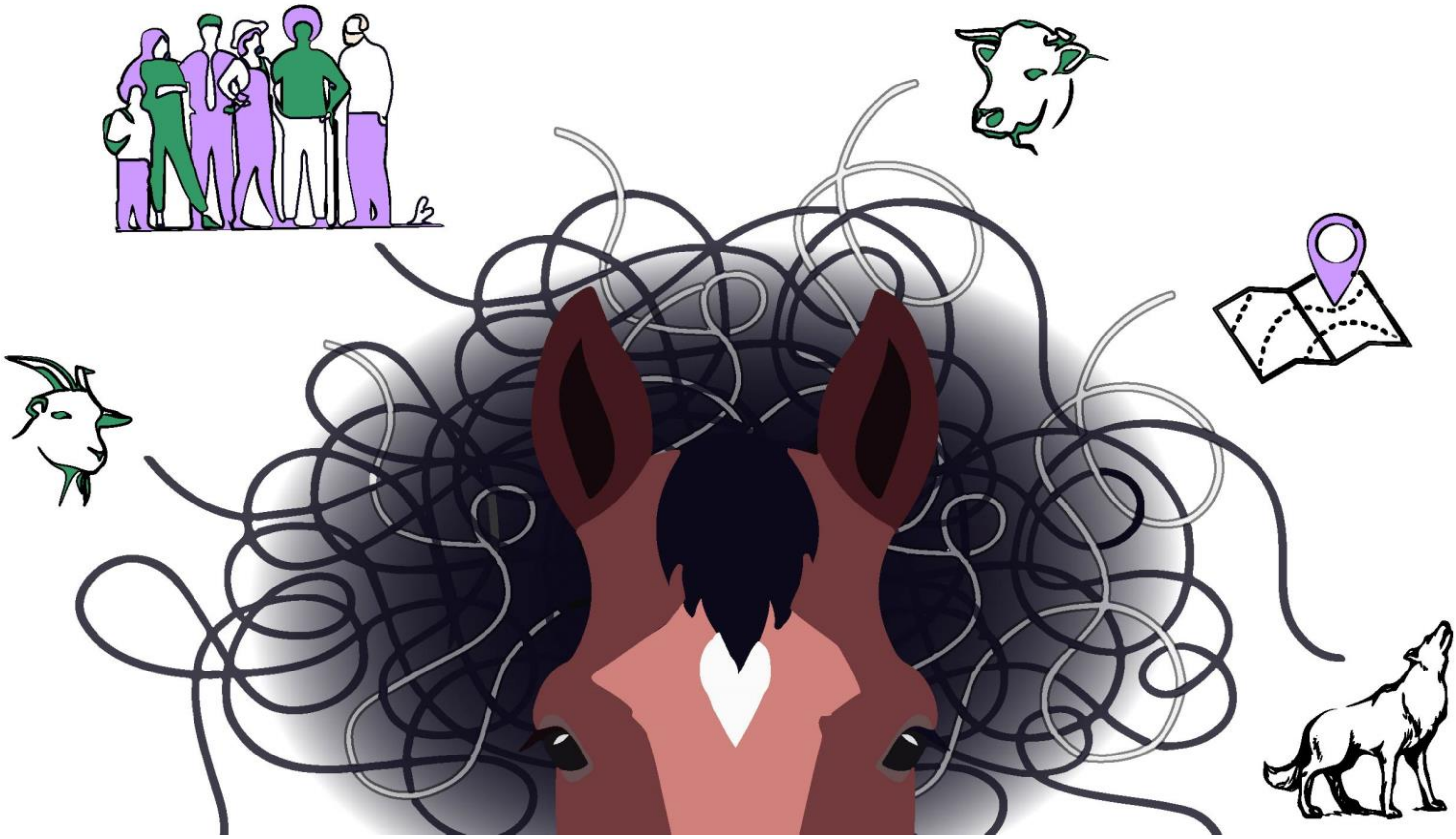

ECOLOGICAL TRAITS OF FREE-RANGING HORSES:

IMPROVING METHODOLOGICAL APPROACHES AND DISENTANGLING DETERMINANTS

Jéssica Gonçalves Abrantes
Mestrado em Biodiversidade, Genética e Evolução
Departamento de Biologia
2021

Orientador
Francisco Álvares, CIBIO/InBIO

Coorientador
Nuno Queiróz, CIBIO/InBIO

Todas as correções determinadas pelo júri, e só essas, foram efetuadas.

O Presidente do Júri,

Porto, ______/______/_________

MSc

Acknowledgements

In the first place, I would like to sincerely thank my supervisor, Dr. Francisco Álvares, not only for all his help and advice throughout this dissertation, but also for his perseverance and never-ending ideas during this hectic year. Every time a new hurdle appeared, Francisco immediately started thinking about plans B, C and D to ensure there was data to be analysed and discussed at the end of the day.

Secondly, I would like to express my gratitude to my co-supervisor, Dr. Nuno Queiroz, and his colleagues over at ELECTRICBLUE for the development of the tail tags used on this thesis and the tagging of the two mares that ended up telling us a bit about the ecology of the *Garranos* in Sta. Luzia; also, I would like to thank him for his help on the treatment and analysis of the spatial data.

I would also like to send some words of gratitude to everyone else that has lent me a hand during this year: to my previous co-supervisor Dr. Raquel Godinho, for all the help she gave me at the very beginning of this thesis; to the Kyoto team for allowing me to spend a few weeks getting to know the *Garrano* horses from Serra D'Arga and their work, specifically Pandora and Maria Inês for being two great companions and tutors while in the field; to Dr. Ricardo Carvalhido and the CM Viana do Castelo for providing me with several datasets documenting the eco-climatic and landscape conditions from Viana do Castelo, as well as to the Joanas (Joana Casimiro and Joana Freitas) for providing me with data and bibliography from their thesis that aided me in composing mine; to Susana Lopes from ACERG and Sílvia Ribeiro for providing valuable suggestions about the questionnaires that were to be distributed to the horse owners; and to Marisa Vedor for being amazing at explaining statistical notions and helping me with presenting my data in the most meaningful way.

Also, thank you to everyone that has kept me company and helped me in a more informal way during this atypical degree. To Paulo and Sónia and the Sm@rt City Lab for keeping me curious and occupied when the horses were not 'cooperating' with me. To my family, for receiving me with open arms when COVID forced me to return to my childhood home and for making this year more enjoyable, despite everything. To my friends, Cindy, Joana, Nuno, Xintong and Hanlin, for being there for me when I needed and making everything more fun, whether it is on my small town of Águeda, on the cities of my *alma mater*s or on the fabulous shopping centres of the populous Beijing – now that we're all vaccinated, let's meet again soon, I miss you guys! And to Carla, for being my (this year, online) companion, for being my go-to thesaurus even when thesaurus.com could not help me, and for being there, always. Let’s see where the next year will take us, hai?

And finally, thank you to me and to my trusty sidekick, Mr. Personal Computer, for not giving out until the end of this dissertation. We did well.

Resumo

A ecologia é crucial para assegurar um maneio adequado de cavalos pastoreados em regime de liberdade e outras espécies de gado. No entanto, a ecologia dos cavalos foi pouco estudada até à data e a grande maioria dos estudos é realizada em populações selvagens ou ferais. Na Península Ibérica, várias raças de cavalos em regime livre pertencem a proprietários particulares, mas ocupam livremente pastagens montanhosas comunitárias com pouca ou nenhuma vigilância. O sistema tradicional de criação de cavalos em liberdade do noroeste da Península Ibérica, juntamente com o facto dos cavalos serem a principal presa do lobo nesta região, requerem estratégias de gestão localmente adaptadas, com base no conhecimento ecológico desta espécie pecuária. No entanto, existem estudos insuficientes sobre a ecologia espacial de cavalos para poder avaliar o modo como os cavalos Garrano usam a paisagem envolvente, assim como a sua interação com predadores, como o lobo, uma vez que a predação em cavalos é pouco comum globalmente sendo, portanto, pouco estudada.

O presente estudo visa descrever melhor os padrões e determinantes relacionados com a ecologia espacial dos cavalos criados em regime de liberdade no Noroeste de Portugal e a sua interação com o lobo ibérico, centrando-se em três objetivos principais: 1) avaliar os padrões regionais e temporais de consumo de cavalo pelos lobos e o efeito de 13 fatores ecológicos e humanos no consumo de cavalos, através da realização de uma revisão de estudos da dieta do lobo em 8 alcateias do noroeste de Portugal desde 1990; 2) avaliar os padrões básicos da ecologia espacial de cavalos em regime de liberdade (por ex., tamanho dos territórios e centros de atividade, seleção de habitat, variação temporal do uso do espaço), através do seguimento de 2 cavalos Garrano da Serra de Santa Luzia (NW Portugal) durante Abril-Junho 2020 usando GPS *tail-tags* desenvolvidas para este estudo; 3) avaliar a eficiência dos aparelhos de seguimento GPS inovadores usados nos cavalos Garrano, através da análise do seu desempenho em termos de durabilidade e sucesso a receber localizações sob a influência de diferentes fatores (por ex., periodicidade de receção de localizações, condições climáticas).

Os resultados sobre a interação predador-presa entre lobos e cavalos em regime de liberdade do noroeste de Portugal mostram que os cavalos foram a principal presa em 42% dos estudos de dieta dos lobos, enquanto outras presas domésticas foram a segunda classe de presas mais consumida. No entanto, nenhuma das variáveis consideradas relevantes para o consumo de equinos pôde ser confirmada como um fator determinante para o consumo de equinos pelo lobo no noroeste de Portugal. Das 13 variáveis consideradas, 5 não apresentaram nenhuma influência na magnitude do consumo de cavalos, e as restantes apresentaram uma possível relação com a intensidade do consumo de cavalos, mas sem efeito significativo (p> 0,05).

