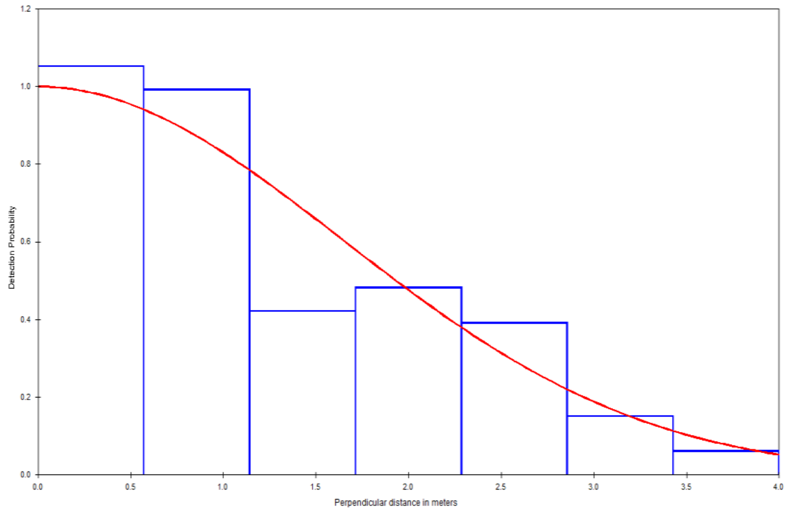

Figure 16 – Histogram of the data using 7 distance categories in perpendicular distance of linear transects. A half-normal detection function (–) fitted to the ungrouped data with w = 4 m, is shown and was used as basis for final inference from these data.

The results obtained from the distance analysis in linear transects showed a higher estimate abundance in summer (15 individuals) and a lower estimate abundance in spring (10 individuals) in the study area (Table 5). Average densities indicate that the roe deer population in the study area has an estimated number of 13 individuals, corresponding to a mean density of 0.42 roe deer/km^2 for all study area.

Table 5 –Estimates of roe deer abundance for each seasonal sampling and for the total sampling period. (D= density per km^2; N=number of individuals; Model: Half-normal/Cosine; see Method section for further details).

		Estimate	%CV	df	95% Confidence Interval	
Spring	D	0.33119	47.26	82.43	0.13559	0.80897
	N	10.0000	47.26	82.43	4.00000	24.0000
Summer	D	0.50212	50.55	69.86	0.19392	1.3002
	N	15.0000	50.55	69.86	6.00000	39.0000
Winter	D	0.42734	42.19	120.83	0.19175	0.95236
	N	13.0000	42.19	120.83	6.00000	29.0000
Total	D	0.42022	36.91	83.78	0.20647	0.85526
	N	13.0000	36.91	83.78	6.00000	26.0000

From the 68 fecal samples collected for genetic analysis at CIBIO/InBIO, 61 (success rate of 90%) were amplified for a mitochondrial marker, allowing their identification as roe deer. Moreover, 25 (41%) out of these 61 samples were amplified for the microsatellite markers, allowing the identification of 13 different individuals, including five females, seven males and one of unknown sex. The number of times each individual was detected in the fecal sampling varied between one and seven, although none of these individuals have been recaptured in more than one 1x1km quadrat (Figure 17; Appendix 4).

