

Universidade de Évora - Escola de Ciências e Tecnologia

Mestrado em Biologia da Conservação

Dissertação

## Influence of the Iberian wolf (Canis lupus signatus) on the activity patterns of a reintroduced population of roe deer (Capreolus capreolus)

João Pedro Mano Ferreira

Orientador(es) | Nuno M. Pedroso Dário Hipólito

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A dissertação foi objeto de apreciação e discussão pública pelo seguinte júri nomeado pelo Diretor da Escola de Ciências e Tecnologia:

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Évora 2025

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## Influence of the Iberian wolf (*Canis lupus signatus*) on the activity patterns of a reintroduced population of roe deer (*Capreolus capreolus*)

#### Abstract

Human-wolf conflicts are one of the greatest challenges for the conservation of the Iberian wolf (*Canis lupus signatus*). These conflicts stem primarily from attacks on livestock, mainly due to the reduced diversity and/or availability of wild prey. To reduce attacks on livestock, a mitigation measure was implemented: the reintroduction of roe deer. Given that such measures require a period of habituation to new locations for the released individuals, long-term monitoring is crucial. In this study, the roe deer (*Capreolus capreolus*) populations reintroduced since 2013 in the Serra da Freita and Arada and in the Serra de Montemuro were monitored to analyse how their activity patterns are affected by the local Iberian wolf population. The activity patterns of the roe deer were determined using camera traps and later analysed by comparing the overlap of their activity patterns. Due to the inherent anti-predatory behaviour of the roe deer, it is difficult to determine the real influence of the Iberian wolf on the diel cycle of the roe deer. These findings highlight the importance of integrating predator-prey dynamics into conservation strategies, as they can improve the success of prey reintroductions and help mitigate human-wildlife conflicts.

Key Words: Diel Cycle; Wildlife Monitoring; Central Portugal; Predator-prey; Ungulate

#### Influência do lobo ibérico (*Canis lupus signatus*) nos padrões de atividade de uma população reintroduzida de corço (*Capreolus capreolus*)

#### Resumo

Os conflitos homem-lobo são um dos maiores desafios para a conservação do lobo-ibérico (Canis lupus signatus). Estes conflitos derivam essencialmente de ataques a gado, devido principalmente à reduzida diversidade e/ou abundância de presas selvagens. De modo a reduzir estes ataques foi implementada uma medida de minimização de conflitos, a reintrodução do corço (Capreolus capreolus). Tendo em conta que este tipo de medidas requerem um período de habituação a novos locais por parte dos indivíduos libertados, a monitorização das populações a longo prazo é crucial. Neste estudo a população reintroduzida de corços desde 2013 nas Serra da Freita e Arada e Serra de Montemuro foi estudada de forma a analisar como os seus padrões de atividade são afetados pela população local de lobo ibérico. Os padrões de atividade do corço foram determinados recorrendo a foto armadilhagem, e posteriormente comparados usando a sobreposição dos seus padrões de atividade entre locais com e sem a presença do seu predador, o lobo ibérico. Complementou-se esta análise com testes estatísticos de análise de padrões de atividade. De acordo com os resultados obtidos, com o comportamento anti-predatório institivo do corço é difícel perceber o quanto este é realmente afetado pelo lobo ibérico. Estes resultados realçam a importância de integrar dinâmicas predador-presa nas estratégias de conservação de forma a melhorar o sucesso de reintrodução de presas selvagens de forma reduzir conflitos entre a população humana e fauna selvagem.

Palavras-Chave:Ciclo diel; Monitorização de vida selvagem; Centro de Portugal;Interacções predador-presa;Ungulados

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#### List of Abreviations

PAC- Plano de Ação para a Conservação do Lobo-ibérico
LCIE- Large Carnivore Initiative for Europe
WP- Wolf Present
WA- Wolf Absent
R- Breeding Season
NR- Non-Breeding Season
CI- Confidence Interval
CKD- Circular Kernell Density Test

#### **1. Introduction**

#### **1.1 Iberian Wolf**

The grey wolf, Canis lupus, is a key predator and an iconic symbol of forests worldwide. In the 20<sup>th</sup> century, the wolf populations, as did the major carnivores of Europe, suffered a dwindling in their numbers due to human persecution, habitat destruction, and prey depletion, occupying small nuclei throughout Europe (Bessa-Gomes & Petrucci-Fonseca 2003; Linell et al. 2010). In recent years, however, people's attitudes towards environmental issues and biodiversity have changed, and the protection and conservation of these species is now seen as a necessity (Boitani 2015). For this reason, wolf populations have rapidly re-established across Europe, their numbers increasing, and are occupying old territories (Chapron et al. 2014; Boitani 2015; Torres & Fonseca 2016). It is estimated that there are currently 19,000 wolves in Europe, inhabiting all countries except the island states (LCIE 2022). However, this positive scenario of an increase in numbers cannot be observed in all wolf populations (LCIE 2022). One of the areas where the recovery scenario was not as evident is the Iberian Peninsula (Torres & Fonseca 2016; Torres 2015). This region is the home of the Iberian wolf (Canis lupus signatus), a subspecies of the European grey wolf confined to the Iberian Peninsula. The Iberian wolf is browner and slightly smaller than the European grey wolf (Torres & Fonseca 2016). It has distinctive white marks on its upper lips and dark marks on the tail and black stripes on its front paws (Torres & Fonseca 2016). The population estimate for this subspecies is around 2,500 wolves with 297 packs (Boitani 2015; Torres & Fonseca 2016; Quevedo 2019; LCIE 2022). The Iberian wolf population in Portugal can be divided into two subpopulations, one residing north of the Douro River and the other south of the same river. North of the Douro River, wolf packs show signs of connectivity, allowing them to move between different territories, including Spanish populations (Rio-Maior et al. 2018). This allows intraspecific interactions and genetic flow across the region, including passing the border from Spain/Portugal or Portugal/Spain (LCIE; Torres & Fonseca 2016). The south of the Douro River subpopulation, due to habitat fragmentation and human persecution, is isolated (Torres & Fonseca 2016). Genetic flow among Iberian wolf subpopulations is extremely important since, with less genetic variability, there is an increased risk of inbreeding and of major diseases (Hindrikson et al. 2017; Silva et al. 2021). Habitat loss and fragmentation are one of the most significant challenges to wolf conservation in Portugal. agricultural expansion, deforestation, and the construction of roads, highways, and urban settlements have drastically altered the landscape, reducing the available habitat for wolves and their prey (Llaneza et al. 2012; Grilo et al. 2020). Infrastructure development, particularly roads and railways, not only limits the connectivity between wolf populations, isolating the central Portugal populations, but also increases mortality rates due to vehicle collisions (Grilo et al. 2019).