Os resultados sobre a ecologia espacial de cavalos Garrano mostraram que os cavalos em regime de liberdade da Serra de Sta. Luzia parecem ocupar pequenos territórios não-exclusivos compostos principalmente por matos localizados a grandes altitudes (440m) e encostas com pouco declive (16%). Em termos de seleção de habitat, as florestas de eucalipto parecem ser altamente evitadas, enquanto os matos são ligeiramente selecionados e as florestas de pinheiro são selecionadas positivamente à noite dentro dos centros de atividade. Não foram observadas grandes mudanças no uso do espaço ao longo de um ciclo circadiano e dos períodos semanais.

Os resultados sobre a eficiência das GPS *tail-tags* mostraram que os aparelhos de seguimento GPS parecem resistentes o suficiente para serem utilizados em estudos de ecologia espacial, mas necessitam ser otimizados antes de serem utilizados em estudos de grande escala. Ambos os aparelhos tiveram um desempenho pouco satisfatório, recebendo, em média, apenas 27% ou 41% de todas as localizações esperadas. A periodicidade de receção de localizações (2 horas ou 30 minutos) afetou significativamente a receção de localizações, mas a hora do dia e as condições climáticas não afetaram a receção de localizações dos aparelhos de seguimento GPS.

Em conclusão, os resultados deste estudo forneceram informações relevantes sobre dois tópicos importantes para a ecologia de cavalos criados em regime de liberdade, bem como sobre procedimentos metodológicos que permitem melhorar o nosso conhecimento sobre a ecologia de cavalos. Com base nos resultados obtidos, várias abordagens são sugeridas para melhorar nosso conhecimento sobre esses tópicos, como a recolha de variáveis adicionais para explorar a predação de cavalos em regime de liberdade por lobos, e o aumento da amostra (tanto no número de indivíduos marcados como período de acompanhamento) usada nos estudos de telemetria.

Palavras-chave: cavalo *Garrano*; predação pelo lobo; telemetria GPS; ecologia espacial; seleção de habitat; otimização metodológica.

Abstract

Ecology can be of crucial importance for an adequate management of free-ranging horses and other livestock species. However, horse ecology is still seldom studied and the vast majority of the studies are performed on wild or feral populations. In Iberian Peninsula, several breeds of free-ranging horses are privately owned but roam freely in communal mountainous pastures with little to no human management. The traditional free-ranging husbandry system of horses in northwest Iberia, along with the fact that in this region horses are a main wolf prey, requires locally adapted management strategies based on ecological knowledge of this particular livestock species. However, there are limited studies on horse spatial ecology to allow an evaluation of how *Garrano* horses use the landscape and on horse-predator interactions since predation on horses is uncommon globally and, thus, scarcely studied.

This study aims to better describe the patterns and determinants related to the spatial ecology of free-ranging horses in Northwest Portugal and their interaction with Iberian wolves, by focusing on three main goals: 1) assess regional and temporal patterns of horse consumption by wolves and evaluate the effect of 13 ecological and human-related factors on horse consumption, by conducting a review of wolf diet studies on 8 packs from northwest Portugal since 1990; 2) assess basic spatial ecology patterns of free-ranging horses (e.g. home range and core area sizes, habitat selection, temporal variation in space use), by following 2 *Garrano* horses from Serra de Santa Luzia (NW Portugal) during April-June 2020 using GPS tail-tags developed for this study; 3) evaluate the field efficiency of the innovative GPS tail-tags deployed in the *Garrano* horses, by analysing their performance in terms of durability and performance in successfully receiving locations under the influence of different factors (e.g. reception schedules, eco-climatic conditions).

Results on predator-prey interactions between wolves and free-ranging horses in northwest Portugal show that horses were the main prey in 42% of the wolf dietary studies, while other domestic prey were the second most consumed prey class. However, none of the variables considered relevant to horse consumption could be confirmed as a determinant of horse consumption by wolves in northwest Portugal. From the 13 variables considered, 5 showed no influence on magnitude of horse consumption and the remaining showed a possible relation with intensity of horse consumption but no significant effect ($p>0.05$).

Results on the spatial ecology of *Garrano* horses showed that free-ranging horses in Serra de Sta. Luzia seem to occupy small, non-exclusive home ranges composed mostly by shrublands located at high altitudes (440m) and flat slopes (16%). In terms of habitat selection, eucalyptus forests seem to be highly avoided while shrublands are slightly selected and pine forests are positively selected at night inside core areas. No major changes in space use across daily and weekly periods were observed.

Results on the field efficiency of the GPS tail-tags showed that the tags seem durable to conduct studies on spatial ecology but need further optimization before being deployed on large-scale experiments. Both tags performed unsatisfactorily, receiving only an average of 27% or 41% of all expected locations. Reception schedule (2hr or 30 min) significantly affected the reception of locations, but time of day and eco-climatic conditions had no effect on the reception of locations by the GPS tail-tags.

In conclusion, the findings of this study provided valuable information on two important ecological traits of free-ranging horses as well as on the methodological procedures to further improve our understanding of horse ecology. Based on the obtained results, several approaches are suggested to improve our understanding of these topics, such as the collection of additional variables to explore wolf predation on free-ranging horses and the increase of the sampling effort (both in the number of tagged individuals and tracking period) used on the telemetry studies.

Keywords: *Garrano* horse; wolf predation; GPS telemetry; spatial ecology; habitat selection; methodological optimization.

Index

Figure index

Table index

Abbreviations

CIBIO-InBio: Research Centre in Biodiversity and Genetic Resources - Research Network in Biodiversity and Evolutionary Biology

CMIA: Centro Metereológico de Interpretação Ambiental

DEM: Digital Elevation Model

DGT: Direção-Geral do Território

EEA: European Environment Agency

FFMS: Fundação Francisco Manuel dos Santos

F.O.: Frequency of Occurrence

GLM: General Linear Model

GPS: Global Positioning System

ICNF: Instituto de Conservação da Natureza e das Florestas

INE: Instituto Nacional de Estatística

LEAF: Research Centre ‘Linking Landscape, Environment, Agriculture and Food’

PNPG: Parque Nacional da Peneda-Gerês

SIC: Sites of Community Importance

VHF: Very High Frequency

1. Introduction

1.1. Importance of ecology on animal management

Ecology can be defined as the study of an organism and its interaction with other organisms and the surrounding environment (Begon *et al.*, 2014; Begon *et al.*, 2006; Rees, 2015). The processes by which organisms affect and are affected by their surroundings at different levels of organization can be generally categorized into four main ecological traits: 1) Trophic Ecology, the study of individual feeding relations and its impact on the flux of energy and matter within the ecosystem (Levin, 2013); 2) Spatial Ecology, the study of the distribution of an individual in a territory over time, according to their requirements in terms of resources, physic-chemical conditions and behaviour, and its impact on the ecosystem (Begon *et al.*, 2006; Fletcher & Fortin, 2018); 3) Intraspecific Interactions, the study of the behaviour and relationships between individuals of the same species (e.g. competition) (Levin, 2013); and 4) Interspecific Interactions, the study of the relationships between individuals of different species (e.g. competition, predation) (Levin, 2013).

These ecological traits are important to define appropriate strategies for animal management, since having a good knowledge on the ecology of a species can help managers to better understand its ecological role and requirements in order to prioritize management actions, formulate solutions based on theorical data, predict population trends and habitat preferences, among others (Begon *et al.*, 2006; Fletcher & Fortin, 2018; Gordon *et al.*, 2004; Newman, 2000). However, the use of ecological studies to support management decisions is different for wild and domestic species. For wildlife, the development of ecologically-based management actions is a common practice worldwide and often associated with conservation goals, for which ecology is considered as extremely relevant to make informed decisions that maximise the preservation of biodiversity and ecosystem processes over time (Gordon *et al.*, 2004; Newman, 2000). In comparison, ecological studies are far less frequent in domestic animal management (Hackmann & Spain, 2010; Koene & Ipema, 2014). In fact, ecological studies tend to be overlooked mainly because of the perceived notion that ecological relationships are less relevant for confined or captive animals (Boyce *et al.*, 2021; Rees, 2015), and therefore, studies on domestic animal management tend to focus more on topics such as physiology, nutrition and welfare (Fraser, 2010; Hackmann & Spain, 2010). Whenever studies on domestic animals are indeed focused on ecology, they target most frequently either free-ranging or feral populations of domesticated animals, such as feral dogs

(Hughes & Macdonald, 2013; Lacerda *et al.*, 2009), stray cats (Denny & Dickman, 2010; Ferreira *et al.*, 2011), and feral livestock species (Girard *et al.*, 2013a; Graves, 1984; Linklater *et al.*, 2000; Meriggi & Pagnin, 1994). This pattern leads to a knowledge gap on the ecology of many domestic species, particularly livestock raised under a free-ranging husbandry system, such as horses. Notwithstanding, ecology can be of crucial importance for an adequate management of free-ranging horses and other livestock species, for example to optimize resilience and resource use in farming systems, develop sustainable husbandry strategies, or mitigate conflicts with wildlife (Begon *et al.*, 2014; Destoumieux-Garzón *et al.*, 2020).

1.2. Horse ecology

Horses (*Equus caballus* Linnaeus, 1758) are equids that have been widely domesticated and currently can be found across the globe, with only few populations considered to belong to wild species, such as the Przewalski's horse (*Equus ferus przewalskii*) originally native to the steppes of Central Asia (Bouman, 1986; Ransom & Kaczensky, 2016). Although domestic horses are commonly found under an intensive husbandry system in which they are strictly confined, there are few populations of domestic horses, particularly in Northern Iberia and Southern United Kingdom, raised by their owners under an extensive and free-ranging system (Bárcena, 2012; Hovens & Rijkers, 2013). Besides, in several regions, such as North America, Australia and Eastern Europe, domestic horses do not have owners and have formed feral populations, living under wild conditions (Ransom & Kaczensky, 2016).

