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**TESTING THE ACTIVITY BUDGET HYPOTHESIS TO
EXPLAIN SEXUAL SEGREGATION IN RED DEER**

Tese de Mestrado em Ecologia, orientada pelo Professor Doutor José Paulo Sousa e pela Doutora Joana 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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Resumo

Como um enigma complexo ainda por desvendar, a segregação sexual tem sido bastante estudada nos últimos anos, sobretudo em espécies de ungulados sexualmente dimórficas. Para uma melhor compreensão deste conceito, várias hipóteses foram propostas ao longo dos anos, em torno de duas componentes principais: segregação de habitat e segregação social. No entanto, nenhuma foi exclusivamente capaz de explicar este fenômeno, o que indica que múltiplos fatores se encontram na base da sua origem.

Segundo a hipótese da sincronia de atividades, machos e fêmeas apresentam diferenças nos padrões de atividade devido a diferenças no dimorfismo no tamanho corporal, necessidades energéticas e eficiência digestiva. Este estudo tem, então, como objetivo avaliar os pressupostos que servem de base à hipótese da sincronia de atividades, de modo a explicar os padrões de segregação sexual.

Através da análise de vídeos e fotos de armadilhas fotográficas, colocadas na Serra da Lousã, foi possível observar os diferentes padrões de atividade e segregação do veado. Os nossos resultados mostraram que machos e fêmeas apresentam uma segregação sexual acentuada ao longo do ano, exceto na época de acasalamento, altura em que machos e fêmeas mostram uma tendência para se agregarem em grupos mistos. Relativamente aos padrões de atividade, os resultados evidenciaram um padrão de atividade crepuscular e noturna por parte do veado. Em termos gerais, o maior grau de sincronização de atividades entre machos e fêmeas foi na época de acasalamento, o que é esperado face aos padrões de agregação e segregação sexual reportados.

Deste modo, os nossos resultados apoiam a hipótese de sincronia de atividades, porém, outros estudos verificaram o oposto, e, por isso devemos ter em conta outros fatores para uma melhor interpretação da segregação sexual.

Palavras-chave: *Cervus elaphus*, segregação sexual, hipótese da sincronia de atividades, padrões de atividade, armadilhas fotográficas

Abstract

As a complex puzzle yet to be unveiled, sexual segregation has been extensively studied in recent years, especially in sexually dimorphic ungulate species. For a better understanding of this concept, several hypotheses have been proposed over the years, around two main components: habitat segregation and social segregation. However, none has been exclusively able to explain this phenomenon, which indicates that multiple factors are at the base of its origin.

According to the activity budget hypothesis, males and females have differences in activity patterns due to differences in body size dimorphism, energy requirements and digestive efficiency. This study, then, aims to assess the assumptions that serve as the basis for the activity budget hypothesis, to explain the patterns of sexual segregation.

Through the analysis of videos and photos from camera traps placed in the Lousã Mountain, it was possible to observe the different activity and segregation patterns of the red deer. Our results showed that males and females show marked sexual segregation throughout the year, except in the mating season, when males and females show a tendency to aggregate in mixed groups. Regarding activity patterns, the results showed a pattern of twilight and nocturnal activity by the red deer. In general terms, the highest degree of activity overlap between males and females was in the mating season, which is expected given the reported sexual aggregation and segregation patterns.

In this way, our results support the activity budget hypothesis, however, other studies verified the opposite, and, therefore, we must take into account other factors for a better interpretation of sexual segregation.

Key words: *Cervus elaphus*, sexual segregation, activity budget hypothesis, activity patterns, camera traps

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Chapter I - General Introduction

1.1) Sexual Segregation: A different kind of romance

Sexual segregation is a phenomenon that has been widely addressed in the last decades, particularly regarding the life history of ungulates (Bowyer, 2004). This event is characterized by the separation of males and females in distinct groups, i.e. when different sexes live separately in time and/or space throughout the year, with the exception of the mating season (aka rut season for ungulates) (Bleich, Bowyer & Wehausen, 1997; Ruckstuhl & Neuhaus 2000; Ruckstuhl & Neuhaus 2005).

In ungulate species with sexual dimorphism, sexual segregation is very common, particularly in cervids (Main *et al.* 1996; Kie & Bowyer, 1999; Bowyer, 2004). Nevertheless, this event also occurs in several other taxa, like, fishes (Sims *et al.*, 2001), birds (Myers, 1981) and reptiles (Parmelee & Guyer, 1995; Shine *et al.*, 2003).

To have a deeper understanding of this concept, two main components within sexual segregation need to be clarified: social segregation and habitat segregation. In one hand, in social segregation males and females may live in the same habitat but in separate social groups. Although the two sexes exploit the same habitat, they must be active at different periods of the day or at the same period but without coexisting (Conradt, 1999). On the other hand, in habitat segregation, males and females differ from one another in the way they are distributed by the different habitats (Kie & Bowyer, 1999), i.e. the classes are separated by the habitats and differ in the way they use it (Clutton-Brock *et al.*, 1982). These two concepts need to be precisely and clearly distinguished (Villaret & Bon, 1995; Conradt, 1998). Although, it is accepted that these two types of segregation can occur independently or simultaneously (Conradt *et al.*, 2005).

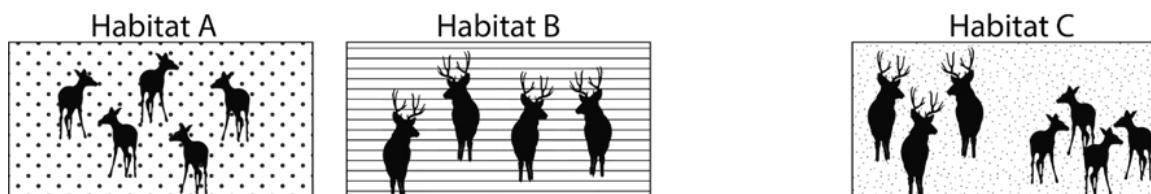


Figure 1 Representation of the main components of sexual segregation; Habitat segregation: Habitat A, used only by females, while Habitat B is used only by males; Social segregation: Habitat C, unisex groups occupy the same portion of habitat but without interconnecting. (Adapted from Ruckstuhl & Neuhaus, 2005).

The phenomenon of sexual segregation is a very complex reality in which the possible factors underlying its origin are still being discussed. Sexual dimorphism leads to differences in energy requirements, reproductive strategies, predation risks, synchronization of activities and social affinities between groups. These may be key factors to explain the great puzzle that is sexual segregation (Ruckstuhl *et al.*, 2005).

Several hypotheses to explain sexual segregation have been proposed over the years (Main & Coblentz, 1990). So far, many were rejected while others have prevailed. Among the most prominent hypotheses to explain sexual segregation in polygamous populations there are: the Reproductive Strategy Hypothesis (RSH), also known as Risk-Predation Hypothesis; the Forage-Selection Hypothesis (FSH), or Sexual Dimorphism Body-size Hypothesis; the Activity Budget Hypothesis (ABH); the Social Affinity Hypothesis (SAH), also known as the “social preferences”(Bon & Campan, 1996).

All the mentioned hypotheses are associated with the concept of sexual dimorphism (body-size) (Bon & Campan, 1996; Bowyer 2004; Ruckstuhl & Neuhaus, 2005). The RSH and FSH try to explain sexual segregation by how distinct classes use different habitats (habitat segregation). The SAH attempt to explain segregation through social behaviour among sexes (social segregation) (Ruckstuhl & Neuhaus, 2001). On the other hand, ABH stands out from the rest, as it tries to explain segregation through the habitat component and the social component.

The RSH states that in polygamous ungulates, sexual segregation results from differences between male and female reproductive strategies to maximize their own reproductive success (Main & Coblentz, 1996). According to RSH, the reproductive success of females aims to ensure the survival and growth of offspring, until they become self-independent (Clutton-Brock, 1982). However, this decision present consequences for the welfare of the females' progenitor, as they opt for safer habitats, avoiding the risk of predation even if it offers lower quality of food. Therefore, females end up valuing the safety of the offspring over their own needs, resulting in a trade-off between the welfare of the offspring and the needs of the mothers (Ruckstuhl & Neuhaus, 2005).

Regarding the males, according to RSH, they prefer habitats with a higher diversity of high-quality food, even when the risk of predation is higher. These types of habitats are essential for the males to increase their energy reserves and body condition, needed for the mating season. After the mating season, weather conditions (e.g. severe winters and dry seasons) may conditioning the food supply, which is why the pre-mating season is so important for survival and reproductive capacity of the males. Their physical condition

is also important for the reproductive success, to increase successful competition with rival males (Mautz, 1978; Clutton-Brock, 1982; Main & Coblentz, 1996; Main *et al.*, 1996; Main & Du Toit, 2005). Thus, we can expect divergent behaviour patterns and habitat choices by both classes, which will be mostly reflected in the reproductive season (Ruckstuhl & Neuhaus, 2005).

Body-size variation regarding sexual dimorphism is a concept closely related to FSH, being related to differences in food intake and digestion, thus contributing to habitat segregation between sexes in ungulates, particularly in ruminants (Clutton-Brock *et al.*, 1987; Gross *et al.*, 1996; Barboza & Bowyer, 2000). To support a morphological/physiological explanation to habitat segregation among sexually dimorphic ungulates, almost all authors have used the rumen-based foundation (Ginnett & Demment, 1997; Main, 1998; Barboza & Bowyer, 2000; Ruckstuhl & Neuhaus, 2002).

The FSH basic principles claim that males have a larger rumen, giving them greater digestive capacity and efficiency (Demment & Van Soest, 1985; Van Soest, 2018). Since males are larger, they also have higher energy requirements and, consequently, prefer habitats with more resources. In comparison, morphologically smaller females and sub-adult males have lower digestive capacity and efficiency, eventually choosing habitats that provide them with good quality and easily digestible food (Beier, 1987; Barboza & Bowyer, 2001).

Habitat segregation can also occur among same-sex individuals with different reproductive status. Despite presenting similar morphology, according to FSH, reproductive and non-reproductive females have different nutritional needs during the late gestation and lactation season which may lead to their segregation (Oftedal, 1985; Gittleman & Thompson, 1988). Regarding this hypothesis, the balance between physiological factors and diet requirements constitute the main reason leading to sexual segregation, aiming to satisfy of the physiological needs of each class.