In Portugal, this subspecies is classified as "Endangered" (IUCN Red book of Mammals in Portugal 2019) and has been protected in Portugal since 1988. According to the Wolf Conservation Plan (PAC) of 2015, there are four population nuclei, Peneda-Gerês, Alvão-Padrela, Bragança, and South of the Douro, with the latter divided into two subnuclei, one more stable in Arada-Trancoso and an unstable one in the border region with Spain called Sabugal. It is estimated that the majority of wolf mortality events is caused by humans, with the main causes being car collisions (35%), shooting (20%), snaring (12%), infectious diseases (6%), poisoning (3%) and attacks by other canines (3%) (PAC 2015). Illegal persecution, including poisoning and shooting, remains a major problem in some rural areas (Linnell & Boitani 2012; Vicente et al. 2019). A study by Blanco & Cortés (2012) found that illegal killing was responsible for a significant portion of wolf mortality in some Spanish regions, especially where human tolerance regarding the species is low. The presence of feral dog populations is also detrimental to the wolf subpopulation south of the Douro River, as they increase the risk of hybridization (Torres & Fonseca 2016; Lino et al. 2023), compete for territory and food resources, and attack wolves (PAC 2015). These packs of dogs are mostly abandoned individuals or strays left to wander, and can increase conflicts between the local population and wolves by attacking livestock (Lino et al. 2023), misidentified as a wolf attack (PAC 2015; Torres & Fonseca 2016). The loss of the Sierra Nevada nucleus in southern Spain, with the existing pack not being found in the 2014 census (Quevedo 2019; LCIE 2022), is a conservation warning for the subpopulation south of the Douro in Portugal.

Food sources are pivotal conservation subjects for the survival of wolves south of the Douro River (Eggermen et al. 2011; Torres et al. 2015). In areas of France and Italy with high availability of wild prey, wolves' diet consists mainly of red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), chamois (*Rupicapra rupicapra*), and wild boar (*Sus Scrofa*), with a low presence of domestic animals (Galaverni 2016; Imbert 2016). In the north of the Iberian Peninsula, in Portugal and Spain, where there are stable populations of wild prey, there is an increase in wild prey consumption, which consists mostly of wild

ungulates such as wild horses (*Equus ferus caballus*), roe deer, and wild boar (Torrena et al. 2016; Lagos & Barcena 2018, Passarinha 2018; Figueiredo et al. 2020). The frequency of occurrence of wild prey species in wolf diet is related to the availability of prey (Barja et al.2024). South of the Douro, wolf diet consists mainly of domestic ungulates due to the low diversity and abundance of wild prey (Álvares 2011; Torres et al. 2015; Imbert et al. 2016; Torreta et al. 2016; Otero et al. 2020).

Currently, the attitude of the local human population towards the presence of the Iberian wolf is mostly positive (Valente et al. 2024), as it is in other parts of Europe where the wolf is present (Arbieu et al. 2019; Torres et al. 2020). The most negative view about these predators comes from livestock owners (Valente et al. 2024). The frequent attacks on livestock inflate conflicts between the predator and the local community (Lino et al. 2023, Valente et al. 2024). These people often rely on livestock for economic subsistence, which leads to negative attitudes when attacks occur (Torres et al. 2015; Imbert et al. 2016; Torres & Fonseca 2016; Otero 2020).

Various mitigation strategies have been implemented to reduce conflicts between wolves and livestock owners. Financial compensation programs for livestock losses are among the most widely used approaches, yet they often do not fully address farmers' concerns, especially when bureaucratic procedures delay payments or do not cover indirect economic losses (Nyhus 2016; Llaneza et al. 2022).

Non-lethal deterrence measures, such as livestock guarding dogs, electric fences, and night enclosures, have been showing effectiveness in reducing predation rates (Landry 2020; Llaneza et al. 2022). Additionally, educational programs to promote coexistence and foster positive attitudes towards wolves are crucial for changing public perceptions (Boitani & Linnell 2015; Chapron & López-Bao 2020).

Ultimately, one of the best ways to prevent attacks on livestock and improve human-wolf relations is to increase and stabilize wild prey populations in the ecosystem through the reintroduction of wild ungulate species such as the roe deer (PAC 2015).

#### **1.2 Roe Deer Reintroduction**

Restoration of wild prey populations, such as roe deer, has been identified as a key strategy to reduce wolf predation on livestock (Torres et al. 2016; Pereira et al. 2022). Studies suggest that in areas where wild prey is abundant, wolves rely less on domestic animals, resulting in fewer conflicts with humans (Ripple et al. 2014; Berger-Tal et al. 2020). However, the success of prey restoration programs depends on habitat availability, human acceptance of increasing ungulate populations, and effective wildlife management policies (Treves & Karanth 2003).

The roe deer is a medium-sized cervid species widely distributed throughout Europe and parts of western Asia. Adults typically weigh between 15 and 35 kg and measure 95 to 135 cm in head-body length. Sexual dimorphism is evident; males possess short, branched antlers that are shed and regrown annually, whereas females lack antlers. The species inhabits a variety of habitats, including mixed deciduous forests, forest edges, and agricultural landscapes, which offer both food and refuge from predators (Imbert 2016), demonstrating notable ecological plasticity (Andersen et al. 1998; Apollonio et al. 2010). They prefer deciduous and mixed forests, with dense tree and shrub cover, providing shelter from both predators and harsh environmental conditions (Freire 2012). In contrast, exotic tree plantations have a negative effect on roe deer habitat selection due to low nutritional value and limited anti-predation benefits (Pereira et al. 2022). Roe deer exhibit primarily crepuscular and nocturnal activity patterns, with peaks at dawn and dusk. Their diet consists mainly of selective browsing on herbaceous plants, shoots, and leaves, which positions them as influential herbivores within their ecosystems (Bartoszewicz et al. 2013; Pagon et al. 2013; Bonnot et al. 2019). Reproduction is seasonal, involving delayed implantation to ensure births occur when resources are most abundant in late spring. During the rutting season, males exhibit territoriality through scent marking and vocalizations to secure breeding opportunities (Andersen et al. 1998).

Roe deer were selected for reintroduction in the area south of the Douro River since, in similar study areas, they are one of the preferred prey species in wolf diet (Mattioli 2004; Torreta et al. 2016; Barja et al. 2024) and are native to the Iberian Peninsula. Known for their digestive plasticity and adaptive foraging patterns, these traits have contributed to their successful recolonisation in Europe (Apollonio et al. 2010; Serrano et al. 2012; Abbas et al. 2013). The reintroduction aimed to increase wild prey diversity and reduce livestock predation (Cruz et al. 2014). From 2013 to 2021, there were multiple

reintroductions in the area of this study, coming from wild populations residing in Spain (Cruz 2014), Montesinho and Gerês (Torres et al. 2018). These reintroductions were made by the Aveiro University, Rewilding Portugal, and LIFE WolFlux project to create stable wild food sources for Iberian wolves to thrive. In total, there were 70 roe deer released in Serra da Freita and Arada from 2013-2019 in 4 different releases, and in Serra Montemuro, twelve individuals were released each year between 2016 and 2018.

The International Union for Conservation of Nature (IUCN) has outlined a set of guidelines for species reintroduction (IUCN, 2013). These guidelines are designed to be applied globally and can be tailored to local conditions and specific species. They address several key areas:

Need Assessment: Reintroduction should only occur if it is the best conservation option for the species, supports other species that benefit from its presence, or is essential for ecosystem restoration.

Involvement of All Affected Parties: The process must involve all relevant stakeholders, including local communities, governments, and NGOs.

Biological and Ecological Assessment: This includes understanding the species' physiological needs, the ecological conditions at the release site, and the reasons for its previous local extinction.

Legal and Ethical Considerations: All reintroductions must comply with legal requirements and consider ethical concerns, such as animal welfare.

