UNIVERSIDADE DE ÉVORA

ESCOLA DE CIÊNCIAS E TECNOLOGIAS

DEPARTAMENTO DE BIOLOGIA

Study of Iberian wolf food habits in Trás-os-Montes, Portugal: Present and Past |

Patrícia Passinha |

Orientação: Prof. Doutor Francisco Petrucci-Fonseca |

Mestrado em Biologia da Conservação

Dissertação

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Resumo

O maior obstáculo para a conservação do lobo são os conflitos com o Homem devido à predação de gado. O estudo dos hábitos alimentares é importante para compreender a variação das presas do lobo em diferentes áreas. As áreas de estudo foram o Parque Natural do Alvão e o Parque Natural de Montesinho. O estudo das diferentes presas do lobo nestas áreas foi feito com base em dejetos. Para avaliar a variação temporal da dieta do lobo analisámos estudos desde 1977 até 2017. Os resultados mostram diferentes tipos de presas: no Alvão, com maior pressão humana, o lobo depende de gado. Contrariamente, em Montesinho este alimenta-se de ungulados silvestres. Ao longo do tempo a dieta sofreu alterações em Bragança (de ungulados domésticos para silvestres). Enquanto que nas restantes áreas não houve alteração. No Alvão/Padrela, Peneda/Gerês e Sul do Douro há grande dependência do lobo ao gado.

Study of Iberian wolf food habits in Trás-os-Montes, Portugal: Present and Past

Abstract

The main difficulty for wolf conservation is the human conflicts due to predation on livestock. The study of wolf food habits is important to understand the variation of wolf prey in different areas. Our study areas were Natural Park of Alvão and Natural Park of Montesinho. The study of the different wolf prey in these areas was based on scats. To evaluate the temporal variation of wolf diet, we reviewed studies from 1977 to 2017. The results show a different type wolf prey: in Alvão, with great human pressure, wolves depend on livestock. While in Montesinho wolf feeds on natural prey. Over time the diet has change in Bragança (from domestic to wild ungulates). While in other areas didn't change. In Alvão/Padrela, Peneda/Gerês and South of Douro River wolf diet is based on livestock.

Introduction

The wolf (*Canis lupus* Linnaeus, 1758) had a great evolution and specialization. From a generalist carnivore that lived 100 million years ago to nowadays species (Nowak, 1995). Wolf species show a wide range of habitats and climate adaptability, for example artic wolf (*Canis lupus arctos* Pocock, 1935) living in tundra environments and Arabian wolf (*Canis lupus arabs* Pocock, 1934) adapted to hot climes (Nowak, 2003). It is possible to understand the great distribution of this species, only located in the North hemisphere (Mech, 1970).

Wolves are territorial animals that mark their territory with scats, urine, ground scratching or anal segregations. In reproductive period there is a larger marking in the territory, by the reproductive adults (Llaneza *et al.*, 2014). Wolves live and hunt in groups, called packs. The number of individuals per wolf pack have a big range of variation, depending on prey availability, territorial space and competition (Mech, 1970). Bigger wolf packs hunt bigger prey, all wolves contributes significantly to the hunt (Mech and Boitani, 2003). This social structure is extremely important for wolf conservation. A wolf pack can have various sexually mature individuals but only a couple contributes for the increase of population. The successful reproduction rate depends on age and experience of the couple (Brainerd *et al.*, 2008; Mech and Boitani, 2003; Packard *et al.*, 1985; Peterson *et al.*, 2002). Wolves do their matting in winter and pups are born between April and May, and by Autumn yearlings can follow the pack (Mech, 1970; Packard, 2003).

The wolf has an extreme important role in the ecosystem, and is a strong influence on it (Mech, 1970). Carnivores have a positive influence in the balance and trophic cascades of ecosystems (Galaverni *et al.*, 2016). Even in low densities large predators have a great ecological effect in controlling herbivores populations and other carnivores by competition. Which makes their conservation a priority due to their role in the ecosystems (Ripple *et al.*, 2014).

Being a large carnivore, wolves need large areas to establish their territories, with sufficient prey, protection from disturbance, and areas for denning and taking shelter. In Iberian Peninsula wolf distribution decreased greatly, being at the present time locally extinct in some regions (Grande del Brío, 1984;

Petrucci-Fonseca, 1990). In Portugal this reduction was gradual until XX century. In 1950 wolves were present in all mainland Portugal. The species disappeared in Southern and western regions due to direct persecution and drastic changes in the soil use (Álvares, 2011; Petrucci-Fonseca, 1990). Since 1990, wolf distribution area stabilized, occupying only 20% of the original area (Petrucci-Fonseca, 1990). Studies show that Iberian Peninsula have about 2000 wolves, with a continuous population in the North and two isolated populations: One in Spain, Andaluzia, and the other one in Portugal, South of Douro River (Blanco *et al.*, 1992; Pimenta *et al.*, 2005; Torres and Fonseca, 2016). At present time, in the North region, the wolf population numbers increased as well as and the distribution area (Blanco *et al.,* 1992; Blanco and Cortés, 2002; Cayuela, 2004).

Wolves are affected by several factors, however none of them has the dimension of conflict with humans. As a habitat generalist species abundance of prey, forest areas and anthropogenic pressure are the main factors to influence the wolf distribution (Cayuela, 2004; Massolo and Meriggi, 1998). Man has a determinant role in the distribution, ecology and behavior of these species. The competition between humans and wolf started when Man settled. Thus, the two hardly have large densities at the same sites (Mech, 1970).

As a generalist predator, wolf change his diet according to the availability of prey (Mech, 1970). In Portugal the wolf main prey are domestic animals (Petrucci-Fonseca, 1990; Vos, 2000). The most common cause of death for wolves is conflict with humans over livestock losses. Since domestic animals have evolved in constant protection of man, they are not suited to a successful defense alone (Mech, 1970). There are at least two factors driving wolf livestock predation in Southern Europe: the use of unappropriated methods of protecting livestock and mismanagement of wildlife, resulting in low density of wild ungulates. When densities of wild herbivores are high, livestock predation decreases substantially (Cozza *et al.*, 1996; Meriggi and Lovari, 1996; Meriggi *et al.*, 1996; Torres *et al.*, 2015). Due to the losses of livestock caused by wolves and to all consequent problems (economic, social), the management of wild prey in its natural habitat is of great importance, in order to reduce the impact of wolves on livestock (Oliveira and Carmo, 2000). The main wild prey of the Iberian wolf are red deer (*Cervus elaphus* Linnaeus, 1758), roe deer (*Capreolus capreolus* Linnaeus, 1758) and wild boar (*Sus scrofa* Linnaeus, 1758). These three species

have a high hunting value in Portugal nowadays. Red deer and roe deer are ruled by Portuguese hunting law (Appendix III of the Berne Convention, 82/72/CEE) and are under the Regime Cinegético Especial law (Oliveira and Carmo, 2000).

The Iberian wolf (*Canis lupus signatus* Cabrera, 1907) conservation status is Endangered (EN) and is protected by national legislation since 1988, granting it the status of protected species (Queiroz *et al.*, 2005). *C. lupus signatus* is also protected under the Habitats Directive (Appendix II and IV). It was also listed in the Berne Convention (Appendix II) and CITES (Appendix II-C2) (ICNF, 2018; Pimenta *et al.*, 2005).

Wolf conservation is highly conditioned by public opinion. Wolves are one of the carnivores most studied by conservationists and the most popular species among urban people, contrary attitudes exist in rural areas (Blanco *et al.*, 1992). The interactions between wolves and humans will depend on the wolf distribution over time. Since persecution is the greatest threat to wolf conservation, to minimize this threat in several European countries compensatory measures were implemented concerning compensation payment to breeders for any livestock lost to wolves (Espirito-Santo and Petrucci-Fonseca, 2017). The methods for verifying damage and compensation are different from a country to another but with the same goal of reducing social tensions and supporting shepherds that lost livestock due to wolf predation (Boitani *et al.*, 2000). In Portugal, the compensation system covers the payment of head of livestock injured or dead. The law covers damages when the livestock is confined, and surveilled by a shepherd (Espirito-Santo and Petrucci-Fonseca, 2017).

The goal of this thesis is to understand the variation of Iberian wolf food habits. The information was gathered in two different areas of Portugal (Alvão and Montesinho Natural Parks. These areas were chosen, due to their different characteristics: levels of human pressure, livestock grazing and diversity and density of wild ungulates. By the analysis of scats collected in the two areas, was possible to study the variation of wolf diet in this Natural Parks. To complement the study, studies were reviewed in four regions of Portugal were wolf is present (Peneda/Gerês, Alvão/Padrela, Bragança and South of Douro River). Therefore, was possible to determinate the temporal variation in the different locations.

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Research article

Study of Iberian wolf food habits in Trás-os-Montes, Portugal: Present and Past

Abstract

The main difficulty for wolf conservation is the human conflicts due to predation on livestock. The study of wolf food habits is important to understand the variation of the wolf prey in different areas with different human pressure and habitat characteristics. Our study areas were Natural Park of Alvão and Natural Park of Montesinho. The study of the different wolf prey in these areas was based on the analysis of 383 scats. To evaluate the temporal variation of wolf diet, we reviewed studies from 1977 to 2017, in the four regions in Portugal were wolf is present. The results show a different type wolf prey: in Alvão area, with great human pressure, wolves depend on livestock. While in Montesinho area wolf feeds on natural prey. Over time the diet has change in Bragança (from domestic to wild ungulates). While in other areas didn't change. In areas of Alvão/Padrela, Peneda/Gerês and South of Douro River wolf diet is based mainly on livestock.
Key words: *Canis lupus signatus*, wolf, food habits, wild ungulates, livestock

Introduction

The wolf have three species, grey wolf (*Canis lupus* Linnaeus, 1758), red wolf (*Canis rufus* Audubon and Bachman, 1851), and Ethiopian wolf (*Canis simensis* Rüppell, 1840), and various subspecies, for example the artic wolf (*Canis lupus arctos* Pocock, 1935) or the Iberian wolf (*Canis lupus signatus* Cabrera 1907) (Nowak, 2003). These different subspecies can be found in a wide range of habitats, from deserts to tundra, in the North hemisphere (Mech, 1970). The wolf lives and hunts in group, a social unit called wolf pack: the reproductive couple and their offspring. The number of individuals per pack vary: the minimum number of wolves required to find and capture prey with efficiency and safety; the maximum number of individuals that can feed successfully from that prey; the

number of neighboring wolf packs; and how much social competition every member of the wolf pack can support (Mech, 1970).

There are several studies carried about wolves because of human activities. The diversity of natural prey decreased from 5/6 species to 2/3 in Eurasia (Peterson and Ciucci, 2003). In areas with human and wolf populations, wolves depend on livestock (Meriggi *et al.*, 1996; Vos, 2000). Otherwise, in areas with a high abundance of wild ungulates, wolves shift their feeding habits, to wild prey over livestock (Cozza *et al.*, 1996; Meriggi and Lovari, 1996; Meriggi *et al.*, 1996; Migli *et al.*, 2005; Torres *et al.*, 2015a). To work towards the wolf conservation, it is mandatory to understand the ecology of humanized habitats. With the increasing of human-dominated landscapes, the food habits of big carnivores, such as wolves need to be studied to avoid conflicts with humans and prevent negative impacts (Lindenmayer *et al.*, 2010; Mech, 2012; Newsome *et al.*, 2016).