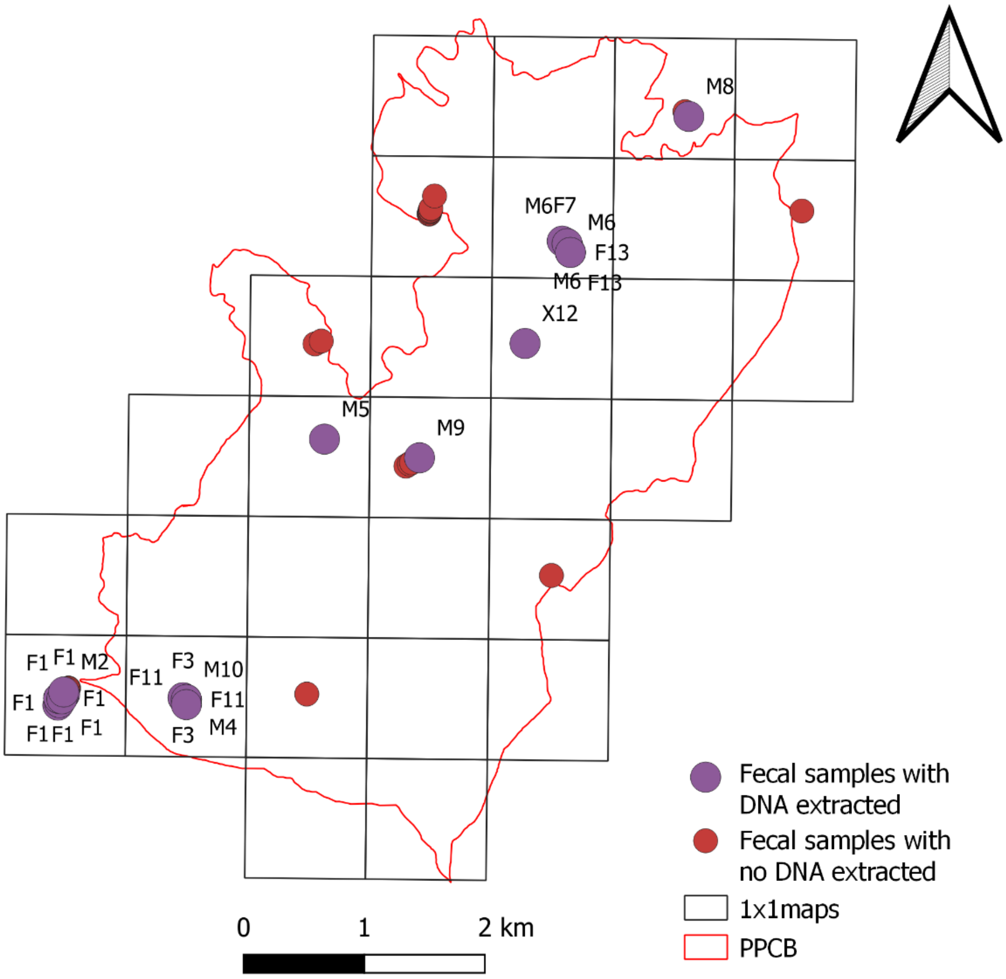

Figure 17 – Location of the fecal samples of roe deer collected for genetic analysis in the study area, including samples without success on DNA extraction (in red) and samples successful on DNA extraction for individual identification (in purple), with reference to the individual ID and sex (F: females; M; males).

Based on the maximum number of different individuals detected by camera-trapping on a single night of sampling, it was possible to estimate roe deer population in 15 individuals.

Unfortunately, clearance plots proved to be useless in this study area, with only 4 (6%) out of 72 clearance plots having fecal pellets deposited during the surveyed period (see

Appendix 5). Considering such low number of detections, no density estimate was performed based on this method.

3.4.1. Comparison of sampling methods

To assess the suitability and accuracy of the methods used for detection and population estimates of wild ungulates, a brief comparison was made considering the number of sampling points that obtained detection of roe deer and wild boar for each of the three methods: clearance plots, linear transects and camera trapping. The method with the most detection points is camera trapping, with 26 quadrats for roe deer and 28 quadrats for wild boar, followed by transects, with 19 points for roe deer and 13 points for wild boar, and clearance plots, with 4 points for roe deer and 0 for wild boar (Figure 18). Clearance plots proved to be inefficient for the study area, as only 4 points with roe deer were detected.