The next hypothesis can not only be incorporated into the social concept, but also in the energy concept arising from synchronization costs. In gregarious species, synchronized activities can help individuals in mixed groups by reducing the risk of predation or increasing the effectiveness of foraging (Rands *et al.*, 2003; Giraldeua & Caraco, 2018). However, synchronization can also present disadvantage to individuals with divergent needs from the rest of the group (Rands *et al.*, 2003). In addition, living in a group has other disadvantages, such as increased competition for resources, pathogenic infections and attraction of predators (Sun 2001; Davies *et al.*, 2012). In sexually dimorphic

species, the needs between males and females differ, and so, the cost of synchronization may be too high for them to aggregate.

In 1998, Conradt (1998) and Ruckstuhl (1998) proposed the ABH, in which they suggested that males and females differ in their activities (e.g. food demand, resting and rumination) due to different energy requirements and digestive efficiency, ending up segregating over time (Conradt 1998; Ruckstuhl & Neuhaus, 2000). Regarding the link of food demand with group cohesion, ABH proposes that social segregation occurs due to differences in body-size, which will determine rumen size and food intake. Such differences will be reflected in the time spent in the active state (e.g. searching for food) or inactive state (e.g. resting or ruminating) between each class (Ruckstuhl, 1998; Conradt & Roper, 2000).

So, ABH is based on two main assumptions: (1) females are smaller in size and, as a result, have lower digestive efficiency (e.g. smaller rumen size and faster food passage) leading to higher time spent foraging, while males, being larger, have higher digestive efficiency and will spend more time ruminating and less time foraging (Demment, 1982); (2) due to differences in the activity patterns between males and females, the costs of synchronize their behaviours, and, to stay together, are too high to be sustainable (Conradt, 1998; Conradt & Roper, 2000, 2003). The predictions that constitute this hypothesis are: (1) as females are smaller in body-size and have a lower digestive efficiency, to compensate these “imperfections” they will stay in the active state for longer period (e.g. feeding or walking) while males with larger body-size will spend more time in the inactive state (e.g. resting or ruminating); (2) individuals with similar activity patterns will tend to group, while others will segregate (Ruckstuhl & Neuhaus, 2002).

Since the hypothesis is based on differences in body size, increased dimorphism in body size (e.g. when the male is 20% bigger than the female) increases the likelihood of sexual segregation (Ruckstuhl, 1998; Ruckstuhl & Neuhaus, 2000). Activity patterns also differ with reproductive status, for example, breastfeeding females spend more time in the active state compared to other females (Hamel & Côté, 2008). Recent studies have recognized that ABH alone cannot explain sexual segregation (Bowyer & Kie, 2004; Yearsley & Pérez-Barberia, 2005). Although it is considered that the threshold of body-size dimorphism that leads to sexual segregation is around 20%, Alves et al. (2013) reported segregation between females and sub-adult males (older than 2 years) of *Cervus elaphus* with only 12% of body-size dimorphism (Alves et al., 2013). Therefore, body-size dimorphism may not be the only mechanism responsible for sexual

segregation, raising the importance of taking into consideration other physiological (e.g. hormonal changes) and behavioural (e.g. social motivations) factors (Alves *et al.*, 2013).

The next hypothesis is referent to social segregation, the possibility that both genders can form separate groups and explore the same places, at the same time or not (Francisci *et al.*, 1985), but without grouping. Jacklin and Maccoby (1978) have suggested that differences in the rhythms of activity and the way both genders engage socially can lead to problems of social compatibility between males and females. According to the SAH, males and females have unmatched ontogenetic behaviour, with different social motivations that may result in a preference to remain in same-sex groups outside the mating season (Bon & Campan, 1996).

SAH also states that groups composed exclusively by females, will be able to develop breeding skills more efficiently; in groups composed exclusively by males, the individuals are going to develop, more effectively, develop their fighting skills, and ability to evaluate potential rivals, and dominance hierarchies will be establish within the group. As for sub-adult males, they will form groups without joining adult males, as they have different social motivations, body-size and fighting skills (Bon *et al.*, 2001). Therefore, this “incompatibility” leads to social segregation between different genders and ages (Geist, 1971).

In addition, SAH also proposes that social segregation between genders is due to the fact that females tend to avoid males, as males exhibit agonistic behaviour (Bon & Campan, 1996; Cransac *et al.*, 1998) and may be a threat to offspring, as well as to the female group cohesion (Weckerly *et al.*, 2001). Social segregation can also occur within females, motivated by the fact that females with offspring become aggressive towards females without offspring (Habibi, 1997). This hypothesis differs from the others mentioned above because it is not directly linked to body size dimorphism, habitat segregation or predation risk to sexual segregation (Ruckstuhl & Neuhaus, 2000). Although, much remains to be debated and explained about sexual segregation, or on which hypothesis is the best to explain sexual segregation.

1.2) The Archetype *Cervus elaphus*

1.2.1) Distribution and Importance

Cervus elaphus, commonly known as red deer (Linnaeus, 1758), represents the archetype of all cervids and, is one of the most widely distributed mammals in the world.

In the last century, populations of diverse deer species have grown considerably across Europe, although they can also be found in Asia, North America and Africa (Gill, 1990; Apollonio *et al.*, 2010). The red deer is a species currently expanding and is currently the largest ungulate present in the Iberian Peninsula (Oliveira, 2013). Due to its great ecological and, economic (mostly as game hunt species) importance in the European continent, it has been extensively studied over the last years, mainly in northern Europe (Clutton-Brock 2001, Clutton-Brock *et al.*, 2004).

In Portugal, populations of endogenous species became extinct throughout the nineteenth century and much of the twentieth century, as direct consequence of hunting over-exploitation and destruction of habitats (Salazar, 2009). The red deer population is geographically distributed from north to south of Portugal and can be found in Montesinho Natural Park, Lousã Mountain, Tagus Natural Park, Moura, Barrancos, Vila Viçosa Tapada and Mafra National Tapada (Sampaio, 2013). The Lousã Mountain was part of a reintroduction process that took place from 1995 to 1999, with the release of 96 animals (32 males and 64 females). This programme aimed to improve the natural heritage of this Mediterranean-type region and enable the practice of hunting and ecotourism (Alves, 2013).

Due to the reintroduction programme, the deer population in Lousã Mountain continues to grow in numbers and distribution, being present throughout the mountain and surrounding areas. It was estimated that the presence of red deer populations, covered a total area of more than 500 km², including the Lousã Mountain (170 km²), but also the surrounding areas (Alves, 2013). During the breeding season, which occurs between mid-September and late October, reproductive males and females are more concentrated in the centre of this mountainous region, in an area of approximately 120 km². During the rut season, between 2005 and 2009, was estimated a density of 5.6 red deer/ km² (Alves, 2013), which did not change much in the last years (Alves da Silva *et al.*, *In prep.*). Although there are no natural predators, wild dogs had taken their place and may qualified as non-natural predators, preying mostly young, sub-adults and adult female deer (Alves, 2013).

The red deer, like other cervids, play an important role in flora dynamics, through selective intake of vegetation, and also play a crucial role in seed dispersal (Bugalho & Milne, 2003). Despite the important role in maintaining the plant community, especially in forest areas, in high densities, the deer populations can cause serious damage in adult

and young trees. These problems are related with negative impacts in plant regeneration, especially of young or slow growing species (Côté *et al.*, 2004).

In terms of conservation, *Cervus elaphus* is an essential prey for the maintenance of populations of some endangered species, such as the Iberian wolf (*Canis lupus signatus*) and the Iberian lynx (*Lynx pardinus*), endangered carnivores in Portugal (Cabral *et al.*, 2005). The red deer has the status of king of forest communities, being one of the mammals of greatest hunting importance, mainly due to the high economic value of the antlers, which constitute a trophy for hunters (Peixoto, 2014) and also for their meat and medicinal products (Milne *et al.*, 1978; Jiang *et al.*, 2008; Salazar, 2009). In east Asia, velvet (nourishing tissue that lines the stems during the growth phase) is considered a stimulating and fortifying medicine, being used as an aphrodisiac medicine (Bugalho, 2002).

1.2.2) Morphology, ecology and behaviour

Cervus elaphus is a species of ungulates (hoofed animals) with polygamy behaviour (males' mate with several females throughout the mating season: Clutton-Brock, 1989 Vanpé, 2007). In taxonomic terms, and according to the classification adopted by the IUCN (Lovari *et al.*, 2008), the red deer is an ungulate belonging to the Cervidae family, order Cetartiodactyla (even-toed ungulates).

Its morphology is characterized by an elongated body, with a broad and robust head and a short tail (Baskin & Danell, 2003). The coat is usually thick, suffering seasonal variation; reddish brown during summer and dark brown during winter (Oliver, 1999). The offspring up to two months old has dorsal white spots, which help them camouflage in vegetation, in addition to this advantage, they also do not release odours, hiding easily from predators (Baskin & Danell, 2003; Wilson & Mittermeier, 2011).

Males have natural bone formations of high value in their heads, the antlers, which fall every year usually in March/April, and then develop at a record speed again within the same year, usually increasing their size and number of tips (Peixoto, 2014). The antlers are the best characteristic to differentiate adult males and females, followed by the body size, being males larger (Peixoto, 2014). In Mediterranean regions, males measure approximately 160 to 220 cm in length and have 80 to 160 kg in weight, while females measure about 160 to 195 cm in length and present a weight from 50 kg to 100 kg. These differences in body level will be reflected in a sexual dimorphism of $37 \pm 3\%$ (Alves, 2013).

Regarding social organization, as mentioned, the red deer is a polygamous species (Clutton-Brock *et al.*, 1982), which exhibits a gregarious behaviour characterized by sexual segregation throughout the year except, during the mating season (Clutton-Brock, *et al.*, 1982; Alves, 2013). In Lousã, the mating season, called rut, occurs from mid-September to late October, when males try to mate with as many females as possible.

Through the body odour and the urine of the females, males recognize when they are ready to mate. To attract their partners, the males emit vocalizations, known as “roaring” that resound all over the forest (sounds similar to snoring), which can last for several minutes. In this magical season, in addition to the roaring signals characteristic of the beginning of the rut, the appearance of patches, where the soil and vegetation are perturbed, is also a presence indicator. Emission of vocalizations is part of the males' routine during the mating season to attract the attention of the females, but they also emit roars with the purpose of defending the territory and making a stand against potential rivals (Pépin *et al.*, 2001; Reby & McComb, 2003 ; Charlton *et al.*, 2007; Hurtado *et al.*, 2012). Vocalizations can also be issued by females and young, especially for identifying their locations or as warning signs in situations of danger, even outside the rut season (Pépin *et al.*, 2001).

