



UNIVERSIDADE D
COIMBRA

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**ACOUSTIC ECOLOGY OF RED DEER IN A
MEDITERRANEAN LANDSCAPE**

Tese de Mestrado em Ecologia, orientada pelo Professor Doutor José Paulo Sousa e pela Doutora Joana Silva Alves (Universidade de Coimbra) e apresentada ao Departamento de Ciências da Vida da Faculdade de Ciências e Tecnologia.

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BUCÓLICA

“A vida é feita de nadas:
De grandes serras paradas
À espera de movimento;
De searas onduladas
Pelo vento;

De casas de moradia
Caiadas e com sinais
De ninhos que outrora havia
Nos beirais;

De poeira;
De sombra de uma figueira;
De ver esta maravilha:
Meu Pai a erguer uma videira
Como uma mãe que faz a trança à filha.”

Miguel Torga

Acoustic Ecology of Red Deer in a Mediterranean Landscape

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

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Abstract

The relationship mediated by sound between animals and the environment around them is designated Acoustic Ecology. Acoustic Ecology is nowadays one of the most emerging and innovative areas of study, and the study of sounds in the habitat in which animals live is extremely important, especially in behavioural studies.

Red deer are animals with a high sexual dimorphism. One of the great differences between the sexes is in the fact that males emit vocalizations at the rut season. During this season, males roar to compete for the attraction of females and defend them from possible rival males. At this point, males begin to adopt increasingly aggressive behaviour moving away from the other males and emitting roars to constitute their harems.

The aim of this study is to understand the spatial and temporal patterns of these vocalizations emitted by males. I expect to find spatial and temporal heterogeneity on the number of roars across the study area and sampling period, respectively. By placing AudioMoths in the Lousã Mountain it was possible to analyse the roars of males during their roaring season, between September to October. The results showed that males have their roaring activity focused at night, showing a nocturnal behaviour. A significant increase in roars was observed during the peak of the roaring season. We also noticed that some climatic parameters, such as temperature and wind, and some environmental parameters, such as altitude and slope, can have a negative impact on the production of vocalizations. Regarding the type of habitat chosen by the red deer to roar in the study area, it was noticeable a higher number of roars in coniferous forests, which are more reserved areas and with lower anthropogenic noise.

Although this study brings new insights on red deer roaring season, the lack of other studies on this subject in other red deer populations does not allow further comparisons and conclusions. Future studies are needed to compare these results with results from other habitats and other red deer populations, as well as the characterization of these vocalizations to truly understand the role of the roars during one of the most important season for red deer, which is the rut season.

Keywords: *Cervus elaphus*, Acoustic ecology, Soundscape, AudioMoths, roars

Resumo

A relação mediada pelo som entre os animais e o ambiente que os rodeia designa-se de Ecologia Acústica. A Ecologia Acústica é nos dias de hoje uma das áreas de estudo mais emergente e inovadoras, sendo de extrema relevância o estudo dos sons nos meios em que os animais vivem, principalmente em estudos comportamentais. Os veados são animais que apresentam um elevado dimorfismo sexual. Uma das grandes diferenças entre os sexos é o facto de os machos emitirem vocalizações na época de reprodução, comumente chamados bramidos. Durante a época de reprodução, os machos bramam para competir pela atração das fêmeas e defendê-las de possíveis machos rivais. Nesta fase, os machos começam a adotar um comportamento cada vez mais agressivo separando-se dos outros machos e emitindo bramidos para constituírem os seus haréns.

Este estudo tem como objetivo estudar os padrões espaciais e temporais destas vocalizações emitidas pelos machos. Os resultados esperados incluem heterogeneidade espacial e temporal no número de vocalizações ao longo da área de estudo e das épocas de amostragem, respetivamente. Através da colocação de AudioMoths, na Serra da Lousã, foi possível fazer uma análise dos bramidos dos machos durante a sua época de brama, que ocorre entre setembro e outubro. Os resultados mostraram que os machos têm a sua atividade de brama concentrada durante a noite, apresentando um comportamento noturno. Um aumento significativo de bramidos foi observado durante o pico da brama. Também notamos que alguns parâmetros climáticos, como a temperatura e o vento, e alguns parâmetros ambientais, como a altitude e o declive, podem ter um impacto negativo na emissão destas vocalizações. Relativamente ao tipo de habitat escolhido pelo veado para bramar na área de estudo, foi notório um maior número de bramidos nas florestas de coníferas, que são zonas mais recatadas e com menor ruído antropogénico.

Embora este estudo proporcione novas informações sobre a época de brama do veado, a ausência de estudos sobre esta temática noutras populações de veado não permite efetuar comparações ou retirar conclusões mais globais. Estudos futuros são necessários para comparar estes resultados com resultados de outros ambientes e outras populações de veado, bem como a caracterização destas vocalizações para entender verdadeiramente o papel dos bramidos durante uma das épocas mais importantes para o veado, que é a época de acasalamento.

Palavras-chave: *Cervus elaphus*, ecologia acústica, paisagem sonora, AudioMoths, vocalizações.

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Chapter I - General Introduction

1.1 Animal Communication

For all animals communication provides a vehicle to convey information (Hauser 1996). Animal communication is the process that involves the transference of information from one or more animals (sender) to other(s) (receiver), that will affect and modify their behaviour. When this information changes the behaviour of the receivers, it is called signal (Davies et al. 2012). These are only evolutionarily stable if both the receiver and sender benefit from the signalling system. Thus, they must contain reliable, interesting, and emphatic information for the receiver (Davies et al. 2012).

Depending on the environment in which the animal lives, some types of signals are more efficient than others, which may or may not be detected by the receiver. The environment in which the animal finds itself has limitations regarding the type of signals that will be sent to the receiver, and whenever possible, these should have a low level of distortion (Halliday and Slater 1983). All communication signals involve some form of energy, it is subjected to the laws of physics, which rules its production and transmission in the environment (Halliday and Slater 1983; Bradbury and Vehrencamp 1998).

Communication can be one of three types: 1) intraspecific communication; 2) interspecific communication; 3) autocommunication. Intraspecific communication occurs between participants of the same species (Lindström and Kotiaho 2002). It usually occurs in social species, which live in groups, playing a fundamental role in their cohesion and organization. Interspecific communication occurs among different species. One of the most studied examples is the prey-predator relationship. Autocommunication is a type of communication in which the sender and receiver are the same individuals. The sender produces a signal that will be changed by the environment, which will then eventually be received, providing him with additional information (e.g. active electrolocation (Albert and Crampton 2006) and echolocation (Jones and Teeling 2006)).

There are several types of signals used by the most diverse animals: chemical signals, tactile signals, visual signals, electrical signals and acoustic signals. Chemical communication is related to a chemical signal secreted by animals. Pheromones are an example and are used to trigger a response in another individual of the same species. Billen and Morgan (1998) stated that many insects have exocrine glands scattered throughout the body that produce and secrete chemical compounds that are used as signals. Tactile communication is widely used by animals in fights, mating, and social inclusion. One example of this is the bees, which perform a complex sequence of tactile signals and vibrations, communicating the location of food to other bees (Frings 1962). Visual communication is one of the most well-known types of communication, it involves signals that can be seen. These signals include gestures, body postures and colouration. For example, several species of fireflies attract females by emitting lights flashing at the bottom of their body (de Crespigny and Hosken 2007). Electrical communication plays a fundamental role in mating rituals, orientation and marking of territory. Some species of fish produce electric pulses and use these skills in their courtship, orientation, communication

and territorial competitions (Baier and Kramer 2007). Finally, there is acoustic communication, which happens when the information transmitted by one or more senders to one or more receivers is made through sound production (Hopkins 1977).

Acoustic communication has evolved to take advantage of the physical properties of sound. Each species has its own well-defined acoustic communication system, which seeks to adapt to the specific needs of information exchange and the propagation requirements imposed by the environment where it lives. Acoustic signals are used by a diversity of groups of animals, mainly insects (Simmons 1986; Zuk 1987), amphibians (Ryan 1982; Robertson 1986), birds (Searcy and Andersson 1986; Catchpole 1987) and mammals (McComb and Reby 2005). In mammals, it has become of particular significant due to the functions it performs at the level of marking territory, mating rituals (Charlton and Reby 2011), alarm calls (Fichtel and Gros-Louis 2005), in disputes (Lindburg 1971), in signs related to food, among others.

In acoustic communication, two types of vocalizations are associated: 1) vocals and 2) non-vocal. Vocal vocalizations can be divided into ultrasounds and audible. Ultrasounds are sounding whose frequency is so high that it exceeds the capacity of human range and are found, for example, in species of bats and dolphins. Bats use ultrasounds as a means of guidance (Griffin 1958) and dolphins and whales use them to communicate with each other (Tyack and Clark 2000). Audible vocal vocalizations are considered sounds produced by the larynx (Fitch 2006) or syrinx (Fee 2002), present mostly in mammals and birds, respectively.

Non-vocal vocalizations are those that are not produced by the vocal apparatus, as the sound emitted in vibrations and stridulation (Masters 1980), for example. Stridulation is the production of sounds when rubbing one part of the body with another, this often seen in insects, such as cricket and cicada (Simmons 1986).

This study will have as main focus the vocalizations emitted by the red deer, these are in general a set of sounds with certain properties that are transmitted, depending on their emitter.

1.2 Sound Properties

Sound is a valuable communication system, used in various situations, taking an important role in the animal kingdom. Sound waves are mechanical waves that propagate through various materials, however, the most important are those that propagate in the air (Serway and Jewett 2014).