From the center to the northeast of Europe, wolf main natural prey are red deer (*Cervus elaphus* Linnaeus, 1758), wild boar (*Sus scrofa* Linnaeus, 1758) and roe deer (*Capreolus capreolus* Linnaeus, 1758). On the other hand, in the south of Europe, characterized by extreme human density and intense livestock raising, wolves depend mostly on anthropogenic food sources, with some exceptions in areas with good populations of wild ungulates (Gazzola *et al.*, 2005; Peterson and Ciucci, 2003; Torres *et al.*, 2015a). When the wild prey abundance is higher, the predation towards domestic ungulates is lower (Cozza *et al.*,1996; Meriggi and Lovari, 1996; Meriggi *et al.*, 1996; Torres *et al.*, 2015a). Food habits studies are carried based on scat analysis, a technique commonly used for studying carnivore diet and frequently used to evaluate the diet of wolves (Putman, 1984). Compared to other techniques such as stomach analysis and direct observation, scat analysis is easy to apply, allows relatively large sample sizes, and is non-intrusive (Ciucci *et al.*,1996).

In Portugal the population of Iberian wolf (*Canis lupus signatus*) reflects the great conservation difficulty of this species in human dominated landscape (Vos, 2000). Wolves are very resilient, and have a long association with human activities (Lopez, 1978; Álvares, 2011). The main threats to this carnivore are degradation and fragmentation of habitat, scarce availability of wild prey and illegal killing by humans. Livestock predation the main reason for human and wolf

conflicts (Lopez, 1978; Naughton-Treves *et al.*, 2003; Torres *et al.*, 2015a). At the present time the Iberian wolf occupies about 20% of its original area, with a population of only about 2000 individuals (Petrucci-Fonseca, 1990; Pimenta *et al.*, 2005). In human dominated habitat wolves are extremely dependent on domestic animals, being the most preyed livestock the goat (*Capra hircus* Linnaeus, 1758), sheep (*Ovis aries* Linnaeus, 1758) and cow (*Bos taurus* Linnaeus, 1758) (Barja, 2009; Capitani *et al.*, 2004; Gazzola *et al.*, 2008; Iliopoulus *et al.*, 2009; Meriggi and Lovari, 1996; Torres *et al.*, 2015a; Vos, 2000). Wolves and pastoralist economies have always been in conflict (Kaczensky, 1998; Boitani *et al.*, 2010). Wolf predation across Europe, in 2000 was estimated to kill around 10 000 livestock heads per year, with a total loss of 7 to 9 million euros (Boitani, 2000). Nowadays, Portuguese government is responsible to pay for wolf attacks towards livestock in order to mitigate the farmer losses. By this law, the damages to livestock are paid if there is at least one guardian dog for 50 livestock heads (Petrucci-Fonseca *et al.,* 2000).

Studies in Portugal and Spain show that the Iberian wolf is very dependent on domestic ungulates (Álvares, 2011; Petrucci-Fonseca, 1990; Vos, 2000). Despite wolf small distribution area in Portugal, there are still clear regional differences in the proportion of each type of prey. Due to wolf damage on livestock and all the related economic and social problems, the management of wild prey populations in their natural habitats is extremely important in order to assure wolf conservation (Oliveira and Carmo, 2000).

The goals of this work are to understand the spacial and temporal wolf food habits variation. The information will be gathered in two different areas of Portugal (Alvão and Montesinho Natural Parks), by conducting an analysis of scats, to evaluate the wolf food habits spacial variation. With these two areas we tested our hypothesis – with different levels of human pressure wolf food habits change. In this study we provide a review of Iberian wolf food habits studies in Portugal. The results reviewed derive from field studies in which Iberian wolf diet was based on scat analysis. To evaluate the different region temporal variation the studies were grouped into location: South of Douro river; Bragança; Alvão/Padrela; and Peneda/Gerês. With the goal to determinate the variation of wolf diet since the first studies done until nowadays in the different locations.

Methods

Study area

To this study were considered two areas in the north of Portugal (*Figure 1*). Both areas have the wolf present since historical times, and is the only large carnivore there (Petrucci-Fonseca, 1990). Both areas have hunting zones, the principal species hunted are wild boar (*Sus scrofa*), red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), fox (*Vulpes vulpes* Linnaeus, 1758), hare (*Lepus granatensis* Rosenhauer, 1856) and European rabbit (*Oryctolagus cuniculus* Linnaeus, 1758).

The Natural Park of Alvão, is located in the district of Vila Real. Climate in Alvão area is Atlantic with Mediterranean influence, with high precipitation in Winter and dry climate in Summer. There's a large influence of human activities, such as cultivated fields, livestock production (*Bos taurus*, *Capra hircus* and *Ovis aries*) and eolic energy production. The vegetation is characterized with chestnut trees (*Castanea sativa*), *Quercus* and *Pinea* species, oak species and riparian forest (ICNF, 2018). The wild ungulates found in Alvão area are wild boar and roe deer.

The Natural Park of Montesinho is located in Bragança. The mountain ranges of Coroa (1273 m) and Montesinho (1486 m) constitute the highest points in the Park. Montesinho climate is Mediterranean, although with Continental influence due to the geographic position (Gonçalves, 1985). This area is known for having a high abundance of wild ungulates (*Cervus elaphus, Capreolus capreolus, Sus scrofa*) which are part of the Iberian wolf diet. The most representative vegetal species are heather (*Erica australis*), gorse (*Chamaespartium tridentatum*) and *Halimium alyssoides*. In the climatic transitions there's woods of holm oak (*Quercus rotundifolia*). The destuction of these forests led to the emergence of another type of vegetation, dominated by rosemary (*Lavandula pedunculata*) and *Cistus* sp. Water lines have typical species of marshes, being frequent alder (*Alnus glutinosa*), ash (*Fraxinus angustifolia*), willow (*Salix* sp.), elm (*Ulmus* sp.) and poplar (*Populus* sp.) (Agroconsultores and Coba, 1991).
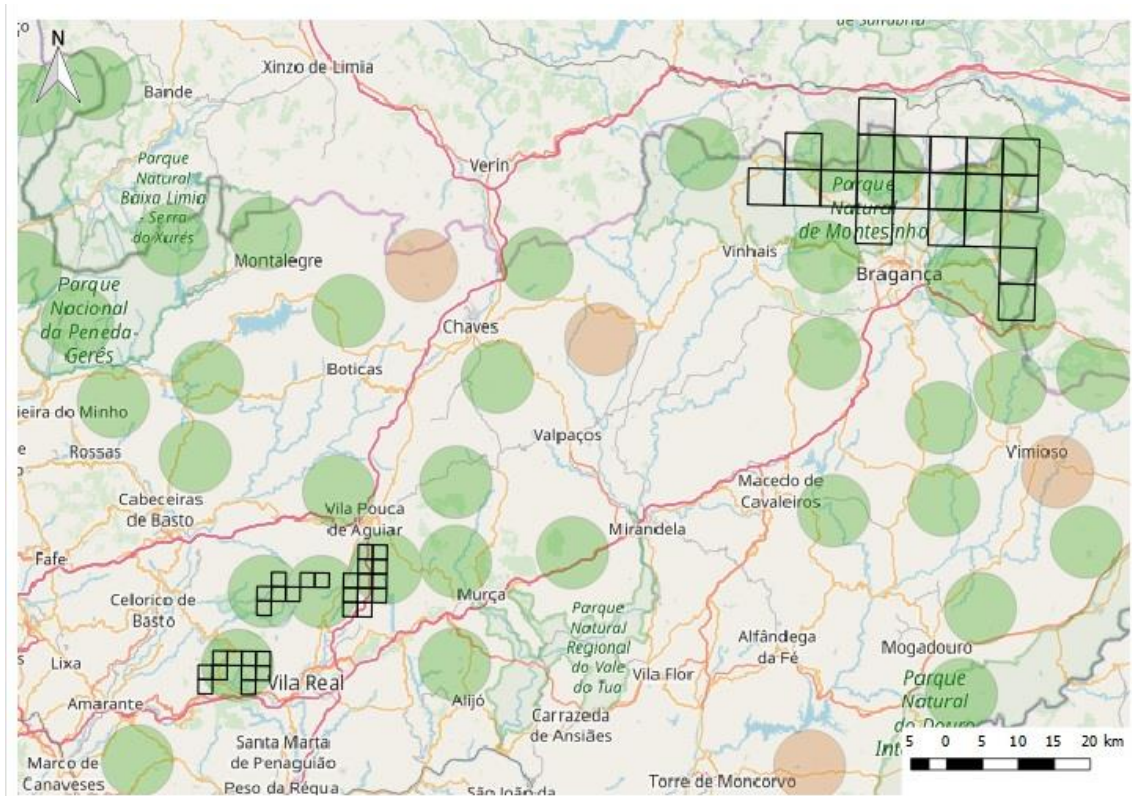
Figure 1 - Study areas with wolf packs location. Natural Park of Montesinho is located in Northeast of Portugal, with quadrats of 5x5km. Natural Park of Alvão is located in Southeast in this map, with quadrats of 2x2km. Green circles represent the confirmed wolf packs and orange circles represent the probable wolf packs, from Pimenta *et al.*, 2005.

Wolf scat collection

Wolf scats were collected along sandy roads (*Figure App. 11*), from August 2016 to December 2017. Transects were made by car with the maximum velocity of 10 km per hour. The cross paths were made by foot at least 100 metros to each side. From September to December, a scat detection dog was used. The use of detection dog is notably helpful to detect the presence of elusive species and low density populations, such as Iberian wolf. Detections dogs, allows to reduce the error of collecting dog (*Canis lupus familiaris*) or fox (*Vulpes vulpes*) scats instead of wolf scats. These ones are brown/black color, in general, bigger than fox or dog scats and have prey hairs and bones (*Figure App. 12*). The odor is another characteristic used to identify canid scats, dog scats have a strong smell, while wolf scat smell with time tends to disappear. Scats were collected in zip locked plastic bags and marked with a code and GPS mark. Later preserved in refrigerator.

Altogether 433 scats were collected, and 383 were used for the study of diet. The 50 scats not used for the analysis were excluded by doubts in the identification or identified as dog or fox, or very old scats that hair could not be identified.

The forward analysis consisted in separate the components (animal, vegetal and mineral material) with special attention to animal hair (*Table 1*). The scats were washed in a sieve and the macro components categorized. First examination of hairs was visual, concerning color, length and thickness. To identify the hair through medullar pattern and cuticular scale identification keys were used (Cornally and Lawton, 2016; De Marinis and Asprea, 2006; Valla-Pinto, 1979) and Teerink, 2003 methods (*Figure App. 13*). When hair identification was impossible as described, cross cuts were made using Rijksirstituut voor Natuurbeheer method in Valla-Pinto, 1979. European rabbit and hare hairs were considered in the same group - Lagomorph, due to the difficulty of a correct identification. Rodents and insectivores were included in the group - Small mammals, due to their scarce presence in scats.

Table 1 - Identified prey of the Iberian wolf scats in the study areas.

Cow	Goat	Sheep
Horse	Red deer	Roe deer
Wild boar	Lagomorph	Dog
Small mammals	Bird	

To evaluate how often an item was eaten the frequency of occurrence (F.O.) method was used, expressed by percentage (Ciucci *et al.*, 1996). This was calculated for each of the identified prey (number of occurrences of each prey on the basis of the number of samples analyzed), having been expressed in relative percentage. In order to determine the importance of each prey species, F.O. values were categorized in: occasional food resource (F.O. < 1%); supplementary (1% ≤ F.O. < 5%); constant (5% ≤ F.O. < 20%); and basic (20% ≤ F.O.). To evaluate statistical differences of seasonal variation in each study area for each season, was used a X^2 test, with a significance level of 5% and Yates correction for 1 degree of freedom (Cochran, 1952).