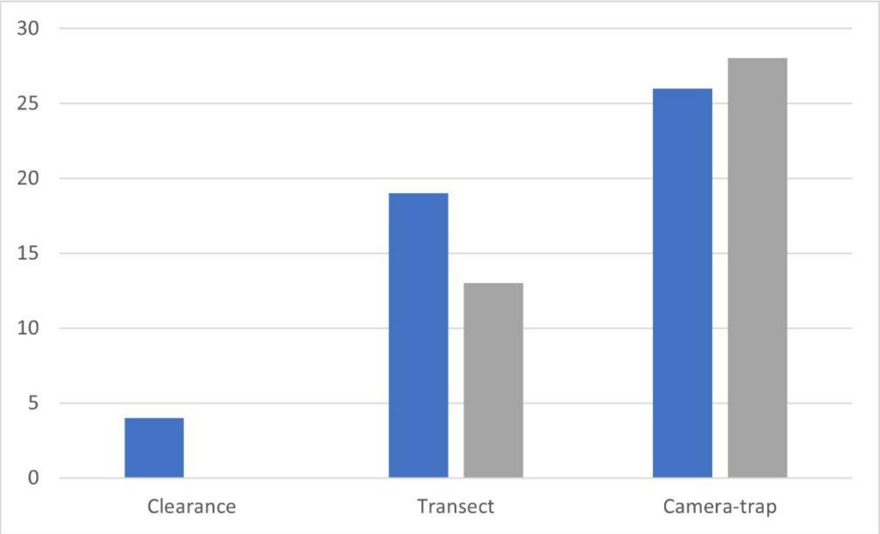

Figure 18 – Number of sampling points with each ungulate species detected by method type. Roe deer is represented in blue ● and wild boar is represented in grey ●.

Regarding population estimates of roe deer resorting to different methods, we obtained high consistency in the results, with 13 individuals based on non-invasive genetic sampling, 13 individuals based in linear transects and 15 individuals based on camera-trapping data.

3.5. Opportunistic data

During the fieldwork conducted in this study, it was possible to detect 8 individuals of roe deer by direct observation, including 4 males, 2 females and 2 with unknown sex, which comprised 7 adults and one fawn (Table 7). However, no wild boar was detected by direct observation in the study area.

Table 6 – Information regarding visual contacts of roe deer in the study area.

Individuals	Date	Sex	Coordinates
1 adult	22/06/2019	Male	41.870653, -8.518077
1 adult	06/07/2019	Female	41.866661, -8.549926
2 adults	02/01/2020	unknown	41.891779, -8.504539
1 adult	04/07/2019	Male	41.890661, -8.495626
1 adult	23/12/2020	Male	41.895579, -8.515335
1 fawn	19/06/2020	Female	41.914157, -8.546533
1 adult	24/03/2020	Male	41.935031, -8.504935

During this study, were also detected 2 mortality records of roe deer (Table 8) in the study area. One of the deaths was an adult female that died by car collision in an area where she was kept with her young (based on camera-trap). The other individual, an adult male, died by drowning in an artificial pond with plastic margins, unsuitable for animals to access.

Table 7 – Information regarding roe deer known mortality in the study area.

Individual	Date	Sex	Coordinate	Cause of death
1 adult	10/10/2019	female	41.878104, -8.558048	Car collision
1 adult	20/01/2020	male	41.914171, -8.505841	Drowning

4. Discussion

Overall, the findings in this study provide valuable knowledge on population estimates, activity patterns and habitat selection of two wild ungulates in a heterogeneous landscape, a topic with high relevance for a proper wildlife management. In particular, the methodological approaches employed here enable to respond to the main objectives of this project, namely to estimate abundances of roe deer and to identity which environmental and human-related factors influence the occurrence and habitat use of roe deer and wild boar. Furthermore, the multi-method approach allowed a compassion in the efficiency and reliability of each method to monitor populations of wild ungulates in northwestern Portugal, which is crucial given their socio-economic and ecological implications.

Roe deer and wild boar use the entire protected area with preference for forest areas, contrary to what was expected by one of our hypotheses. In fact, we expected a higher use of agricultural fields by wild boar as documented in previous studies (Dardaillon, 1986; Fonseca, 2008; Fernanda Cuevas *et al.*, 2013), however our results showed a higher use of oak forests, probably due to the high availability and suitable resources (particularly for refuge) of this habitat type in our study area. As roe deer is a forest-dependent animal (Putman, 1988; Lovari and San José, 1997; Morellet *et al.*, 2011), it is possible to conclude that its wide distribution in Corno do Bico protected area is related to the landscape composition, where forest is the dominant habitat type present in most quadrat, providing the necessary food and refuge for roe deer survival.