Sound is a very relevant information transfer system that can avoid obstacles and achieve long distances (Sueur and Farina 2015). The sound is characterized by oscillating waves. These waves can propagate at different speeds depending on the environment. Several environments can be involved, as the water, (1484 m/s to 20°C), soil ground (~5000 m/s depending on porosity) or even air (343 m/s to 20°C). All these speeds are temperature-dependent (Obrist et al. 2010).

The basic properties of sound are frequency, amplitude, and duration. Amplitude is the temporal oscillation of a wave, usually expressed in decibels (dB). Frequency is the number of cycles per unit of time, expressed in Hertz (Hz). This property allows distinguishing bass sounds, with low frequency, and high-pitched sounds, with high frequency. The sound spectrum is the set of all frequencies of sound waves, which is divided into three categories: the frequencies between 0 Hz and 20 Hz designated infrasounds, between 20 Hz and 20000 Hz, which have audible sounds and frequencies greater than 20000 Hz that are designated ultrasounds (Resnick and Halliday 1983).

1.3 Acoustic Ecology

Acoustic ecology is the study of the relationship mediated by sound, between animals and the environment in which they live (Westerkamp 2002; Sueur and Farina 2015). This area of study is related to the term “sound landscape”, which is the environmental sounds of a particular site.

Today, interest in Acoustic Ecology is on the rise mainly due to the activities of the World Forum on Acoustic Ecology (WFAE), which was founded during the First International Conference on Acoustic Ecology in 1993 (Wrightson 2000).

The use of these animal vocalizations focuses on several areas, such as nature conservation, education, recreation and tourism (Alström and Ranft 2003). In nature conservation, they serve as a reference for species identification and as a measure of biodiversity (Pereira 2011).

With the increasing interest in the monitorization of the effect of climate change on biodiversity, acoustic ecology emerged as a method for evaluating and controlling species as it may be indicative of habitat changes (Pereira 2011). Moreover, it is a non-invasive method that does not have implications on the natural behaviour of the animal, allowing posterior analysis of the records to retrieve different types of information (Marques et al. 2011). It can also be used for nocturnal species and for ones that are active over a 24 hours period.

The study of this area has assisted researchers to develop species management and conservation projects, allowing for the study of phenology and geographical distribution of species and animal populations. Also, the identification of important areas for the biodiversity and for the evaluation of long-term changes related to climate change, habitat modification and anthropogenic activities (Pereira 2011) were improved with the development of this area.

In Portugal, acoustic ecology is focused on the investigation of fish ecology (Amorim et al. 2010) and in conservation (Pereira 2011). The main animals studied are groups of birds (Saraiva 2005), marine mammals (Faustino 2010), bats (Rainho 2007; Rebelo and Rainho 2009), and anurans (Márquez et al. 2008). Studies on this area are relatively recent and there are no studies on terrestrial mammals in Portugal (Pereira 2011).

1.4 Soundscape ecology

Sound is defined by Pijanowski et al. (2011) as “perpetual and dynamic property of all landscapes”. The soundscape ecology is a junction of two important fields of study: landscape ecology and acoustic ecology. The relationship between the landscape and its sound is called soundscape (Pijanowski et al. 2011).

This is an emerging area and, since it is a multidisciplinary research topic, it can provide new perspectives on the acoustics of landscapes. During recent times, studies involving sound became increasingly common, mainly due to their interdisciplinarity and for transmitting valuable information about changes in landscapes.

A soundscape is a powerful tool for monitoring biodiversity and plays an important role in understanding animal acoustic communication. The Sender-Propagation-Receiver (SPR) model is behind several studies, which focus on animal acoustic communication. This model is mainly used to describe elements of information propagation. It is able to clarify the influence of physical environment in modelling the signal, the purpose of the message and the characteristics of biophysical sender, and also the interpretation the receiver makes with the signal (Pijanowski et al. 2011).

Soundscape ecology aims at sort out several challenges, being thus classified as a transdiscipline. Quantifying parameters as species competition, mutualistic behaviour or biotic diversity based on sound are examples of its purpose (Hansen and DiCatri 1992). Further, it is known to contribute to solving problems related to ecological resource management (Barrett 1985); and also to be able to decipher ecosystem/landscape patterns based on spatial-temporal scales, apprising sound as acoustic processes (Truax and Barrett 2011).

This study aims to gather aspects of animal communication, acoustic ecology and soundscape ecology to decipher aspects of red deer’s ecology.

1.5 Study Species – *Cervus elaphus*

1.5.1 Specie distribution and habitat

The red deer *Cervus elaphus* (Linnaeus 1758) is one of the most widely distributed cervids in the world, occurring in America, Europe, Asia and North Africa (Salazar 2009). At European level, this mammal has had an increase in its population over the years (Lovari et al. 2008), being present in almost all countries except Iceland, Finland, and Albania (Blanco 1998).

The red deer was almost extinct in Portugal, but with the efforts of different reintroduction plans and the abandon of rural areas (Alves 2013), nowadays red deer has stable populations throughout the country. The largest populations in Portugal are in the Montesinho Natural Park, São Mamede Mountain Natural Park, Tagus Natural Park and Lousã Mountain (Salazar 2009).

This species has high ecological plasticity, using preferably ecotones between forestry areas and open areas of shrublands or herbaceous vegetation (Blanco 1998; Carranza 2007; Alves et al. 2014). Open areas are often used for food, while forestry areas function as places of refuge and rest (Lovari et al. 2008).

1.5.2 Morphology, ecology and behaviour

Cervus elaphus is a ruminant mammal belonging to Ungulata superorder and the Cervidae family, is the largest cervid of Portuguese Fauna (Peixoto 2014). Characterized by side-eyes, elongated snout and wide ears on top of the head (Baskin and Danell 2003). The red deer changes its colour depending on the season, during the summer it presents reddish-brown fur and on winter presents dark brown fur (Oliver 1999). The offspring have a dark brown colour with whitish-yellow stains on the back and flanks (Barroso and Rosa 1999)

The red deer presents high sexual dimorphism. Males and females present distinct characteristics that allow individual recognition, those being: 1) the presence of antlers in males; 2) size and body shape; 3) coat colouring and 4) the male's roar in rut season (Clutton-Brock et al. 1982; Blanco 1998; Barroso and Rosa 1999; Alves 2013).

The most noticeable characteristic for differentiating the sexes are the antlers present on the heads of males, bone formations of high hunting value (Peixoto 2014). The formation of these antlers is a process energetically demanding (Geist 1991), since it falls every year in March and April (Blanco 1998), renewing every year after four months. In the first year, young males already have two unbranched antlers (Ferreira 1998). The body dimensions and weight of this species vary depending on the geographical location (Blanco 1998). Females are smaller than males, and in

Mediterranean regions, males weigh 130 kg to 180 kg and measure 175 to 250cm long, while females weigh approximately 80 to 120Kg and measure 160 to 210cm long.

Another big difference is the fact that males are the only ones who emit vocalizations in the rutting season (McComb 1991), which occurs from September to October in our study area, and that serve to intimidate rival males, compete for the attraction of females and to mark their territory (Alves 2013). When only one sex of a species vocalizes means that it is directly linked to its reproductive success (Frey and Gebler 2010), which leads that these vocalizations have evolved due to sexual selection, transferring reproductive advantages to the Cervidae family. The rut season represents a phase of great effort and wears for males (Barroso and Rosa 1999). In the group of these cervids, vocalizations are costly in energetic terms, so only males in great shape can vocalize (Weissenrubler et al. 2002). These vocalizations in the breeding season are of low frequency (Weissenrubler et al. 2002).

Red deer is a polygamous species (Clutton-Brock 1989), presenting a gregarious behaviour, significantly influenced by sexual segregation (Clutton-Brock et al. 1982; Alves et al. 2013). For most of the year, males and females live separately, establishing unisex groups (Blanco 1998; Carranza 2007). Outside the rut season, females have a matriarchal organization, with groups being composed by at least one adult female and offspring, i.e. calf, sub-adult females and sub-adult males (Clutton-Brock et al. 1982). In males, juvenile leaves the parent group from the age of three, forming groups with other males of similar age (Carranza 2007). In pre-rut, males start to become intolerant to each other, moving to the rutting areas to defend the females (Alves 2013). At this point, males start to join to females, forming their harems and initiating mating activities (Clutton-Brock et al. 1982).

The reproductive cycle of the deer is considered highly synchronized, with all births concentrated in a short period (Festa-Bianchet 1988; Coulson et al. 2003; Alves 2013). The reproductive cycle is divided into three main phases: 1) pregnancy (September to May); 2) lactation (June to September) and 3) rut (September to October) (Alves et al. 2013). Both sexes have different breeding strategies, males depend on their physical condition during the rut season, while females depend entirely on the survival of the offspring's (Clutton-Brock et al. 1982).

1.5.3 Rut Season

The mating season, called rut season, is one of the most important stages for red deer and is the moment when their organization becomes more varied. During this season, the mature male competes for the hind's attention and try to defend them from other males. For this species, the rut season begins with changes on a set of factors, which includes body condition, photoperiod and testosterone levels (Goss 1968; Lincoln et al. 1970; Lincoln 1971; Alves 2013). For that, during spring and summer, the males improve their physical condition to enhance their performance during the rut season.

This season can be divided into three phases: 1) pre-rut (August-September), 2) rut (September-October) and 3) post-rut (November-December) (Alves 2013). In the pre-rut, males leave their groups and progressively adopt an individualistic behaviour towards other males (Blanco 1998; Barroso and Rosa 1999) and territorial behaviour (Carranza et al. 1990, 1995), and at this time they still do not emit roars very frequently. In the rut, they begin to regularly emit roars, attracting the females to mate and to constitute their harems, defending them from possible competitors. When the peak of the rut occurs, males emit a greater number of roars. The time of the rut represents a phase of great effort and wear for males, in which an adult individual can lose about 40 kg of his body weight (Barroso and Rosa 1999). In the post-rut, they return to their unisex groups' formation, being segregated and performing the cycle again. At this point, the roars cease completely.