Independently of the ecological trait in question (trophic, spatial, intra or interspecific interactions), horse ecology is still seldom studied (Ransom & Kaczensky, 2016) and the vast majority of the studies are performed on wild or feral populations. In fact, a review of 33 available studies focusing the spatial ecology of horses worldwide (Fig. 1; Appendix I, Table S1), revealed that only 4 of those studies (12%) were performed on free-ranging domestic horses; additionally, most of the data is not recent (only 9 studies – 27% – had data collected on the last decade) and target horse populations with no natural predators (n=21; 64%), which provides managers with an outdated and incomplete depiction of horse spatial ecology (Fig. 1; Appendix I, Table S1).

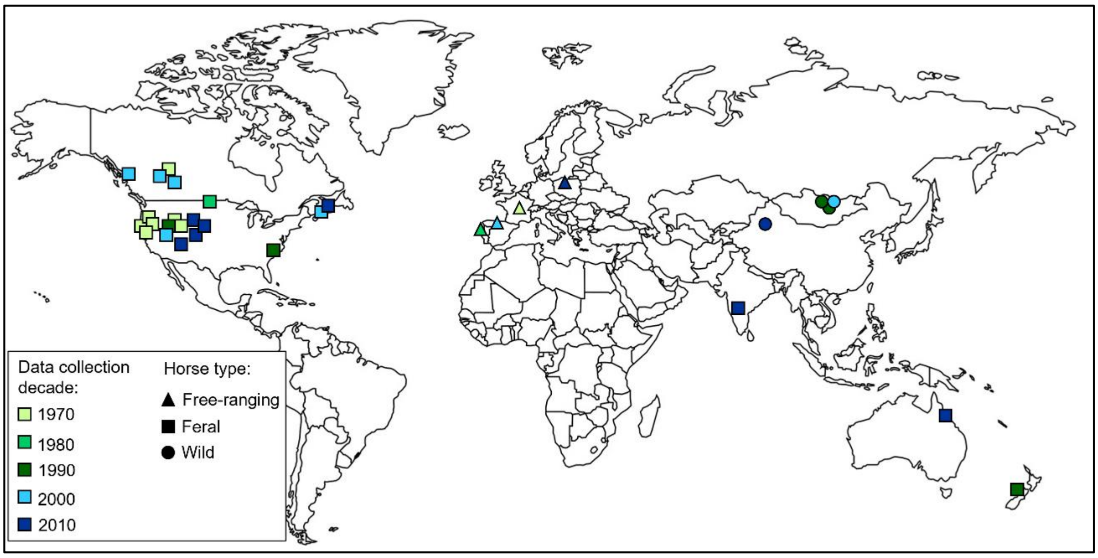

Figure 1. Location of available studies addressing the spatial ecology of horses worldwide, categorized according to decade of data collection (data collected < 2000 identified by a green gradient while ≥ 2000 identified by a blue gradient) and horse type. Bibliographic references and additional information for each study in Appendix I, Table S1.

Regarding horse spatial ecology, space use is mainly determined by forage availability and varies moderately depending on the season and region being studied (Duncan, 1983; Keiper, 1986; Ransom & Kaczensky, 2016). Generally, horses tend to occupy open landscapes (e.g. grasslands, steppes, meadows) at high elevations and low slopes (Ganskopp & Vavra, 1986; Keiper, 1986; Wockner *et al.*, 2004). Forested areas are generally avoided, especially in populations with natural predators, due to increased predation risk (Namgail *et al.*, 2007; Van Duyne *et al.*, 2009). However, during some specific seasons horses can select steeper slopes, lower altitudes (Linklater *et al.*, 2000), or more forested habitats (Girard *et al.*, 2013a; Wockner *et al.*, 2004). Water availability is another determinant for habitat selection, with horses preferring sites closer to rivers or water sources (Ransom & Kaczensky, 2016; Stevens, 1988). Home ranges, defined as the area occupied by an individual for their daily activities, such as feeding and refuge (Keiper, 1986; Linklater *et al.*, 2000), are demarcated by the social group in which the individual belongs and tend to be constant over time (Berger, 1977; Girard *et al.*, 2013a), frequently overlapping between different herds (Ganskopp & Vavra, 1986; Linklater *et al.*, 2000; Miller, 1983b). Similarly to space use, the size of home ranges are primarily determined by the availability and quality of resources (King & Gurnell, 2005; Ransom & Kaczensky, 2016), but can also be influenced by band size (Berger, 1977; Linklater *et al.*, 2000) or exposure to disturbance (Girard *et al.*, 2013a). In areas poor in resources, horses travel longer distances in search of forage or water, occupying larger home ranges (Ganskopp & Vavra, 1986; King, 2002). Larger bands require higher forage biomass thus requiring larger home ranges (Linklater *et al.*, 2000; McCort, 1984).

Likewise, horses can use larger areas to avoid human interaction (Girard *et al.*, 2013a; Laliberte & Ripple, 2004). In general, home range sizes vary widely between populations, ranging from 0.9 Km^2 to over 300 Km^2 according to the available literature (Keiper, 1986; McCort, 1984; Ransom & Kaczensky, 2016).

Regarding intraspecific interactions, namely horse social ecology, studies show that horses live in stable social groups composed by one or two stallions and several mares (harems), which tend to occupy non-exclusive home ranges and be influenced by resource availability, predation, or the population's sex-ratio. Besides, temporary groups formed by several lone male horses (bachelor groups) can also be observed (Feh *et al.*, 1994; Ganskopp & Vavra, 1986; Krueger, 2008; Linklater *et al.*, 2000). Intraspecific competition has been mostly reported between males, as a way of defending their respective harem (intergroup competition) or asserting dominance over other stallions of the harem (intragroup competition) (Keiper, 1986). In terms of cooperation, sometimes several harems can share similar movement patterns and home ranges, forming a higher social unit (herd) that can help minimize predation risk and bachelor harassment, or shelter against harsh climatic conditions (Krueger, 2008; Miller, 1983b).

Regarding trophic ecology, horses spend most of their time foraging (Oom & Reis, 1986; Salter & Hudson, 1979) and prefer to feed in areas with multiple forage species available (Saastamoinen *et al.*, 2012). Graminoid species such as sedges (*Carex sp.*), fescues *(Festuca* sp.) or needlegrasses (*Stipa* sp.) constitute the primary feeding resource of most horse populations (Crane *et al.*, 1997; King & Gurnell, 2005; McInnis, 1984; Saastamoinen *et al.*, 2012; Salter & Hudson, 1979). Shrub and forb species such as gorse (*Ulex* sp.), heather (*Erica* sp.) or willows (*Salix* sp.) are consumed less regularly, but they can become important in the diet when high quality grass is less available, for example during colder seasons (Ransom & Kaczensky, 2016) or in habitats with higher shrub content (e.g. heathlands; Fagúndez, 2016). Feeding habits tend to change seasonally, according to the seasonal availability of local plant species (Crane *et al.*, 1997; Ransom & Kaczensky, 2016; Salter & Hudson, 1979).

Regarding interspecific interactions, horses can play an important role in shaping the landscape where they occur, according to its interaction with other species. For plant species, horses act as seed dispersers, increasing plant diversity in some regions (Linnartz & Meissner, 2014; Lopéz-Bao *et al.*, 2013). However, high grazing pressure from horses, namely in regions with no natural predators, can damage crops and wild vegetation as well as limit forest regeneration (Ransom & Kaczensky, 2016). Furthermore, feral horses are reported to compete with domestic and wild herbivores for water and feeding resources (Gooch *et al.*, 2017; Hennig *et al.*, 2018; Perry *et al.*, 2015), which can threaten some wildlife species and lead to conflict with humans by decreasing cattle productivity (Nimmo & Miller, 2007; Ransom & Kaczensky, 2016). Finally, in some regions of their distribution range, horses are reported to suffer predation from several carnivore species, such as grey wolves, mountains lions, leopards and brown bears (Freitas *et al.*, *in press; see Fig. 2*), reflecting one of the main but less studied interspecific interactions involving horses. The role of predation on horses involves important ecosystem services by providing a stable food source for carnivores, as documented for Iberian wolves (Lopéz-Bao *et al.*, 2013), but when it comprises domestic horses it may lead to important economic losses to horse owners, increasing human-predator conflict (Álvares, 2020; Lagos *et al.*, 2019). In fact, horses are often a valuable livestock species for humans due to their cultural, economic and social importance (Nimmo *et al.*, 2007; Ransom & Kaczensky, 2016), which makes predation the ecological interaction with the biggest impact on horse management and with the higher need for knowledge on its determinants. Most studies reporting horse predation are found in Europe and Asia (Fig. 2), which does not coincide with the region of most studies on horse spatial ecology (North America) (Fig. 1). This pattern results in a lack of knowledge regarding the impact of predation on horse ecology and the ecological determinants influencing predation on horses specifically, since most available studies are focused on damages to livestock in general (e.g. Cozza *et al.*, 1996; Davie *et al.*, 2014; Pimenta *et al.*, 2018). In terms of predation determinants, current knowledge suggests that livestock depredations can be influenced by availability of alternative domestic and wild prey (Figueiredo *et al.*, 2020; Gazzola *et al.*, 2005; Gula, 2008; Imbert *et al.*, 2016), habitat selection (Davie *et al.*, 2014; Van Duyne *et al.*, 2009), wolf abundance (Freitas, 2019; Pimenta *et al.*, 2018), husbandry practices (Meriggi & Lovari, 1996; Namgail *et al.*, 2007; Pimenta *et al.*, 2017), herd size (Cozza *et al.*, 1996) and seasonality (Cozza *et al.*, 1996; Freitas, 2019; Pimenta *et al.*, 2017). In terms of effects of predation on horse ecology, the few available studies report the establishment of male-female bonds (Feh *et al.*, 1994), increase in herd size (Feh *et al.*, 1994; Kaczensky & Walzer, 2003) and changes in habitat selection (Van Duyne *et al.*, 2009), as a response to predation risk by wolves. Overall, these effects of predation are similar to what has been observed in other domestic (Breck *et al.*, 2012; Howery & DeLiberto, 2004; Kluever *et al.*, 2009; Meriggi & Pagnin, 1994) and wild ungulates (Childress & Lung, 2003; Creel *et al.*, 2005; Creel & Winnie Jr, 2005; Hebblewhite & Pletscher, 2002).