In this study, roe deer showed avoidance of areas with higher elevation, probably because lower areas are more dense in terms of vegetation, constituting more protected refuges and with higher humidity (San José *et al.*, 1997). The avoidance for higher slopes may also be linked to protection and security, as in the previous explanation, however it can also be explained by the difficulty to escape from possible predators in these areas. The avoidance of slopes facing north and west, may be related with thermal protection but also plant phenology, since those areas have lower temperatures and as consequence delayed phenology (Chakrabarti *et al.*, 2011). Furthermore, since the slopes facing north do not receive as much sunlight as the rest of the areas and to the west the study area receives masses of oceanic air from the Atlantic Ocean, the thermal hypothesis seems to fit our findings. However, although contradicting our results, is important to note that areas facing north and east may have greater food availability, since there is a higher abundance of grasses and forbs (Baber and Coblentz, 1986) due to lower sun exposure, promoting nitrogen availability and consequently its growth (Wilson and Wild, 1991). Regarding the influence of the human-related factors, the results point out to an avoidance of agricultural areas and roads and, consequently, avoidance of areas with higher human presence and activity, which is in accordance with previous studies (Hewison *et al.*, 2001; Bonnot *et al.*, 2013; Oberosler *et al.*, 2017; Wevers *et al.*, 2020). Despite the activity patterns of wild ungulates being almost opposite from domestic animals and humans, the prevalence of some diurnal activity by roe deer seem to be associated to the secure conditions provided by forest areas (Bonnot *et al.*, 2013), in opposition to other areas more exposed to anthropogenic pressures.

The higher use of forest habitats by wild boar, although contrarily to the expectations, may have several explanations, including the wide extent of oak forests and consequent abundance of acorns, which is an important food item for this species, particularly in some populations (Fournier-Chambrillon *et al.*, 2014). Another aspect to consider is that in this landscape, although there are numerous agricultural fields, few are used for farming, and only those closest to the villages are cultivated, while the others are used as pastures for livestock, resulting in low food availability for wild boar, and high human disturbance. This avoidance of human disturbance can also explain the absence of diurnal activity by wild boar in our study area, as also documented in other human dominated landscapes (Ohashi *et al.*, 2013), and can also explain the avoidance by roads, which also represent anthropogenic pressure. In our study area, wild boar avoided low elevations, reduced slopes and areas facing south and facing west. Some of these results are contrary to other studies, where wild boar presented a preference for low elevations and reduced slopes (Baber and Coblentz, 1986). The avoidance of areas facing south and west can be explain by the higher abundance of grasses and forbs on north and east slopes (Baber and Coblentz, 1986).

Roe deer and wild boar has a marked activity pattern in this study area, with roe deer being a crepuscular species and wild boar being a markedly nocturnal. This behavior by roe deer was also documented by Cederlund (1989) and Pagon *et al.* (2013). Wild boar activity patterns seem to differ depending on the level of human presence. In an area free from hunting, wild boar showed mostly a nocturnal activity pattern but with some activity also during daylight, and this was the results of the absence of human activity during daylight in the studied area (Russo *et al.*, 1997). The wild boar activity is influenced by human activity, i.e., sites or time of the day with more activity by humans are avoided by the wild boar (Russo *et al.*, 1997; Ohashi *et al.*, 2013) and this can explain their activity pattern in our study area as well as the avoidance of areas with high anthropogenic activity. Another factor that significantly influences the activity patterns of wildlife and indirectly linked to humans is free-ranging livestock, which often occupies the same territories than wild animals (Pudyatmoko, 2017). Accordingly, the absence of wild ungulates from livestock-occupied territories (de Leeuw *et al.*, 2001; Pudyatmoko, 2017) was also detected in our study area, with both roe deer and wild boar avoiding places with high activity of livestock and humans.

Regarding the comparison in the suitability of different methods to estimate roe deer density in Corno de Bico, our results showed wide consistency in terms of the number of roe deer estimated by each method. The expectations in terms of the abundance fluctuations across seasons as already previously described (Romani *et al.*, 2018) was supported by the results, with the lowest abundance observed in spring, and the highest abundance in summer, which can be explained by the birth season that occurs between May and June. Regarding the obtained population estimates of roe deer, line transects with distance sampling estimated a mean abundance of 13 individuals in the study area, while the results from molecular analysis proves the existence of at least 13 individuals, which corroborates the results obtained using fecal pellet counts with distance sampling, indicating its suitability to estimate roe deer abundance in this type of environment. However, molecular analyses only allowed the genetic identification of individuals in 7 quadrats and fail to extract DNA from fecal samples collected in 5 additional quadrats where this species was detected by camera-trapping, which considering the obtained short distance between recaptures of genetically identified individuals may suggest to an underestimation of the roe deer population based on non-invasive genetics. Furthermore, in the analysis of fecal pellet counts with distance sampling data, the used decay rates were based on reported values from bibliography due to the lack of decay rates specific for our study area, which may cause an over or underestimation of the population estimates. On the other hand, the clearance plots proved to be inefficient in the study area since insufficient data were obtained to make any estimate, probably due to the low density of animals in the study area. In fact, clearance plots are proven to provide very accurate density estimates, mostly for not requiring disappearance rates, which are a major source of bias due to difficulty to obtain (Van Etten and Bennett Jr, 1965; Neff, 1968). The accuracy of line transects and clearance plots has already been assessed for red deer in a Mediterranean environment, and the results obtained were similar to ours (Alves *et al.*, 2013).