The wolf diet seasonal diversity was calculated by the Shannon-Wiener index (H'):

$$H' = -\sum p_i \, (\ln p_i) \quad \text{for } i = 1, \ldots, n$$

where p_i is the number of prey i occurrences in function of the total number of identified prey occurrences (where $\sum p_i = 1$), and n is the total number of identified prey categories (Krebs, 1999). The sampled months were divided in four seasons: Winter (January to March), Spring (April to June), Summer (July to September) and Autumn (October to December). The Equitability Index (H') was also calculated (H'/H_{max}), tending to 0 when the diet is based by a single prey, and to 1 when prey classes are equally represented (Krebbs, 1999; Pité and Avelar, 1996).

Niche overlapping (N.O.) between the two study areas and seasons was calculated with Pianka Index (Patalano and Lovari, 1993).

$$N.O._{jk} = \Sigma(p_{ij} \cdot p_{ik}) / \sqrt{(\Sigma p_{ij}2 \cdot \Sigma p_{ik}2)}$$

where p is the proportion of a prey species i in the season j and k. The index varies from 1 to 0, where 1 is the maximum value of overlapping prey classes and 0 represents no equal species along the seasons.

Wolf food habits temporal variation:

Literature on Iberian wolf food habits in Portugal, from 1977 to 2017, was reviewed and the results were compared with the ones obtained in the present study. The information available on these studies considered the frequency of occurrence and number of scats collected, thus all studies can be compared. Frequency of occurrence (F.O.) is the most consistently used measure of the relative importance of prey (Klare *et al.*, 2011; Newsome *et al.*, 2016). The F.O. was used for comparisons among studies. The average of F.O. was calculated in each region to evaluate the evolution of the wolf's predatory habits. The studies reviewed were grouped by region (Bragança, Alvão/Padrela, Peneda/Gerês and South of Douro) and grouped in intervals of time of 10 years.

In Portugal the wolf distribution is divided in two sub-populations: North of Douro River and South of Douro River (*Figure 2*). The North sub-population is stable, connected to Spain population and is divided is three nuclei: Peneda/Gerês, Alvão/Padrela and Bragança. This sub-population has altogether

45 confirmed wolf packs and 9 probable, in an area of 12 500 km^2. South of Douro area is isolated and have only one nucleus, with 6 confirmed wolf packs and 3 probable, in an area of 3 800 km^2 (Pimenta *et al.*, 2005). The mean number considered of wolves per pack is 5 individuals. The studies reviewed didn't include the total area of the different nucleus.
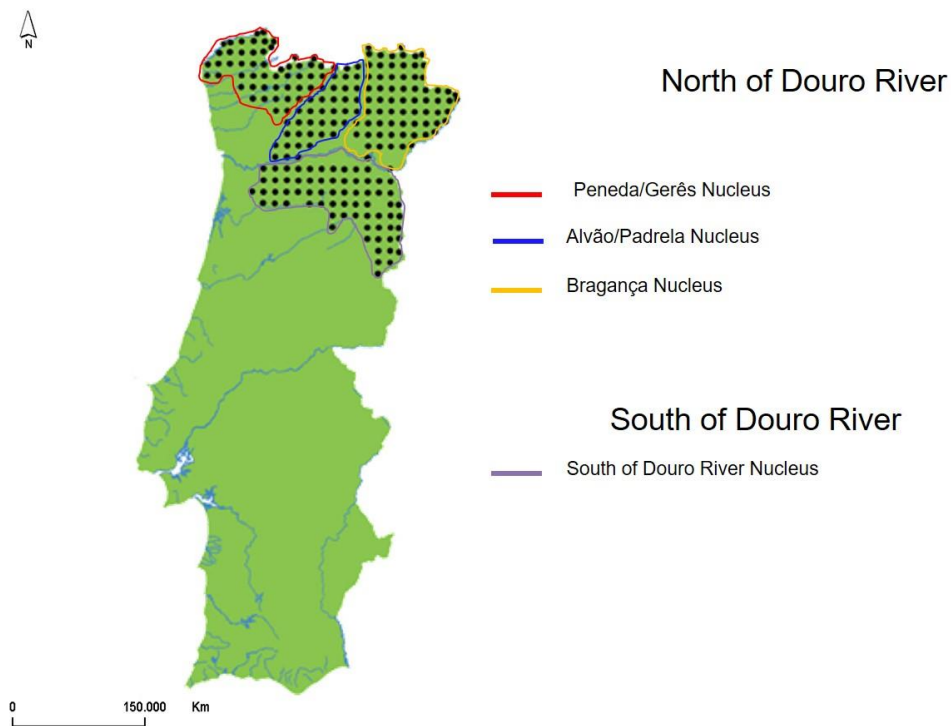
Figure 2 - Total area of wolf nucleus in Portugal. The areas are divided in two zones, North of Douro River and South of Douro River. Black circles represent the quadrants 10x10km. Adapted from Pimenta *et al.*, 2005.

Results

Wolf food habits spatial variation

A total of 383 wolf scats were collected from August 2016 to December 2017, of these 146 were from Parque Natural de Montesinho (Montesinho study area) and 237 from Parque Natural do Alvão (Alvão study area) (*Table 2*). For both study areas, hair was the primary item found in scats, followed by vegetal matter and bones. The percentage of items in each scat visually was calculated, animal hair had a mean value of 60,5% for every scat analyzed, vegetal matter had a mean value of 17,9% and bones 13,5%. Of the 383 scats, 199 contained domestic ungulates (51,95%), 162 wild ungulates (42,29%), 1 carnivore (0,26%),

21 small mammals (5,49%) and 1 bird (0,26%). There are evident differences between the consumption of domestic and wild prey in the two areas (*Figure 3*).

In Montesinho, wild ungulates were the basic prey (97,95%), while in Alvão livestock, especially domestic goat, corresponded to 82,70% of all identified prey, representing a basic resource for wolves in the area (*Table App. 4*). Alvão had a more heterogeneous diet, having unique prey items, absent from Montesinho, such as dog (*Canis lupus familiaris*), lagomorphs, small mammals, and birds.

Prey	Nº Scats	F.O. (%)	Nº Scats Montesinho	F.O. (%)	Nº Scats Alvão	F.O. (%)
Domestic ungulates	**199**	**51,95**	**3**	**1,68**	**196**	**82,7**
Goat	159	41,51	0	0	159	67,09
Cow	28	7,31	1	0,68	27	11,39
Sheep	8	2,09	2	1	6	2,53
Horse	4	1,04	0	0	4	1,69
Wild ungulates	**162**	**42,29**	**143**	**97,95**	**19**	**8,02**
Roe deer	57	14,88	52	35,62	5	2,11
Red deer	39	10,18	39	26,71	0	0
Wild boar	66	17,23	52	35,62	14	5,91
Carnivores	**1**	**0,26**	**0**	**0**	**1**	**0,42**
Dog	1	0,26	0	0	1	0,42
Lagomorpha	**15**	**3,92**	**0**	**0**	**15**	**6,33**
Small mammals	**5**	**1,31**	**0**	**0**	**5**	**2,11**
Bird	**1**	**0,26**	**0**	**0**	**1**	**0,42**
Total	383	100	146	100	237	100

Table 2 - Wolf diet in the two study areas, scats (n = 383) were collected between August 2016 and December 2017. Frequency of occurrence and number of scats per item.
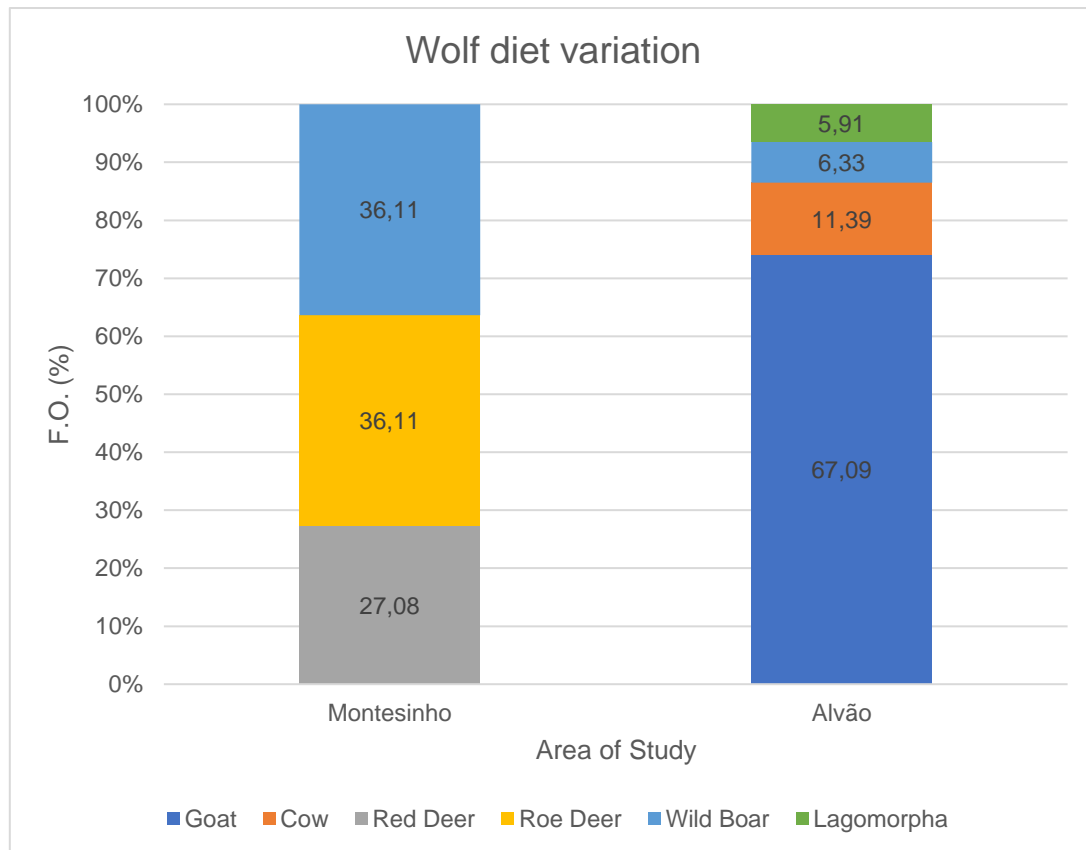
Figure 3 - Importance of prey species in the two study areas. Wolf food habits spatial variation in Montesinho and Alvão. Values expressed as Frequency of Occurrence. Prey that represent a supplementary or occasional resource (F.O. < 5%) are not represented in the figure. In Montesinho prey not represented are cow (0,68%) and sheep (1%), in Alvão are sheep (2,53%), horse (1,69%), roe deer (2,11%), dog (0,42%), small mammals (2,11%) and bird (0,42%).

In Alvão the diversity of the wolf's diet among seasons was expressed by Frequency of Occurrence, Shannon-Wiener index, Equitability index and Niche overlapping. No evident differences were found among seasons. Domestic ungulates were present all year in every season, with the exception of horse that was absent in Winter and Summer (*Figure 4*). For goat, values expressed by Frequency of Occurrence showed a statistically difference (0,05>p>0,01) in Spring-Autumn (X^2 = 6,11, p = 0,013) and in Winter-Autumn (X^2 = 4,07, p = 0,044). For horse only in Winter-Spring (X^2 = 4,72, p = 0,029) the values were statistically significant.

Least important prey resources (horse, wild boar, lagomorphs and small mammals) showed a lower seasonal variation due to less scats with their presence.