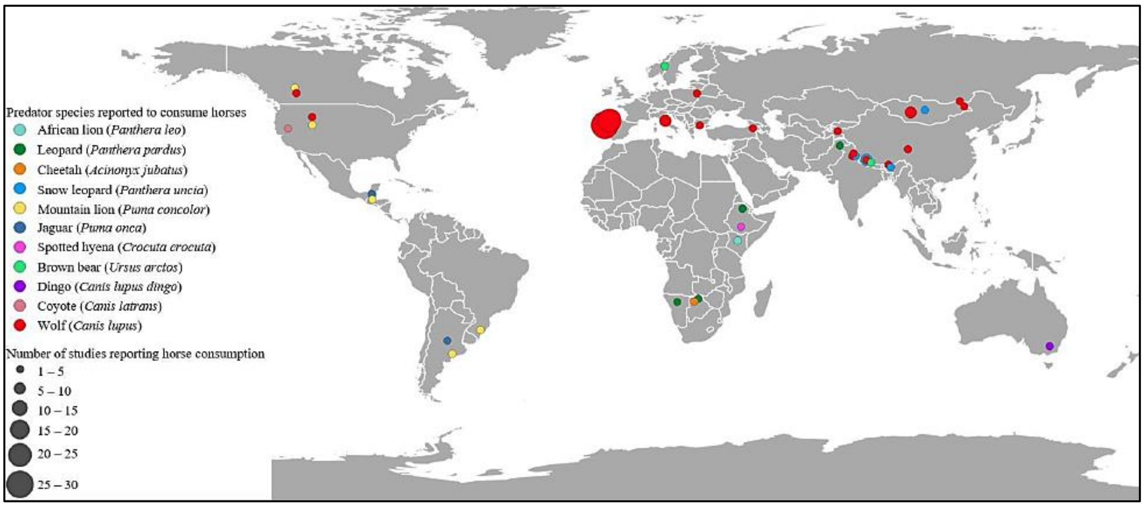

Figure 2. Location of studies reporting horse predation worldwide, categorized according to predator species and number of studies for a given geographic area (identified by circles encompassing 1 to 30 studies per location) (adapted from Freitas *et al.*, *in press*).

1.3. Methodological approaches used on horse ecological studies

In order to understand the different ecological traits of horses, available studies have relied mostly on two types of methodological approaches to collect data: direct observations and telemetry.

Direct observation has been frequently used by researchers to describe the spatial ecology, feeding habits, behaviour, social structure and intraspecific interactions of free-roaming horses (e.g. Linklater *et al.*, 2000; Mendonça *et al.*, 2020; Oom & Reis, 1986; Ringhofer *et al.*, 2017). However, it has a few setbacks, such as the fact that: 1) researchers are only able to observe diurnal activity; 2) no data can be collected if the observer is not on the field or horses are not located; and 3) it can yield vague and unprecise data because it relies on the observer's measurements (Cagnacci *et al.*, 2010; Millspaugh & Marzluff, 2001; Oom & Reis, 1986). In contrast, telemetry and tracking devices, which began to be used for animal studies in the 1960s (Kaczensky *et al.*, 2010), are a tool that is able to overcome these problems by providing unbiased, accurate location coordinates of animals at a given time without the need to see them, and have been successfully used to track animals and make inferences about distribution, home ranges, movement patterns, habitat selection, among others (Hebblewhite & Haydon, 2010; Millspaugh & Marzluff, 2001). The first trackers in use for animal studies were VHF (Very High Frequency) transmitters, which emitted signals that were collected by receptor devices (either in a fixed location or carried by researchers) located within the range of the tagged animal (Kaczensky *et al.*, 2010; Lagos & Bárcena, 2010). In recent decades, GPS (Global Positioning System) transmitters became the most popular type of transmitter, because of its ability to store more robust and precise locations at pre-defined time intervals, without the need of an external receptor or personnel in the field (Cagnacci *et al.*, 2010; Collins *et al.*, 2014; Hennig *et al.*, 2018). Despite this, the use of telemetry is extremely rare for studying horses, unlike other animals (Collins *et al.*, 2014; Hennig *et al.*, 2018; Schoenecker *et al.*, 2020). In fact, very few studies have used telemetry to characterize the ecology of free-roaming horses, and most of the information available still relies on observational data. For example, considering the publications on horse spatial ecology available worldwide (n=33), telemetry methods were only applied on 8 (24%) of these studies (Fig. 3; Appendix I, Table S1). Furthermore, telemetry has been used in horses mostly in feral populations from North America without the presence of natural predators and involving VHF or GPS transmitters located in collars (Appendix I, Table S2). Although collars (Fig. 4A) are the most common tag type for horses and wildlife, in general, they have been reported to be inappropriate and harmful if wrongly placed on the neck (causing chaffing, wounds, or even death), or if used in certain individuals (e.g. juveniles, whose neck diameter can increase during the tracking period) (Collins *et al.*, 2014; Hennig *et al.*, 2018). Other tag types that can be used in horses and do not pose this problem include: 1) ear tags (Fig. 4B) but which, contrastingly, can tear apart from the ear quite easily and, thus, are less successful at colleting locations (Kluever *et al.*, 2012; Lagos & Bárcena, 2010); or 2) tail tags (Fig. 4C), which are not exposed to impact caused by grooming or fighting among individuals (Collins *et al.*, 2014; Keiper, 1986), but are yet to be used in ecological studies with free-roaming horses since they have only been tested in a captive setting (Schoenecker *et al.*, 2020).

Figure 3. Location of available studies addressing the spatial ecology of horses worldwide, categorized according to the methodological approach used and presence of natural predators in the study site. Bibliographic references and additional information for each study in Appendix I, Table S1.

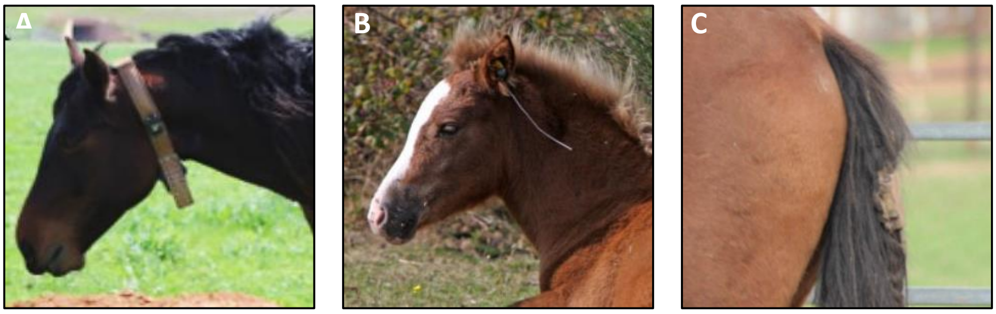

Figure 4. Different transmitter locations for telemetry tags used in horses: a) collars; b) ear tags; c) tail tags (adapted from Kluever *et al.*, 2012; Schoenecker *et al.*, 2020).

Studies focused on horse trophic ecology and interspecific interactions with predators rely strongly on dietary data from scat analysis of horses and carnivores, respectively (Martinson *et al.*, 2017). This means that most of the available information regarding predation on horses is obtained from carnivore diet studies. In fact, a recent review of studies reporting horse consumption by carnivores (Freitas *et al.*, *in press; see Fig. 2*), show that only few studies focus primarily on this prey species (Fico *et al.*, 1993; Namgail *et al.*, 2007; Turner Jr & Morrison, 2001). Furthermore, many of the feral horse populations that have been subject of demographic and ecological studies do not have natural predators, which limits the current knowledge on this important topic. Finally, when the ranges of predators' and free-roaming horses do overlap, horses tend to comprise only an occasional prey in most areas, in which their consumption by carnivores is either minimal or non-existent (Freitas *et al.*, *in press;* Newsome *et al.*, 2016). All these factors contribute to a limited knowledge regarding the effects and determinants related to predation on free-roaming horses, albeit losses caused by wolves constitutes one of the main mortality causes for horses in some worldwide regions, such as northwest Portugal (Freitas & Álvares, *in press;* Gomes, 1996).

Lastly, it is important to highlight the crucial influence of human-related factors in ecological studies of horses. For instance, the importance of human attitudes and perceptions on horse management has been widely addressed in North America and Australia, where populations of feral horses constitute an alien species detrimental to native biodiversity but any management efforts for horse population control are met with pushback from animal welfare groups and the general public due to their cultural value (see Chapple, 2005; Nimmo & Miller, 2007; Nimmo *et al.*, 2007). Also, for free-roaming populations of mountain ponies in North Iberian Peninsula or South United Kingdom, owners are frequently subjected to legislations (e.g. related to grazing areas or damage compensation) that are unfit for the traditional husbandry system in place due to horses being legally considered domestic despite living in a semi-wild state (Fagúndez *et al.*, 2017; Milheiras & Hodge, 2011; Ransom & Kaczensky, 2016). However, the importance of humans on horse ecology is rarely addressed, especially in ecological studies focusing free-roaming domestic horses that are still under some degree of management by their owners, and in which human decisions and attendance can have a strong impact in horse ecology. For example, in Iberian Peninsula the frequent removal of male foals by horse owners has been documented to influence the herd social structure, by forcing the formation of bigger harems or an increase in the number of lone individuals (Álvares, 2011; Freitas, 2019; Keiper, 1986). Furthermore, it has been suggested that the traditional unconfined husbandry of free-roaming horses together with the level of management by horse owners seem to be the main cause explaining the high levels of horse predation by wolves in northwest Portugal (Freitas & Álvares, *in press;* Pimenta *et al.*, 2018). Therefore, future studies on horse ecology should take into account the potential effect of human-related factors, particularly in areas where domestic horses are raised under a free-roaming husbandry system, such as in northern Iberian Peninsula.