1.5.4 Importance

The red deer is an economically and ecologically valuable species, being increasingly studied over the years (Clutton-Brock et al. 1982). This species plays an important role as prey of populations of threatened carnivorous species, such as wolf *Canis lupus* L. and lynx *Lynx* spp. (Jedrzejewski et al. 1993; Cabral et al. 2005; Nilsen et al. 2007).

Cervus elaphus is extremely valuable due to hunting, for their trophies; and ecotourism (Peixoto 2014; Alves 2013). In Portugal, the main deer hunt process is called *montaria* (Alves 2013). However, this should be controlled through proper management of the existing populations.

At the ecological level, these mammals influence the structure and composition of vegetation of the ecosystems in which they are present (Palacios et al. 1984; Gill 2000) since they influence soil nutrients, soil biota and animal communities (Mohr et al. 2005).

1.6 Study area

1.6.1 Location, topography and climate

The study area is Lousã Mountain, which is located in the central region of Portugal (40°3'N, 8°15'W) and presents an area of approximately 170 km² (Alves 2013). This mountain presents an altitude between 100 and 1205 meters above the sea level, being its highest point, the top of the Trevim (1205 meters). Characterized by deep valleys and pronounced hilltops (Alves 2013), this area is involved by two drainage basins, the Mondego, and Tejo Rivers basins (Alves 2013).

Lousã Mountain plays a role in the climatic characteristics of the region. Its climate is typically Mediterranean, characterized by hot and dry summers and almost snowless rainy winters (Archibold 1995; Alves et al. 2013). Temperature and precipitation patterns vary due to the mountainous topography (Alves 2013).

The ecological importance of this region has been recognized internationally being integrated into Natura 2000 Site. The geological, geographical, climatic and hydrographic influences from the region originate habitats with very particular conditions, presenting high selectivity on fauna and flora. Natura 2000 Site includes 13 priority habitats, these habitats present high importance for the conservation of plant and animal species (Cabral et al. 2005).

This mountainous region has a vast road network with more than 500 km of length, featuring a high traffic volume, at the beginning and end of the day, in the surrounding areas (Alves 2013).

1.6.2 Flora e fauna

Lousã Mountain has a wide diversity of protected plant and animal species, of high synergetic value at a regional and national level. The land cover of the Lousã Mountain is mainly composed by a mixture of coniferous and broadleaf trees combined with large areas of shrublands (Alves 2013). The coniferous forests are dominated by some species of pine trees, essentially *Pinus pinaster*, *Pinus sylvestris*, *Pinus nigra*, *Pseudotsuga menziesii* and *Cupressus lusitanica*. The broadleaf trees are less common and consist of *Quercus* spp., *Castanea sativa*, *Prunus lusitanica* and *Ilex aquifolium*. These coniferous trees together with some broadleaf trees represent the mixed habitats. In the shrubland areas, the dominant species are *Erica* spp., *Calluna vulgaris*, *Ulex minor*, *Rubus ulmifolius* and *Pterospartum tridentatum*. Outside the mountainous region, is common to find plantations of eucalyptus and pine trees and agricultural fields with fruit trees and crops (Alves 2013).

The Lousã Mountain hosts important communities of amphibians (e.g. *Chioglossa lusitanica* Bocage, *Rana iberica* Boulenger), reptiles (e.g. *Vipera latastei* Boscà, *Lacerta schreiberi* Bedriaga), a wide diversity of avifauna (e.g. *Circus cyaneus* L., *Circus pygargus* L., *Caprimulgus europaeus* L.) and mammals (highlight the carnivore populations of *Vulpes vulpes* L., *Genetta genetta* L., *Mustela putorius* L., *Martes foina* Erxleben). With a focus in the ungulates, in addition to red deer,

there are roe deer *Capreolus capreolus* L. and wild boar *Sus scrofa* L. populations. There are no natural predators, yet abandoned dogs act as non-natural predators (Alves 2013).

1.7 Aims

The main goal of this study is to understand the spatial and temporal patterns of males' vocalizations in red deer at Lousã Mountain. For that, it is important to understand the spatial patterns of the roaring, more specifically, whether vocalizations occur uniformly throughout the study area and if their number are related to environmental parameters (e.g. habitat, proximity to infrastructure, altitude, slope). On the other hand, it is also relevant to analyse roaring temporal patterns. To achieve this goal, it is relevant to understand whether the vocalizations occur mostly during the day and how it changes during the roaring season, from September to October, understanding if the number of vocalizations are somehow related to climacteric parameters (e.g. temperature, wind).

Chapter II – Acoustic Ecology of Red Deer in a Mediterranean Landscape

2.1. Introduction

The animal communication system has evolved so that individuals can make decisions, through signals, based on the behaviour, physiology and morphology of other individuals. Signals are changes in the environment caused by one individual (emitter) to another (receiver) transmitting information of various types. These are transmitted differently depending on the medium they cross, either air, water, or substrate, and can be interpreted by the receiver or receptors in various ways (Endler 1993).

Animal communication uses signals that exploit various sensory modalities, such as visual, chemical, electrical, or acoustic signals (Frings 1962; Billen and Morgan 1998; Baier and Kramer 2007; de Crespigny and Hosken 2007). Acoustic communication is especially important since the sound is a signal with high flexibility and that transmits a lot of information at long distances and in a short time. Sound signals play a fundamental role in the choice of the sexual partner in several animals and can be emitted by one or both sexes (Ryan 1990). Mammals preferably use these signals for sexual selection, territory defence, alarm calls and for communication in dense habitats (Frey and Gebler 2010).

For most of the year, the males and females live separately, establishing unisex groups (Blanco 1998; Carranza 2007), except at the mating season, called rut season. The rut season is a fundamental time for its reproductive biology because the breeding success of the male depends exclusively on this season (Clutton-Brock et al. 1982; Main and du Toit 2005). In the Lousã Mountain, it is possible to verify two types of reproductive strategies adopted by adult males, some defend a group of females and others defend some sites (Alves 2013). The rut season occurs between September and October in our area of study and consists of vocalizations emitted by the males to attract the females and as a signal of competition with other stags (Reby et al. 2005).

According to some authors, the rut season starting is related to males' body condition, photoperiod and testosterone levels (Goss 1968; Lincoln et al. 1970; Lincoln 1971). When the photoperiod changes from September on, the pineal gland is stimulated to secrete a hormone. This hormone is also known to lead to an increase in testosterone, as it is directly linked to its secretion (Baratelli 2014). Males also increase their physical condition during spring and summer as a way of improving their performance during this season. Since this season is highly demanding, as it implies a high effort and wears for them, that can imply an adult male losing of about 40kg of weight (Barroso and Rosa 1999).

It is also at this phase that their social organization becomes more varied. For most of the year, outside the mating season, males and females of red deer live separately, establishing unisex groups (Blanco 1998; Carranza 2007). Then, during rut season, mixed sex groups are formed. This season can be divided into three main phases: 1) pre-rut 2) rut and 3) post-rut (Alves 2013). In our study area, in the pre-rut, between August and September 15th, the roars begin to be emitted by males to keep distant other rivals. At pre-rut, the stags progressively adopt an individualistic (Blanco 1998; Barroso and Rosa 1999) and territorial behaviour towards other males (Carranza et al. 1990, 1995), and they move to the

core areas of Lousã Mountain, starting to mark their territories and roaring. After the establishment of males' ranking, the proportion of mixed groups increase and starts the mating season, where males follow a group of females, trying to keep them together and protecting them from other males (Alves 2013). In rut, between September 15th and early October, they begin to regularly emit roars, appealing the females to mate and to constitute their harems, and defending them from possible contestants. At this period occurs the roaring peak, and the males emit a high number of roars throughout the day, for several days. In the post-rut, in November-December, the rut ends, and the groups return to being separate and performing the cycle again.

The adult red deer males produce loud low-pitched vocalizations (Fitch and Reby 2001). The low frequency and lowered vocal tract resonances, calling formants, are characteristic from the roaring (Frey and Gebler 2010). Some studies indicate that the momentary retractions of the larynx, during the rutting calls, probably evolved through selection pressure. This allows to acoustically exaggerate the body size in contests, especially at night since visualizing a rival can be difficult under twilight or at night (Fitch and Reby 2001). The low formant values suggest that these mammals can elongate their vocal tracts during the roaring. The larynx has a low resting position (Fitch and Reby 2001) and retracts down to the sternum during rutting roars (Reby and McComb 2003). The atmospheric absorption and sound scattering increase with a high frequency, so low frequencies propagate more (Richards and Wiley 1980), which may be the strategy used by various animals, including the red deer, to diffuse their message to the receptor. Nevertheless, red deer emit calls close to the ground, and the destructive interference between the direct and reflected waves in the ground increases to low frequencies, thus decreasing the spread (Fitch and Reby 2001). Soon, the stag is significantly more likely to roar when competing stags are nearby than when they are distant (Clutton-Brock and Albon 1979).