The main goal is to secure offspring for the next generation, that is, the ultimate evolutionary prize. At rut, males become more active and less careful, becoming more aggressive when facing rivals. It is in this short exclusive period that mixed groups can be spotted, where males aggregate with females and mark their position, in a phenomenon called harems formation (Clutton-Brock *et al.*, 1982).

Outside the mating season, females live in a matriarchal society, where adult females (older than 2 years) live together with sub-adults (between 1-2 years in females and 1-3 years in males) and young (less than 1 year), separated from groups of males (Alves, 2013). On the other hand, males form groups of the same age group, but regularly show a more reserved behaviour, remaining mostly solitary (Clutton-Brock *et al.*, 1982). Red deer's life cycle is highly synchronized to increase reproductive success and survival of future generations (Clutton-Brock *et al.*, 1982). It is divided into several main stages: (1) gestation, which occurs between September and May (about 34 weeks); (2) birth, which occurs between May and June, where females usually have one offspring per year; (3) lactation, period that takes place after birth, ending in September; (4) then a new breeding season begins in September (Alves *et al.*, 2013).

Deer's are mammals that easily adapt to new environments and conditions (Clutton-Brock *et al.*, 1982). Red deer is considered to be one of the species with highest plasticity, as they live in a wide variety of habitats. They prefer shrubland areas, which have more open space and a higher food availability, but also use forest areas of coniferous and deciduous species, which are closed areas that offer greater protection and refuge (Alves *et al.*, 2006; Lovari *et al.*, 2008; Skog *et al.*, 2009).

Red deer are very flexible regarding their diet, using various habitat types to meet their nutritional needs. The feeding behaviour alternates between browsing (i.e., eating woody and non-woody dicotyledonous plants) and grazing (i.e., eating grass and sedges), thus shows an intermediate behaviour (Hofmann 1989) This behaviour differs depending on whether food availability is mainly herbaceous, shrubby or arboreous (Hofmann 1989; Garin *et al.*, 2001). The red deer diet during the dry summer is mostly made up of leaves from trees and shrub species, with a predominantly grass diet throughout the rest of the year, according to the available food (Bugalho & Milne, 2003). This species can also eat fruits and seeds when they are available (Hofmann 1989; Gebert & Verheyden-Tixier, 2001).

1.3) Study area - Lousã Mountain

1.3.1) Location, climate and topography

Lousã Mountain is located in the Central Portugal region (40°3'N, 8°15'W) and is characterized as a Mediterranean type region (Rivas-Martinez, 2004). Its typical climate is characterized by hot and dry summers and rainy winters, almost without snow (Archibold, 1995). It covers an area of 170 km² with altitude between 100 and 1205 m (the highest point is called Pico do Trevim) and consist of a very rugged terrain with deep valleys and rounded tops (Alves, 2013).

The annual temperature ranges from -4.1° to 35.9°C, reaching an average annual temperature of 12°C and, with an annual precipitation of 827 mm, which in higher altitudes can reach 1600 mm. In Lousã Mountain, a dense hydrographic network is embedded, which includes the Mondego River and Tagus River drainage basins. The ecological importance of this region has been internationally recognized and has been included in the Natura 2000 National List regarding the priority of some habitats (according to the Habitats Directive) to be conserved in the flora and fauna levels.

1.3.2) Land cover and flora

The mountainous region of Lousã is essentially woodland, characterized by the predominance of coniferous forests, with dominance of pine trees such as, *Pinus pinaster*, *Pinus sylvestris* and *Pinus Nigra*. Broadleaf trees are found in smaller patches, being particularly composed by species such as: Chestnut (*Castanea sativa*), *Prunus lusitanica*, *Ilex aquifolium* and *Quercus sp.*, (*Quercus pyneraica*, *Quercus robur* and *Quercus suber*), which together with the coniferous species represent a mixed forest, present in various areas of the mountain.

In Lousã mountain, the studied population is known to consume shrub species as their main food composition. The most consumed plant species, regarding shrub species, are the *Pterospartum tridentatum* and *Ulex minor* (Garcia & Alves da Silva (*In prep*)), plant species that are highly available in the study area. Nevertheless, they can also consume other plant groups, as herbaceous and arboreous, consuming also species of graminoids as *Hordeum murinum* or *Agrostis castellana* (Garcia 2016; Garcia & Alves da Silva (*In prep*)). The study in this population also showed a higher consume of arboreous plant species by males, comparing to females or calves (Garcia 2016). Considering the arboreous, species like *Acacia melanoxyton* or *Castanea Sativa* are among the most consumed plants.

1.4) Aims

The main objective of this project is to determine whether the differences between male and female activity patterns may explain the full pattern of sexual segregation of red deer at the Lousã Mountain. For this, it is important to evaluate the activity patterns of males and females, both in general terms and for specific activities throughout the life cycle of red deer. It is also important to realize if the overlap in terms of activity patterns of both sexes are capable of explaining the patterns of sexual segregation. Several hypotheses attempt to explain the complex phenomenon of sexual segregation, however, my work focuses on the Activity Budget Hypothesis (ABH), which stands out from the others hypotheses, since it encompasses both the habitat component and the social one. Thus, through our objectives, we will be able to partially test ABH and its predictions.

Chapter II - Could the differences between activity patterns of males and females explain sexual segregation patterns?

2.1) Introduction

Although all love stories begin with “Once upon a time...”, this is not one of them. Sexual segregation is a very complex phenomenon, defined by the separation of females and males into different groups all year, with an exception at the mating season (Bowyer, 2004). This unique type of romance occurs mainly in sexually dimorphic species, particularly in the ones belonging to the superorder Ungulata, such as cervids (Main *et al.*, 1996; Kie & Bowyer, 1999).

Sexual segregation can be divided into two main components, based on differences in the use of the habitat by the different genders (habitat segregation) (Clutton-Brock *et al.*, 1987; Kie & Bowyer, 1999) or by an association between same sex individuals, males or females, forming separated social groups within the same habitat, without interrelating (social segregation) (Conradt 1998; Ruckstuhl & Neuhaus, 2005). Despite the countless studies carried out to try to identify an universal explanation for this phenomenon, it is known that there is not only a single factor behind this event, since a vast combination of factors may be involved - such as risk of predation, digestive efficiency, social factors, and synchrony of activities - and be the cause of sexual segregation. (Bon *et al.*, 2001; Ruckstuhl & Kokko, 2002; Ruckstuhl & Neuhaus, 2005). Therefore, we should not be guided by the thought of an exclusive hypothesis, but by the combination of the various hypotheses that have been proposed over time (Bonenfant *et al.*, 2004; Loe *et al.*, 2006; Ciuti & Apollonio 2008) Around this great puzzle yet to be deciphered – sexual segregation - several hypotheses have been proposed over the years (Main & Coblentz, 1990).

Conradt (1998) and Ruckstuhl (1998) suggested the Activity Budget Hypothesis (ABH). According to this hypothesis, differences in activity patterns translate into a high cost of synchrony for individuals with different energy needs and digestive efficiency, leading to sexual segregation between the sexes (Conradt 1998; Ruckstuhl & Neuhaus, 2000). So, the cohesion of males and females in mixed groups becomes unstable and expensive, leading to a split from mixed groups to unisex groups, and in turn to sexual segregation (Conradt, 1998; Conradt & Roper, 2000, 2003).

Due to differences in body size dimorphism, energy requirements and digestive efficiency, males and females differ considerably in time spent on activity patterns. As males are larger, they have a greater rumen and a slower passage of food, making their digestion more efficient compared to females (smaller rumen and faster passage/absorption of food) (Demment, 1982). Consequently, differences in the activity

budget, for example, feeding or ruminating will be noticeable between the sexes (Hudson 1985; Berger and Cunningham, 1988). As the first ABH prediction, females will spend more time in the active state (e.g. feeding or walking) to compensate physiological characteristics above-mentioned, while males, as they have a larger size, do not need to stay in the active state as long, spending more time resting or ruminating (inactive state). In a second prediction, individuals with similar activity patterns will tend to group, while others will segregate (Ruckstuhl & Neuhaus, 2002).

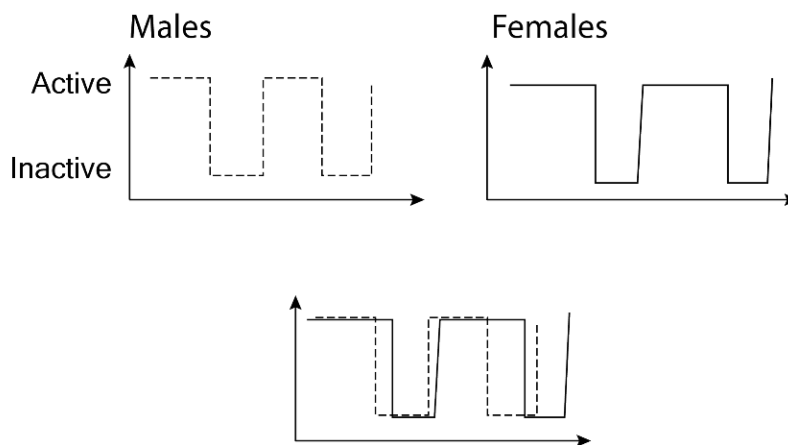


Figure 2 Exemplary scheme of the Activity Budget Hypothesis. (Adapted from Ruckstuhl & Neuhaus, 2005).

The amount of time each animal spends in each activity can be named time budget or activity budget (Fawzy *et al.*, 2019). The activity budget can be influenced by several external factors, that can be abiotic, as temperature, precipitation, light levels, and food availability, and biotic, like interaction or ecological competition with others from the same or different species (Schoener, 1974; Frey *et al.*, 2017). Additionally, to the natural factors mentioned, this can also be influenced by anthropogenic factors.