Preparation and Release: Careful preparation of both the animals and the release site is essential, with close monitoring before, during, and after release.

Post-reintroduction Monitoring and Evaluation: Continuous monitoring is vital to assess success and make necessary adjustments.

Education and Awareness: Public education and stakeholder engagement are key to fostering support for the reintroduction.

Documentation: Detailed records must be kept at every stage, from planning to monitoring.

Thorough planning is crucial for a successful reintroduction, and several factors need to be considered. The most important of these is the availability of suitable habitat for the species' establishment and proliferation (Cruz et al. 2014; Pereira et al. 2022). Such habitats must provide sufficient food and shelter, especially during the initial release phase (Laundré et al. 2009). Refuge availability is critical, although the "landscape of fear" experienced by prey changes as animals become familiar with the environment over time (Laundré et al. 2009).

Population size is another critical factor in the success of a reintroduction. Small populations are more vulnerable due to low genetic diversity, making the number of individuals released an important determinant of reintroduction success (Torres et al. 2016). Larger populations increase the likelihood of long-term success (Berger-Tal et al. 2014). Reintroduced species typically begin by occupying densely vegetated forest areas, which provide protection from predators, and gradually expand their range as they become more established (Maor-Cohen et al. 2021). Reintroduced prey often lacks predator awareness since they have grown up in a predator-free environment and therefore exhibit a certain naivety towards predators. This naivety towards predators leads to underdeveloped anti-predator skills, limiting their ability to escape or respond effectively to predators (Banks & Dickman 2007). Therefore, understanding interspecific interactions is critical, as predation significantly influences reintroduction success (Berger-Tal et al. 2020).

Monitoring is essential for assessing reintroduction success (IUCN 2013). Post-release monitoring should assess key factors such as home ranges, core areas, activity patterns, and both intra- and interspecific interactions (Berger-Tal et al. 2014). This helps to identify threats to the reintroduced population, such as excessive predation or poaching (Moseby 2015).

There are several methods for monitoring wildlife populations. These include direct visual observations (Zero et al. 2013), assessing wildlife activity based on signs such as scats, tracks or fur (Feffer et al. 2018; Sentilles, 2021), tracking individuals via GPS collars (Hooven et al. 2022), and camera trapping (Foster et al. 2013; Feffer et al. 2018), which is one of the most widely used methods globally.

#### **1.3 Camera Trapping**

In recent years, wildlife research and monitoring, particularly related to mammals, has undergone a technological revolution with the emergence of camera trapping as a key tool (Burton et al. 2015; Feffer et al. 2018). This method has advantages over other noninvasive techniques (Foster et al. 2013).

The efficiency of camera traps also extends to their continuous operation, which runs for 24 hours and requires minimal human intervention. These devices can be left unattended for weeks or even months, resulting in more intensive and prolonged data collection (Feffer et al. 2018), which is highly beneficial to detect and record rare and elusive species

like jaguars (*Panthera onca*), pumas (*Puma concolor*), and Mexican wolves (*Canis lupus baileyi*) (Foster et al. 2013; Russo et al. 2022). Not only is it cost-reductive, but by being a non-invasive study method, it aligns with ethical considerations involving handling wildlife (Russo et al. 2022; Wearn et al. 2017). This method allows for to study of complex dynamics like predator-prey interactions (Feffer et al.2018). Camera traps have proven particularly effective in multi-species systems by providing clear evidence of species identity, circumventing the challenges and human error associated with traditional dung counts (Feffer et al. 2018). They include records of an animal's presence and the exact time and date of the observation, allowing studies of species activity patterns, population density, coexistence, and animal behaviour (Monterroso et al. 2014; Feffer et al. 2018), like predator and prey dynamics.

#### **1.4 Predator-Prey Dynamics**

Predators have a significant impact on the structure and dynamics of ecosystems by influencing prey density, spatio-temporal distribution, and behaviour (Gaynor et al. 2019). This effect extends to social behaviour (Lima et al. 2009), feeding activity (Zhao et al. 2019; Randler et al. 2020), and even parental care (Bongi et al. 2008), creating a "landscape of fear" in which prey change their behaviour to reduce interaction with predators (Gaynor et al. 2019; Zhao et al. 2019). Prey species must constantly balance the risk of predation and often trade optimal resources or behaviours for increased safety (Zhao et al. 2019; Randler et al. 2020). This avoidance strategy can lead individuals to stay away from areas recently used by predators, even if they have optimal conditions (Smith et al. 2019; Randler et al. 2020).

Predation success depends on the predator's ability to intersect with its prey, leading to spatial and temporal overlap (Foster et al. 2013; Yang et al. 2018). In response, prey adapt their behaviour to avoid predators by avoiding detection and interaction (Murphly et al. 2011; Naderi et al. 2021).

Prey species may alter their behaviour to enhance survival by hiding, shifting their temporal or spatial activity, or using physical traits to avoid predation (Bongi et al. 2008; Murphly et al. 2011; Yang et al. 2018; Ross et al. 2011). This includes adjusting daily activity patterns to avoid peak periods of predator activity, switching from diurnal to nocturnal habits, or increasing activity during twilight when predators are less active (Murphly et al. 2011; Monterroso et al. 2013; Ross et al. 2013). Prey can also change

their spatial niche and occupy areas with lower predator presence or higher refuge characteristics (Bongi et al. 2008; Smith et al. 2019).

Both predators and prey adapt their behaviour to factors such as abiotic conditions, human presence, and social dynamics, all of which influence their activity patterns (Wereszczuk & Zalewski 2023). Human presence influences predator-prey dynamics by creating temporal refuges for prey in areas with high human activity. Prey often use human-populated areas as a buffer against predators and benefit from higher human tolerance towards herbivores compared with predators (Murphly et al. 2011; Berger 2017). Additionally, light pollution can alter predator-prey interactions. For example, it has been found that a population of mule deer tends to move closer to human settlements at night and use the increased visibility to avoid predators such as cougars (Ditmer et al. 2021).

Wolves are opportunistic predators that adapt their hunting strategy and preference to the availability of prey in their territory (Barja et al. 2024). Their main prey are wild ungulates such as red deer and wild boar (Torreta et al. 2016; Churski et al. 2021). Roe deer are one of the main prey of wolves due to their limited physical advantages against predators (Torretta et al. 2016; Barja et al. 2024). They are mainly predated during the breeding season (Torretta et al. 2016) since fawns are more vulnerable (Mattioli et al. 2004). One of the wolves' hunting strategies during the breeding season is to separate the fawns from the adult female (Linell et al. 1998). The maternal strategies adopted by these species to protect their offspring add complexity to the predator-prey dynamic. Lactating roe deer females reduce their home range during the presence of fawns, a behaviour that enhances vigilance (Bongi et al. 2008). As fawns grow and become more independent, they occupy highly vegetated habitats during the day to hide and avoid predators (Bongi et al. 2008).

#### **1.5 Objectives**

In the context of conservation biology and species management, understanding the dynamics between predators and their prey is pivotal. In the Freita and Arada and the Montemuro mountain ranges, reintroduced populations of roe deer coexist with the native Iberian wolf. Given the intricate relationships between these two species, this study aims to comprehensively assess the effect of the extant Iberian wolf population on the activity pattern of the reintroduced roe deer. We aim to shed light on any emerging patterns and potential ecological impacts of their interactions, providing valuable data for ongoing conservation and management efforts in the region.