1.4. Free-ranging horses in Iberian Peninsula

In Iberian Peninsula, several breeds of short, stocky ponies (< 300kg) are raised under a semi-wild husbandry system in the mountains of northwest Portugal (namely in Trás-os-Montes and Alto Minho) and northern Spain (namely in Galicia, Asturias and the Pyrenees) (Bárcena, 2020; Giesteira, 1999). These mountain ponies are privately owned but roam freely in communal mountainous pastures with little to no human management: owners go to the mountains occasionally to locate their herds (Faria, 2012) and, generally, collect the animals only once a year (normally in October), to perform sanitary controls, identify new-born animals, and remove selected individuals (e.g. foals) for selling (Bárcena, 2012; Lagos *et al.*, 2019; Leite, 2012; Serôdio, 1992). Historically, these Iberian breeds were useful for agriculture, transportation or for meat production (Bárcena, 2020; Lopéz-Bao *et al.*, 2013), although nowadays, they are mostly raised for their cultural value, and used for sports, tourism and, more rarely, meat production (Faria, 2012; Lagos *et al.*, 2019; Perez, 2012). The free-ranging mountain ponies occurring in northwest Portugal belong to an autochthonous breed – the *Garrano* horse. Its origin dates back to the Pleistocene (Leite, 2012; Portas, 2020; Vieira e Brito & Silva, 2020), but in the last decades population numbers have been dwindling, mainly due to the loss of utility of *Garrano* horses – caused by the mechanization of agriculture and rural exodus –, crossings with other non-native horse breeds, and high pressure from wolf predation (Álvares, 2020; Giesteira, 1999). Similarly to other domestic species, most of the available knowledge about this horse breed is related to its morphology (D'Andrade, 1938; Lima, 1872), husbandry practices (Serôdio, 1992) and demography (Gomes, 1996; Oom & Reis, 1986). Ecology has been far less studied and behavioural ecology studies focusing intraspecific interactions (Oom & Reis, 1986; Ringhofer *et al.*, 2020; Ringhofer *et al.*, 2017) represent most of the publications to date.

Garrano horses can have a significant positive impact on mountain landscapes and habitats, since they can eat a wide variety of vegetation, including thorny and harsh shrubs (e.g. *Ulex* sp.), which, sometimes, other livestock species cannot easily digest (Saastamoinen *et al.*, 2012; Serôdio, 1992). As a consequence, *Garranos* play a key role in maintaining heathland habitats, increasing biodiversity and habitat heterogeneity, and decreasing risk of wildfires due to an efficient control of shrub biomass (Bárcena, 2020; DeSilvey & Bartolini, 2019; Fagúndez, 2016; Lopéz-Bao *et al.*, 2013; Saastamoinen *et al.*, 2012). However, these horses can sometimes move near human settlements due to their unguarded husbandry, which frequently lead to damages on crops or horse-vehicle collisions (Lagos & Bárcena, 2020; Lagos *et al.*, 2019).

Despite the documented positive and negative effects that the feeding habits and movements of *Garrano* horses can have on ecosystems and human activities, very little is known about their trophic and spatial ecology. Regarding trophic ecology, only few diet studies have been done focusing the free-ranging horses in northwest Spain (Aldezabal *et al.*, 2013; Bas-López, 2017). Regarding spatial ecology, although some studies infer preferred habitats from the most common vegetation consumed by *Garranos* (Faria, 2012; Portas, 2020), and this breed is reported to live and being physiologically adapted to mountainous landscapes in general (Gomes, 1996; Leite, 2012), information beyond that is very scarce and obtained in older studies based on direct observations (D'Andrade, 1938; Oom & Reis, 1986). For example, information on the seasonal movements of *Garranos* in Portugal are derived from a single ecological study performed in only one herd from Peneda-Gerês National Park (PNPG) (Oom & Reis, 1986), or general descriptions of the horse breed (D'Andrade, 1938; Leite, 2012). In terms of quantitative data, horses are known to travel up to 40Km per day in search of water (D'Andrade, 1938), but there is no knowledge on the sizes of home ranges for reference (Oom & Reis, 1986). This lack of knowledge prevents owners and managers of knowing how horses use their surrounding landscape. Therefore, more detailed information about the space use of different populations of *Garrano* horses, under different management practices, can be useful to understand the ecology of this horse breed and minimize human-related risks associated with *Garrano* horses near settlements or roads.

Regarding intraspecific interactions, free-roaming horses in the Iberian Peninsula show similar patterns to their feral counterparts in other worldwide regions (Lagos & Bárcena, 2020; Oom & Reis, 1986). In Portugal, a long-term study on *Garrano* horses' behaviour and social structure has been started at Serra D'Arga (Viana do Castelo) in 2016 and based on direct observations (Inoue *et al.*, 2020; Inoue *et al.*, 2019; Mendonça *et al.*, 2020; Ringhofer *et al.*, 2020; Ringhofer *et al.*, 2017). Before that, the only other Portuguese studies focusing *Garranos*' behaviour and demography were conducted during the last decades of the XX century, in the PNPG region (Gomes, 1996; Oom & Reis, 1986). Concerning interspecific interactions, the relationship between Iberian wolves and free-ranging horses has been explored in several studies (see Álvares, 2020; Lagos & Bárcena, 2020), showing that horses are widely predated and positively selected by wolves (Freitas, 2019; Lagos & Bárcena, 2018, 2020) and reflecting an ancient prey-predator relationship (Bárcena, 2012, 2020). The presence of unattended ponies in the mountains provides wolves with a steady feeding supply, minimizing wolf predation risk on other livestock with higher economic value, such as cattle (Álvares, 2020; Lagos, 2013; Lopéz-Bao *et al.*, 2013). However, wolf predation, particularly on foals, results on a limited productivity in most *Garrano* horse populations, which strongly contributes for the endangered status of this horse breed and leads to conflicts between wolves and horse owners (Álvares, 2020; Lagos *et al.*, 2019).

In order to minimize the economic impact of wolf damages, the Portuguese government compensates owners for livestock losses due to wolf predation (Law no. 90/88, Iberian Wolf Protection Law), but the strict eligibility requirements are not compatible with the traditional husbandry system of free-ranging horses (Freitas & Álvares, *in press*). For example, the presence of adequate sanitary conditions and damage prevention measures (e.g. shepherds, guarding dogs, fences) are required to obtain full compensation for wolf damages; additionally, carcasses need to be timely verified by a technician to attribute the cause of death to wolf predation although horse owners might take note of a wolf attack too late, or even not at all, due to poor herd vigilance and the complete consumption of young foals by wolves; finally, the compensation procedure is often slow, bureaucratic and insufficient to cover all losses (Álvares, 2020; Álvares *et al.*, 2019; Leite, 2012; Milheiras & Hodge, 2011; Pimenta *et al.*, 2017). Wolf predation on free-ranging horses in Portugal involves an average yearly value of approximately 300 horses/year declared as killed by wolves, corresponding to a paid compensation ranging from 30.000 and 80.000€/year (Fig. 5). However, as a result of inadequate procedures and requirements for damage compensation of free ranging horses, compensation values for horses have been gradually decreasing in the past decade, despite the fact that the number of declared horses killed by wolves remains fairly constant (Fig. 5). This pattern is more evident since 2017, when a sharp decline in the annual compensation value for horses killed by wolves occurred due to a legislation change that negatively affects owners of free-ranging horses, since it only assures a partial compensation for livestock raised without the required damage prevention measures and no compensation for animals younger than one year old (Álvares *et al.*, 2021).

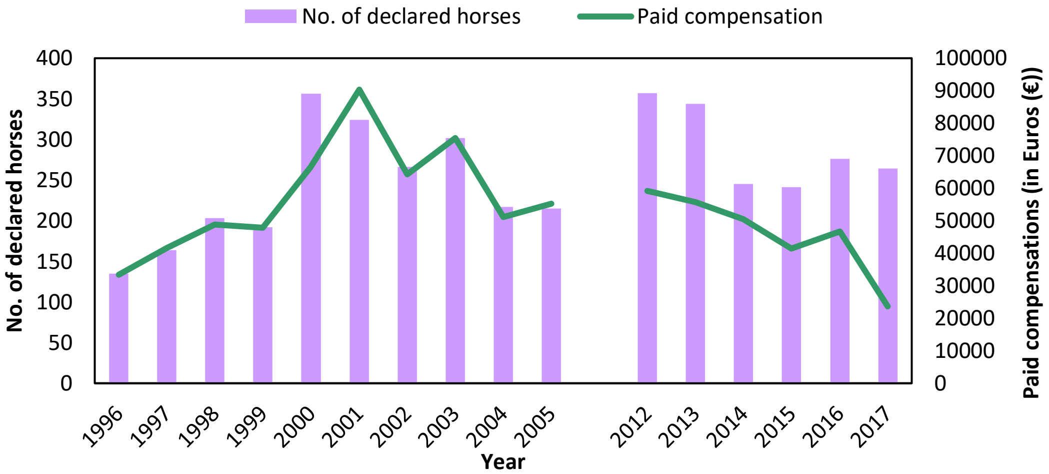

Figure 5. Yearly number of declared horses attacked by wolves (purple bars) and compensation values attributed to horse owners (green line) in Portugal between 1996-2005 and 2012-2017, based on data from Instituto de Conservação da Natureza e das Florestas (ICNF).