The main purpose of this study was to understand the vocalizations of red deer males in the Lousã Mountain, both spatially and temporally. Thus, we analysed the spatial patterns of the roaring, more specifically, whether the number and duration of roars are related to environmental parameters and if vocalizations occur uniformly throughout the study area. We hypothesize that vocalizations will not occur evenly throughout the study area, as males show a preference for certain habitats or even patches of certain habitats, such as shrublands. Based on analysis of the roaring temporal patterns, we expect that vocalizations throughout the day also do not happen evenly in the Lousã Mountain, since red deer is described as having bimodal activity patterns, with peaks of activity at sunrise and sunset. It is also expected that the stags do not have uniform vocalizations throughout the breeding season, since there are a few days when males will roar uninterrupted, when the roaring peak occurs, decreasing considerably after this peak until the end of the roaring season. The purpose of this analysis is also to verify if the number and duration of the vocalizations are in any way related to the climacteric parameters. Thus, we predict that the increase in temperature will cause a decrease in the number of roars of the males due to the expenditure effort and energy of a roar at higher temperatures. The same

pattern is expected for the wind because the propagation trajectory of the roar will be altered, constituting an obstacle to the sound.

2.2 Methods

2.2.1 Study area

The fieldwork was conducted in the Lousã Mountain (40°3'N, 8°15'W), a mountainous Mediterranean area, characterized by deep valleys and pronounced hilltops (Archibold 1995; Alves 2013). Its climate is predominantly Mediterranean, with hot and dry summers and rainy winters, where temperature and precipitation vary according to topography (Alves 2013).

This region is quite humanized, with small agricultural fields in areas of lower latitude, where sometimes conflicts between red deer and humans can occur. This region also has a large network of roads over 500km long, with intense traffic at the beginning or the end of the day, in the surrounding areas (Alves 2013).

In the Lousã Mountain, hunting events are organized, called "montaria", which take place every year between October and February outside the core area of the Lousã Mountain.

2.2.2 Data collection

To understand the spatial and temporal patterns of red deer vocalizations in the Lousã Mountain 41 AudioMoths were used. This equipment is used for acoustic monitorization and can have various applications such as monitorization of specific species, soundscape analysis, environmental surveillance and as a hobby for wildlife enthusiasts (Hill et al. 2019)

AudioMoth consists of a single credit-card sized (58 x 48 x 15 mm) circuit board (PCB), which includes a side-mounted switch, universal serial bus (USB) port and microSD card port. This is a low-cost, full-spectrum acoustic logger, it can be configured to record in different types of sampling, making it suitable for monitoring sounds from various types of sources. These include anthropogenic noise (8 kHz sample rate); audible wild animals (sampling rate 48 kHz); and ultrasonic wild animals (384 kHz sample rate) (Hill et al. 2019).

We deployed 41 AudioMoths (version 1.1.0) from 4 September to 8 October 2019, during rut season in an 1km² sampling grid (Figure 1).

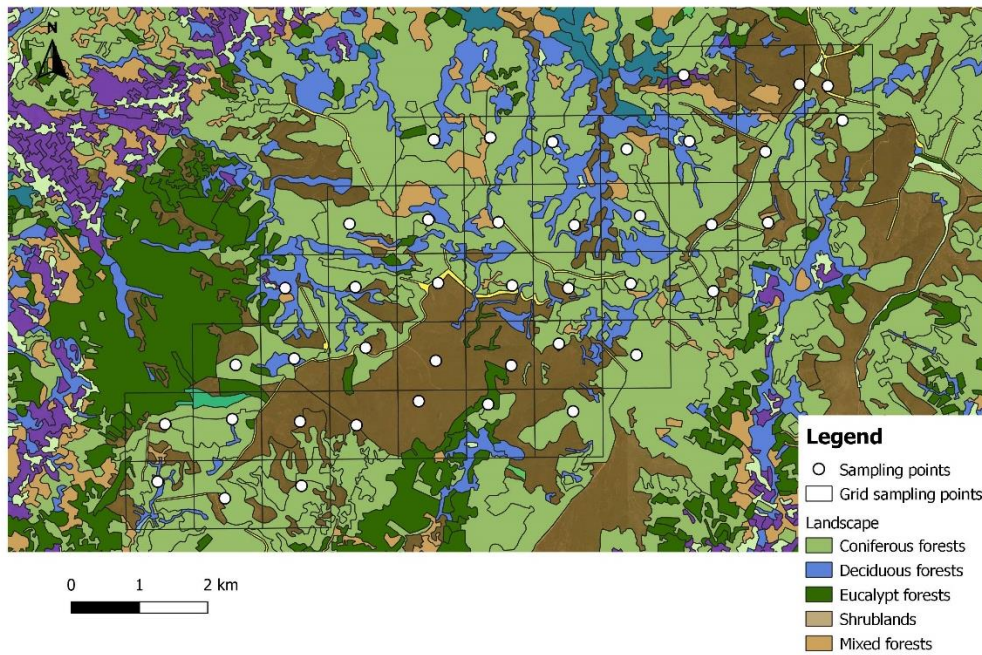


Figure 1. Map of the study area with the positioning of the sampling points and land cover units.

To protect the devices from rain and moisture, we sealed them inside a zipped bag and used tapes to hold the bags to the trees (Figure 2A). To complement the information of AudioMoths, when possible, camera traps were placed in the same sampling points (Figure 2B).

As a proxy of red deer density, the number of fecal pellet counts was determined in each sampling point using linear transects of 200 m with distance sampling (adapted from Garcia et al. *In prep*).



Figure 2. A) AudioMoth deployed in the Lousã Mountain, Coimbra, Portugal, during 4 September – 8 October 2019; B) AudioMoth and camera-trap deployed in the Lousã Mountain, Coimbra, Portugal, during 4 September – 8 October 2019.

The AudioMoths were configured using the configuration app, and the defined configurations were a sample rate of 48 kHz, gain at the medium level, sleep duration of 600 seconds, recording duration of 300 seconds. The real-time clock kept track of time and date in UTC.

In each sampling points the following parameters were recorded: GPS coordinates, date and time of placement and collection of AudioMoth, habitat type (coniferous, shrub and mix) and its diameter (tree/branch), AudioMoth placing height (cm) and bearing.

2.2.3 Recording analysis

The analysis of the audio recording was performed using Audacity and Raven Pro. For the preliminary analysis, Audacity was used to test which were the best parameters later applied in Raven Pro. Raven Pro is an automatic program with flexible visualization and measurement tools for analysing audio recordings of any duration, however, its results need to be fully validated manually due to the high proportion of false positives and missing detections for this type of recordings.

In Raven Pro, 635436 detections were presented, of which 557135 were false and for that reason eliminated (87.7%) and 78301 were true contacts (12.3%). To the contacts found by Raven Pro were added 24923 (24.1%), premaking a total of 103224 roarings.

2.2.3.1 Audacity

Audacity is a free, easy-to-use program that contains tools useful for analysing the physical properties of music and sound, which can be used to record multiple audio tracks (Hill et al. 2018; Smith 2011). In this program, the recordings of the 41 AudioMoths from September 4 to September 17 2019, were heard and the presence of roaring's registered.

All acoustic recordings were processed on a computer using Audacity 2.4.2 software (<https://www.audacityteam.org/>, accessed 10 Aug 2020). To analyse the audio files with the representative call's spectrograms, we set the following configuration: maximum frequency 2000 Hz, minimum frequency 0 Hz and Hann bandpass.

In this program, we can observe that the most intense red zones correspond to the zone where contacts will be most likely to occur (Figure 3A). We also can see the wave format (dB) of a contact (Figure 3B).

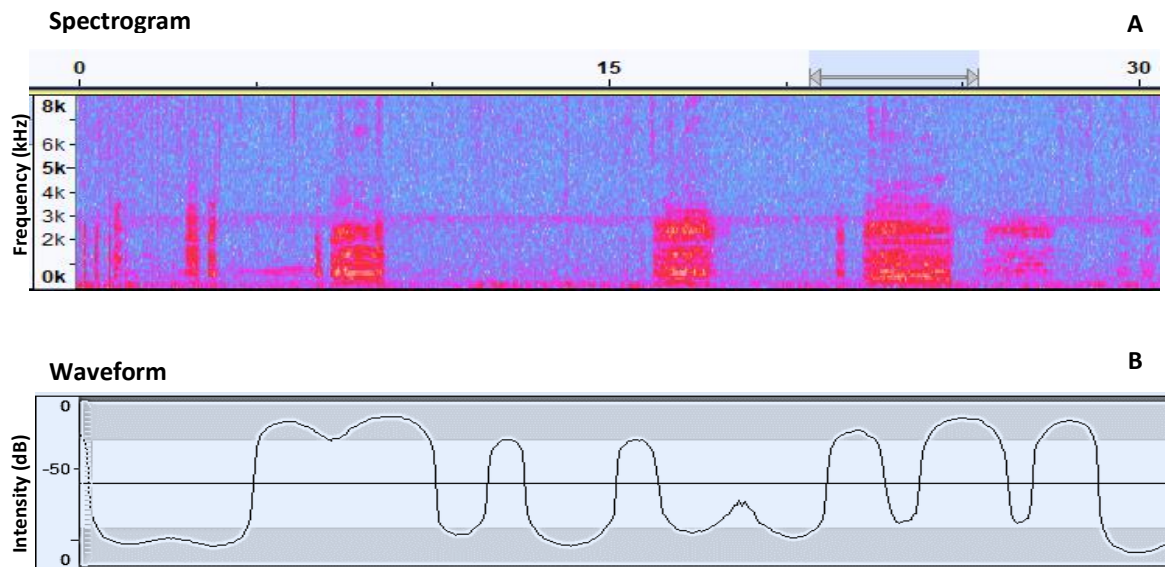


Figure 3. A) Spectrograms generated in Audacity 2.4.2; B) Waveform generated in Audacity 2.4.2

The preliminary results of this analysis will not be presented since the goal of this step was the determination of the parameters to be set in Raven Pro for the automatic detection analysis.