The hypotheses of this study are:

i) The reintroduced roe deer population changes its activity patterns in response to the presence of a potential predator;

ii) Female roe deer, more sensitive to predation during the reproductive season due to the presence of fawns, will have bigger shifts in their diel cycle than the males

#### 2. Material and Methods

#### 2.1 Study Area

The study area (Fig. 1) encompasses two regions defined in the Natura 2000 Sectorial Plan, named Serras da Freita and Arada (PTCON0047) and Serra de Montemuro (PTCON00025). These mountains are situated within the municipalities of Arouca, Canelas, Cinfães, Alvarenga, São Miguel do Souto, Urrô, São Pedro do Sul, Lamego, Castro Daire, and Baião. With an area of 750 km<sup>2</sup>, these sites correspond to 30% of the wolf population habitat south of the Douro River and are the habitat of two confirmed packs: Arada and Montemuro (Pimenta et al. 2023)



Fig. 1- Map of the study area (Scale: 1:1990323)

The Freita and Arada mountain range (Fig.2), henceforth denominated Arada, spans a total area of 116 square kilometres, characterised by rugged mountainous terrain, with prominent ridgelines associated with granite and quartzite formations. The landscape also features significant escarpments and deeply incised river valleys shaped by tectonic activity. The highest ridgelines exceed 1050 meters in altitude, while the lowest areas are found in the northern extremities, along the bed of the Paivô River, a tributary of the Paiva

River. The Arada mountain ranges have a temperate oceanic climate with strong Atlantic influences, leading to cool, wet winters and mild summers. Average winter temperatures range between 0°C and 7°C, while summer temperatures vary from 15°C to 25°C. Precipitation is abundant, often exceeding 1,500 mm annually, with the highest rainfall occurring in autumn and winter, contributing to the lush vegetation and numerous waterfalls in the region. (Alfa 2004).

Vegetation cover is dominated by shrublands and heathlands, along with coniferous forests, together representing nearly 74% of the land use. Shrublands are primarily concentrated on ridges and upper slopes, while forested areas are distributed at midelevations and on some valley floors. The area also boasts a substantial forest cover, with various forest types occupying over 45% of the total area, though native deciduous forests account for less than 7% of the total area (Alfa 2004).

Other notable land use categories include agricultural areas (3.79%) and sparsely vegetated zones (2.67%). Agricultural lands are typically located near forested areas and villages, while sparse vegetation is often intermixed with shrublands. The agroforestry mosaic covers 1.19% of the ZEC, and all other land use types, including urbanised areas, have minimal coverage (Alfa 2004).

Human settlements in the two areas are primarily small towns and remote parishes spread across rugged valleys, with population densities below 150 inhabitants per square kilometre (Valente et al. 2024). The local population is ageing, and population density has been steadily declining. For example, Arouca's population density decreased from 68 inhabitants/km<sup>2</sup> in 2011 to 64 in 2021, while São Pedro do Sul experienced a reduction from 48 inhabitants/km<sup>2</sup> in 2011 to 43 in 2021 (Valente et al. 2024).



Fig. 2 -Arada Landscape

The Montemuro mountain range (Fig.3), henceforth called Montemuro, is characterised by a rugged mountainous landscape with significant elevation changes, ranging from approximately 250 to 1300 meters. Montemuro has a temperate climate with Atlantic influences, featuring cold, wet winters and warm, dry summers. Average winter temperatures range between 2°C and 8°C, while summer temperatures vary from 18°C to 28°C. Precipitation is higher on the western slopes, exceeding 1,000 mm annually, whereas the eastern areas receive less rainfall. The main watercourses that traverse the area include the Balsemão River, a tributary of the Douro River, which flows directly into the Douro. In addition, several smaller streams, such as Corvo, S. Martinho, and Carvalhosa, also contribute to the area's hydrological network (Alfa 2004).



Fig. 3- Montemuro landscape

The landscape is predominantly covered by low-lying vegetation, such as shrublands and heathlands, which account for over 53% of the territory. These vegetation types are mainly found along ridges, plateaus, and slopes, deeply influencing the region's visual character. Forested areas represent the second most significant land cover, comprising nearly 25% of the ZEC. The majority of these are native deciduous forests, covering 14.4% of the area, followed by coniferous forests (approximately 7%). Non-native forests, mainly composed of eucalyptus plantations, occupy 3.4% of the ZEC and are primarily concentrated on the southwestern slopes of the Montemuro mountain range (Alfa 2004).

The population residing in these two areas is very dependent on livestock and agriculture for their livelihood and economic sustainability; therefore, there is a high density of domestic ungulates, including goats (*Capra hircus*), sheep (*Ovis aries*), horses (*Equus caballus*), and cattle (*Bos taurus*). The only wild ungulate species present in the study area are wild boar and the recently reintroduced roe deer. The roe deer was reintroduced to increase wild prey diversity to reduce the attacks on domestic ungulates (Torres et al. 2018). As it was referenced in the introduction, there were reintroductions in Serra da Freita and Arada, and Serra de Montemuro. In Serra da Freita and Arada, twelve roe deer individuals were released in 2013, twenty-four in 2014, twenty-two in 2015 (Torres et al. 2018), and twelve in 2019. In the Serra Montemuro, twelve were released in 2016, twelve in 2017, and twelve in 2018.



Fig. 4- Roe deer being processed for release (Photo taken by Dário Hipólito).

#### 2.2 Data Collection

Camera-trapping campaigns were conducted in two different regions situated within the borders of Arada and Montemuro, from May 2022 to the end of April 2023. Cameras were placed on roads and trails where previous observations and/or signs of activity like

footprints or scats were found. Cameras were placed to avoid detection by humans, due to the high risk of theft, while providing appropriate images for identification.

In total, 85 cameras (Browning BTC-5HDPX and Bushnell NatureCAM) were deployed at various locations for this study. The camera positions were adjusted if any signs of theft or vandalism attempts were detected. The cameras continuously recorded the time, date, and temperature at the time of each occurrence, and were set to operate 24/7, capturing photos continuously whenever movement was detected. GPS coordinates were also logged for each camera location.



Fig. 5- Camera-trap placed in the study area.

#### 2.3 Data Analysis

The Wildlife Insights online platform was used for identification of species captured in camera trap images. This tool was developed for efficient wildlife monitoring and processing of extensive photo datasets (Ahumada et al. 2019).

Image bursts were created with a maximum time interval of 600 seconds (10 minutes) between photos to ensure accurate identification without duplicating information. The Wildlife Insights AI system detected animals and outlined them with a red square to facilitate species identification. For each burst, the species names and the number of individuals detected were recorded. For wolves and roe deer, sex and age class (juvenile or adult) were recorded when possible. Juvenile roe deer were excluded from activity pattern analyses, as this age group typically remains with mothers during early life stages

(Dainikin & Hewison 1996). In addition, roe deer with ear tags were identified as reintroduced individuals.

All data was compiled and processed using Google Sheets and RStudio (R version 4.1.1, 2021-08-10). To prevent overestimation of species presence due to prolonged individual stays, records of the same species within a 30-minute interval were considered as one independent event (Wearn et al. 2017).