In species with high body size dimorphism, such as red deer (*Cervus elaphus*), females and males substantially differ in their activity budget (Ruckstuhl & Neuhaus, 2000). For a better understanding of the social and behavioural dynamics of gregarious herbivores, of their adaptations to new temporal changes, and for the definition of effective strategies towards their conservation (e.g. conservation of the surrounding wild fauna) and management, studies on the density of populations and activity patterns need to be carried out, allowing us to obtain possible future answers about the species in question (Singh & Kaumanns, 2005; Caro, 2007).

Our study focused on determining whether males and females' activity patterns are different enough over time to explain sexual segregation patterns of red deer. To this end, three specific objectives were defined: (1) to determine the sexual segregation pattern of red deer; (2) to determine the activity patterns for each gender; (3) quantify the temporal overlap of the activity patterns between the genders.

This study will be focused on red deer, which is considered the largest ungulate of the Portuguese fauna and has a wide distribution in Lousã Mountain. In terms of sexual segregation and aggregation patterns, we expect that the level of overlap in terms of activity patterns of males and females change according to the segregation level. As females are smaller in size and have a lower digestive efficiency, they will spend more time in the active state (e.g. feeding or walking). Males with a large body-size, will spend more time in the inactive state (e.g. lying). This first prediction will occur when the different sexes are segregated and it is expected to find different activity patterns, which may indicate support ABH. In a second prediction, when the different sexes are aggregated (in the mating season), we expect to find similar activity patterns for both sexes. During the mating season, males focus on attracting females and, to do so, they will adjust their activity pattern to theirs, especially in terms of movement, eventually failing to satisfy their own needs, bringing a high energy cost and probably future consequences at the interpersonal level of physical condition.

2.2) Materials and methods

2.2.1) Study area

This study took place in Lousã Mountain, located in the Centre of Portugal (40°3'N, 8°15'W), characterized as a Mediterranean region. Its typical climate is mainly hot and dry during the summer, with rainy winters, almost without snow (Archibold, 1995; Alves *et al.*, 2014). The flora is mostly composed by coniferous forests, mixed forests (evergreen forests and deciduous forests), and large shrublands areas (Figure 3). It was estimated that the presence of red deer populations covers a total area of 500 km², including the Lousã Mountain (170 km²) and surrounding areas (Alves, 2013).

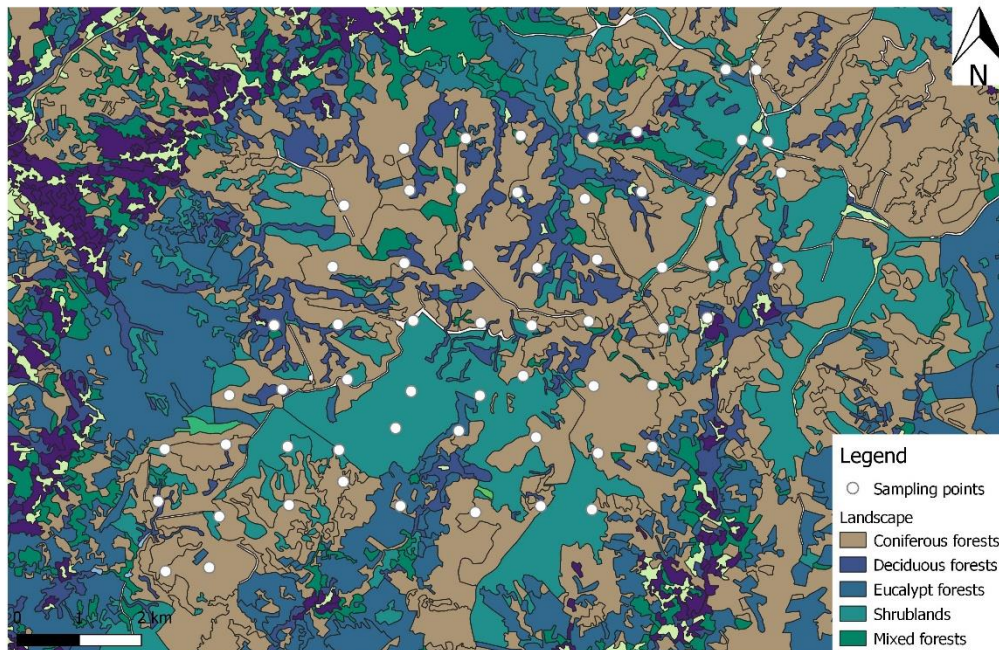


Figure 3 Location of the camera traps on the study area in Lousã Mountain.

In relation to the fauna, a variety of species can be found in addition to study species, red deer, as other ungulates, like roe deer (*Capreolus capreolus*) and wild boar (*Sus scrofa*). Wild dogs had taken the place of non-natural predators of these species, as there are no natural predators, preying mostly young, sub-adults and adult female deer (Alves, 2013).

2.2.2) Field methods and sampling design

2.2.2.1) Camera traps

The fieldwork took place between April 2019 and January 2020. 60 sampling points (camera stations) were selected in the central region of Lousã Mountain, approximately 1 km away from each other, in a stratified sampling design (Figure 3). As in previous studies for the same population, three types of habitat were considered: shrublands, coniferous forests and mixed forests, based on aerial photographs, followed by confirmation in the field. At the 60 points, we can find 20 points covering coniferous forests (presence only of trees of the genus *Pinus* or *Chamaecyparis*), 20 points that constitute mixed forests (presence of more than one species of tree) and, finally, 20 points corresponding to shrublands.

For a better understanding of the behaviour, composition and typology of the group of the red deer population, we analysed videos and photos obtained by camera-traps. 20 Bushnell Natureview Cam HD were deployed, using a rotation scheme, over six established sampling times (Birthing - May 1 to June 15; Lactating - June 16 to July 31; Pre-rut - August 1 to September 15; Rut - September 16 to October 31; Post-rut - November 1 to December 15; Winter dispersal - December 16 to January 31). Each camera was programmed to record for two weeks (24h/per day), and were configured as follows: (1) when triggered take three photos, followed by a video with a duration of 60s (set to time interval of 6s between three photos and the video); (2) higher image quality (14 MP); (3) the sensitivity of the sensor was set to medium, after checking the results of a pilot survey. The cameras were activated whenever the motion sensor was activated, also recording the date, hour and temperature. Every two weeks, the SD cards (32GB) were recovered and replaced, together with the batteries.

2.2.2.2) Data collection

For each group/animal captured by the camera-traps, sex, age-class, group composition and behaviour were recorded. The sex and age-class of the individuals were determined based on their body size, antlers size, and colour of the coat. Calves have smaller bodies with white spots on the dorsal part during the first two months; sub-adults were considered all animals from one to three years old (our four in case of males), which are notorious smaller than adult animals (older than four years, or five case of males) (*e.g.* Bonnet and Klein 1991).



Figure 4 Records of red deer showing the different characteristics between the sexes and among ages in the Lousã Mountain. (A) Female with calf; (B) Matriarchal group; (C) Group of sub-adult males (bachelor group); (D) Solitary male adult.

The beginning of each behaviour will be noted and the duration of that behaviour until a possible change. Each behaviour was classified into eight categories (Table 1), and only registered when the photos and videos were noticeable, otherwise were classified as non-identifiable (NI).

Table 1: Ethogram of red deer behaviours.

Code	Behaviour	Description
FE	Feeding	Animals have the head down and are eating or chewing plants. Contrary to olfactory control the head movements are jerky due the plucking of plants.
WA	Walking	Animals are moving, either walking or running.
ST	Standing	The animal stands without vigilance behaviour.
LY	Lying	All kind of behaviour in which the animal is lying on the ground, i.e. resting or sleeping.
AS	Alert state	Animals interrupt their previous behaviour and raise their head and hears staying static.
OC	Olfactory control	This behaviour is defined as using the sense of smell. The mouth stays closed and chewing motion is missing.
RO	Roaring	When males emit vocalizations.
OT	Others	Other types of behaviour: ruminating, scraping, urinating, defecating, drinking, jumping, fighting, breastfeeding, among others.

2.2.3) Data analysis

We used SSAS to test for changes in temporal patterns of sexual segregation and aggregation between adult males and females. SSAS is a statistical test, which was developed by Bonenfant *et al.* (2007), which aims to test a null hypothesis (random association between the sexes), based on an estimate of the distance between the observed and the expected distribution of males and females. The SSAS results accurately demonstrate, when males and females (or between any age and sex classes), are segregated, aggregated or simply randomly distributed. When SSAS is equal to 1, we are facing complete sexual segregation (animals appear mainly in unisex groups; the observed SSAS is greater than expected). When the SSAS is equal to 0 (complete aggregation), the animals occur mainly in mixed groups (the observed SSAS is less than expected). Therefore, SSAS is an index that varies between 0 and 1 and needs to be interpreted according to the confidence intervals of the expected values.

The synchronization of adult males and females' activities was calculated using the approach developed by Ridout and Linkie (2009). To calculate the overlap of activity patterns between the sexes, we used the kernel density analysis (Ridout & Linkie, 2009). The main goal was to adjust a kernel density activity function to the daily temporal pattern of the males for each type of behaviour, and at each season, and to adjust another kernel

density function for the females' activity. Subsequently, we measured the overlap between the two kernel density functions, which correspond to the grey area. The overlap coefficient Δ varies between 0 (without overlapping) and 1 (complete overlapping). The confidence intervals of the overlap were estimated using bootstrap. The statistical analyses were performed using R 3.5.0 (R Core Team 2019) with the package “overlap” (Meredith & Ridout, 2016).

3) Results

During the nine-month study period, 63, 471 photographs and 21, 157 videos were collected, and subsequently analysed. The captures included 6, 696 photographs and 2, 232 videos of red deer, and species of non-target ungulates, such as wild boar (2, 190 photos and 730 videos) and roe deer (972 photos and 324 videos). We also detected the presence of other mammals, such as red fox (*Vulpes vulpes*) (570 photos and 190 videos) and common genet (*Genetta genetta*) (27 photos and 9 videos).

3.1) Patterns of sexual segregation

Throughout the biological cycle, the social structure of the deer has changed, with sexual segregation being possible cause behind the variation in the group's composition, as reflected in the following image (Figure 5). Throughout the year, with the exception of the mating season, the red deer tends to form groups with individuals belonging to the same sex. In that time frame (signalled by the birthing, lactation and winter dispersal), males and females were totally segregated (SSAS = 1) (Figure 5A).