2.2.3.2 Raven Pro

The full analysis of the recordings, from 4 September to 8 October 2019, was performed through the software Raven Pro 1.6.1 (<https://ravensoundsoftware.com/>, accessed 20 Aug 2020). Raven Pro is a powerful research application for scientists needing flexible visualization and measurement tools for analysis of audio recordings of any length.

After the automatic process of roar detection, all the roaring detected were validated through visual inspection. The detections where doubts subsisted after visual inspection were listened to confirm the existence of roar or to delete the register. This process allows to delete all the false positive detection, and also to register all the missing detections by the automatic detection procedure.

When operating the program, spectrograms of the various audio files were obtained. These spectrograms indicated the form of red deer calls, as can be seen in Figure 4. Of these files only calls of good quality, with clearly visible spectral structure and not superimposed by wind, were used for the acoustical analyses.

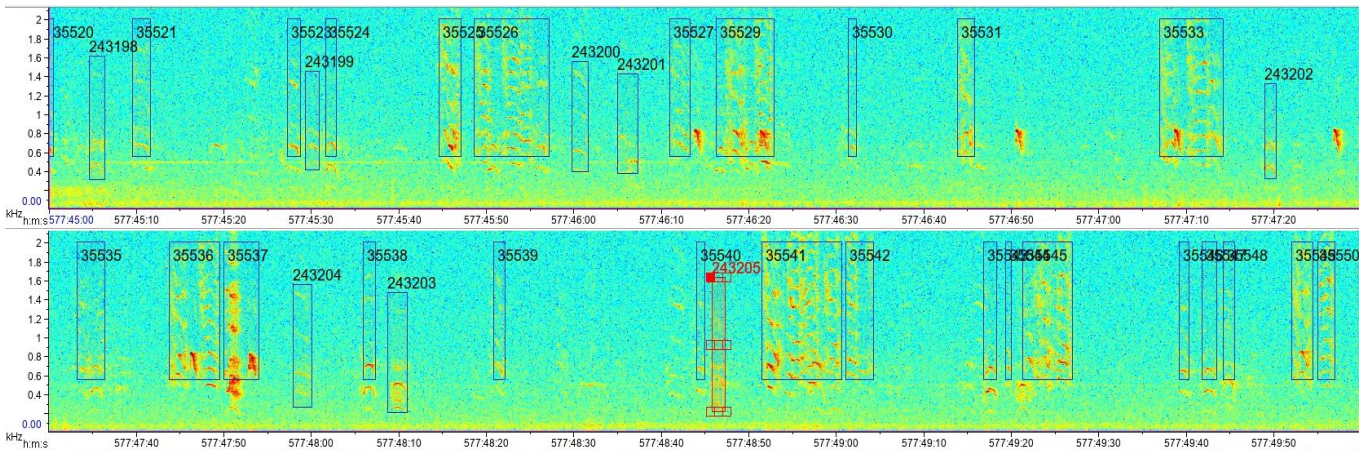


Figure 4. Spectrogram of an audio recording file with several roars detected using the Raven Pro 1.6.1

2.2.4 Statistical analyses

The spatial analysis of the number of roars and number of pellets was performed through inverse distance weighted (IDW) interpolation using a smooth neighbourhood search with a major semiaxis of 2950m, a minor semiaxis of 2950m and a smoothing factor of 0.2.

To test our predictions, generalized linear mixed models (GLMM), with Poisson distribution and log link function, were used to analyse the potential effect of environmental (Model 1) and climatic variables (Model 2) on the number of roars (response variable). Sampling point was included as random factor. The environmental variables included were habitat type (coniferous, mixed forest and shrubland), distance to wind turbines (m), distance to roads (m), altitude (m) and slope (degrees), after standardization. Regarding climatic variables, temperature (°C) and wind speed (km/h) were considered.

The activity roaring patterns were analysed using kernel density functions into a temporal daytime scale (Ridout and Linkie 2009).

The statistical analyses were performed using IBM.SPSS, version 23, ArcMap 10.6 and R 3.5.0 (R Core Team 2019) using the package “overlap” (Meredith and Ridout 2016). All statistical analyses were considered significant when $P < 0.05$. The results are expressed as mean \pm standard error (SE) unless otherwise stated.

2.3 Results

2.3.1 Roaring Spatial Patterns

2.3.1.1 Vocalizations occurrence in the study area

According to the spatial interpolation analysis performed using IDW is possible to verify that the distribution of vocalizations is heterogeneous throughout the study area, being evident the presence of hotspot zones and coldspots (Figure 5). From this analysis, three hotspots are easily identified, areas with a high number of roars (35.53 – 51.36), located at Northeast region of Lousã Mountain.

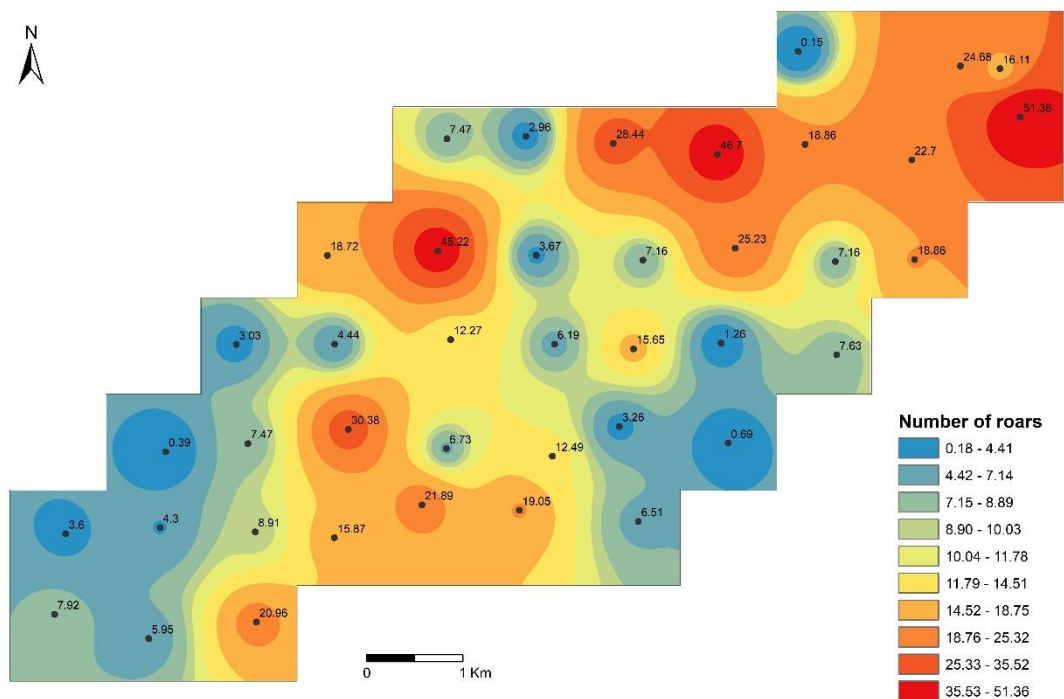


Figure 5. Inverse distance weighted interpolation map of the number of roars in each sampling point in the study area.

The habitat type of hotspot areas is mostly dominated by coniferous and some shrubland patches, highlighting the preferential use of these habitats for roaring behaviour (Figure 1)

A comparison between the interpolation map of roars with an interpolation map of faecal counts (adapted from Garcia et al. *In prep*), revealed that the areas where higher density of roars were detected did not correspond to the areas where a higher number of faeces were counted, which is a proxy of red deer density (Figure 5). Considering the pellet counts, red deer exhibits a preference for areas of shrublands (Figure 6).

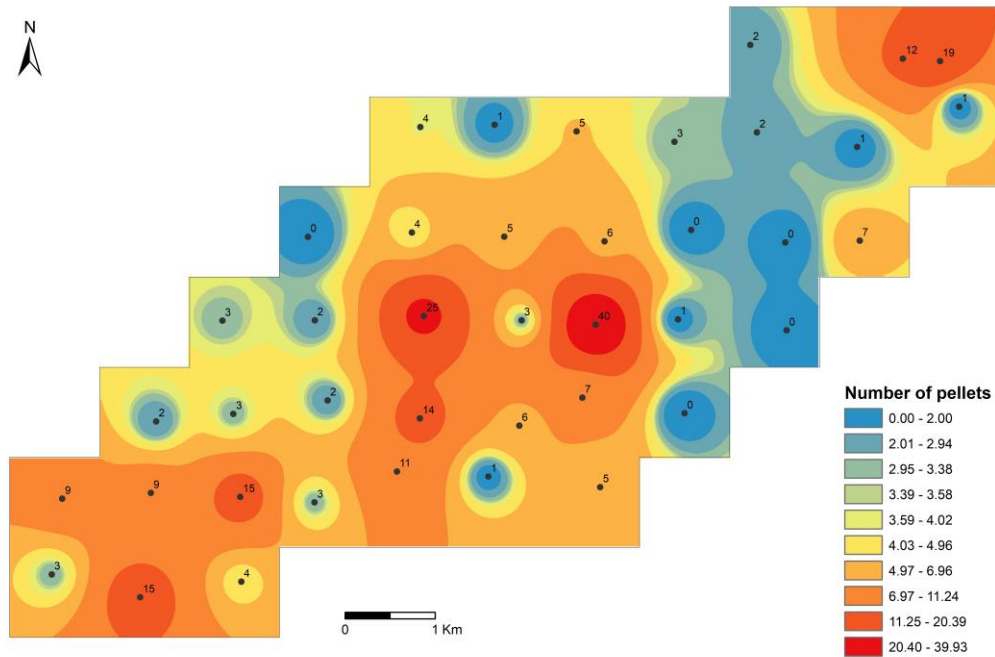


Figure 6. Inverse distance weighted interpolation map of the number of faecal counts in the study area

2.3.1.2 Relationship of numbers of roars and environmental parameters

The number of roars per sampling time was significantly affected by the habitat type ($F_{(2,73634)}=233.138$, $p<0.001$), being higher in coniferous forests ($\beta=0.249$) when compared with shrublands, and lower in mixed forests ($\beta=-0.525$). It was also noticeable an increase of the number of roars as the distance to roads ($F_{(1,73634)}=95.705$, $p<0.001$; $\beta=0.152$) and wind farms ($F_{(1,73634)}=103.100$, $p<0.001$; $\beta=0.187$) increased (Table 1).