Data analysis involved various RStudio packages, including: "Readxl" (Wickham & Bryan 2023); "camtrapR" (Niedballa et al.2016); "dplyr" (Wickham et al.2023);" tidyr" (Wickham & Henry 2023);"lubridate" (Grolemund & Wickham 2011); "solaR" (Rowcliff 2023); "latticeExtra" (Andrews & Sarkar 2023); "suncalc" (Naggy 2023); "writexl" (Csardi & FitzJohn 2023); "overlap" (Meredith & Ridout 2014); "circular" (Agostinelli & Lund 2012); "CircStats" (Lund & Agostinelli 2012); "activity" (Rowcliff 2023).

Activity patterns were analysed by area (Arada and Montemuro), due to the different times of reintroduction, the population in Arada had more time to ajdust to its predator therefore it is likely its activity patterns are influenced differently in both populations and by season (breeding and non-breeding), due to differente behaviours roe deer take on the two different seasons. The breeding season was defined from May 1st to October 31st, which is consistent with the birth and development periods of roe deer (Mateos-Quesada et al., 2011; Stache et al., 2013) and wolves (Oliveira et al., 2020). Wolf activity data allows for to distinction between locations with wolf presence (WP) and absence (WA). The periods of dawn, daytime, dusk, and nighttime were determined using <u>https://timeanddate.com</u>. Dawn was defined by the beginning of astronomical twilight. Standard time was converted to solar time using the suncalc package and <u>https://kochtcm.ch/en/uhrzeit-sonnenzeit-rechner/</u> for averaging each period of the day. The resulting solar time was converted into a range of 0–1 and converted into radians (time\*2 $\pi$ ) using the solar package (Rowcliff 2023; Meredith & Ridour 2024).

The overlap, circular, and activity packages were used to analyse roe deer activity patterns and assess the impact of wolf presence. The overlap package uses kernel density functions to calculate overlap coefficients, ranging from 0 (no overlap) to 1 (identical activity patterns) (Riddout & Linkie, 2009). The overlapEst function provides quantitative measures of overlap based on sample size. For datasets smaller than 50, Dhat 1 is recommended; for larger datasets, Dhat 4 is more suitable (Meredith & Ridour 2024). Overlap was categorised into three levels: low overlap ( $\Delta 1/4 < 0.50$ ), moderate overlap ( $0.50 < \Delta < 0.75$ ), and high overlap ( $\Delta > 0.75$ ), indicating similar or identical activity patterns (Monterroso, 2014).

Overlap confidence intervals were estimated using a bootstrap approach. In this method, the original dataset is considered representative of the population, and a new dataset is generated by resampling (Efron, 1985). The resample function was used to draw 1,000 random observations from the collected data. Confidence intervals were calculated using the BootCllogit function from the overlap package, using basic values as this is an uncorrected estimator (Ridout & Linkie, 2009).

The compareAct and compareCkern functions, from the activity package, were used to correlate activity patterns. The compareAct tool uses a Wald test to assess whether the differences between population estimates are significantly different from zero, based on a chi-squared distribution with one degree of freedom, at the 5% significance level (Ridout and Linkie, 2009; Rowcliff, 2023). Conversely, compareCkern calculates an overlap index for the two distributions and generates a null distribution sampled from the collected data, functioning as a kernel density-based test (Rowcliffe 2023). A p-value < 0.05 indicates that activity patterns are significantly different between the datasets (Rowcliff 2015).

Finally, the visualization of activity patterns was performed using the overlapplot function. By including the average sunrise, daytime, sunset, and nighttime during the study period, periods of the day with increased activity were identified within their circadian cycles.

#### **3. Results**

#### 3.1 Data Organization

In the course of the study, 394 independent events of roe deer were recorded. There were 220 recorded males, 149 females, and 25 unknown sex (Table 1).

A total of 154 independent events of Iberian wolf were recorded allowing to confirm wolf presence. Of the 394 roe deer events, 41% were recorded in areas with wolf presence (161 independent events), while 59% were recorded in areas with wolves absent (233 independent events) (Table 1).

In locations with wolf presence in Arada, 81 male and 48 female roe deer events were recorded. In Montemuro, only 29 male and 3 female roe deer events were observed. Due to the low number of roe deer events in locations with wolf presence in Montemuro, it was not possible to perform further analysis using this dataset. In locations with wolf absence, 59 male and 49 female roe deer events were recorded in Arada. In Montemuro, 51 male and 49 female roe deer events were recorded in Montemuro. All 25 events where it was not possible to identify the sex were recorded in areas with wolf absence (Table 1).

AREA	MALE	FEMALE	UNKNOWN	TOTAL
Arada	140	97	20	257
WP	81	48	0	129
WA	59	49	20	128
Montemuro	80	52	5	137
WP	29	3	0	32
WA	51	49	5	105
Total	220	149	25	394

Table 1-Roe deer independent events in each study area by sex class and wolf presence (WP)

Regarding season:

During the breeding season, there were 204 events recorded, of which 125 were males and 79 were females. Of the males, 43 were recorded in areas where there were wolves present and 82 wolf absent. As to the females during the breeding season, there were 18 events in wolf present areas and 61 in wolf absent areas (Table 2). In the non-breeding season 165 independent events were recorded (Table 2), 95 males and 70 females. The males were divided between 55 wolf present areas and 40 wolf non-present areas. The female events were in 34 wolf present areas and 36 wolf absent areas.

SEASON	MALE	FEMALE	TOTAL
REPRODUCTIVE	125	79	204
WP	43	18	61
WA	82	61	143
NON REPRODUCTIVE	95	70	165
WP	55	34	89
WA	40	36	76
Total	220	149	369

Table 2- Roe deer independent events in each study season by sex class and wolf presence (WP)

#### 3.2 Data analysis

#### **3.2.1 Roe Deer Diel Cycle**

Both male and female roe deer individuals (Fig.7) show a crepuscular, bimodal diel cycle, having 2 activity peaks during dawn and dusk, and decreased activity during the day (Fig.6).



Figure 6-Female (left) and male (right) diel cycles

#### **3.2.2 Wolf Diel Cycle**

The Iberian wolf in this region has a diel cycle with one activity peak; it has a strong nocturnal presence, and it has a peak in its activity from around the start of the night until dawn, where it significantly lowers its activity during the day. Its activity dwells and rises during the crepuscular times, hours when the roe deer activity peaks (Fig.7)



Fig. 7- Wolf Diel Cycle

#### 3.2.2 Area analysis

The activity patterns of both genders in the Arada subpopulation are highly overlapped, and there are no significant changes in their activity (Fig.8).

In Arada, male and female roe deer show a high activity overlap (Dhat 4 =0.8408[CI:0.812-0.942]) and similar overall activity (Kernell Density test: p=0.623), and the activity distribution is very similar (Fig. 8). In Montemuro, male and female roe deer show a high activity overlap (Dhat 4=0.933[0.864-0.972]) and similar overall activity (Kernell density test p=0.981) as well.



Fig. 8-Activity Patterns overlap between female and male in Arada (left side) and in Montemuro (right side). Red and green lines represent female and male roe deer activity patterns, respectively.

When comparing areas, female roe deer present high overlap as well (0,81[CI:0.689-0.905]) and similar overall activity (Kernell density test: p=0.399) although the activity distribution shows slight changes during the dusk hours at 18:00 (Fig.9). Male roe deer present the same high overlap (Dhat 4= 0.8374 [CI: 0.748-0.919]) and a similar activity (Kernell density test: p=0.327) (Fig.9).