Alvão's season variation

	Goat	Cow	Sheep	Horse	Wild Boar	Lagomorpha	Small mammals
Winter	78,57	7,14	3,57	0,00	0,00	7,14	0,00
Spring	68,42	10,53	5,26	15,79	0,00	0,00	0,00
Summer	47,13	13,79	1,15	0,00	5,75	13,79	1,15
Autumn	80,39	10,78	2,94	0,98	0,00	0,00	0,00

Figure 4 - Wolf food habits spatial variation in Alvão (Winter, Spring, Summer and Autumn). Values are expressed by Frequency of Occurrence for 237 scats.

In reference to diet diversity, Summer showed the highest values (H' = 1,53; E = 0,66), while Autumn showed the lowest values (H' = 0,75; E = 0,32) (*Figure 5*).
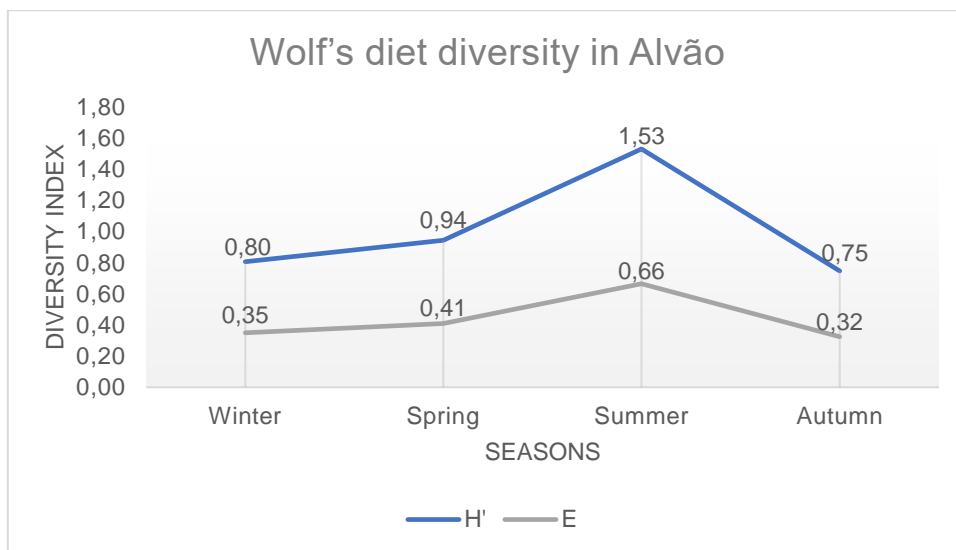
Figure 5 - Wolf food habits diversity in Alvão area. H' and Equitability (E) by Shannon-Wiener index.

Relatively to Niche Overlap (Pianka index) in this area there was almost a complete overlap among seasons. Spring-Summer showed the lowest value, nevertheless it remained closer to 1 (N.O. = 0,89) (*Table 3*).

Table 3 - Niche Overlap values among seasons in Alvão.

	Winter	Spring	Summer	Autumn
Winter	-			
Spring	0,97	-		
Summer	0,94	0,89	-	
Autumn	0,99	0,98	0,92	-

In Montesinho area only Summer and Autumn were analyzed. Values expressed by Frequency of Occurrence showed statistically difference (0,05>p>0,01) for roe deer (X^2 = 4,26, p = 0,039) and red deer (X^2 = 4,03, p = 0,045), while wild boar (X^2 = 14,39, p = 0,00015) was the main prey in Autumn, but the difference is not statistically comparable (*Figure 6*). Livestock prey were not included in this figure due to their very low presence in scats.

Montesinho's season variation

F.O. (%)

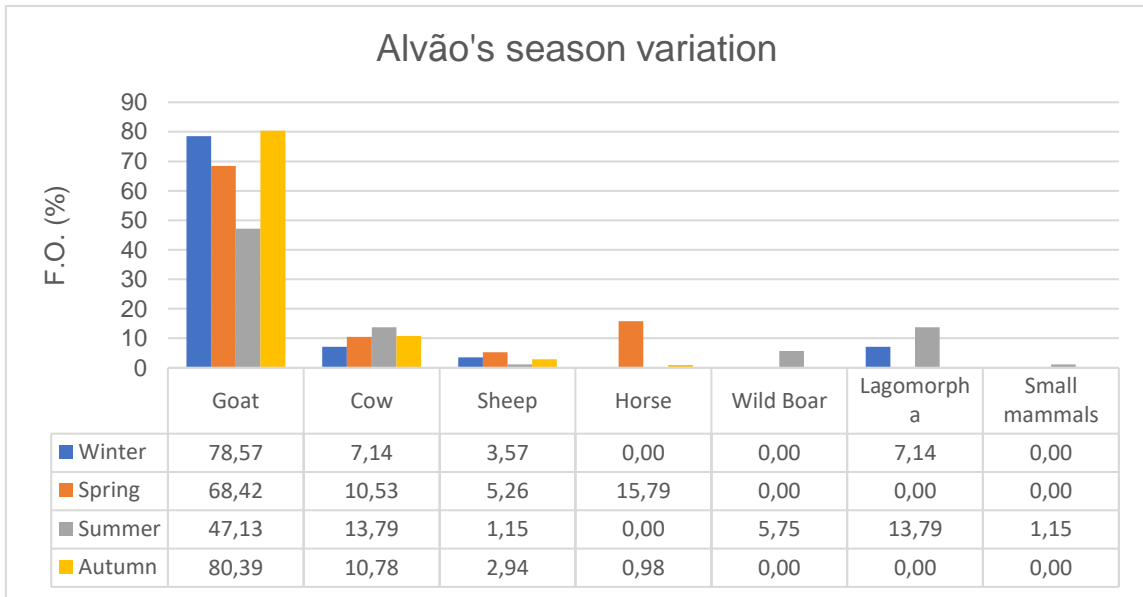|	Roe Deer	Red Deer	Wild Boar
Winter	0,00	0,00	0,00
Spring	0,00	0,00	0,00
Summer	44,44	33,33	22,22
Autumn	32,00	24,00	42,00
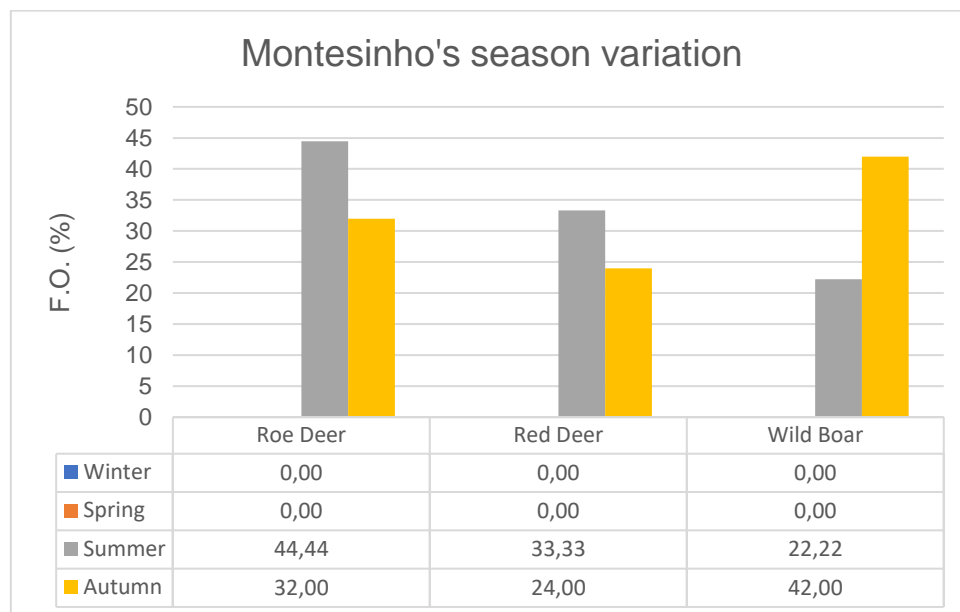
Figure 6 - Wolf food habits spatial variation in Montesinho (Winter, Spring, Summer and Autumn). Values are expressed by Frequency of Occurrence for 146 scats.

The diversity of the wolf diet is virtually equal in the two seasons (*Figure 7*). This shows that wolves prey the same species in these two seasons. Between these two seasons the value of niche overlap was nearly total (N.O. = 0,91).
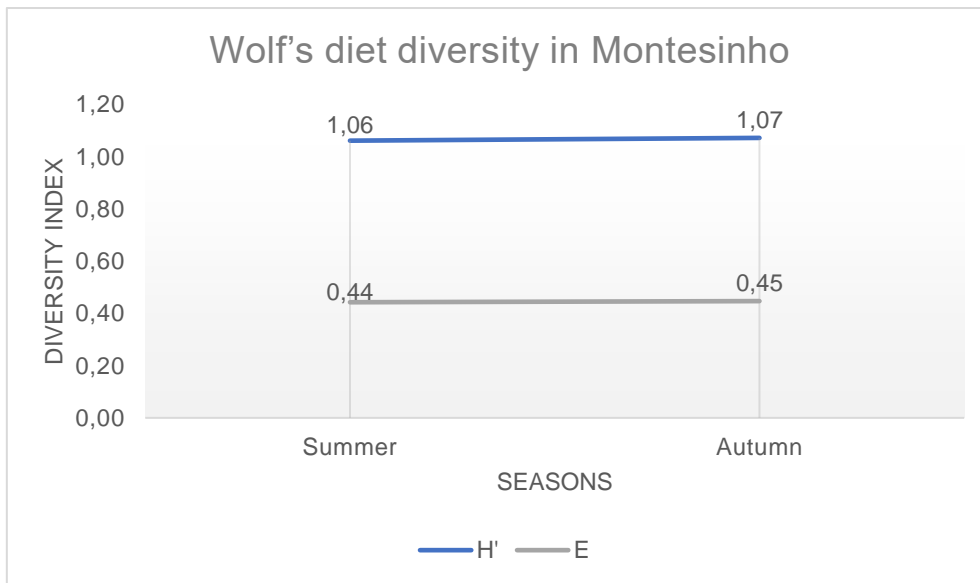
Figure 7 - Wolf food habits diversity in Montesinho area. Values of H' and Equitability (E) were calculated by Shannon-Wiener index.

The Niche Overlap between the two study areas, in Summer and Autumn seasons, was almost null (N.O. = 0,087). Showing a very different diet in the two areas.