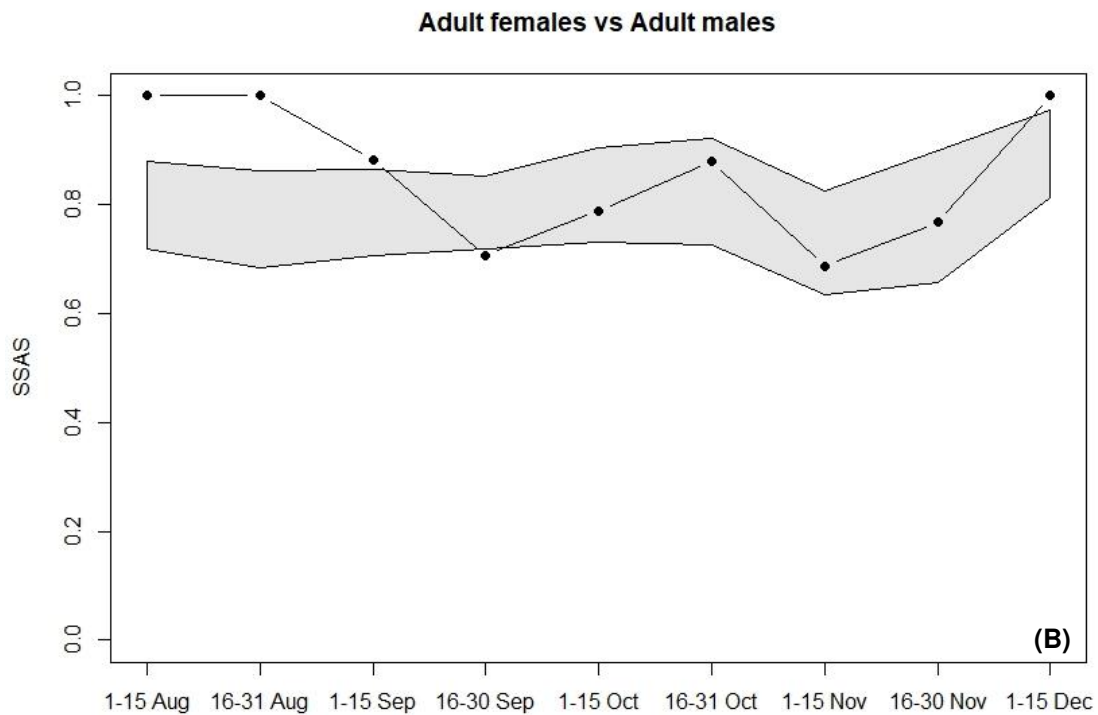
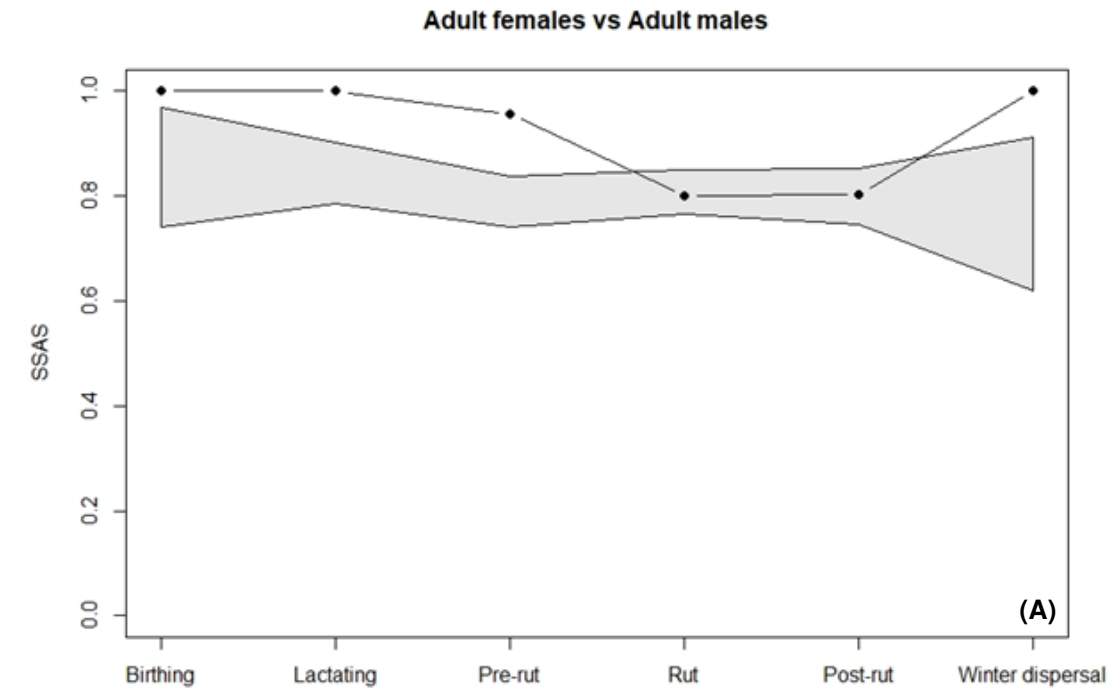


Figure 5 Seasonal patterns of sexual segregation and aggregation for adult males and females of red deer. (A) Seasonal timescale and (B) 15-day interval timescale at pre-rut (August 1 to September 15), rut (September 16 to October 31) and post-rut (November 1 to December 15). The SSAS indicates significant sexual segregation or aggregation when the observed value (black point) falls above or below the SSAS expected interval (grey area), respectively.

To detect more clearly the dynamics of sexual segregation and aggregation, the association between the sexes was analysed on a finer timescale (15-day intervals) (Figure 5B). For the finer timescale, we analysed the complete breeding season, which includes the pre-rut, the rut and the post-rut, giving a more detailed information on how SSAS varies withing this season. The pre-rut marks the beginning of the rutting season, during which there is a movement of animals from their original groups leading to a change on the SSAS from a complete segregation (1) to lower values (Figure 5B). Although some males start roaring on the first and second week of September, is only after the September 15th that aggregation between males and females occurs (Figure 5B), period that marks the peak of the rut season. After a short period of aggregation, males and females assume a random association, that will persist until almost the end of the post-rut (16-30 November) (Figure 5B). After this date, males and females gradually separate, forming unisex groups again, reaching the maximum SSAS value during the winter dispersal.

3.2) Activity patterns of red deer

To clarify the seasonal changes in the daily activity rhythm, the male and female curves were compared at each defined season. A relative dissimilarity in the activity patterns was observed during the phases of the life cycle in which they are segregated, except in the rut, when we can observe a basically identical activity pattern (Figure 6). The kernel density estimates showed that, over the study period, the red deer revealed a bimodal activity pattern, that is, showed by a pattern of twilight activity (with peaks around dawn and dusk).

The comparison of the activity patterns observed over the sampling period allowed to reveal differences in the daily rhythm for each sex. Both at birthing (Figure 6A) and at lactating (Figure 6B) seasons, females exhibited greater activity in the early morning (between 05:00h and 09:00h) and in the late afternoon (between 18:00h and 00:00h). On the other hand, males at birthing season were more active between 03:00h and 11:00h, and between 21:00h and 02:00h. In the lactation season, the males' activity period took place between 02:00h and 12:00h, and between 16:00h and 00:00h. In both seasons, the activity overlap was at their lowest (Birthing: $\Delta=0.58$; Lactating: $\Delta=0.72$).

In the pre-rut, the formation of mixed groups begins to be detected, and this is noticeable in the form of the daily activity patterns of the presented graph (Figure 6C). At this time, the peaks of activity of females vary between 03:00h and 11:00h, and between 17:00h and 00:00h, while the daily rhythm of males was concentrated between 04:00h and

11:00h, and between 18:00h and 03:00h. This pattern resulted in an increase in the overlap ($\Delta=0.82$) when compared with the two previous seasons. In the rut (Figure 6D), we can see very similar patterns in both sexes. Here, we observe a greater synchrony of activity throughout the day ($\Delta=0.88$), where the males are more active compared to the other seasons (between 01:00h and 12:00h, and between 16:00h and 00:00h), and in turn, the females present a daily rhythm approximately coincident (between 00:00h and 11:00h, and between 16:00h and 00:00h). After the rut, the mixed groups began to disperse, and the formation of a unisex group occurred again, which is notorious, due to the appearance of differences in the activity patterns exhibited by males and females, and consequently of the activity overlap ($\Delta=0.80$) (Figure 6E).

During the winter (time of year when they disperse), there was a drop in the daily activity overlap of males and females ($\Delta=0.62$), with a greater asynchrony in the activity patterns (Figure 6F). They maintained their bimodal activity pattern, with peaks at sunrise and sunset, where females concentrate their daily activity (between 07:00h and 12:00h, and between 15:00h and 21:00h), and males between 05:00h and 14:00h, and between 16:00h and 22:00h). In general, females showed a greater proportion of activity, being more active especially during the day. In contrast, males showed greater activity at night. Total activity was higher during the birth season, lactation, pre-rut, intensifying during the rut, post-rut, and being lower during the winter.