From the few available studies, horse-wolf interactions in Iberia appear similar to what is observed in other regions worldwide (e.g. Central Asia), where horses raised extensively and occupying areas with low densities of wild prey are highly predated (Freitas, 2019; Hovens & Tungalaktuja, 2005; Lagos & Bárcena, 2020). In fact, wolf predation on *Garrano* horses seem to be mainly influenced by the traditional free-ranging husbandry system, which leaves horses highly vulnerable to predation when compared to other livestock species (Álvares, 2020; Freitas, 2019; Pimenta *et al.*, 2018), as well as by the availability and diversity of alternative prey, given the fact that wild prey species are usually scarce in northwest Iberia (Llaneza & López-Bao, 2015; Milheiras & Hodge, 2011). Nevertheless, despite extensive knowledge regarding the relevance of horses in wolf diet and wolf damages, specific information focusing the regional and local factors determining the predation of *Garrano* horses by wolves is still limited (Álvares, 2020; Pimenta *et al.*, 2018).

1.5. Goals and hypotheses

The traditional free-ranging husbandry system of horses in northwest Iberia (Lagos *et al.*, 2019; Serôdio, 1992), along with the fact that in this region horses are a main wolf prey

(Lagos & Bárcena, 2018), requires locally adapted management strategies based on ecological knowledge of this particular livestock species. Among horse ecological traits, spatial ecology and interspecific interactions with predators might be the two most important traits for the appropriate management of *Garrano* horses. However, there are limited studies on horse spatial ecology to allow an evaluation of how *Garrano* horses use the landscape and wolf predation on horses is uncommon globally and, thus, scarcely studied. Therefore, this study aims to better describe the patterns and determinants related to the spatial ecology of *Garrano* horses and their interaction with Iberian wolves, in order to provide owners, managers, and other stakeholders with updated and detailed knowledge to allow informed decisions regarding the local management of this autochthonous breed of mountain ponies. This approach was done by focusing on three studies with different goals.

In **Study I**, a review of wolf diet studies in several packs from northwest Portugal since 1990 was conducted, in order to assess horse consumption by wolves in relation to several ecological and human-related factors, such as availability of alternative prey, horse abundance, wolf density, habitat features and reported wolf damages to horses. In particular, this chapter has the following objectives: 1) assess regional and temporal patterns of consumption of free-ranging horses by wolves in northwest Portugal; and 2) determine which ecological or human-related factors can influence the intensity of horse consumption by Iberian wolves. Based on these objectives, it is hypothesised that there is a higher relevance of free-ranging horses on wolf diet in areas with higher abundance of horses, lower availability of alternative prey and larger number of wolves (Álvares, 2011, 2020; Oom & Reis, 1986); additionally, it is hypothesized that human-related factors, such as the level of herd management and vigilance, is also a main determinant for the magnitude of horse consumption by wolves (Pimenta *et al.*, 2018).

In **Study II**, GPS telemetry in *Garrano* horses from Serra de Santa Luzia (NW Portugal) was performed by using innovative and newly developed GPS tail tags, in order to assess basic spatial ecology of free-ranging horses. In particular, this chapter has the following objectives: 1) determine the size of home ranges and core areas as well as habitat selection by using GPS telemetry on free-ranging horses; and 2) evaluate temporal variation in space use patterns. Despite the limited knowledge on this topic, it is hypothesised that *Garrano* horses will follow the general patterns documented in other horse populations, such as the use of non-exclusive home ranges and the selection of open landscapes at high altitudes with low slopes (Ganskopp & Vavra, 1986; Keiper, 1986; Ransom & Kaczensky, 2016).

In **Study III**, the field efficiency of the innovative GPS tail-tags deployed in *Garrano* horses from Serra de Santa Luzia (in the scope of Study II) was evaluated, in order to assess the influence of different factors (namely reception schedules and eco-climatic conditions) on the performance of these newly developed tags and use the technical information derived from these tests to optimise new future devices. In particular, this chapter has the following objectives: 1) determine the durability of these new GPS tail-tags focusing on lifespan (i.e. time tag stays attached to the animal) and integrity (i.e. time tag continues emitting an useful signal); 2) examine the performance of the GPS tail-tags considering two different reception schedules (e.g. locations each 30min or each 2h); and 3) determine the effect of circadian periods (e.g. day and night) and eco-climatic factors (e.g. precipitation, humidity and land cover) in the field efficiency of these GPS tail-tags. Based on these objectives, it is hypothesized that tags will remain attached to the horse without any compromise in integrity for a minimum of 221 days (Schoenecker *et al.*, 2020) and have collection rates similar to the average rate for terrestrial mammals (75%) (Hofman *et al.*, 2019). Additionally, shorter reception schedules (e.g. 30 min) are hypothesised to perform better than longer schedules (e.g. 2hr) (Cain *et al.*, 2005). Time of day and eco-climatic conditions are hypothesised to have no effect on the performance of these GPS tail-tags, since their effect is usually associated with behavioural changes (Parraga Aguado *et al.*, 2017) and has not been reported to affect tag performance in studies focusing horses (Collins *et al.*, 2014; Kaczensky *et al.*, 2010; Kluever *et al.*, 2012; Schoenecker *et al.*, 2020).

Hopefully, the findings of this study will provide innovative information on two important ecological traits of free-ranging horses (space use and factors influencing predation by wolves), as well as on the methodological procedures to further improve our understanding of horse ecology [1], which overall are key topics with important management implications.

[1] Besides the above goals described in this chapter, the original working plan had additional objectives that could not be completed in time for the submission of this Master thesis due to constraints caused by COVID-19 pandemic and by conflicts with horse owners. These additional goals included: 1) to GPS tag several horses in Serra de Santa Luzia and other neighbouring regions with different ecological conditions (e.g. Serra de Arga and Paredes de Coura) for a longer period of time, in order to increase the telemetry dataset to better describe horse spatial ecology across different landscapes and seasons, and better evaluate the performance of the GPS tail tags; and 2) to conduct a semi-structured questionnaire to horse owners (see **Appendix II**), in order to gather detailed data in several regions of NW Portugal regarding management practices, benefits and costs of free-ranging horse husbandry, with important ecological implications and which then was supposed to be used as additional human-related factors in the analysis of horse consumption by wolves conducted in current Study I.

2. Study areas: Northwest Portugal and Serra de Sta. Luzia

This work was conducted in two different study areas, comprising all northwest Portugal for the analysis of factors determining horse consumption by wolves (**Study I**) and only Serra de Sta. Luzia for the analysis of *Garrano* horses' spatial ecology and methodological assessment of GPS tail-tags (**Study II** and **Study III**, respectively) (Fig. 6).

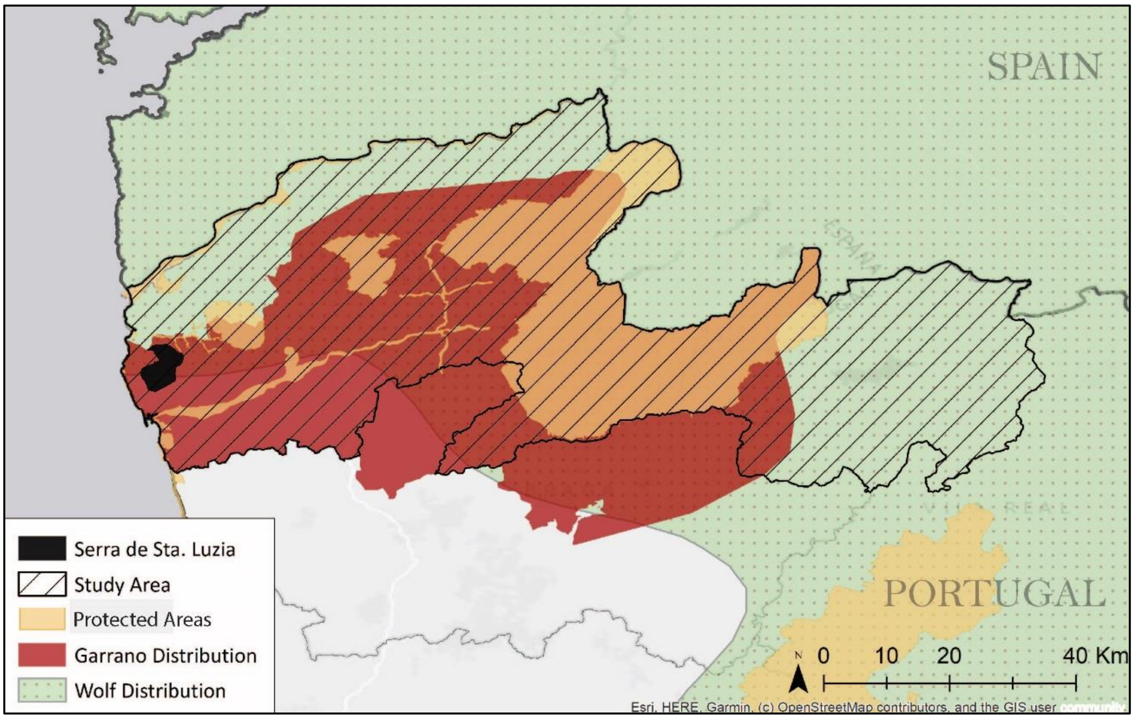

Figure 6. Location of the study areas in northwest Portugal (for Study I, represented by a dashed pattern) and in Serra de Sta. Luzia (for Studies II and III, represented in black), including protected areas (in orange) and the distribution area of both *Garrano* horses (in red) and Iberian wolves (in green).