Wolf food habits temporal variation

The temporal analysis of the wolf diet was based on compiled results from previous studies. Four different areas were considered for this analysis: Peneda/Gerês, Bragança, Alvão/Padrela and South of Douro River. Studies were gathered from 1977 to 2017. The comparison of the wolf food habits across all areas and among years was done by collecting the values of Frequency of Occurrence (*Figure 8*). Generally, the basis of wolf diet are domestic animals, except in Bragança which are wild ungulates since the last decade. South of Douro is the area with a more heterogenous diet, with high presence of lagomorphs and birds, prey species that in other areas were found to be least important.
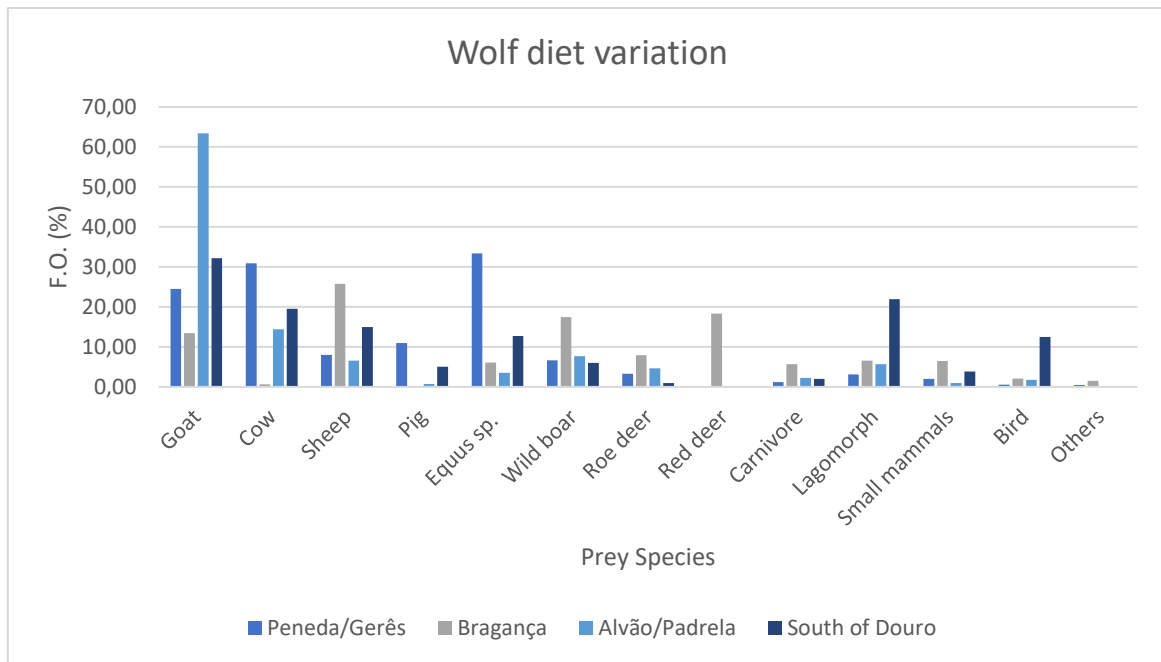
Figure 8 - Wolf food habits temporal variation (n = 20 studies). Values were expressed in Frequency of Occurrence (F.O.). The "Carnivore" group included dog (*Canis lupus familiaris*), red fox (*Vulpes vulpes*) and European badger (*Meles meles*). The "Others" group comprised of trash and insects. Values obtain from: Álvares, 2011; Álvares, 1995; Barreto, 2009; Bastos, 2001; Carreira, 1996; Casimiro, 2017; Duarte, 2005; Ferrão da Costa, 2001; Guerra, 2004; Moreira, 1992; Petrucci-Fonseca, 1990; Pinto, 2008; Rauel, 2002; Roque *et al.*, 2001; Santos, 2008; Silva, 2006; Silva, 2007; Sobral, 2006; Torres *et al.*, 2015a; Vos, 2000.

Livestock consumption (F.O.) by wolves was higher in Peneda/Gerês and Alvão/Padrela, when compared to the other two areas: South of Douro, where a slight decrease occurred from 1988 to 2017; and Bragança, in which a drastic decline was observed between 1988 and 2007 (*Figure 9*). Contrary, the wild ungulates consumption greatly increased in Bragança since 1998. While in the other areas had low and similar values over the years (*Figure 10*).
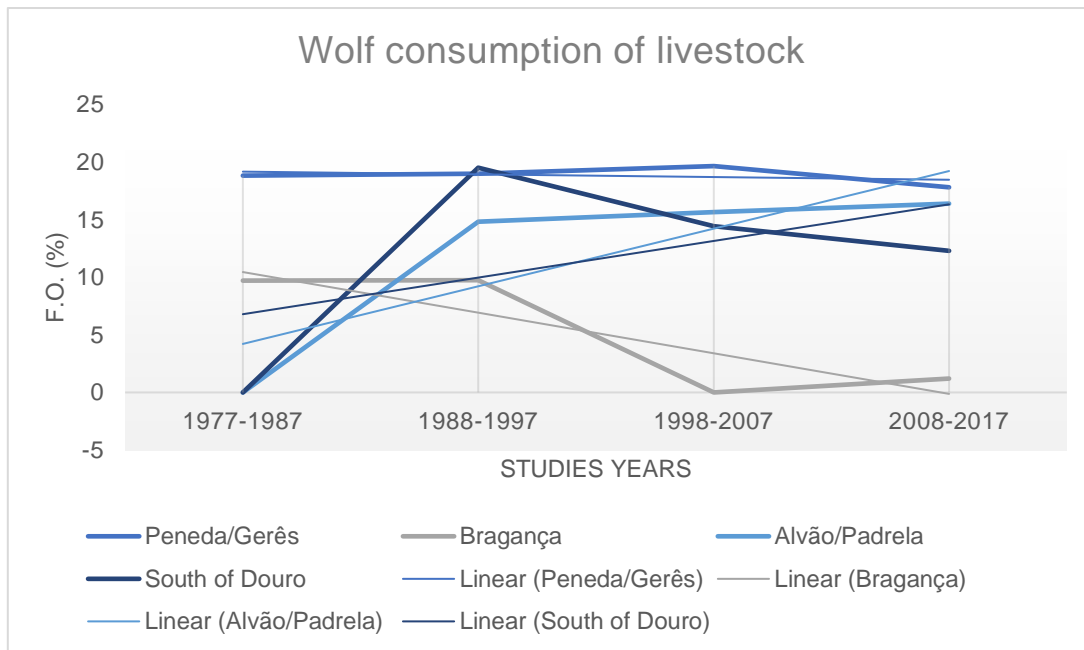
Figure 9 - Temporal variation of the livestock consumption by wolves across four regions of Portugal (Peneda/Gerês, Bragança, Alvão/Padrela and South of Douro), from 1977 to 2017. The 20 studies analyzed were aggregated in four 10-year groups. Livestock included goat (*Capra hircus*), cow (*Bos taurus*), sheep (*Ovis aries*), pig (*Sus domestica*) and horse (*Equus* sp.). Values were expressed in Frequency of Occurrence. Values obtain from: Álvares, 2011; Álvares, 1995; Barreto, 2009; Bastos, 2001; Carreira, 1996; Casimiro, 2017; Duarte, 2005; Ferrão da Costa, 2001; Guerra, 2004; Moreira, 1992; Petrucci-Fonseca, 1990; Pinto, 2008; Rauel, 2002; Roque *et al.*, 2001; Santos, 2008; Silva, 2006; Silva, 2007; Sobral, 2006; Torres *et al.*, 2015a; Vos, 2000.
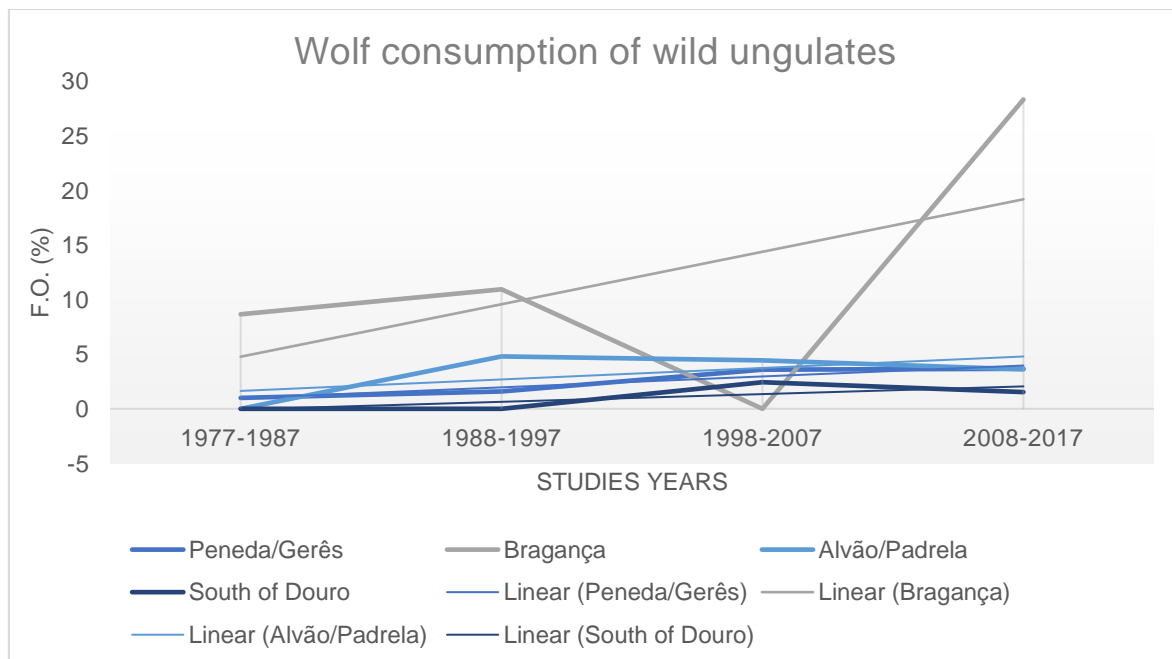
Figure 10 - Temporal variation of the wild ungulates consumption by wolves across four regions of Portugal (Peneda/Gerês, Bragança, Alvão/Padrela and South of Douro), from 1977 to 2017. The 20 studies analyzed were aggregated in four 10-year groups. Wild ungulates included wild boar (*Sus scrofa*), roe deer (*Capreolus*

capreolus) and red deer (*Cervus elaphus*). Values were expressed in Frequency of Occurrence. Values obtain from: Álvares, 2011; Álvares, 1995; Barreto, 2009; Bastos, 2001; Carreira, 1996; Casimiro, 2017; Duarte, 2005; Ferrão da Costa, 2001; Guerra, 2004; Moreira, 1992; Petrucci-Fonseca, 1990; Pinto, 2008; Rauel, 2002; Roque *et al.*, 2001; Santos, 2008; Silva, 2006; Silva, 2007; Sobral, 2006; Torres *et al.*, 2015a; Vos, 2000.

Discussion

The predation of livestock and wild ungulates by wolf is a universal concern (Fritts and Mech, 1981; Torres *et al.*, 2015a; Vos, 2000). The present study revealed the dependence of wolves on livestock in Alvão area and on wild ungulates in Montesinho, from 2016 to 2017. The diversity found in the diet support our hypothesis, that in areas with different levels of human pressure the wolf diet varies. The different studies support the present study. Bragança showed a change in diet over time, from livestock to wild ungulates. Meanwhile, in Alvão/Padrela, Peneda/Gerês and South of Douro River wolf predation is based on domestic animals.

This study provides information about the Iberian wolf's diet: its diversity, seasonal and temporal variation, due to human conflicts and management implications.

Wolf food habits spatial variation

Wild prey are expected to be the main food resource for wolves and helpful in preventing livestock depredation, when existing in high abundance and diversity (Meriggi and Lovari, 1996; Boitani, 2000; Barja, 2009; Meriggi *et al.,* 2011). In the two study areas there are differences in livestock and wild ungulates diversity. There are also differences in the livestock's grazing between the two areas. In this study wolf diet is based on goats and wild ungulates (*Table App. 4; Figure App. 14*).

In Alvão area, livestock predation has been problematic for many years, since large ungulates, like red deer, are absent or in low density (Torres *et al.*, 2015b, Pimenta *et al.*, 2017). In Alvão the diet is based on goats, the presence of a great number of animals and their tendency to spread all over the area makes them an easier prey. In this area livestock is raised extensively in mountainous

regions with a shepherd and his guardian dogs. With less wild ungulates in Alvão due to human density and habitat quality, wolves rely on livestock to survive.

In Montesinho area, livestock stay near to villages. In this area there is less livestock and less human pressure in natural areas. In Montesinho there is a bigger diversity of wild ungulates and better habitat quality for this species. Important prey species for wolf are present, such as roe deer, red deer and wild boar. This explains the decrease of wolf predation on livestock in this area, and the constant predation in Alvão (*Figure App. 20*). Several studies report an association between a high availability of wild prey and the decrease of livestock predation (Meriggi and Lovari, 1996; Meriggi *et al.*, 1996; Boitani, 2000; Barja, 2009).