Density estimates of wild animals using line transects with distance sampling has been regularly used, both with direct observations or fecal counts (e.g. Marques *et al.*, 2001; Calambokidis and Barlow, 2004; Acevedo *et al.*, 2007; Zerbini *et al.*, 2007; Acevedo *et al.*, 2008), turning its suitability for population estimates unquestionable in most conditions. However, considering the costs and benefits in terms of estimating species richness and occurrence, camera-trapping proved to be more appropriate, as it allows a rapid and wide sampling in any type of environment and weather (Silveira *et al.*, 2003). Accordingly, in this study the camera-trap proved to be the more efficient method, since it enables to confirm the presence of roe deer and wild boar in quadrats where no fecal pellets were encountered in line transects.

Regarding the results from the molecular analysis of fecal pellets, it is possible to verify a high consistency with the results from the other methods employed, namely linear transects and camera trapping. In fact, as widely recognized, non-invasive genetic sampling constitutes a reliable method to assess population parameters, particularly for rare and elusive species (Beja-Pereira *et al*., 2009). However, and considering the high cost associated with molecular techniques, and the fact that the samples used were fecal pellets detected along the line transects with distance sampling, the additional laboratory procedure needed did not prove to be necessary to increase the accuracy of population estimates in this particular case study, although it allowed to identify the sex of individuals. It is important to highlight that this conclusion cannot be drawn to other species or study areas, where environmental conditions may decrease the accuracy and bias of distance sampling estimates (Buckland *et al.*, 1993). Moreover, the use of distance sampling in combination with camera-trapping may be a adequate approach not only to estimate populations, but also to obtain additional information in terms of sex ratio, age structure and even behavior of wild populations of ungulates (Romani *et al.*, 2018).

5. Conclusions and management implications

The main objectives of this study were to evaluate the habitat use and activity patterns by roe deer and wild boar, and how different environmental characteristics may influence these parameters, as well as to perform a multi-method approach to estimate the abundance of roe deer, by focusing Corno de Bico Protected Landscape as a study area. Overall, we found that that roe deer and wild boar are widely distributed in the study area, and both species showed a preference for forest areas, while avoiding agricultural lands. We also found a strong influence of anthropogenic activities (e.g. livestock and human presence) on habitat selection and activity patterns of both roe deer and wild boar. Finally, population estimates of roe deer were widely coherent between different methods, with estimated 13 individuals based on linear transects with distance sampling and non-invasive genetic analysis, and 15 individuals through camera-trapping. Considering the results obtained, we can conclude that case studies, such as this one, provide useful information for the conservation and management of roe deer and wild boar in heterogeneous landscapes with a high anthropogenic pressure, a topic with important implications for the conservation of their main predator, the Iberian wolf.

Regarding the comparison between methods to estimate roe deer density, DNA sampling is the only one that provided unequivocal results on species and sex identification (Beja-Pereira *et al.*, 2009). However, this approach is highly dependent on the sampling scheme of fecal samples, which may affect its accuracy and precision. To allow better accuracy and precision of the molecular approach, a higher number of fecal samples with DNA extraction success would be necessary to calculate individuals accumulation curves, and so estimate more accurately the number of individuals of the population (Beja-Pereira *et al.*, 2009). In turn, linear transects of fecal counts with distance sampling, provide not only density estimates but also measures of accuracy, which clearly indicates the reader on the minimum and maximum number of roe deer present in the study area, without further surveys. One of the main constrains of this method is the need for local decay rates, which may have a huge influence on the density estimates(Buckland *et al.*, 1993; 2001). Even so, the results obtained from this method showed coherence with the results obtained by the other methods, confirming its suitability for the roe deer estimates in the study area.