The study area in northwest Portugal (**Study I**), with an area of approximately 3700 Km^2, comprise the districts of Viana do Castelo (all municipalities), Braga (only the municipalities of Amares and Terras de Bouro) and Vila Real (only the municipalities of Boticas and Montalegre) (Fig. 6). This area is where most wolf attacks on livestock are documented at national level (Campos, 2018; Freitas, 2019) and encompasses the main distribution range of *Garranos* horses under a free-ranging husbandry system particularly vulnerable to wolf predation (Portas, 2020), making it the perfect location to explore the predator-prey relationship between wolves and horses. This study area has an average human population density of 90.4 inhabitants/Km^2, where the highest population densities can be found near the coastal zone and along river valleys (FFMS, 2021). This region is one of the areas in Portugal in which agriculture and livestock husbandry are still one of the main human activities (Campos, 2018; FFMS, 2021). Northwest Portugal is mostly composed by agricultural areas, heathlands and shrublands, with small patches of broad-leaved, conifer and mixed forests dominated by native oaks (*Quercus robur*; *Quercus pyrenaica*) as well as plantations of pines (*Pinus pinaster*; *Pinus sylvestris*) and eucalyptus (*Eucalyptus globulus*). Furthermore, it includes three main river basins – Minho, Lima and Cávado –, which shape the characteristic rugged orography comprised by several mountainous areas, namely Serra de Arga (825 m), Corno do Bico (883 m), Serra do Soajo-Peneda (1416 m), and Serra do Gerês (1508 m) (Álvares, 2011; EEA, 2018). In biogeographic terms, northwest Portugal is located in a transition zone between the Eurosiberian and Mediterranean regions, which along with its proximity to the Atlantic Ocean, results in a mediterranean climate with an oceanic influence towards the western limit of the study area – characterized by mild seasons and high precipitation levels – and a mediterranean climate with a continental influence towards the eastern limit – characterized by colder winters with high precipitation and warm summers with low precipitation (Costa *et al.*, 1998; Costa, 2001). These environmental conditions allow a high diversity with several plant and animal species typical from both biogeographic regions (Álvares, 2011). Given the considerable biological importance of this region, there are over 1000 Km^2 of protected areas, including the Peneda-Gerês National Park (695.94 Km^2) and the site of community importance (SIC) of Serra D'Arga (44.75 Km^2) and Corno do Bico (51.44 Km^2) (ICNF, 2021).

Large mammals are mostly represented by wild boar (*Sus scrofa*), roe deer (*Capreolus capreolus*) and the apex predator Iberian wolf (*Canis lupus signatus*). Although there is no available information on the specific densities of wild boar and roe deer for the study area, wild boar seems to occur in high densities (10 ind./Km^2) over the Portuguese territory and roe deer seems to occur at low densities (1.6 ind./Km^2) within and near the Peneda-Gerês National Park (Ferreira, 2003; Terras de Sicó, 2017). In some areas inside Peneda-Gerês National Park there are also recently reintroduced populations of red deer (*Cervus elaphus)* and Iberian wild goat (*Capra pyrenaica*). Wolves occurring in this region belong to the Portuguese wolf subpopulation of Peneda-Gerês nucleus, which is currently estimated in approximately 20 breeding packs (Nakamura *et al.*, 2019). Several livestock species are abundant and raised under extensive grazing, including goats and sheep always protected by shepherds or guarding-dogs; cattle confined in stables during the colder months but free-ranging in mountain pastures during summer;

and *Garrano* horses traditionally raised in a free-ranging system during all year, with little to no vigilance and protection. This husbandry system makes *Garrano* horses particularly vulnerable to wolf predation, comprising up to 80% of the wolf's diet in this area (Álvares, 2011; Casimiro, 2017; Freitas, 2019).

The study area in Serra de Sta. Luzia (**Study II** and **Study III**) includes a small mountainous range located near the Atlantic coast, in the western limit of northwest Portugal (Fig. 6). Serra de Sta. Luzia (41°45'40.00" N, 8°49'19.00" W) is a coastal plateau of approximately 23 km^2, comprising the parishes of Afife, Areosa, Carreço, Freixeiro de Soutelo, Outeiro and Perre, from Viana do Castelo municipality. This mountainous range, with altitudes ranging from 140m to 550m, is included within the Eurosiberian biogeographic region, being characterized by a climate with mild seasons and high annual precipitation values (Costa *et al.*, 1998). For the year of 2020, the average temperature was of 15.5ºC, the relative air humidity was 83.4%, and the annual precipitation surpassed 1000 mm^3 (CMIA 2020). Unlike other mountainous ranges within northwest Portugal, Serra de Sta. Luzia is not included in any protected area and has a degraded human-dominated landscape composed mostly by scrublands (which cover more than half of the study area) and forest plantations of exotic species, such as pines, eucalyptus and acacias (*Acacia* sp.) (DGT 2018). Nonetheless, it has several values of biological importance, such as the permanent humid pastures dominated by several heath species (e.g. *Erica ciliaris*) which harbour a high biodiversity of insects and amphibians, and provide an important water source for wildlife and livestock (CM Viana do Castelo, 2017; ICNF 2021). In Serra de Sta. Luzia a large population of *Garrano* horses managed in a free-ranging husbandry system exists, although there is no available data on the specific horse numbers present in the plateau. Regarding wolf presence, which was considered occasional during the last decades, in 2018 this area was recolonized by a new breeding pack with an estimated size of 2-4 adult wolves per year (Nakamura *et al.*, 2019).

3. STUDY I: Horse-wolf interactions in northwest Portugal

3.1. Methodology

3.1.1. Literature review and data collection

To assess the regional and temporal patterns of horse consumption by wolves in northwest Portugal and evaluate the effect of different ecological and management-related factors on horse consumption, a literature review was performed focusing both scientific articles and grey literature (e.g. thesis, technical reports) documenting wolf diet since 1996 (Appendix III, Table S3). For this review, only studies that included 1) quantitative data on wolf diet, expressed as Frequency of Occurrence (F.O.), discriminated by wolf pack per sampling period; 2) free-ranging horses as a prey item; 3) horse consumption by wolves attributed mostly to predation and not to other reasons (e.g. scavenging); and 4) enough information to retrieve data on independent variables either from the original study or supplementary sources were selected. This resulted in the final selection of 12 wolf dietary studies covering 8 different packs located in northwest Portugal (bibliographic references on Appendix III, Table S3). Is important to note that the packs Vez and Soajo, despite being different breeding groups, were considered as a single sampling unit in all dietary studies that were reviewed, since the configuration of their territories have been quite dynamic during the last decades and overlap extensively in some areas (Casimiro, 2017).

3.1.2. Regional and temporal variation on horse consumption by wolves

The wolf dietary information obtained from the selected studies was classified by wolf pack per sampling period (comprising one or more years of data collection), in order to fully discriminate the information at the spatial and temporal scales. Regional variation was assessed by considering data from different wolf packs. Additionally, information on the temporal variation of wolf diet was considered when available in the selected studies, in order to perform a finer analysis on the yearly and seasonal variation of horse consumption by wolves. Yearly variation was assessed by considering 7 studies from 3 packs: Arga (2017), Arga (2018), Gerês (1996), Gerês (1997-1998), Vez/Soajo (1999-2000), Vez/Soajo (2004) and Vez/Soajo (2008-2010). Seasonal variation was assessed by considering five studies focusing three wolf packs: Gerês (1997-1998), Pitões (1998), Vez/Soajo (1999-2000), Vez/Soajo (2004) and Vez/Soajo (2008-2010). Seasonal periods were categorized as: Spring/Summer (March-August); Autumn/Winter (September-February). This data was used to perform a descriptive analysis characterizing temporal variations on horse consumption by wolves, by considering different years across the last three decades and seasonal periods.

3.1.3. Ecological and human determinants for horse consumption by wolves

Based on the review of 12 studies on wolf diet, a dataset composed by one dependent variable (magnitude of horse consumption by wolves), and 13 ecological and human-related independent variables considered relevant to influence wolf predation on free-ranging horses according to literature (Álvares, 2011, 2020; Lagos, 2013; Pimenta *et al.*, 2018) was built. Ecological variables obtained for each dietary study included the representativeness of alternative prey in wolf diet, level of wolf selection towards horses, availability of horses and alternative prey as well as wolf abundance and proportion of forest cover inside the pack territory, as a proxy for predation risk. As a human-related variable, the number of horses killed by wolves declared by owners for compensation was used, which reflects the level of horse vigilance and management. Detailed information regarding each variable is represented in Table 1 (see Appendix III, Table S3 with data for each reviewed study).

Table 1. Variables selected to evaluate the determinants for horse consumption by wolves in northwest Portugal.

Variable type	***Variable name: description and metrics***	***Source(s)***
Magnitude of horse consumption **(Dependent variable)**	**F.O. (Horses):** Values of Frequency of Occurrence for horses as a prey item in wolf diet	Reviewed studies
Consumption of alternative prey **(Ecological determinant)**	**F.O. (Livestock):** Sum of F.O. values for domestic species (cattle, goats, sheep, pigs and others)	Reviewed studies
	F. O. (Wild prey): Sum of F.O. values for wild ungulates (roe deer and wild boar)	Reviewed studies
Diet diversity **(Ecological determinant)**	**Prey Diversity (Livestock):** No. of domestic prey species in wolf diet	Reviewed studies
	Prey Diversity (Wild prey): No. of wild ungulate species in wolf diet	Reviewed studies
	Prey Diversity (Total): Total no. of prey species in wolf diet	Reviewed studies
	Trophic Niche Breadth: Value of Standardized Levin's Index	Reviewed studies; calculated when missing
Wolf selection for horses **(Ecological determinant)**	**Ivlev's Index (Horses):** Value of Ivlev's selectivity index for horses	Reviewed studies; calculated when missing
Availability of horses and alternative prey **(Ecological determinant)**	**Population Density (Horses):** No. horses/km^2 inside the pack territory	Reviewed studies; agricultural censuses (INE, 1999-2019)
	Population Density (Cattle): No. cattle/km^2 inside the pack territory	Reviewed studies; agricultural censuses (INE, 1999-2019)
	Population Density (Sheep+Goat): No. sheep+goats/km^2 inside the pack territory	Reviewed studies; agricultural censuses (INE, 1999-2019)
Wolf abundance **(Ecological determinant)**	**Wolf Pack Size:** Minimum no. of wolves estimated inside the pack territory (per sampling period)	Wolf monitoring studies (Álvares, 2011; Nakamura *et al.*, 2019)
Availability of forested habitats **(Ecological determinant)**	**Forest Cover:** Proportion of all land cover classes related to forests included in the pack territory	Corine Land Cover (2000-2018)
Predation on horses declared by owners **(Human-related determinant)**	**No. of declared horse kills:** No. of horses killed by wolves declared to ICNF for compensation (per wolf pack territory and sampling period), as indicator of horse management by owners	Official statistics from ICNF