In general, what concerns seasonal variation (Winter, Spring, Summer and Autumn), the wolf diet is more diverse in Summer and Autumn (*Figure App. 15*). This variation was due to the fact that Spring and Winter were not sampled in Montesinho (*Table App. 5*).

Alvão area showed no evident seasonal variation. Domestic ungulates, that represent important resources (*Table App. 4*), were present all year, supporting the high dependence of wolves on livestock in this area. When domestic animals are grazing, without any protection, or with lower number of guardian dogs they become more vulnerable to wolves (Pimenta *et al.*, 2017). Wolf diet show almost no variation. Only in Summer was slightly different due to the presence of wild boar, lagomorphs and small mammals, less frequent prey in other seasons. Despite some differences in prey per season there was niche overlap between seasons, showing the basis of the diet is monotonous.

Due to weather conditions in Montesinho area, only Summer and Autumn were studied. In this area the main prey were wild boar, roe deer and red deer. A higher prey consumption was roe deer and red deer in Summer. Roe deer and red deer calve in warmer weather, starting in the Spring (García *et al.*, 2006; Mateos-Quesada and Carranza, 2000). Hunting younger individuals is easier, and requires less energy from wolves, than an adult. Calve season may explain the higher consumption of this prey in Summer. The wolf prey size is related to the wolf pack size (Hayes *et al.*, 2000; Jêdrzejewski *et al.,* 2002; Schmidt and Mech, 1997; Thurber and Peterson, 1993). So, roe deer can feed a wolf pack, but a red deer will give energy for a longer time (Jedrzejewski *et al.*, 2002; Meriggi

et al., 2011). Wild boar was the main prey in Autumn, due to higher number of scats with wild boar presence found in this season, but was not statistically comparable with Summer. The diversity of wolf diet did not change in Summer and Autumn, due to the prey were always the same. Being so, the niche overlap can be considered total.

Comparing the niche overlap for Alvão and Montesinho, between Summer and Autumn, the value was very low. The reason for this is that the wolf main prey are very different in the two areas. Since 2006 the number of livestock killed by wolves is decreasing in Montesinho area, contrary in Alvão that is constant (*Figure App. 20*).

Wolf food habits temporal variation

Previous studies, in Peneda/Gerês, Bragança, Alvão/Padrela and South of Douro River, showed the variation of wolf diet over the years. Mostly, the basis of wolf diet are domestic animals, except in Bragança since the last decade which are wild ungulates. In South of Douro River there is a slight decrease of wolf livestock predation, which may be due to a more flexible diet (*Figure App. 24*). In this area wolves have a more variable predation, which include lagomorphs, small mammals and birds. These studies showed that wolf predation of goats is constant and a basic resource in Alvão/Padrela area (*Figure App. 23*), while in Peneda/Gerês, *Equus sp.* are the main prey (*Figure App. 21*). The changing of wolf food habits in Bragança is clear (*Figure App. 22*). The decrease of wolf livestock predation and the increase of wild ungulates predation is visible, even considering the number of livestock animals (*Table App. 6; Figures App. 16, 17, 18 and 19*).

While livestock continues to be killed by wolves in Peneda/Gerês, Alvão/Padrela and South of Douro, in Bragança this have been declining over the years. The fact that the three main wild prey species are present in Bragança area, explains the declining of killed domestic animals. The factors that influence wolf predation on livestock is their availability and vulnerability. To minimize this predation guardian dogs, electric fences and confined shelter should be used (Barja, 2009; Iliopoulos *et al.,* 2009; Musiani *et al.,* 2003; Shivik *et al.,* 2003; Torres *et al.,* 2015a). Understanding wolf prey preferences in areas where

different potential prey species coexist may be extremely useful in reducing the number of attacks in livestock (Barja, 2009).

Study limitations

The scats collected in each season were not in the same number due to logistic or climate complications. Additional scats from Winter and Spring in Montesinho area would be helpful to a more complete diet study. However, with the review comparing anterior studies the diet didn't change much.

To decrease the error between canids scats, genetic analysis should be used, especially in areas with a low density of wolves or when the technicians are less experienced.

Conclusions and Conservation Implications

This study adds information on Iberian wolf food habits and temporal evolution. The variation in areas with different levels of humanization show what must be done to minimize wolf predation on livestock and contributes to the coexistence between humans and wolves.

One of the greatest obstacles to Iberian wolf conservation is the concern about predation on livestock and consequent persecution of wolves. With extensive grazing systems decreasing, this may represent a threat to wolves dependent on livestock (Vos, 2000). To decrease the predation on livestock, maintaining and restoring wild ungulate populations should be a priority (Newsome *et al.*, 2016; Ripple *et al.*, 2014). The main threats to wild ungulates include un-sustainable hunting by humans, competition with livestock for food, and habitat loss (Newsome *et al.*, 2016; Ripple *et al.*, 2015). Wolf will consume more human-provided foods, such as domestic animals or garbage, due to the depletion of wild prey populations (Newsome *et al.*, 2016).

It is critical to increase the knowledge on Iberian wolf ecology and feeding habits, so human attitudes and management decisions can be improved towards wolf conservation. Without this understanding we will continue to be focused on lowering predation on livestock, by killing wolves, rather than incorporate the benefits of wolf presence. Wolf, as a large carnivore, have a great function in controlling wild ungulates populations such as wild boar, that has been growing and destroying cultivated fields.

Conservation of wolves in areas with human dominated landscapes will depend on the restoration of native ungulates and management of predation on livestock. Plans to preserve natural landscapes and reintroduce wild ungulates have an important role to wolf conservation.

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Final remarks

Wolf have different food habits depending the prey availability in the area. The prey availability will change depending the human pressure and habitat quality. With intense grazing in Portugal, wolves tend to depend on livestock to survive (Pimenta *et al.*, 2017; Vos, 2000). This study showed the dependence of wolves to livestock in Natural Park of Alvão and wild prey as the base of wolf diet in Natural Park of Montesinho. Which was supported by the studies reviewed, and literature from Europe.

To continued this study, the time period should be longer than one year, to cover all seasons. Have enough information from prey species, such as density in the different areas is also significant to a more complete study. Include a genetic analysis is very important to decrease the error in scat identification.

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Appendix

Figure App. 11 - Work team looking for wolf scats on sandy roads.

Figure App. 12 - Iberian wolf scat with key lock for scale.

Figure App. 13 - Three examples of wolf prey hairs found in different scats, with different medullar patterns.
Identification from left to right: hair from roe deer, hair from red deer and from domestic goat.

Table App. 4 - Global and specific patterns of Wolf diet in Alvão and Montesinho. In the prey item column all identified preys are shown, Frequency of Occurrence and resource type by food item.

Prey item	Global F.O. (%)	Resource	Montesinho F.O. (%)	Resource	Alvão F.O. (%)	Resource
Domestic ungulates	**51,95**	**-**	**1,68**	**-**	**82,7**	**-**
Goat	41,51	Basic	0	-	67,09	Basic
Cow	7,31	Constant	0,68	Occasional	11,39	Constant
Sheep	2,09	Supplementary	1	Occasional	2,53	Supplementary
Horse	1,04	Supplementary	0	-	1,69	Supplementary
Wild ungulates	**42,29**	**-**	**97,95**	**-**	**8,02**	**-**
Roe Deer	14,88	Constant	35,62	Basic	2,11	Supplementary
Red Deer	10,18	Constant	26,71	Basic	0	-
Wild Boar	17,23	Constant	35,62	Basic	5,91	Constant
Carnivores	**0,26**	**-**	**0**	-	**0,42**	**-**
Dog	0,26	Occasional	0	-	0,42	Occasional
Lagomorphs	**3,92**	Supplementary	**0**	-	**6,33**	Constant
Small mammals	**1,31**	Supplementary	**0**	-	**2,11**	Supplementary
Birds	**0,26**	Occasional	**0**	-	**0,42**	Occasional
Total	**100**		**100**		**100**	

Wolf prey preferences

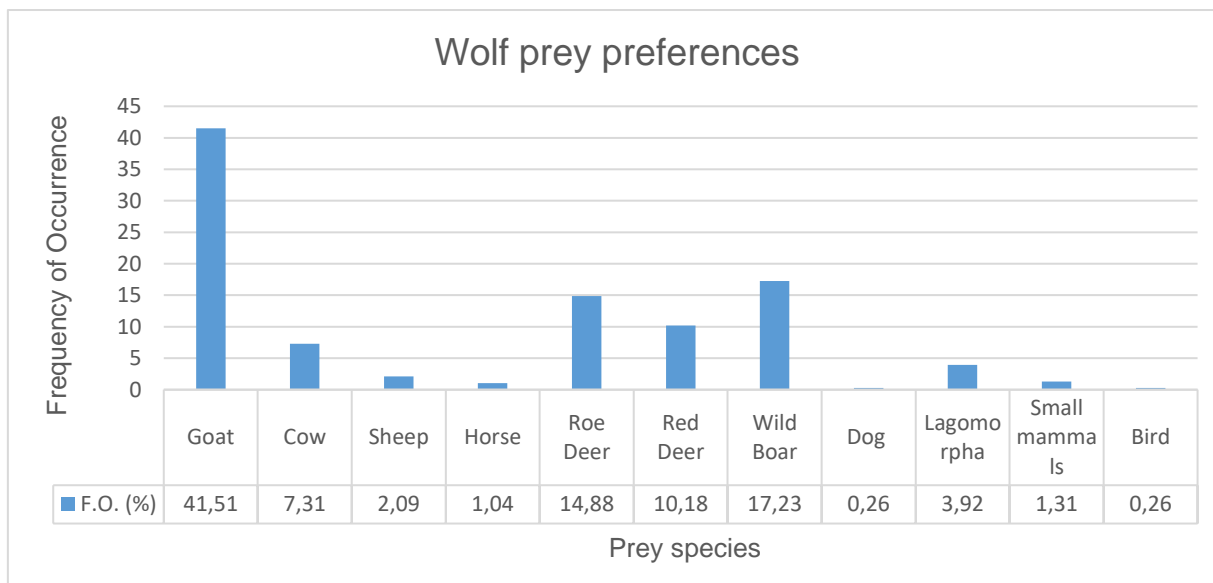| F.O. (%)	Goat	Cow	Sheep	Horse	Roe Deer	Red Deer	Wild Boar	Dog	Lagomorpha	Small mammals	Bird
F.O. (%)	41,51	7,31	2,09	1,04	14,88	10,18	17,23	0,26	3,92	1,31	0,26

Figure App. 14 - Global wolf diet. Goat (*Capra hircus*) is the most consumed item, followed by wild ungulates (*Capreolus capreolus*, *Cervus elaphus* and *Sus scrofa*).

Table App. 5 - Number of scats analyzed in each season for Montesinho and Alvão area.