Fig. 9- Female roe deer (left) and male roe deer (right) comparison between both areas of study.

#### **3.2.3 Season analysis**

It's important to do a comparative seasonal study as the male and female roe deer have specif behaviours associated with reproduction, so it's expected for their activity patterns to change depending on the time of year.

During breeding season there is a high overlap between male and female roe deer (Dhat 4- 0.8489[CI:0.849-0.958]) and similar activity patterns (Kernell density test: p=0.863), both activity distributions are very similar (Fig.10). During the non breeding season, there is high overlap (Daht 4-0.8456[CI:0.833-0.956]), similar activity overlap (Kernell density test: p=0.767). (Fig.10)



Fig. 10-Activity patterns overlap between breeding season (left) and non-breeding season (right). Red and green lines represent female roe deer and male roe deer, respectively.

When comparing seasons, for the female roe deer, the activity remains highly overlapped (Dhat 4-0.909[CI:0.833- 0.956]) and similar activity pattern (Kernell density test-p=0.767). The males have the same relation, high overlap (Dhat 4- 0.782[CI:0.67- 0.87]); however, there is evidence of some changes in the activity patterns (Kernell density test: p=0.003). There are noticeable changes during the night period (Fig.11).



Fig. 11- Different seasons overlap. Female(left) and male (right), red and green lines represent non-breeding season and breeding season, respectively.

#### 3.2.4 Wolf presence analysis

Hypothesis: Female roe deer will display a bigger shift in the activity patterns than the males.

When analysing the results of how the male and female roe deer activity patterns dffer in the presence of the iberian wolf, it's expected that the female roe deer are more influenced over time than the male roe deer due to thier reproductive needs.

Wolf presence (Fig.12) did not significantly affect activity patterns of female and male roe deer (Females-Dhat4=0.886[CI: 0.79-0.95]; Males-Dhat 4=0.860[CI: 0.77-0.93]) (Table). Despite the results, it is possible to identify an increase in activity in the first hours of the day in areas with wolf presence (Fig.13). Despite that, there is still high overlap with no significant statistical changes (Table 3).



Fig. 12- Iberian wolf (picture captured from one of the cameras in the study)

	Table 3 - W	/olf influence on	male and	female roe	deer
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Analysis	Estimator	Overlap Estimation	95% CI	CKD
<b>Female Roe Deer – Wolf Present</b> (WP) vs Wolf Absent (WA)	Dhat 4	0.8857667	0.79- 0.95	p- value:0.877
Male Roe deer – Wolf Present (WP) vs Wolf Absent (WA)	Dhat 4	0.8607535	0.77- 0.93	p- value:0.337



Fig. 13 - Female roe deer(left) and male roe deer (right) activity patterns influenced by wolf. Red and green lines represent wolf presence and wolf absence respectively.

#### 3.2.4.1 Wolf Area Comparison

Hypothesis: The Arada population will have a bigger change in their activity patterns than the Montemuro population.

By comparing the areas, some changes are expected in the adaptations of both roe deer populations. The Arada population, being the population that has been established the longest (the reintroductions started in 2013), will have more adaptability towards its predators, so their diel cycle changes will be more evident. The Montemuro population, as the newest of the two ( reintroductions 2016-1018), will not display such plasticity in their activity patterns and will not display such evident changes in their diel cycle.

In Arada, the male roe deer population has moderate to high overlap (Dhat4=0.77[CI: 0.64-0.87]), and similar activity patterns (Kernell density test: p=0.10). While looking at the activity distribution, there are minor changes although not statistically significant, the activity peaks in the early day, as opposed to the period wolves are absent, the activity is more evenly distributed with a peak at 18.00 during dusk (Fig.14). The female roe deer display high overlap (Daht 4- 0.876[CI: 0.77-0.94]), and similar activity patterns (Kernell density test: p=0.815). (Table 4; Fig.14). In the Montemuro region, the male roe deer have high overlap (Dhat4-0.903[CI: 0.80-0.96]) and very similar activity patterns (Kernell density test: p=0.915) (Table 4) (Fig.15). It's not possible to analyse the female roe deer population as there isn't enough events (check Table 1).

Analysis	Estimator	Overlap Estimation	95% CI	CKD
Arada Male roe deer	Dhat 4	0.7702438	0.64-0.87	p-value:0.10
Arada Female Roe Deer	Dhat 4	0.8764102	0.77-0.94	p-value:0.815
Montemuro Male roe deer	Dhat 4	0.9027644	0.80-0.96	p-value:0.915

Table 4-Area wolf influence analysis



Fig. 14-Female (Left) and male (right) roe deer activity patterns influenced by wolf in Arada.



Figure 15-Male roe deer activity patterns influence by wolf in Montemuro.

#### 3.2.4.2 Wolf Season Comparison

Hypothesis: Female roe deer activity will be more impacted by the Iberian wolf than the males.

Regarding the seasonal analysis, some differences are expected as well. Wolves focus their predation on females when they are most sensitive to predation, the reproductive season. With the birth of calves, the female roe deer will have to change its activity patterns to even more strict activity peaks for maximum avoidance of its predator while remaining with its young. Because of it, it's expected that the female roe deer will have a bigger activity pattern change during the breeding season than during the non-breeding season. As to the males, we expect no differences in the activity pattern changes from one season to the other.

In areas with wolf presence, breeding and non-breeding female roe deer show high activity overlap (Dhat 4 = 0.866 [CI: 0.73–0.94]), with similar activity patterns across seasons (Kernel density test: p = 0.95) (Table 5). However, activity distribution differs during the breeding season; there is one main peak and two smaller peaks (midday and

sunset), whereas in the non-breeding season, activity is concentrated in a single peak with a minor increase at dusk (Fig. 16).

Without wolves, females still adjust their activity patterns seasonally (Fig. 16). During the breeding season, two peaks occur (early morning and dusk), while in the non-breeding season, activity is more uniform with one peak (Fig. 16). Overlap remains high (Dhat 4 = 0.852 [CI: 0.74-0.93]), and patterns are similar (Kernel density test: p = 0.453) (Table 5).

Male roe deer also shift their activity, with an additional dusk peak during the breeding season (Fig. 17). Overlap is moderate (Dhat 4 = 0.705 [CI: 0.56–0.84]), and significant differences exist between seasons (Kernel density test: p = 0.02) (Table 5). Without wolves, males exhibit two peaks—mid-morning and dusk—compared to a more uniform pattern in the non-breeding season (Fig. 17). Despite moderate overlap (Dhat 4 = 0.64 [CI: 0.48–0.79]), activity differences remain significant (Kernel density test: p = 0.007) (Table 5).

Table	5-Season	Wolf	Analysis
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Analysis	Estimator	Overlap Estimation	95% CI	СКД
Female NR X R Wolf Present	Dhat 4	0.8657929	0.73-0.94	p-value:0.950
Female NR X R Wolf Absent	Dhat 4	0.8350950	0.74-0.93	p-value:0.453
Male NR X R Wolf Present	Dhat 4	0.7047194	0.56-0.84	p-value:0.019
Male NR X R Wolf Absent	Dhat 4	0.6456392	0.48-0.79	p-value: 0.007



Fig. 16- Female roe deer comparison between seasons with wolf presence (left) and wolf absence (right). Red and green lines represent breeding season and non-breeding season, respectively.