Despite the protection status and biological relevance of our study area, several human activities were recorded during the field work, including hunting, forestry and recreational activities. Regarding recreational activities, it frequently involved people with no care for the need to keep this landscape preserved, for example riding motorcycles on wildlife trails or making loud noise, which can represent an added impact on wild ungulates, and all wildlife in general. The efficient conservation of suitable habitats occupied by roe deer and wild boar, implies a better conciliation between wildlife purposes and human activities, which is usually quite difficult to achieve (Corradini *et al.*, 2021).

Corno do Bico protected area would benefit of clear and explicit indications and outdoors explaining to local communities the importance of preserving nature and all the ecosystem services associated to it. Besides awareness, also a strong vigilance and penalties for unappropriated human behavior or activities within the protected area would be advisable. In fact, and considering our results on habitat use and circadian activity, a reduction of human activity at night in the natural patches of the landscape, particularly in forest areas, may help to reduce disturbance on both wild ungulates. Our findings also highlight the need for several management actions to increase the local population of roe deer, namely: i) the promotion of natural vegetation cover, particularly oak forests, either by managing scrublands or planting wide areas with seedlings, as it increases the availability of suitable habitat for wild ungulates; ii) a proper livestock management to prevent free-ranging husbandry systems of cattle and horses in certain areas with oak forest, and iii) reducing causes of human-related mortality, such as measures to prevent traffic collisions and drownings in artificial water tanks with slippery plastic edges. Finally, further studies on socio-ecological analysis of the human-wildlife conflict would be very beneficial to promote biodiversity preservation on this protected landscape, but also to gather the necessary knowledge to stablish a suitable management plan for this region, respecting its social and cultural identities, without compromising their natural values.

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7. Appendixes

Appendix 1 – Total number of contacts by camera-trapping for each species per month in the study area, enhancing roe deer and wild boar (months in which offspring were registered are marked with *).

Contacts/month	**ago**	**set**	**out**	**nov**	**dez**	**jan**	**fev**	**mar**	**abr**	**Total**
Roe deer	**118***	**100***	**126**	**163***	**97**	**54**	**62**	**98**	**48**	**866**
Wild boar	**93***	**295***	**465***	**178***	**208***	**174***	**96**	**128**	**40***	**1677**
Wolf	1	6	2	3	6	9	5	10	4	46
Human	126	195	157	147	94	36	18	89	114	976
Cattle	85	338	273	160	93	105	119	149	83	1405
Goat	9	32	7	1	1	2	1	6	5	64
Sheep		7			1	8	2	6	1	25
Horse	15	14	31	38	23	14	39	25	7	206
Badger			2	1		2	4	1	1	11
Domestic cat	5	5	38	25	18	15	8	8	21	143
Domestic dog	6	10	18	8	19	9	10	4	11	95
Beech marten	6	1	14	11	9	4	7	10	3	65
Common Genet	11	18	15	6	10	9	15	16	4	104
Red Fox	121	161	228	104	114	115	165	147	100	1255
Weasel			1		1					2
Hedgehog		2	3	1			1	3	2	12
Squirrel		2	6							8
Rodentia			7				1		1	9
Other			2	1	3	1		2	1	10
Total	596	1186	1395	847	697	557	553	702	446	**6979**

Appendix 2 – Total number of contacts by camera-trapping for each species per sampling point and habitat type.

ID		Cattle	Horse	Human	Roe deer	Wild boar	Wolf	Grand Total
L1C3	Shrubland	69	6		46	301		422
L1C4	Shrubland	39	20		10	31	2	102
L2C1	Oak forest	10		7	50	72		139
L2C2	Oak forest	37	4	1	108	96	1	247
L2C3	Oak forest	128		8	47	42	2	227
L2C4	Shrubland	110	14		14	19		157
L2C5	Oak forest	335	46	1	75	242	5	704
L3C1	Oak forest	5		1	12	4		22
L3C2	Oak forest	17	2	5	28	64	1	117
L3C3	Agricultural area	2	2	8		1		13
L3C4	Oak forest	99		21	11	41		172
L3C5	Oak forest	31	11	3	8	6		59
L4C2	Shrubland	16	78	9				103
L4C3	Agricultural area			1	3	4		8
L4C4	Agricultural area			1	31	16		48
L4C5	Agricultural area	164		792				956
L4C6	Oak forest	40		6	17	48		111
L5C3	Agricultural area				2	1		3
L5C4	Agricultural area			1	15	1		17
L5C5	Oak forest	33		13	83	12	6	147
L5C6	Agricultural area	21		4	14	19		58
L5C7	Shrubland	150	7	12	20	450		639
L6C4	Oak forest			1	166	38		205
L6C5	Oak forest			11	55	24		90
L6C6	Shrubland		1	5	16	20		42
L6C7	Oak forest	10		2	25	19		56
L7C4	Agricultural area		2	51		1		54
L7C5	Oak forest				4	78		82
L7C6	Shrubland	6		1	2	8		17
L7C7	Shrubland	83	13	11	4	19	29	159
Grand Total		1405	206	976	866	1677	46	5176