Table 1. Generalized linear mixed model (GLMM), with a Poisson distribution, of the effect of environmental variables on the number of roars per sampling time (response variable, N=73641). * is the reference category and SE is the standard error of the model coefficient.

	Coefficient	SE	t test	P value	95% Confidence Interval	
					Lower	Upper
Intercept	-5.574	0.022	-251.754	<0.001	-5.618	-5.531
Habitat						
Coniferous forest	0.249	0.030	8.224	<0.001	0.189	0.308
Mixed forest	-0.525	0.038	-13.795	<0.001	-0.599	-0.450
Shrubland*	0*
Distance to windfarms	0.187	0.018	10.154	<0.001	0.151	0.223
Distance to roads	0.152	0.016	9.783	<0.001	0.121	0.182
Altitude	-0.093	0.019	-4.940	<0.001	-0.129	-0.056
Slope	0.058	0.0144	4.161	<0.001	0.030	0.085

The number of vocalizations also increased at lower altitudes ($\beta=-0.093$) and higher slopes ($\beta= 0.058$), although with low influence on roaring spatial patterns, as they have a low coefficient value (Table 1).

2.3.2 Roaring Temporal Patterns

2.3.2.1 Temporal patterns of vocalizations during the roaring season

During the roaring season, the red deer males at the beginning emit a low number of roars, increasing in number on day 12th. From the 12th to the 25th day, we can observe that the number of roars increases considerably, observing a peak on the 20th (approximately 0.93 roars/min). The number of roars decreases again from the 26th forward. On the 18th day of sampling, the results show a considerable low number of roars, which coincides with an extreme atmospheric event (Figure 7).

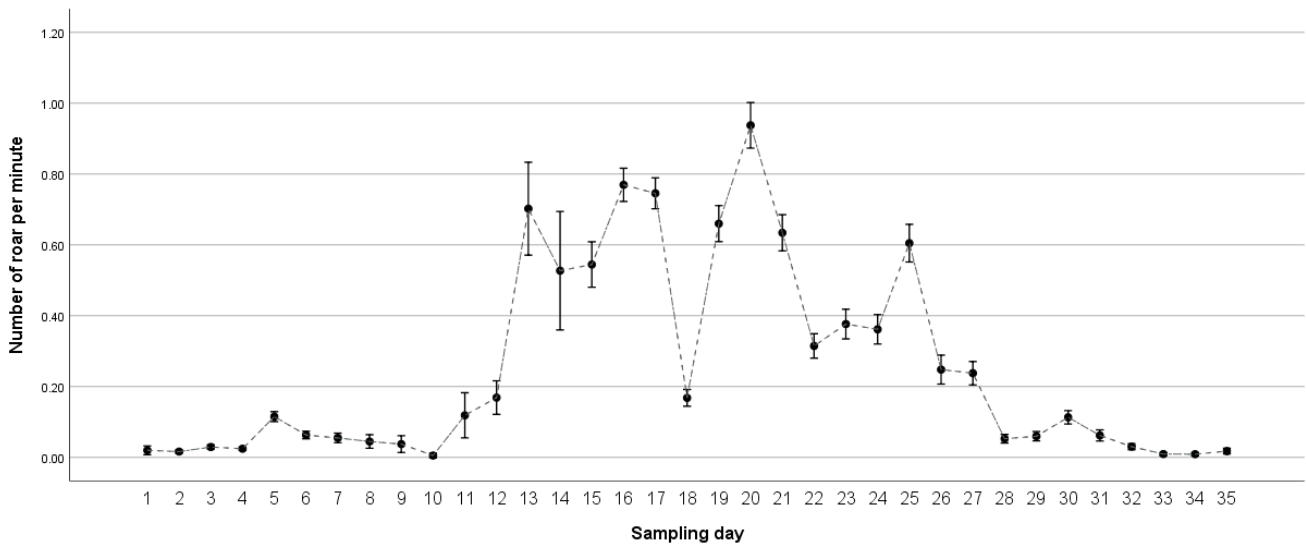


Figure 7. The number of roars per minute for each sampling day of the roaring season. The roaring season begin at 1st day of sampling that corresponds to 04.09.2019 until 35th day of sampling that corresponds to 08.10.2019.

Analysing the roaring time per minute, we can also observe that there is a significant increase from 10th day of sampling, with a maximum on the 13th day of sampling (4.30s of roaring). After that, there is a decrease in roaring time per minute, although there are some peaks on the 20th and 25th (Figure 8).

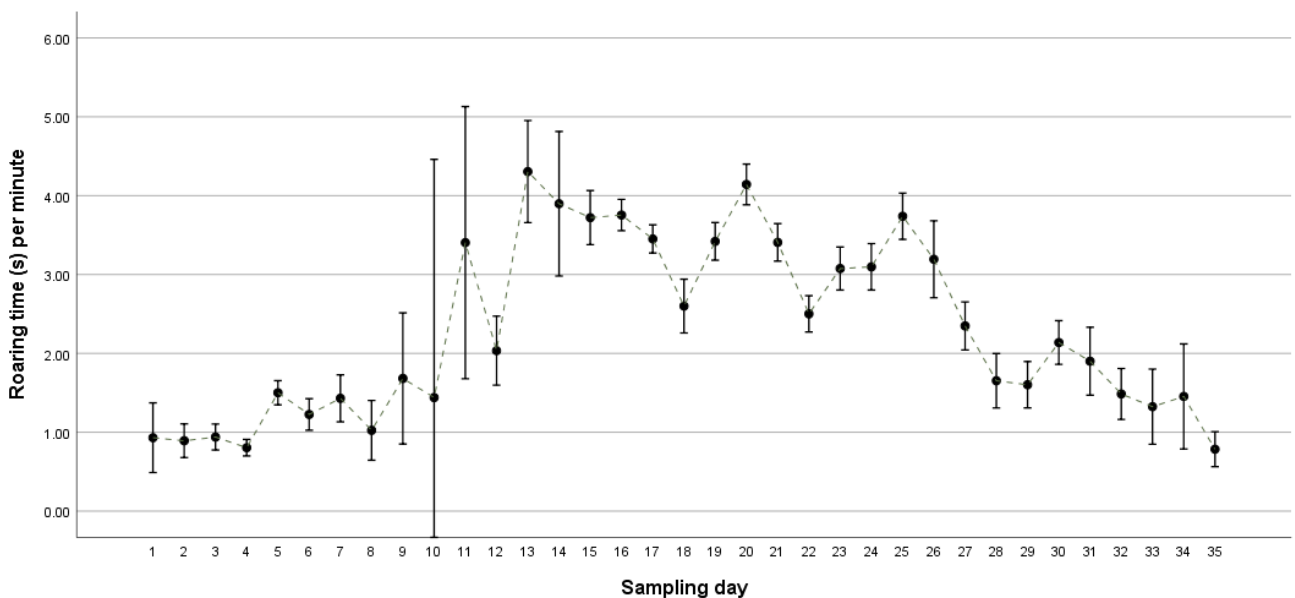


Figure 8. Roaring time (s) per minute for each sampling day of the roaring season. The roaring season begin at 1st day of sampling that corresponds to 04.09.2019 until 35th day of sampling that corresponds to 08.10.2019.

Considering the full sampling season, we can clearly distinguish three phases based on roaring numbers and duration, a roaring begin (day 1 to 12), roaring peak (day 13 to 25) and roaring end (day 26 to 35).

The results showed significant differences between the roaring phases in the number of roars per minute ($\chi^2=4980.80$, d.f=2, $p< 0.001$). In roaring peak, the number of roars was significantly higher (approximately 0.57 roars/min) than the ones observed in the roaring begin and roaring end, where the number of roars per minute was quite lower (Figure 9).

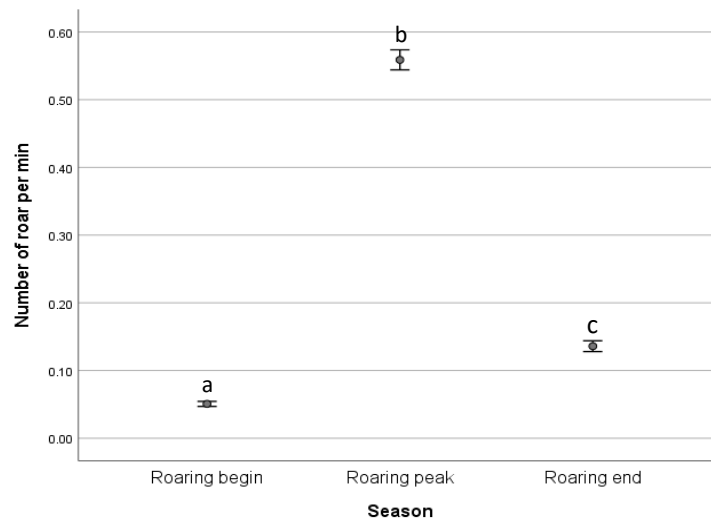


Figure 9. Number of roars per minute during the three periods of the roaring season. The roaring season begin at 1st day of sampling that corresponds to 04.09.2019 until 35th day of sampling that corresponds to 08.10.2019. Different letters (a,b,c) represent significant statistical differences between seasons ($\chi^2=4980.80$, d.f=2, $p< 0.001$).