Fig. 17-Male roe deer comparison between seasons with wolf presence (left) and wolf absence (right)

#### 3.2.4.3 Breeding Season

During the breeding season, populations in wolf-present and wolf-absent areas show high overlap (Dhat 4 = 0.824 [CI: 0.67–0.92]), with similar activity patterns (Kernel density test: p = 0.681) (Table 6). However, differences emerge in activity distribution (Fig. 18).

In females, activity peaks twice daily: once shortly after sunrise and again at dusk (~18:00), with a nighttime drop in wolf-present areas. In wolf-absent areas, the pattern is similar but lacks the nighttime decrease (Fig. 18).

For males, overlap is moderate (Dhat 4 = 0.67 [CI: 0.51–0.81]), but activity patterns differ significantly (Kernel density test: p = 0.012) (Table 6). In wolf-absent areas, activity remains more uniform with a single peak in the early morning, whereas in wolf-present areas, two peaks occur (Fig. 18).

Analysis	Estimator	Overlap Estimation	95% CI	СКД
Female R Season WP X WA	Dhat 4	0.8241124	0.67-0.92	p-value:0.681
Male R Season WP X WA	Dhat 4	0.6701159	0.51-0.81	p-value:0.012

Table 6-Breeding Season Analysis



Fig. 18-Female (left) and male (right) individuals breeding season analysis. Red and green lines represent wolf presence and wolf absence, respectively.

#### 3.2.4.4 Non-Breeding Season

Both male and female roe deer show changes in activity. In females, as in the breeding season, an additional activity peak occurs before 18:00 (Fig. 19). Males in wolf-absent areas exhibit a major peak at dusk (after 18:00) (Fig. 19). The difference in activity patterns is significant (p = 0.017) (Table 7).

Female roe deer display high overlap (Dhat 4 = 0.835 [CI: 0.69–0.93]) with similar activity patterns (Kernel density test: p = 0.702) and minimal distribution changes (Fig. 19). In contrast, males show moderate overlap (Dhat 4 = 0.669 [CI: 0.51–0.82]), but activity patterns differ significantly (Kernel density test: p = 0.01) (Table 7). In wolf-absent areas, males exhibit a second activity peak at dusk (Fig. 19).



Fig. 19-Female (left) and male (right) individuals non-breeding season analysis.

Table 7- Non breeding season analysis

Analysis	Estimator	Overlap Estimation	95% CI	CKD
FemaleNRSeason WP X WA	Dhat 4	0.8350950	0.69-0.93	p-value:0.702
Male NR Season WP X WA	Dhat 4	0.6689246	0.51-0.82	p-value:0.017

#### 3.2.4.5Arada season comparison

By comparing area and season in Arada, we expect to see significant differences during the reproductive season between wolf present areas and wolf absent areas and in between seasons in the areas where the wolf population is present.

In the Arada region, wolf presence is associated with high overlap (Dhat 4 = 0.843 [CI: 0.73–0.92]) and similar activity patterns (Kernel density test: p = 0.567) (Table 8) (Fig. 20). In wolf-absent areas, overlap is moderate (Dhat 4 = 0.593 [CI: 0.72–0.93]), with similar activity patterns (Kernel density test: p = 0.384) (Table 8). Activity distribution shifts seasonally, with two distinct peaks during the breeding season, though these changes are not statistically significant (Fig. 20) (Table 8).

During the breeding season, overlap remains high (Dhat 4 = 0.855 [CI: 0.75–0.93]), with similar activity patterns (Kernel density test: p = 0.39) and no meaningful distribution changes (Fig. 21). In the non-breeding season, overlap is moderate (Dhat 4 = 0.673 [CI: 0.51–0.83]), and activity patterns are only slightly similar, failing to reject the null hypothesis (Kernel density test: p = 0.068) (Table 8). Distribution patterns differ, with a second activity peak in wolf-absent areas, whereas in wolf-present areas, roe deer exhibit only one peak (Fig. 21).

Data from the Montemuro region were insufficient for comparative analysis.

Tuble of Hadd and Season analysis						
Analysis	Estimator	Overlap Estimation	95% CI	CKD		
Arada WP NR X R	Dhat 4	0.8429958	0.73-0.92	p-value:0.567		
Arada WA NR X R	Dhat 4	0.5935225	0.72-0.93	p-value:0.384		
Arada R WP X WA	Dhat 4	0.8547341	0.75-0.93	p-value:0.39		
Arada NR WP X WA	Dhat 4	0.6733646	0.51-0.83	p-value: 0.068		

Table 8-Arada and season analysis



Fig. 20-Arada roe deer wolf present (left) and wolf absent (right) season analysis. The red and green lines represent the non-breeding season and breeding season, respectively.



Fig. 21-Arada roe deer breeding season (left) and non-breeding season (right). The red and green lines represent wolf presence and wolf absence.

#### 4. Discussion

To make it easier to understand the results and to notice a possible trend in them, we made a table to consult all the graphics and the most important statistical test results (Table 9).



Table 9- Comparative Table of Important Graphs and Tests



One of the hypotheses for this study was that the female roe deer, due to their vulnerability in the reproductive season, a result of their maternal responsibilities, would have a greater shift in the activity patterns than the males would. That hypothesis is refuted by the data, as surprisingly, the opposite occurs: female roe deer display high overlap between areas with and without wolf presence, and there were no significant changes in their activity patterns. However, the male red deer displayed significant plasticity, especially between seasons, alternating diel cycles from areas with and without wolf presence.

As to the other hypothesis, that roe deer would change activity patterns in the presence of it's predator, the iberian wolf is more dubious, as there are no significant changes in the overall acticity pattern, but the population present in this area of central Portugal displays a bimodal crepuscular activity pattern, the normal activity pattern observed in wild non reintroduced roe deer population (Pagon et al. 2013; Bonnot et al. 2019). Both females and males have similar activity patterns, with one activity peak shortly after dawn and one activity peak during dusk, keeping their activity distribution low outside of those two time spans. This conduct has the purpose of temporarily avoiding predators (Murphly et al. 2011; Ross et al. 2013; Monterroso et al. 2013; Torretta et al. 2016).

When looking at the activity patterns without accounting the presence of the Iberian wolf and comparing between areas there is a big overlap between each subpopulation's life cycles, showing that despite the high plasticity of the roe deer to change their behaviour and activity patterns when faced with different pressures (Sönnichsen et al. 2013; Jasinska et al. 2022), there was no need to significantly change their activity patterns to thrive in the different environments. Regarding changes in diel cycles during the seasons, which are normal to differ to fulfil their reproductive drives (Bongi et al. 2008; Mattioli 2004; Jarnemo 2011), like protecting young cubs and setting territories (Bongi et al. 2008; Pagon et al. 2013), the female roe deer still showed no changes, contrary to the males, which showed significant changes between them.