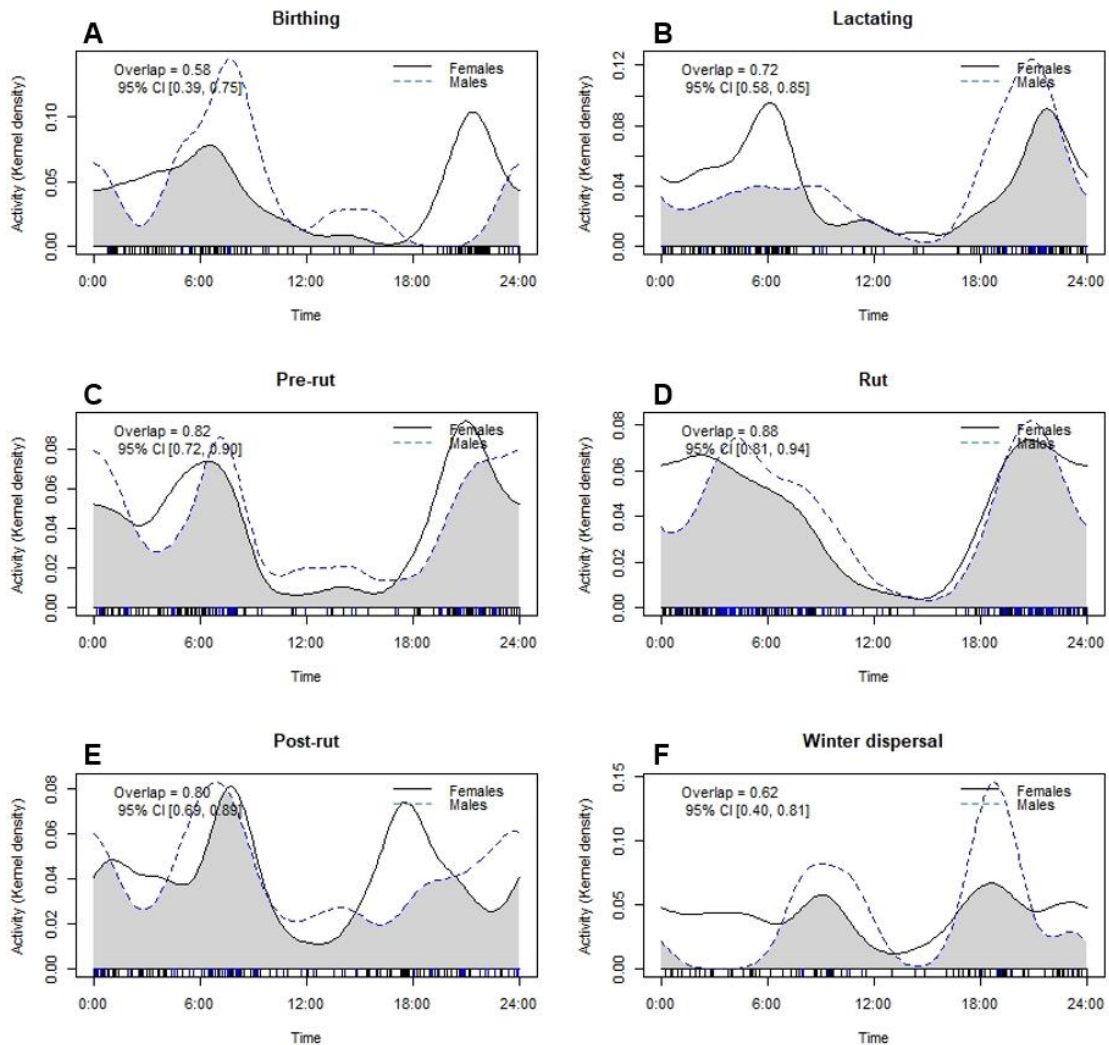


Figure 6 General activity patterns of red deer males (dashed blue line) and females (solid black line) in Lousã Mountain, for each season.

The results showed that solitary females spend most of their activities walking (18.21%) and feeding (50.52%). The remaining registered activities occurred in a smaller percentage (Table 2). Solitary males showed a lower percentage of time spent walking behaviour (11.10%) and feeding behaviour (36.18%), compared to females (Table 2).

When each gender is in groups with individuals of the same sex, in the case of females (matriarchal groups) and males (bachelor groups), the feeding and walking behaviour is maintained with approximately the same percentage as the one observed when they were in solitary groups (Table 2). However, in lying behaviour, it was found that males have greater inactivity compared to females, and this inactivity is higher when males are grouped together. Regarding alert state behaviour, females are more vigilant than males, and this alertness is superior when they occur in matriarchal groups (Table 2).

When males and females are aggregated, *i.e.* during rut season, a discrepancy in the time spent on each type of behaviour is still noticed. The alert state of males is practically non-existent, as is the time spent feeding. The lying behaviour is null. Females maintain their alertness, however, as in males, lying behaviour was not seen. Regarding the feeding behaviour, there was a very sharp decrease in the proportion of time spent for this activity. When mixed groups are present, both males and females, have showed to spend most of their time walking or standing and less time feeding (Table 2).

Table 2: Percentage of time spent by adult males and females performing each behaviour by groups typology.

	Solitary		Bachelor group	Matriarchal group	Mixed group	
	Males (%)	Females (%)	Males (%)	Females (%)	Males (%)	Females (%)
Feeding	36.18	50.52	36.24	47.92	3.89	7.75
Walking	11.10	18.21	12.66	12.01	17.90	23.24
Standing	8.03	3.01	4.33	6.10	40.53	26.91
Lying	3.68	0.57	20.60	6.56	0	0
Alert state	6.91	6.02	7.66	11.83	0.13	6.75
Olfactory control	3.40	2.61	0.40	1.60	2.40	6.16
Roaring	2.93	---	0.56	---	14.85	---
Others	3.00	1.73	6.07	1.66	0	0
Non-identifiable	17.66	24.44	11.47	12.26	20.30	29.20

The activity patterns of adult males and females in six seasons allow us to visualize the degree of activity overlap for specific behaviours at each sampling season. Our results focused only on the most frequent behaviours, which were feeding and walking behaviour.

At birthing and during lactation, females showed a twilight activity, both in feeding and walking behaviours, with inactive hours in the middle of the day. In turn, the males showed multiple peaks through the day. From the birthing to the time of lactation, there was a slight decrease in the daily rhythm of the females for both studied behaviours, with the opposite being verified in the males. The degree of synchrony between males and females, remained approximately the same for feeding and walking ($\Delta=0.65$ and $\Delta=0.66$ for feeding behaviour for the birthing and lactating season, respectively; $\Delta=0.62$ and $\Delta=0.65$ for walking behaviour for the birthing and lactating season, respectively) (Figure 7 and Figure 8).

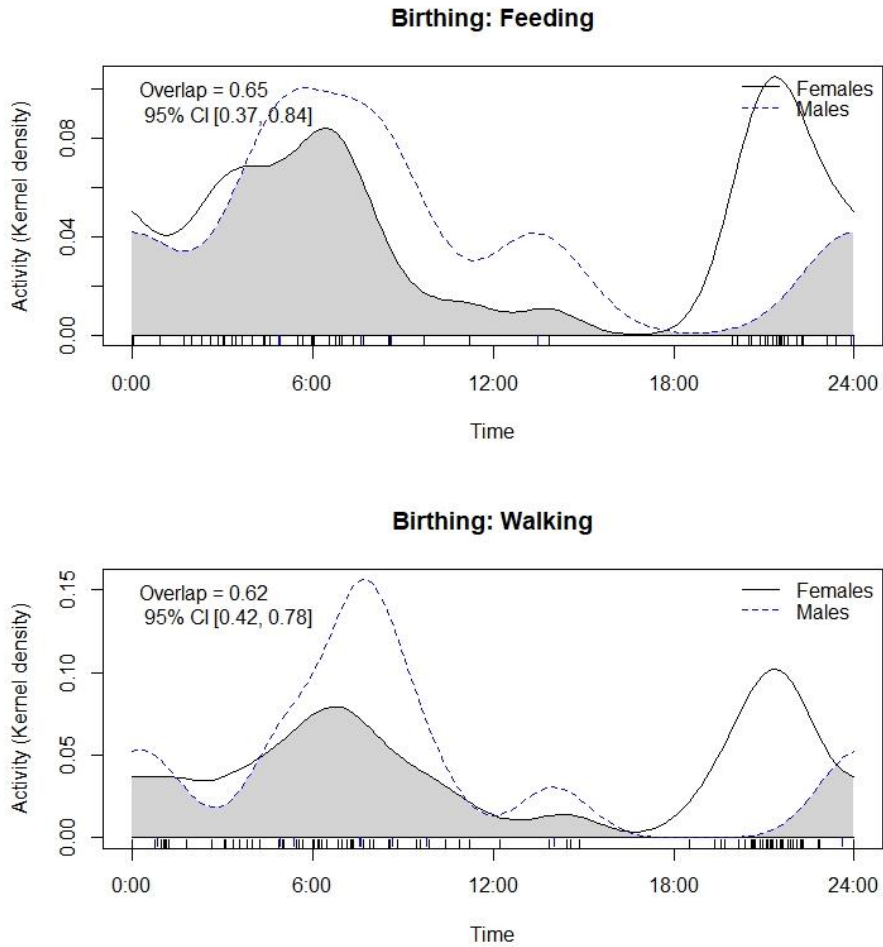


Figure 7 Feeding and walking activity patterns of red deer males (solid line) and female (dashed line), during birthing season. Grey areas represent times when males and female overlap in activities. The overlap values range from 0 (no overlap) to 1 (complete overlap).

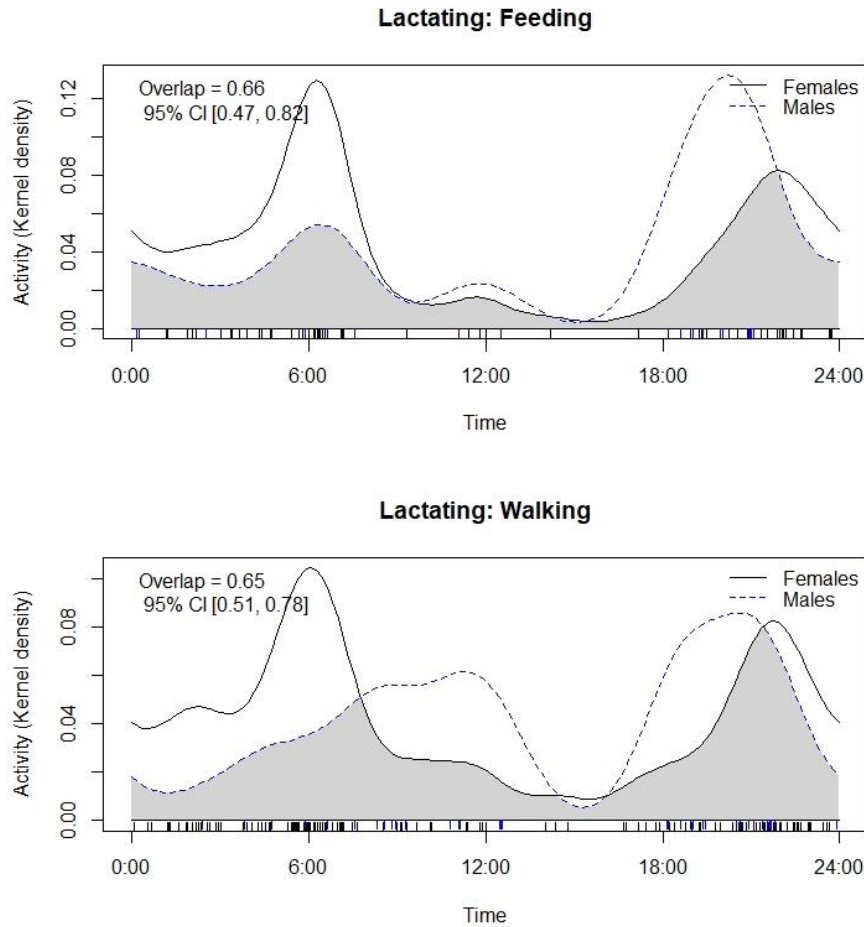


Figure 8 Feeding and walking activity patterns of red deer males (solid line) and female (dashed line), during lactating season. Grey areas represent times when males and female overlap in activities. The overlap values range from 0 (no overlap) to 1 (complete overlap).