Season	Montesinho Nº of scats	Alvão Nº of scats
Winter	0	28
Spring	0	19
Summer	45	87
Autumn	100	102
Total	**145**	**236**
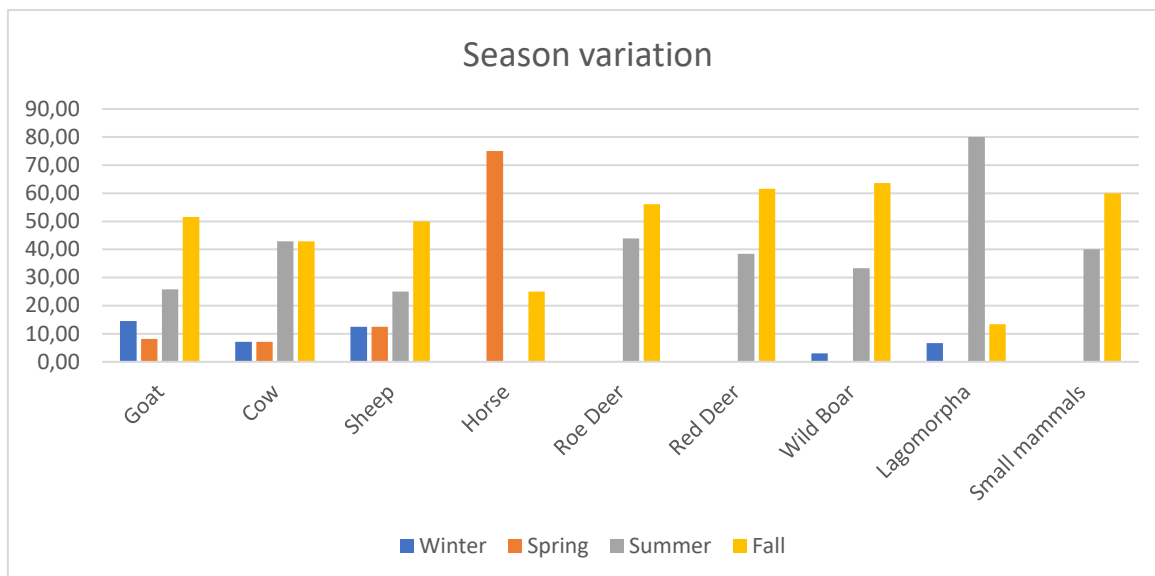
Figure App. 15 - Global seasonal variation. Values are expressed by Frequency of Occurrence.

Table App. 6 - Number of livestock animals in 2009 in three regions of Portugal, Douro and Minho, Trás-os-Montes and Beira Interior. Peneda/Gerês is located in Minho region, Bragança and Alvão/Padrela in Trás-os-Montes region and South of Douro in Beira Interior. Adapted from Recenseamento Agrícola, 2009.

| Region	Livestock	Nº of animals (2009)
Douro and Minho	*Bos taurus*	260 772
	Ovis aries	129 148
	Capra hircus	54 819
	Equus sp.	9 187
Trás-os-Montes	*Bos taurus*	64 502
	Ovis aries	269 726
	Capra hircus	57 006
	Equus sp.	15 579
Beira Interior	*Bos taurus*	61 844
	Ovis aries	359 200
	Capra hircus	66 172
	Equus sp.	6 190
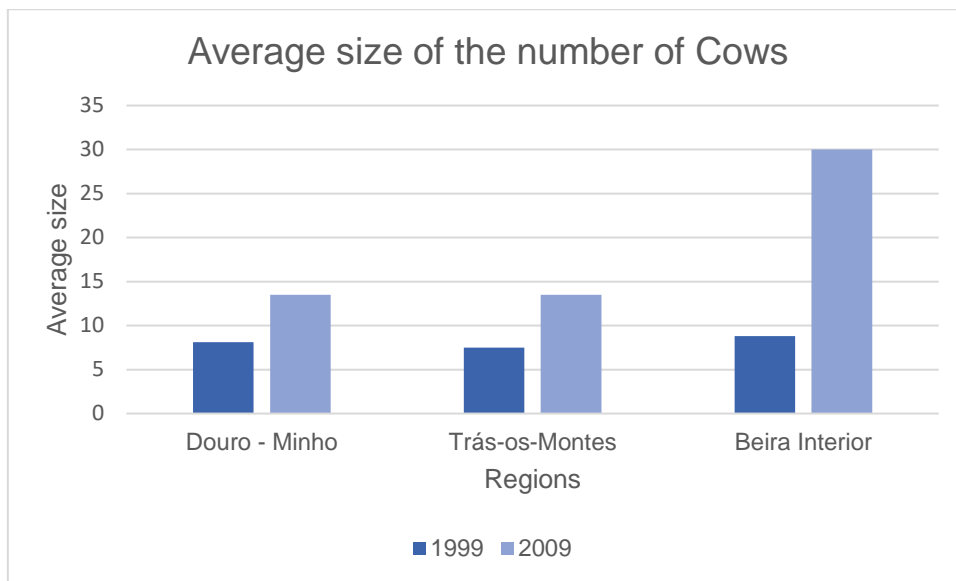
Figure App. 16 - Average size of cows (*Bos taurus*) numbers (calculated by number of cattle head / number of animal explorations) in 1999 and 2009 in three regions of Portugal. Peneda/Gerês is located in Minho region, Bragança and Alvão/Padrela in Trás-os-Montes region and South of Douro River in Beira Interior. Adapted from Recenseamento Agrícola, 2009.
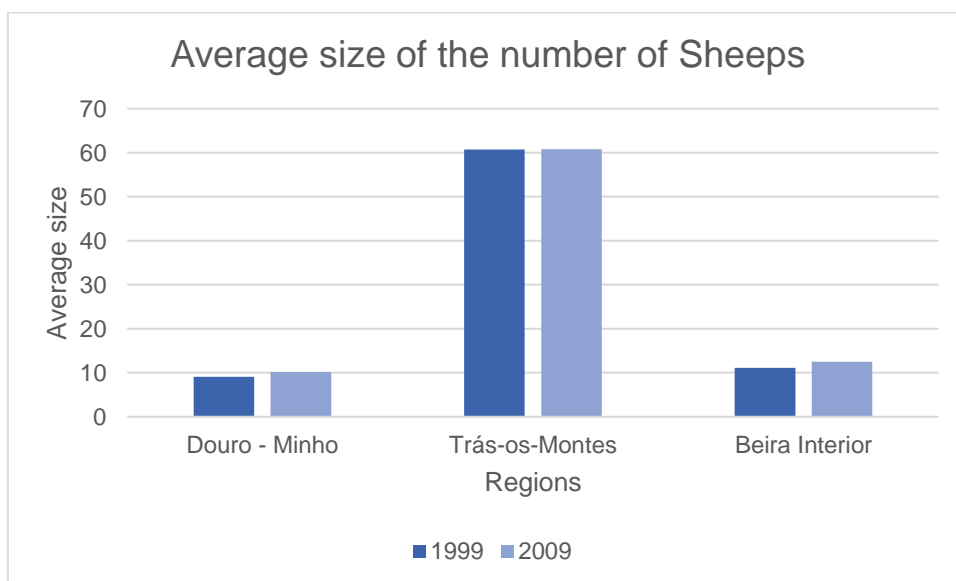
Figure App.17 - Average size of sheep (*Ovis aries*) numbers (calculated by number of cattle head / number of animal explorations) in 1999 and 2009 in three regions of Portugal. Peneda/Gerês is located in Minho region, Bragança and Alvão/Padrela in Trás-os-Montes region and South of Douro River in Beira Interior. Adapted from Recenseamento Agrícola, 2009.
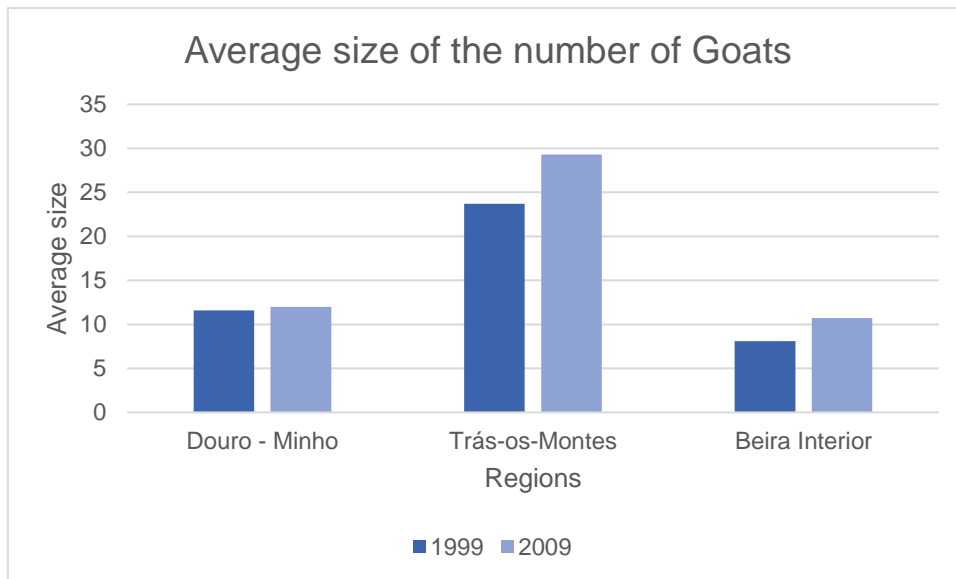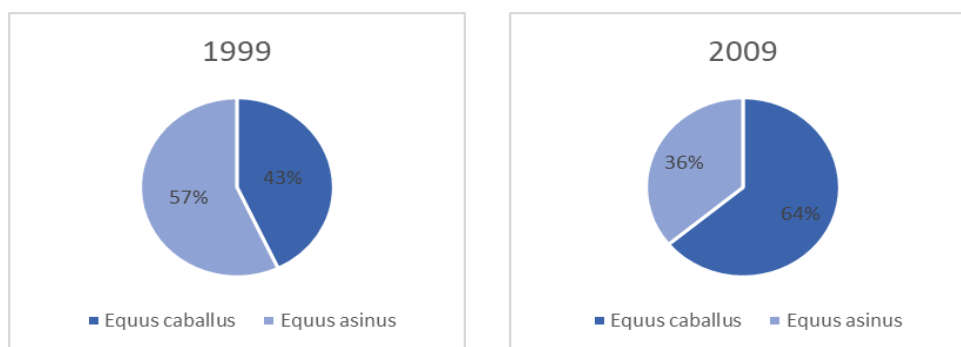
Figure App. 18 - Average size of goats (*Capra hircus*) numbers (calculated by number of cattle head / number of animal explorations) in 1999 and 2009 in three regions of Portugal. Peneda/Gerês is located in Minho region, Bragança and Alvão/Padrela in Trás-os-Montes region and South of Douro River in Beira Interior. Adapted from Recenseamento Agrícola, 2009.