When considering the roaring time per minute, we also observed a significant difference between the three periods of the roaring season ($\chi^2=1444.03$, d.f=2, $p< 0.001$). In roaring peak, there was a higher roaring time (approximately 3.50s of roaring per minute) when compared with the roaring begin and roaring end (Figure 10).

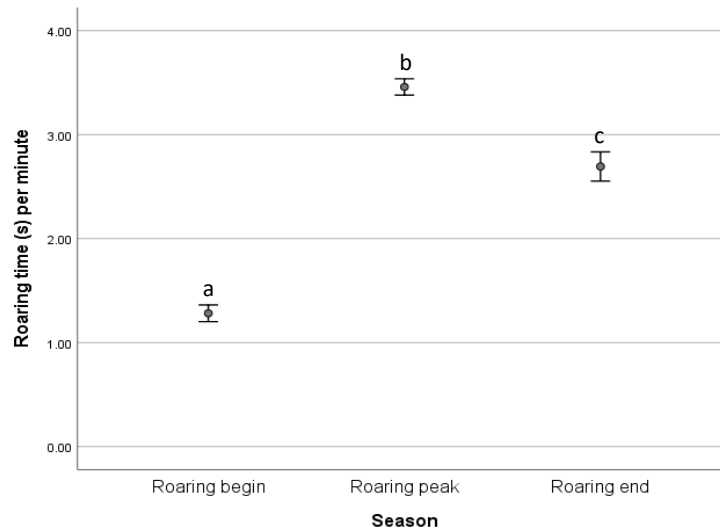


Figure 10. Roaring time (s) per minute during the three periods of the roaring season. The roaring season begin at 1st day of sampling that corresponds to 04.09.2019 until 35th day of sampling that corresponds to 08.10.2019. Different letters (a,b,c) represent significant statistical differences between seasons ($\chi^2=1444.03$, d.f=2, $p < 0.001$; N=73641).

When considering the average duration of each roar, significant differences were found between the begin and the two other roaring phases of the roaring season ($\chi^2=21.342$, d.f=2, $p < 0.001$) (Figure 11).

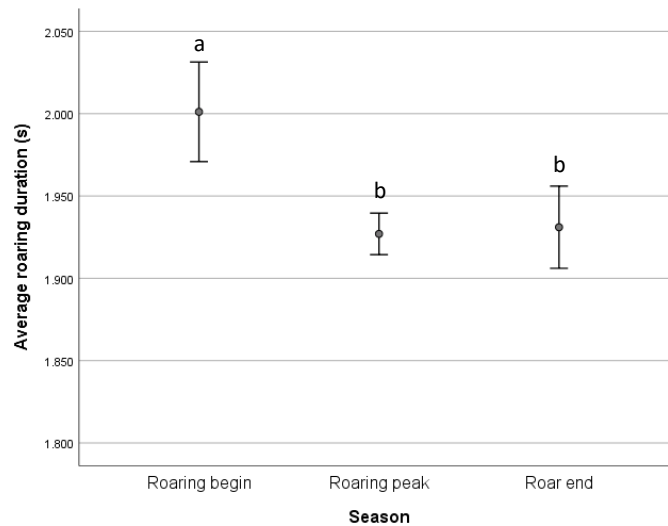


Figure 11. Average roaring duration (s) during the three periods of the roaring season. The roaring season begin at 1st day of sampling that corresponds to 04.09.2019 until 35th day of sampling that corresponds to 08.10.2019. Different letters (a,b,c) represent significant statistical differences between seasons ($\chi^2=21.342$, d.f=2, $p < 0.001$; N=73641).

2.3.2.2 Diel patterns of red deer vocalizations

The kernel density showed that activity patterns of red deer males, in Lousã Mountain, have their highest intensity at night (Figure 12). This analysis indicates a higher activity of roars after the sunset until sunrise, showing that males have a pattern of nocturnal activity since their vocalizations decrease considerably during the day.

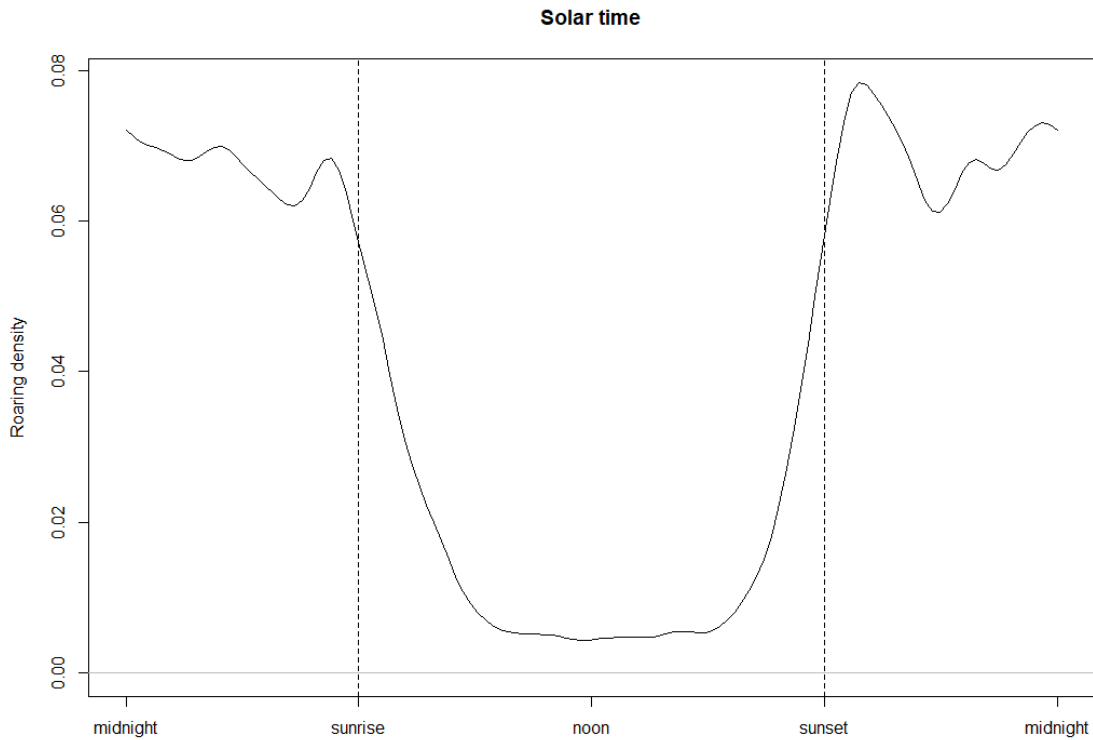


Figure 12. Roaring density patterns of males in Lousã Mountain, throughout the day.

2.3.2.3 Relationship of number of roars and climatic parameters

When analyzing the influence of climatic parameters on the number of roars, the results showed a significant effect of temperature ($F_{(1,73638)}=51694.19$, $p<0.001$) and wind speed ($F_{(1,73638)}=42181.48$, $p<0.001$) (Table 2).

Table 2. Generalized linear mixed model (GLMM), with a Poisson distribution, of the effect of climate variables on the number of roars per sampling time (response variable, N=73641). SE is the standard error of the model coefficient.

	Coefficient	SE	t-test	P value	95% Confidence Interval	
					Lower	Upper
Intercept	-1.724	0.323	-5.332	<0.001	-2.358	-1.090
Temperature (°C)	-0.208	0.001	-227.364	<0.001	-0.210	-0.206
Wind speed (km/h)	-0.142	0.001	-205.381	<0.001	-0.144	-0.141

According to the results, an increase in temperature leads to a decrease in the number of roars by red deer males ($\beta=-0.208$). There was also a decrease in the number of roars with the increase of the wind speed ($\beta=-0.142$) (Table 2).

2.4 Discussion

2.4.1 Spatial patterns of red deer vocalizations in roaring season

By analysing the spatial patterns of the deer's vocalizations in the roaring season, we conclude that there is a heterogeneity in the distribution of these vocalizations throughout the mountain. We were able to verify that there are areas of greatest interest for males to emit roars, being these located in Northeast region of Lousã Mountain. These areas consist mostly of coniferous habitats and some shrubland patches. Comparing the areas where the roars were more intense with the areas where the number of faecal pellets is higher (which indicate a higher density of red deer), we found that the areas that males choose to emit their roars do not correspond to the places where the higher deer densities were achieved, since the areas with the highest number of faecal pellets are in areas of shrubland. Therefore, these results combined suggest that the density of roars does not represent a proxy of the density of red deer.

One explanation that may be behind these results is that males are changing their reproductive strategy. Males in Mediterranean climates use as a strategy characterized by the protection of a site instead of the protection of a group of females (Alves 2013). Although this phenomenon is only described for red deer populations in Spain (Carranza et al. 1990, 1995), it may also be a strategy used by red deer in our area of study.

Further, in forest habitats, sound waves suffer more refractions because they hit vegetation. This causes the sound to reach the receiver by progressively longer paths, and some information of the signal emitted

by the male may be lost (Richards and Wiley 1980). However, in open areas there is greater atmospheric turbulence (Brenowitz 1986), so vocalizations can also be degraded. As a strategy to avoid these difficulties, males produce low-frequency vocalizations, because the high frequencies are affected by scattering and absorption (Brenowitz 1986). Females also prefer males that produce lower minimum frequencies, as they are likely to have a higher body and have higher fitness (Reby et al. 2005).