Appendix 3 – Resume of number of detections of roe deer (CC) and wild boar (SS) in linear transects per sampling season.

	1st season		2nd season		3th season		4th season		Total
	CC	SS	CC	SS	CC	SS	CC	SS	
L1C3	1			1					2
L1C4	1						2		3
L2C1	2	2	15		1		6		26
L2C2	3	3	5	2			6	1	20
L2C3			1				1		2
L2C4									0
L2C5				3			2	3	8
L3C1				1			1		2
L3C2		2		1	2	2			7
L3C3									0
L3C4						1	1		2
L3C5	8		1				1		10
L4C2				1					1
L4C3			1						1
L4C4	5		4	2	1		5	1	18
L4C5									0
L4C6							2		2
L5C3			1		3				4
L5C4									0
L5C5									0
L5C6									0
L5C7									0
L6C4	5	1	11	8	4		5	1	35
L6C5	1		5				1		7
L6C6				1					1
L6C7							7		7
L7C4									0
L7C5					1				1
L7C6	5		3	2	7	2			19
L7C7		1							1

Appendix 4 – Results from DNA analysis for individual and sex identification in fecal samples of roe deer, comprising 13 different individuals.

Transect	Latitude	Longitude	Micros indiv ID	Sex indiv
L2C1	41.868706	-8.561596	**1**	**F**
L2C1	41.868507	-8.561757	**1**	**F**
L2C1	41.868365	-8.561871	**2**	**M**
L2C1	41.867895	-8.562264	**1**	**F**
L2C1	41.868083	-8.562102	**1**	**F**
L2C1	41.868406	-8.562191	**1**	**F**
L2C1	41.868406	-8.562191	**1**	**F**
L2C1	41.868406	-8.562191	**1**	**F**
L2C1	41.868881	-8.561692	**2**	**M**
L2C2	41.868481	-8.549766	**3**	**F**
L2C2	41.868495	-8.549776	**3**	**F**
L2C2	41.867980	-8.549442	**4**	**M**
L2C2	41.868363	-8.549445	**10**	**M**
L2C2	41.868363	-8.549445	**11**	**F**
L2C2	41.868363	-8.549445	**11**	**F**
L4C3	41.887861	-8.535774	**5**	**M**
L4C4	41.886501	-8.526272	**9**	**M**
L5C5	41.895075	-8.515785	**12**	**x**
L6C5	41.902731	-8.512115	**6**	**M**
L6C5	41.902637	-8.511859	**7**	**F**
L6C5	41.902547	-8.511622	**6**	**M**
L6C5	41.901890	-8.511291	**6**	**M**
L6C5	41.901890	-8.511291	**13**	**F**
L6C5	41.901890	-8.511291	**13**	**F**
L7C6	41.912091	-8.499524	**8**	**M**

Appendix 5 – Resume of fecal accumulation in clearance plots per sampling point.

Point ID	Nº of Clearances	Nº of fecal accumulation
L1C3	3	
L1C4	3	
L2C1	3	1
L2C2	3	
L2C3	3	2
L2C4	2	
L2C5	3	
L3C1	2	
L3C2	1	
L3C3	0	
L3C4	3	
L3C5	3	
L4C2	3	
L4C3	1	
L4C4	1	
L4C5	0	
L4C6	3	
L5C3	3	
L5C4	3	
L5C5	3	1
L5C6	3	
L5C7	3	
L6C4	3	
L6C5	3	
L6C6	3	
L6C7	3	
L7C4	1	
L7C5	3	
L7C6	3	
L7C7	1	