In the pre-rut there was an increase in the synchrony rate of walking behaviour ($\Delta = 0.76$). However, males and females showed a lower proportion and synchrony in feeding behaviour ($\Delta = 0.57$) (Figure 9). In rut, we are already facing mixed groups, with the joining of males and females just for mating. We can observe a very sharp decrease in the synchrony of feeding activity ($\Delta = 0.26$). During this short period, males only fed exclusively between 8:00h and 12:00h, and between 18:00h and 00:00h, and even at these hours only a few were observed eating. Females maintained an equal distribution all over the day, with peaks around dawn and dusk. Concerning walking behaviour, a very synchronized daily rhythm was observed ($\Delta = 0.90$), with the presence of very similar activity patterns (Figure 10).

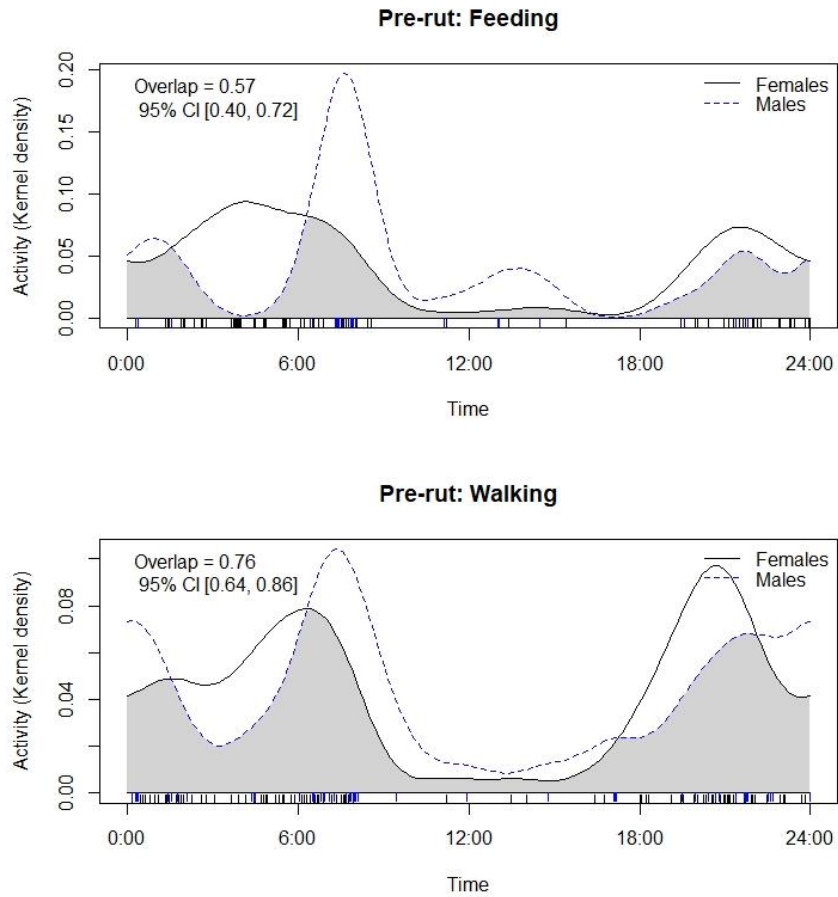


Figure 9 Feeding and walking activity patterns of red deer males (solid line) and female (dashed line), during the pre-rut season. Grey areas represent times when males and female overlap in activities. The overlap values range from 0 (no overlap) to 1 (complete overlap).

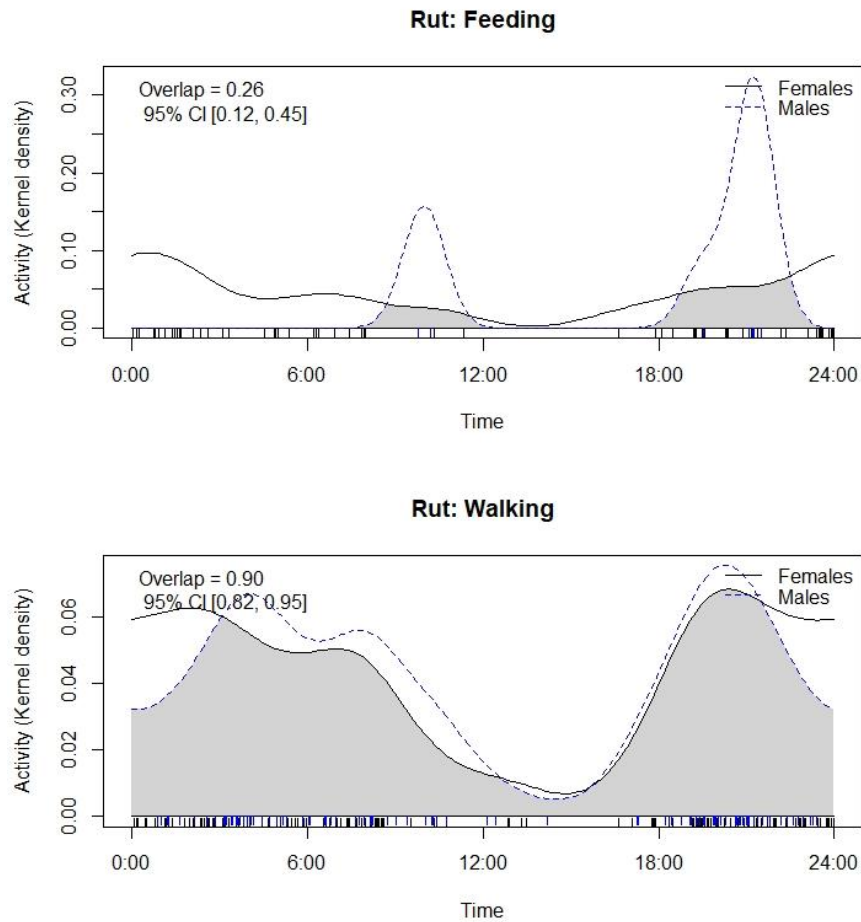


Figure 10 Feeding and walking activity patterns of red deer males (solid line) and female (dashed line), during rut season. Grey areas represent times when males and female overlap in activities. The overlap values range from 0 (no overlap) to 1 (complete overlap).

In the post-rut, the daily rhythms of males and females regarding feeding behaviour increased again, showing multiple peaks during the day, and a higher activity overlap ($\Delta=0.41$). However, with respect to walking behaviour, we can see a noticeable dispersion of daily activity and an increase in the asynchrony between both sexes ($\Delta=0.79$) (Figure 11). With the arrival of winter, males and females are totally segregated, presenting an increasingly greater asynchrony of activities ($\Delta=0.50$ and $\Delta=0.68$ for feeding behaviour and for walking behaviour, respectively) (Figure 12).

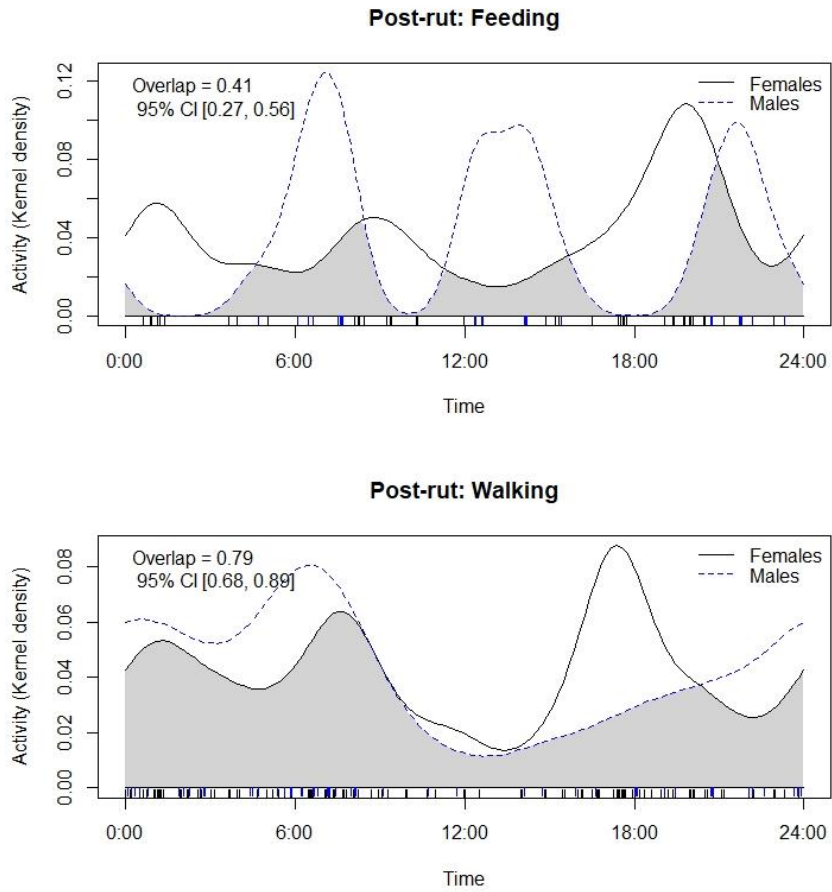


Figure 11 Feeding and walking activity patterns of red deer males (solid line) and female (dashed line), during post-rut season. Grey areas represent times when males and female overlap in activities. The overlap values range from 0 (no overlap) to 1 (complete overlap).