Another strategy used by males in the Lousã Mountain and that we can verify in the results is the fact that they choose low altitudes to emit more roars. This can happen as a strategy of males, since they emit the sound near the ground (Fitch and Reby 2001) and therefore the signal may not be received by the receiver, they look for places with lower altitudes to increase the spread of their roars. As the Lousã Mountain is a place with deep valleys and rounded hilltops (Alves 2013), what the red deer can be using as an approach is to move to these places to emphasize their roars, because the echo will help increasing the intensity of the sound emitted. At higher altitudes the airspeed is higher, which causes sound waves to be refracted and directed to the ground (Brenowitz 1986). The same happens with the slope, in which higher slopes lead to an increase in the number of roars and we can conclude the same as with the altitude. In the valleys there is sound resonance, this hits an object and propagates over longer distances, increasing the roar.

According to our results, we see that males selected areas of coniferous forests, which are areas that confer greater protection and with greater distance to anthropogenic noise. Being this more isolated, the cost of vocalizing will also be lower, delivering the signal more clearly to the receiver, in this case, to females. As it is visible in the results, males emit more roars away from wind farms and roads, which indicates an avoidance from disturbed areas and areas with higher anthropogenic noise, in the same way as the villages. This may also be proof of the preference for sites with less atmospheric absorption and more hidden such as coniferous forests.

Previous studies suggest human activities may influence red deer (Edge and Marcum 1985, Lyon 1983). The males in the mating season begin to go to areas farther from humans or anthropogenic noises, moving to areas that give them greater coverage (Jiang et al. 2008). When the habitat is characterized by open and close patches, ungulates use as a strategy to move to closed areas for protection or to open areas to search for food (Kie 1999). Since there are no natural predators in Lousã Mountain (Alves 2003) it is possible that humans are being considered behaviourally for red deer as predators (Ripple and Beschta 2004), making them display a protective behaviour by selecting more closed habitats. Although Lousã Mountain is part of the Natura 2000 Site, there is hunting and ecotourism (Peixoto 2014; Alves 2013), which can justify the strategy displayed by males in terms of spatial selection of habitats to roar. Indeed, in the Lousã Mountain there has been an intensification of ecotourism, mainly in September and October, to visualize the roaring season. People usually go to open areas to get a better view. This may be exerting pressure on males, making them to choose places with greater isolation and forest density.

2.4.2 Temporal patterns of red deer vocalizations in roaring season

The rut season is the most varied time in terms of the social organization of the deer. Most of the year, outside the breeding season, males and females are in unisex groups (Blanco 1998; Carranza 2007), having the females a matriarchal organization (Ferreira 1998) and the males forming groups with other males (Carranza 2007) or being solitary. At the time of reproduction, these groups aggregate, constituting harems (Clutton-Brock et al. 1982). This season can be divided into three different phases, 1) a pre-rut 2) a rut and 3) post-rut (Alves 2013). In Lousã Mountain, we consider that the pre-rut takes place between August to September 15th, the rut between September 15th to early October and a post-rut in November.

The roaring season is divided into three phases: 1) roaring begin, 2) roaring peak and 3) roaring end. At the beginning of the roaring season, the deer begin to progressively emit a few roars, shifting for certain areas, and marking their territory. After the establishment of males' ranking, the proportion of mixed groups raise (Alves 2013). After this first phase, the number of roars increases considerably with a peak in the two last weeks of September. In this period, the males emit a high number of roars throughout the day, for several days. The males are more aggressive and have their harems constituted, protecting them and pushing away opponents (Alves 2013). At the roaring end, the roars subside until they cease completely. Starting the post-rut in November, in which the groups are again segregated, and repeating the cycle.

Considering the full sampling season (04.09.2019 to 8.10.2019), the results were as expected. Analysing the number of roars per minute for each sampling day of the roaring season, we were able to observe that there was a progressive increase in the number of roaring up to the 12th day of sampling (roaring begin). From that day until the 25th day of sampling the number of roars is quite high (roaring peak) being the peak of roars observed on 20th day of sampling (approximately 0.93 roars/min). However, on the 18th there was an abrupt drop in the number of roars per minute. This day coincides with a big storm with high rainfall that happened in the study area. This may have negatively influenced the number of roars emitted, but rainfall may also have interference on the ability of detection roars from of the audio files, due to the high level of noise. When considering the duration of roaring time per minute, the decrease observed on the 18th day is not so accentuated, indicating that although a lower number of roars was detected, the detected ones had a higher duration than in other days. This may indicate that males choose specific time windows with less noise to roar for a longer time.

Considering the average duration of each roar, the results indicate that in the beginning of the roaring season the males invest in longer roars but less frequent ones, which changes as the roaring season progress. This may be justified by the need of the males to establish social ranks, making them invest in longer vocalizations to ensure their breeding success (Clutton-Brock et al. 1982; Main and du Toit 2005). After the formation of the harem, the males decrease the duration of each roar, increasing the number of roars as warning signs. Young males who own harems may also decrease the duration of the roars as a strategy to avoid the attention of older males (Clutton-Brock and Albon 1979).

The diel patterns of red deer vocalization demonstrate that they are not uniform on Lousã Mountain. The roaring activity of males is concentrated at night, and this might be related to the energy cost of emitting a roar, that is lower during the night when the temperature is lower and environmental noise is also lower. During the night there is no noise, which makes the signal emission of the male with a low level of distortion, transmitting an honest signal to the females and other males. Given that these signals have as their main objective to attract females (Charlton et al. 2007) and to oppose possible rivals (Reby et al. 2005), it will then be expected that the male strategy will be to vocalize at night, as we obtained in the results. This can be explained by the fact that males depend exclusively on this time to reproduce (Charlton et al. 2007). Previous studies reported that females can distinguish the roars emitted by males (Alcock 2009) and that these vocalizations have experienced selective pressure to evolve acoustically to emphasize the male's body size (Fitch and Reby 2001). Therefore, roar at night will be an effective strategy, because during the night visibility is reduced and the possible competitors will have difficulty identifying the body size of the male (Fitch and Reby 2001).

Another explanation may be that the changes in the photoperiod usually occur in September. These changes lead to stimulation of the pineal gland, which will lead to melatonin secretion, increasing the level of testosterone in males (Baratelli 2014; Alves 2013). During the night, melatonin secretion is higher, inducing the aggressive behaviour of males and, consequently, an increase in vocalizations (Baratelli 2014; Lincoln et al. 1972).

When analysing the influence of climatic parameters on the number of roars, results showed that temperature and wind have a negative influence on the number of roars emitted by deer on Lousã Mountain. When there is an increase in temperature, there is a decrease in the roars emitted. This is because when the temperature is higher, the energy cost of roaring for a male is higher. Being the red deer a thermosensitive animal, and the roaring season a time of so much wear for males (Barroso and Rosa 1999), we can conclude that the increase in temperature increases its energy effort.

With the wind, the pattern is similar, since the wind speed will interfere with the amplitude fluctuations (Richards and Wiley 1980). Animals benefit from emitting vocalizations at a time of day with a smaller amount of noise (Brenowitz 1986). As there is a high distortion of sound waves and a higher noise, the signal emitted by the male will be degraded and will not reach the receiver, which will also imply an increase in the energy cost of the vocalizations.

Concerning rainfall, in this work, we could not include this parameter, since we did not have detailed data on this parameter available for the studied period. However, and as presented in the results in the day where heavy rainfall was detected, the number of roars were significantly lower which may be due to lack of detection on the audio recordings due to noise, or a real avoidance of males to roar in extreme weather conditions. This pattern was less noticeable when analysing the roaring duration per minute, which may indicate that during less suitable weather conditions red deer may choose to emit longer roars but less

frequently, as a way of maximizing the energy expenditure, and as a strategy to prevent degradation of sound waves (Richards and Wiley 1980). As noted with the wind, with rain males will have to produce stronger and more intense roars to be audible for females or male competitors, due to the higher surrounding noise.

Although our results provide already a deep inside on the roaring season, more studies on the specificities of the roars are needed to better understand this so particular time for red deer. There is also uncertainty about the anthropogenic impacts on this red deer population and would be important to direct more studies toward this subject to understand if our results on the avoidance of roads and wind farms are representing an avoidance to humans in general or only to more noisy areas.

Even if the results are in line with what is expected, the nonexistence of studies on the temporal and spatial patterns of red deer vocalizations during the roaring season, in other places and conditions do not allow us to draw a generalization of specie level. Not allowing us to compare whether what happens in our area of study is the same as in other red deer populations.

Chapter III – General conclusion

In this study, we conclude that in the roaring season the red deer's have a concentrated roaring activity at night, so they have a nocturnal behaviour. The vocalizations of red deer's do not occur uniformly through of roaring season. The number of roars in this season are higher, having a peak the two last weeks of September. In our study area, we also find that they select certain areas of the mountain range for roaring, mainly in coniferous habitat. We also noticed that some climatic parameters such as temperature and wind can have a negative impact on the emission of these roars.

This study is one of the few studies of sound production *in loco* and the first, to our knowledge, of animal behaviour in vocalizations of mammals in Portugal. This study proves that temporal and spatial patterns are crucial to understanding red deer behaviour during the roaring season. Although the results agree with the expected, more studies on the characterization of these roars should be done. Some uncertainties have remained concerning some of the results that need also to be explored, such as the anthropogenic impact on their behaviour. Also, more studies in other areas with different habitats and different environmental and climatic characteristics are necessary to enable the comparison with our results, enabling to make more general conclusions, which are fundamental to better understand the role of roaring during the red deer mating season.

There are few revisions on this methodology with AudioMoths in Acoustic Ecology. The importance of these low-cost equipment for the development of these studies is noticeable, as they allow a large-scale deployment, thus increasing the existing knowledge in the area. More studies on the subject need to be published in the coming years.

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