Figure App. 19 - Average size of *Equus caballus* and *Equus asinus* numbers (calculated by number of cattle head / number of animal explorations) in 1999 and 2009. Adapted from Recenseamento Agrícola, 2009.
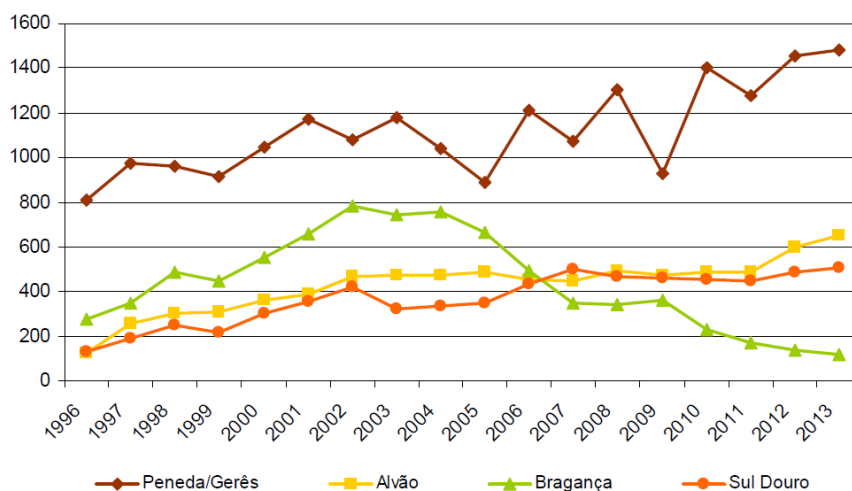
Figure App. 20 - Number of livestock killed by wolves, from 1996 to 2013 in Peneda/Gerês, Alvão, Bragança and South of Douro (Sul Douro). From Álvares *et al.*, 2015.
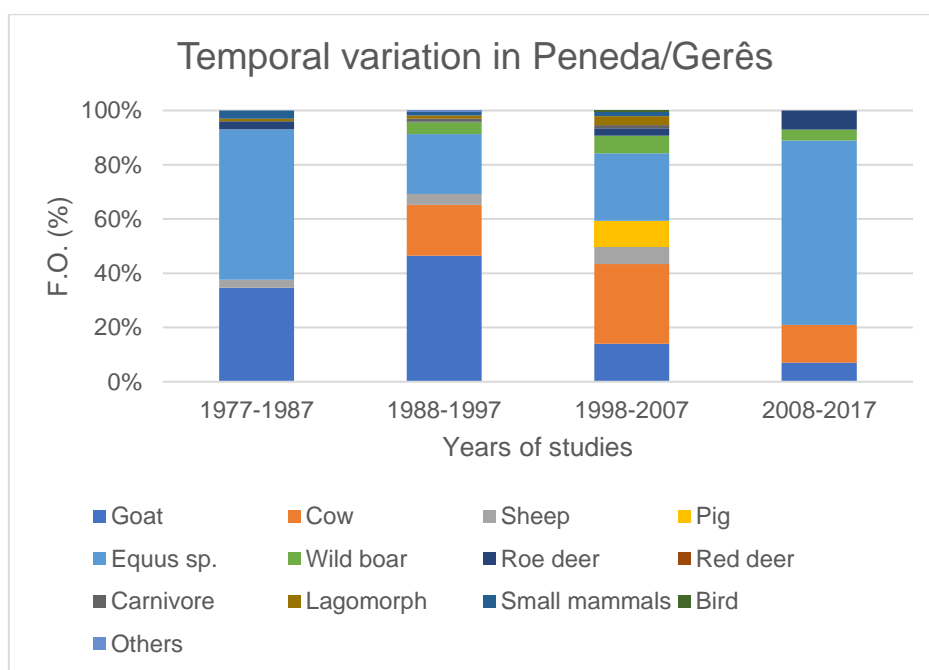

Figure App. 21 - Wolf food habits temporal variation in Peneda/Gerês. Values expressed as Frequency of Occurrence from studies since 1977 to 2017. Carnivore group includes domestic dog (*Canis lupus familiaris*), red fox (*Vulpes vulpes*) and European badger (*Meles meles*). Others group includes trash and insects. Values obtain from: Álvares, 2011; Álvares, 1995; Casimiro, 2017; Duarte, 2005; Ferrão da Costa, 2001; Guerra, 2004; Petrucci-Fonseca, 1990; Roque *et al.*, 2001; Vos, 2000.
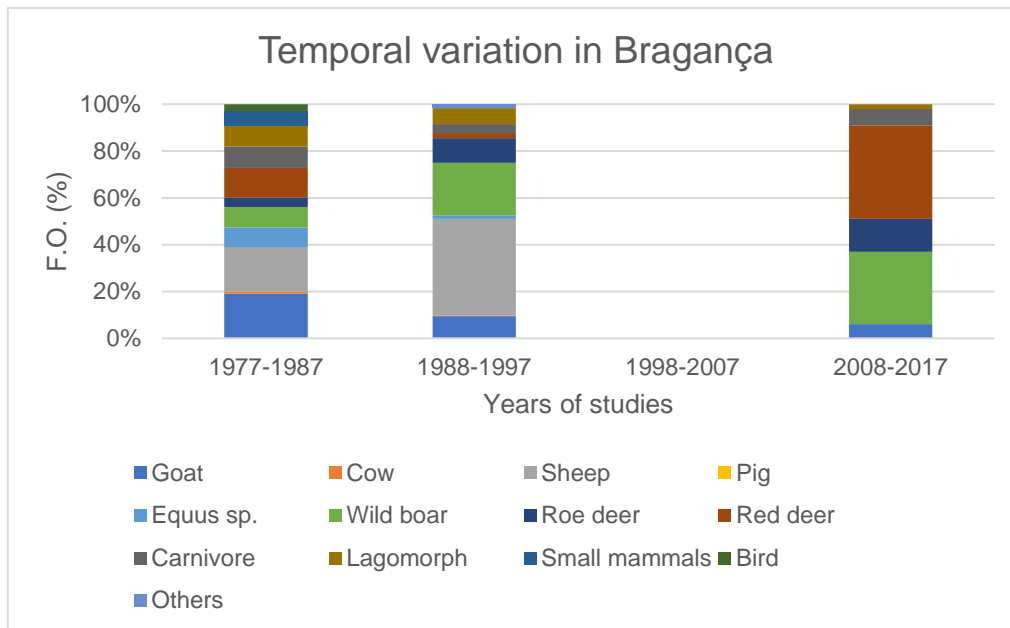
Figure App. 22 - Wolf food habits temporal variation in Bragança. Values expressed as Frequency of Occurrence from studies since 1977 to 2017, years of 1998 to 2007 were not present in any study. Carnivore group includes domestic dog (*Canis lupus familiaris*), red fox (*Vulpes vulpes*) and European badger (*Meles meles*). Others group includes trash and insects. Values obtain from: Casimiro, 2017; Moreira, 1992; Petrucci-Fonseca, 1990.
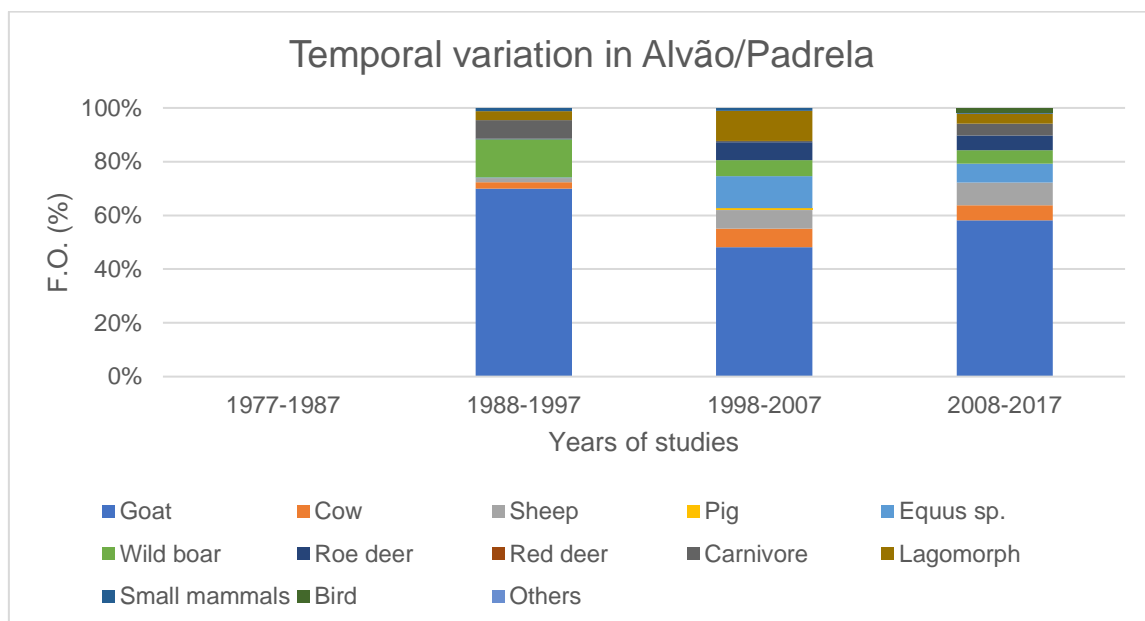

Figure App. 23 - Wolf food habits temporal variation in Alvão/Padrela. Values expressed as Frequency of Occurrence from studies since 1988 to 2017, years of 1977 to 1987 were not present in any study. Carnivore group includes domestic dog (*Canis lupus familiaris*), red fox (*Vulpes vulpes*) and European badger (*Meles meles*). Others group includes trash and insects. Values obtain from: Barreto, 2009; Casimiro, 2017; Santos, 2008; Silva, 2006
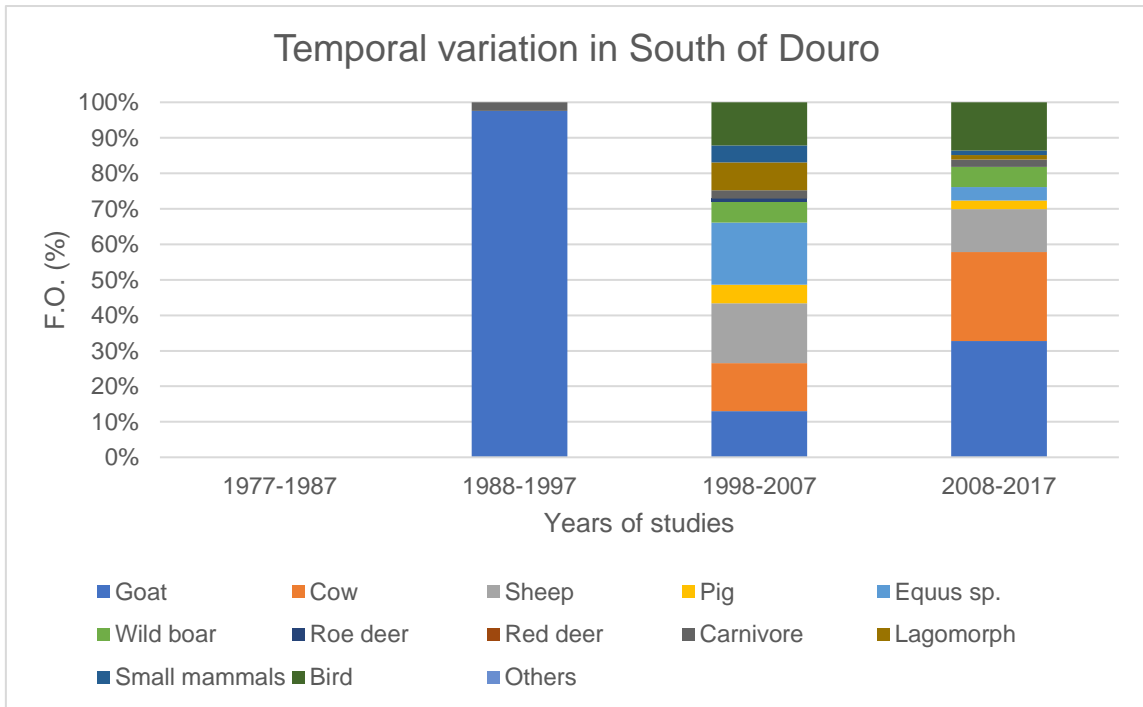
Figure App. 24 - Wolf food habits temporal variation in South of Douro. Values expressed as Frequency of Occurrence from studies since 1988 to 2017, years of 1977 to 1987 were not present in any study. Carnivore group includes domestic dog (*Canis lupus familiaris*), red fox (*Vulpes vulpes*) and European badger (*Meles meles*). Others group includes trash and insects. Values obtain from: Bastos, 2001; Casimiro, 2017; Pinto, 2008; Rauel, 2002; Silva, 2007; Sobral, 2006; Torres *et al.*, 2015a; Vos, 2000.