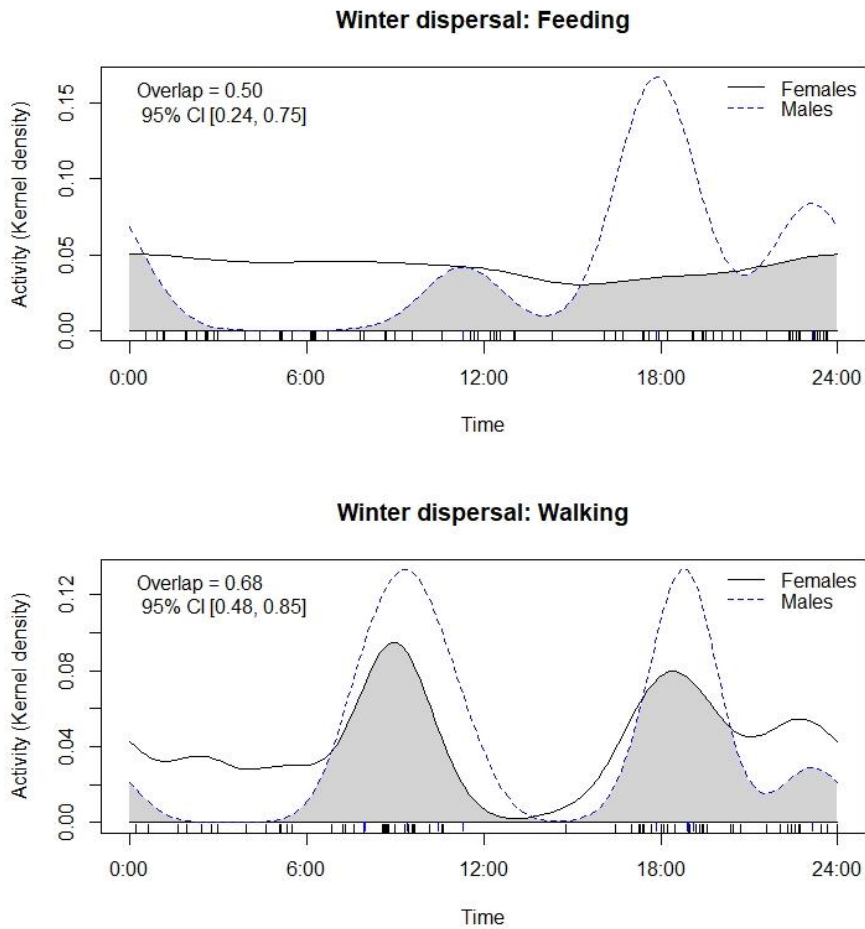


Figure 12 Feeding and walking activity patterns of red deer males (solid line) and female (dashed line), during winter dispersal. Grey areas represent times when males and female overlap in activities. The overlap values range from 0 (no overlap) to 1 (complete overlap).

3.3) Discussion

As an important aspect of behaviour ecology, studies of activity patterns provide crucial information about wildlife, allowing us to better understand and manage species conservation (Singh & Kaumanns, 2005; Caro, 2007). Through the camera-traps it was possible to study the behaviour and activity patterns of the red deer. Although the results obtained only reflect the camera's field of view, the fact that the study was performed over a long sampling period and, with a large coverage of the study area, it is possible to obtain representative data (Ogurtsov *et al.*, 2018).

In our study, the SSAS obtained was in line with expectations. Sexual segregation reached its maximum outside the breeding season (pre-rut, rut and post-rut), that is when, males and females lived in separate groups in the Lousã Mountain (Alves *et al.*, 2013), as it is predicted for most sexually dimorphic wild ungulates, such for example, white-tailed deer (*Odocoileus virginianus*) (Bleich *et al.*, 1997; Ruckstuhl & Neuhaus, 2005), European mouflon (*Ovis musimon*) (Bourgoin *et al.*, 2018) (Kie & Bowyer, 1999; Bowyer, 2004; Main *et al.*, 1996). In a more detailed way, it was observed on a finer timescale, the variation of sexual segregation and aggregation during the breeding season. Pre-rut marks the beginning of the dispersion of males (they begin to become progressively intolerant and aggressive towards each other) for the establishment of their harem, and their relentless pursuit of attracting females (Clutton-Brock *et al.*, 1982). In our results, this is indicated by the decrease in the SSAS value.

During the rut, the association between males and females is notorious according to our results. It marks an exclusive time where mixed groups occur just for mating. The annual patterns of habitat segregation between red deer females and males that we reported on here, with a minimum during September 16 to September 31 (the peak of rut), is in agreement with a previous study on the same species in Lousã Mountain (Alves *et al.*, 2013). After the rut (post-rut), our results showed a split between the association of males and females. This can be verified by the progressive increase in the segregation pattern.

Dimorphism in body size is one of the main pieces of the puzzle associated with trying to unravel sexual segregation (Main *et al.*, 1996). According to ABH, sexual dimorphism in body size is closely linked to digestive efficiency. Indeed, as one of the main factors, dimorphism in body size, leads to sexual differences between males and females causing differences in activity budgets, and therefore, the greater these differences, the greater the differences in activity rhythms (Ruckstuhl 1999; Ruckstuhl & Kokko, 2002;

Ruckstuhl & Neuhaus, 2002). This hypothesis has been widely debated over the years, however, despite many studies supporting ABH (Conradt, 1998; Ruckstuhl 1999; Calhim *et al.*, 2006; Bourgoïn *et al.*, 2018), there are many others that are not in favor of it (Bowyer & Kie, 2004; Pérez-Barbería *et al.*, 2005; Michelena *et al.*, 2006).

As described for other ungulates, such as roe deer (Georgii 1981; Georgii & Schröder 1983; Boyce *et al.*, 2010) or mouflon (*Ovis aries*) (Ciuti *et al.*, 2008), red deer also showed a daily twilight activity pattern, with distinct peaks, one at dawn and another at dusk. This is a typical behaviour performed by deer's (Náhlik *et al.*, 2009; Krop-Benesch *et al.*, 2013). In our study, activity patterns varied greatly by season and during the day. Overall, red deer males and females showed substantially less activity during winter dispersal than in spring/summer (including birthing and lactating, respectively) and fall (including the rut season) (as also showed by Georgii & Schröder, 1978; Green and Bear, 1990).

The synchrony of activity between the sexes was relatively lower in times when they were totally segregated, such as the birthing, lactating and winter dispersal, as expected. In spring and summer (including the birthing and lactating), red deer spent most of their time on feeding behaviour. This may be due to the fact that at this time of year there is a greater availability of forage, either low or high quality. Males take the opportunity to maximize their reserves to be at the maximum of their body condition for the rut season and for the unfavourable winter conditions. Females also had a high feeding activity, due to the great nutritional needs required for breastfeeding (Shank 1985; Lovari & Cosentino, 1986; Bourgoïn *et al.*, 2018).

With the arrival of the rut, our findings showed a summer-autumn transition in feeding and walking activity and in the grouping patterns of males and females. This is because activity patterns are influenced by feeding behaviour, rut's behaviour and by environmental factors (as decreased food availability, temperature and daylight duration) (Ikeda *et al.*, 2019). Consequently, the activity patterns between the sexes during the rut, showed a higher synchrony of activities, compared to the other seasons, as expected. This indicates that at this specific time activity budgets did not contribute to sexual segregation, and therefore, the social behaviour of the rut was the most relevant factor to explain the activity patterns displayed.

Still considering the rut, walking behaviour showed a high degree of overlap between genders. However, regarding feeding behaviour, there was a decrease in activity performed by the two genders, which coincided with the low synchrony of activities

presented. In rut, males are known as non-eaters, decreasing considerably the proportion of time spent feeding activity, and investing all their time in roaring activity, to attract the maximum number of females (Clutton-Brock *et al.*, 1982). These patterns are supported by our results, with a very low proportion of males observed feeding during rut. The females possibly reduced their time spent in feeding activity to get in tune with the males, to be able to give the opportunity to mate and assure offspring. However, both decisions of males and females were not advantageous since the cost of synchronization turned out to be very expensive.

On the other hand, winter is a critical time for ungulates that live in temperate zone, and to deal with environmental restrictions (e.g. food availability and snowfall), red deer reduce their daily activity (Georgii 1981; Renecker & Hudson 1986; Roberts & Dunbar 1991; Owen-Smith 1994; Ruckstuhl *et al.*, 2003). To escape these conditions, males can migrate great distances, while females end up not moving away from their home range (Gonzalez and Pépin 1996; Szemethy *et al.*, 1998). This was reflected in our results, with the low activity rate and synchronization observed between the sexes, since few males were detected during the winter, as they possibly migrated to lower altitudes to search for better quality feeding resources (Alves *et al.*, 2013). Therefore, red deer has shown that its bimodal activity patterns are strongly influenced by feeding behaviour, which is characterized by the rumen morphology and digestion physiology (Hofmann 1989).

With our results we can conclude that the differences found between the patterns of activity, and the patterns of segregation of males and females, it is possible that ABH supports in part sexual segregation. So, answering our main question: "Could the differences between activity patterns of males and females explain sexual segregation patterns?", although our results appear to be sufficient to explain the great phenomenon of sexual segregation, further studies must include other factors to more accurately complement ours.

Chapter III- General Conclusions

The main objective of this study was to understand if the differences in activity patterns between males and females could explain the sexual segregation patterns. Our results allowed us to confirm the proposed predictions, supporting Activity Budget Hypothesis in the red deer population in the Lousã Mountain. Males spent more time resting (inactive state) than females. In turn, females spent more time in the active state (e.g. feeding or walking) as predicted. Differences in activity budgets are generally interpreted as a consequence of body-size dimorphism and digestive efficiency (rate of bite and digestion), leading to an asynchrony of activity, and consequently the formation of unisexual groups by individuals with similar activity budgets (Ruckstuhl & Kokko, 2002).

Dimorphism in body size allows us to relate the habitat and social components. However, in our present study, we only focused on the social component, and in future studies we should focus on exploring differences at the level of habitat to complement these results and to test for the other predictions associated to ABH, and thus understanding better the sexual segregation of red deer.

In conclusion, as mentioned earlier, some studies did not support ABH (Bowyer & Kie, 2004; Pérez-Barbería *et al.*, 2005; Michelena *et al.*, 2006), and therefore differences in activity budgets might not be the only factor responsible for sexual segregation. This being said, we must consider other factors, such as predation risk, forage selection and different social motivations when trying to interpret all the patterns of sexual segregation in the different species or different populations of the same species (Bon *et al.*, 2001; Ruckstuhl & Kokko, 2002; Ruckstuhl & Neuhaus, 2005).

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