Now, comparing the roe deer activity patterns with the wolf activity patterns, there is an interesting revelation. The roe deer activity patterns between wolf present areas and wolf absent areas remain always highly overlapped and with no signs of an adjustment towards the predator by the roe deer, concentrating its activity around dawn and dusk suggesting an attempt to minimize encounters with predators during high-risk periods, which are more active during the night period (Zhao et al. 2019; Rio-Maior et al. 2018; Dietmer et al. 2021; Frey et al. 2022) like we see in our data. Regionally, there are still changes to be seen. In Arada and Montemuro, the activity patterns remain the same in wolf present

and wolf absent areas which can mean the predator population is not exerting enough predatory pressure to make the roe change its diel cycle and the temporal avoidance by the roe deer continues to be effective (Murphly et al. 2011; Monterroso et al. 2013; Ross et al. 2013), or that the reintroduced prey population remains naive towards the iberian wolf, as they are a more recent reintroduced population, not settling to their new habitat yet (Maor-Cohen et al. 2021). The lack of female roe deer in wolf-present areas in Montemuro can be a sign that the population remains naive and therefore suffers severe casualties, especially with the females, possibly during breeding season (Bongi et al. 2008). This might be a result of prey behaviour that modifies their spatial distribution to avoid high-risk areas where predator presence is more likely (Maor-Cohen et al. 2021). It is by doing a seasonal analysis that we see the biggest changes in activity patterns, and it is possible to see a trend in the results. Female roe deer continue to show high overlap their activity patterns, surprisingly, changing very little from breeding season to nonbreeding season and from wolf present areas and wolf absent areas, the breeding period is a time of heightened energy demands and increased vulnerability, as females are in charge of taking care of the fawns (Mattioli 2004; Bongi et al. 2008); Jarnemo 2011), so it would be expected for changes in their behavior wich did not occur. On the contrary, it's with the males that we see the biggest changes in the activity patterns. During the breeding season,

in areas where the wolves were absent, the male individuals reduced their activity to one activity peak during dawn and the early hours of the day, something that happens normally as the males spend most of their activity during this time marking, patrolling and defending their territories (Pagon et al. 2017). The contrary happened in wolf present areas where the males did not reduce their activity and continued on the normal crepuscular bimodal activity pattern observed for the rest of the year (Kamler 2007; Pagon et al. 2013; Bonnot et al. 2019), which shows the wolves present in these areas affected their temporal distribution over time, disrupting the normal roe deer diel cycle. The shift in activity towards dawn and dusk in wolf-present areas may reflect a strategy to avoid physical overlap with wolves, whose activity is often concentrated during crepuscular and nocturnal time zones (Rio-Maior et al. 2018; Dietmer et al. 2021; Frey et al. 2022), which means the need to avoid predators was higher than the need for reproduction (Torsekar et al. 2022). While in the non-breeding season, in the areas where wolves were present, the male roe deer exhibit a major activity peak during dusk, which is a noticeable departure from the bimodal pattern seen during the breeding season. This change suggests that

males may be more focused on avoiding wolves during the non-breeding season, as there is no reproductive pressure to maintain activity during risky periods. The significant drop in activity during the night reflects an avoidance of nocturnal predators, while the concentration of activity in the late afternoon may coincide with safer foraging opportunities (Randler et al. 2020).

#### **5** Conclusion and Final Remarks

This study aimed to investigate the influence of wolf presence on the activity patterns of a reintroduced roe deer population in central Portugal, analysing variations across sex, geographic region, and season. The findings suggest a complex dilemma: on one hand, this roe deer population displays natural behaviors associated with predator avoidance in other regions where roe deer populations have to interact and adapt to wolves and other predators (Pagon et al. 2013; Bonnoit et al. 2019), probably a result from the interactions in their previous habitats as these reintroduced populations come from wild roe deer. On the other hand, when comparing the activity patterns with wolf influence, there were no significant changes in the activity patterns to confidently determine that the Iberian wolf population in this area is actively influencing roe deer diel cycles. Furthermore, the roe deer's anti-predatory strategies, including their ability to detect and avoid predators, may play a significant role in maintaining their behavioural patterns despite potential threats from wolves (Bongi et al. 2008).

There is a high overlap in activity patterns between wolf-present and wolf-absent areas, suggesting that the presence of wolves does not exert enough selective pressure to drive a significant temporal shift in roe deer diel cycles. While minor nighttime adjustments were observed in wolf-present areas, these shifts were not substantial enough to confirm a correlation between wolf presence and changes in activity patterns.

Regional differences also provided insight into how roe deer may respond to predation pressure. In the Arada region, both male and female roe deer exhibited concentrated activity around dawn and dusk, a pattern consistent with previous research on prey avoidance behaviors. However, there was still high activity overlap and a lack of significant behavioural shifts. In Montemuro, where roe deer populations are more recently reintroduced, the lack of behavioral adjustments in females could be linked to lower population densities or a higher mortality rate due to predation, but there is no data to support it.

By looking at the seasonal analysis that we can see some changes influenced by the Iberian wolf. The female roe deer surprisingly show very little sensitivity towards their predators, by displaying always high degrees of overlap and steady absence of significant results displaying any time of influence in their activity patterns. However, when looking at the males, we can see adaptability and changes in their diel cycle, a result of their interactions with the Iberian wolf. During the breeding season in areas without the presence of the wolf they have 1 activity peak and in areas where there is a wolf they still have the same bimodal diel cycle, and in the non breeding season in areas with the Iberian wolf they display activity with just one activity peak and outside of them they have a bimodal activity. This indicates a considerable impact on the wolf in the male roe deer diel cycle, showing signs of temporal avoidance (Murphly et al. 2011; Monterroso et al. 2013; Ross et al. 2013).

Taking all this into consideration, it is clear that, despite being a reintroduced population, the roe deer in this study displayed similar diel cycles and anti-predatory strategies as wild populations of roe deer have (Pagon et al. 2013), avoiding high risk times like the day and night, and prioritising crepuscular time zones like the dawn and dusk. It is also clear by the data we gathered that the male roe deer are more affected by the Iberian wolf than the female roe deer, causing them to shift their diel cycle between seasons and causing them to have different activity patterns in regions where the wolves are present. The female roe deer, despite their maternal responsibilities and probable need for adjustment of their activity patterns (Bongi et al. 2008), show no signs of being affected by the Iberian wolf.

Due to the inherent anti-predatory behaviour of roe deer, it remains challenging to determine, based on the available data, whether the presence of the Iberian wolf significantly influences their activity patterns. The findings presented in this dissertation do not allow for a conclusive assessment of predator-driven behavioural shifts within the reintroduced roe deer populations south of the Douro River. The crepuscular diel cycle of this roe deer population presents evidence that the Iberian wolf is affecting roe deer activity patterns, and the notable evidence of seasonal variation in the diel activity cycle of male roe deer points to an influence of the predator on the roe deer diel cycle. However, the absence of more significant results in other analyses, particularly regarding female activity, prevents the establishment of a strong and consistent link between predator presence and changes in temporal behaviour, and therefore we cannot conclude a direct correlation of the iberian wolf in the diel cycle of the roe deer in this region.

This study underscores the complex interplay between predator and prey in the region south of the Douro River and emphasizes the importance of continuous monitoring of reintroduced ungulate populations in ecosystems where apex predators like the Iberian wolf are present. Understanding these dynamics is crucial for ensuring the long-term stability and resilience of both species and the entire ecosystem. We also suggest studies involving this reintroduced roe deer population further elaborate on their behavior and how it's driven by other outside forces.

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