The magnitude of wolf consumption for horses and other alternative prey was quantified by using the values of Frequency of Occurrence (F.O.) for these prey reported in wolf diet studies. Although the measure of Consumed Biomass for each prey item is considered to be more reliable to evaluate consumption of large and frequent prey by wolves (Ciucci *et al.*, 1996), ultimately F.O. was used for the analysis since Consumed Biomass was not available for all reviewed studies and is difficult to calculate for all prey items when missing. Besides, F.O. represents well the relative intensity of prey consumption by wolves that is required for the analysis. The sum of the F.O. values for the main livestock species (e.g. cattle, sheep, goats, pigs and donkeys) were considered as “Livestock Consumption”, while the sum of the F.O. values for the main wild ungulates (e.g. roe deer and wild boar) were considered as “Wild prey Consumption”. Additionally, diet diversity and the relative relevance of free-ranging horses as a prey in the overall diet, was assessed by: 1) the total number of prey species represented in each dietary study (namely, total number of species for livestock, wild ungulates, and all prey items);

2) the value of trophic niche breadth for each dietary study using the Standardized Levin's index (Krebs, 1989), which was either obtained from the reviewed studies or calculated when missing and reflects the degree of diet specialization, varying from 0 to 1 (0 indicates the diet is composed by only one prey and 1 indicates all prey are consumed equally); and 3) the value of Ivlev's Selectivity Index (D) modified by Jacobs (1974) (Krebs, 1989 in Álvares, 2011) as a measure of wolf selection towards horses (i.e. comparing level of consumption with local availability), which was either obtained from the reviewed studies or calculated when missing. This index varies between -1 and 1 and determines the selection of a given prey species by the predator, (negative values mean the prey is avoided, 0 means it is consumed according to its availability and positive values mean it is preferred).

To evaluate the effect of prey availability on wolf predation, the density (expressed as no. of animals per Km^2) of horses and other livestock species inside the territory of each studied pack was obtained either from data reported on the reviewed studies or, when missing, by calculating abundance using official population size estimates from the agricultural censuses at parish level (INE, 1999; 2019). For calculating local densities, the sum of all the parishes overlapping at least 10% with wolf pack territories as defined according to literature (Álvares, 2011; Casimiro, 2017) was considered. The availability of alternative prey was only focused on other livestock species (categorized as cattle density and small ruminants (sheep/goat) density). The availability of wild prey was not considered since there is no updated information regarding the local densities of wild boar and roe deer at a pack territory level, given that only coarse estimates for all Portuguese territory or for the entire area of PNPG (Ferreira, 2003; Freitas, 2019; Terras de Sicó, 2017) are available. Regarding a measure of wolf abundance inside the territory of each studied pack as a proxy for predation risk, available information on the minimum number of estimated wolves per pack and per each year or sampling period of dietary studies was considered. This data on yearly pack sizes is available in the scope of wolf monitoring studies conducted in northwest Portugal since 1996 (Álvares, 2011; Nakamura *et al.*, 2021). In wolf dietary studies comprising sampling for more than one year, the average of the estimated pack size per year was considered.

Additionally, in order to assess the influence of habitat features, the relative proportion of forest cover (from 0 to 1) inside the territory of each studied pack was calculated, since forested areas are documented to increase the risk of horse predation by wolves (Álvares, 2020; Namgail *et al.*, 2007; Van Duyne *et al.*, 2009). The proportion of forest cover was obtained by calculating the total area of forested habitats (both native woodlands and forest plantations) inside each pack territory in relation to the overall territory size. The forest cover information was obtained from Corine Land Cover maps (EEA, 2000, 2006, 2018), by considering the year closest to the sampling period of each reviewed study, and the land cover classes defined as “Broad-leaved forest”, “Coniferous forest” and “Mixed forest”. Finally, as a proxy for the level of management and vigilance by horse owners in the different studied packs, official data provided by ICNF regarding the number of declared wolf attacks to horses per year at the parish level was used. This information was obtained by calculating the total number of killed horses declared for compensation on all the parishes comprising at least 10% of a pack territory, during the same sampling period of each reviewed study. In all studies with sampling periods longer than one year, the average of the total number of compensated horses for each year was considered. This was the only indicator related to horse management included in the analysis, due to the lack of detailed and updated information on the local husbandry practices of free-ranging horses in northwest Portugal.

Data analysis was firstly conducted by a linear regression relating F.O. values for horses in wolf diet with the different independent variables, to identify which determinants have an influence on horse consumption levels by wolves in northwest Portugal. Based on this initial analysis, the statistical significance of variables demonstrating an apparent positive or negative relation with horse consumption variation was tested using GLMs (Generalized Linear Models) with binomial distribution and logit-link function (Nelder & Wedderburn, 1972). Namely, the variables related to prey consumption (F.O.), prey diversity, prey density and horse selectivity (Ivlev’s index) were selected. The models constructed included the individual variable and other variables considered in the linear regression graph. The cut-off level considered for significance was $p < 0.05$. The statistical analyses were performed in R 4.0.4 (R Core Team, 2021), a language and program for statistical computing, and RStudio 1.4.1106 (RStudio Team, 2021), an open-source support interface program for R. All spatial analysis were performed on ArcGIS 10.8 (ESRI 2019), a Geographic Information System program.

3.2. Results

3.2.1. Regional and temporal patterns on horse consumption by wolves

Information regarding the consumption of free-ranging horses by wolves was available for 8 different wolf packs from northwest Portugal, namely Arga, Boulhosa, Cruz Vermelha, Vez/Soajo, Gerês, Pitões, Larouco and Leiranco, according to a W-E gradient (Fig. 7). Considering the total of 12 wolf dietary studies covering a time period of more than 30 years (1996-2018), the feeding habits of some packs were studied for more than one sampling period, namely Arga pack (2017 and 2018), Vez/Soajo pack (1999-2000, 2004 and 2008-2010) and Gerês pack (1996 and 1997-1998) (Fig. 7; Appendix III, Table S3).

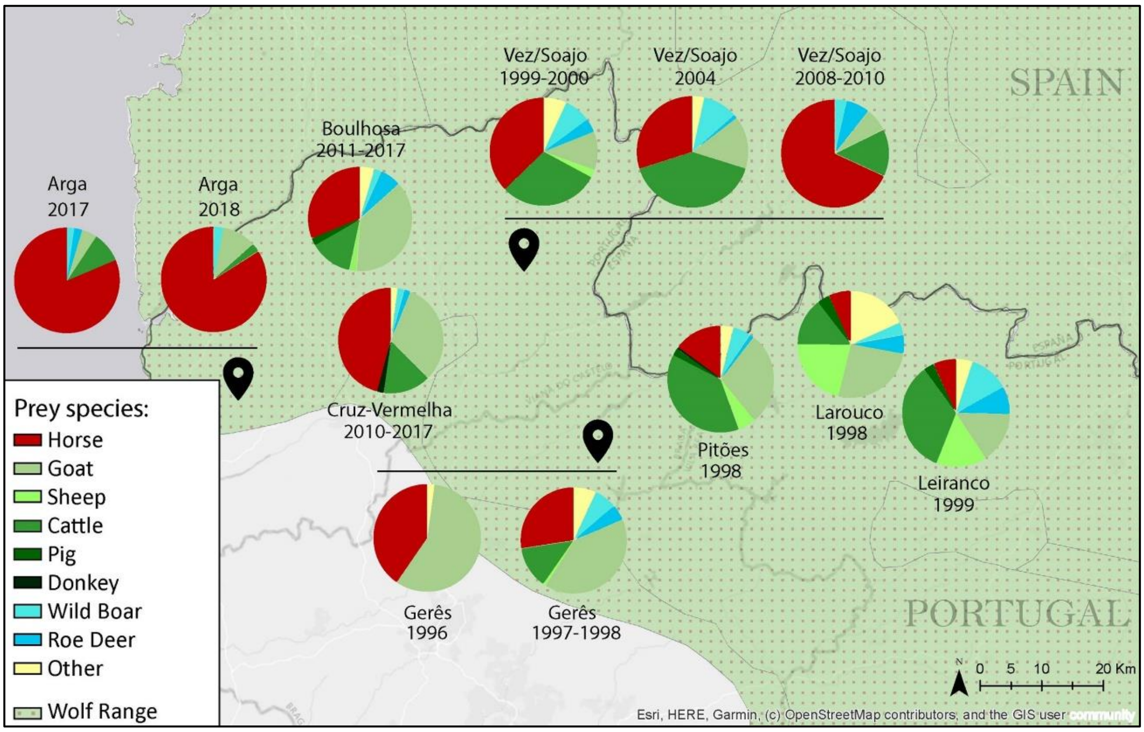

Figure 7. Regional and temporal variation in the diet of 8 wolf packs in northwest Portugal, considering the magnitude of prey consumption (expressed as F.O.) for free-ranging horses (in red), livestock species (in green) wild ungulates (in blue) and other prey (in yellow). See Appendix III, Table S3 for detailed information for each dietary study.

Regarding regional patterns, there was a wide variation of the magnitude of free-ranging horses as a wolf food resource throughout northwest Portugal. Horses were the main prey in 5 (42%) of the wolf dietary studies, while other domestic prey, such as goat and cattle, were the second most consumed prey class, with wild ungulates (represented only by wild boar and roe deer) and other prey (e.g. lagomorphs and other carnivores) being much less consumed. In general, there was a trend for a West-East gradient in the relevance of free-ranging horses as a wolf prey, reaching more than 80% of wolf diet in the western limit (e.g. Arga pack) while comprising less than 10% of wolf diet in the eastern packs (e.g. Larouco and Leiranco packs). However, it’s important to note that collection of dietary data from western packs is generally more recent than in the eastern packs, which may reflect an influence between regional and temporal patterns of variation. In fact, there seems to be an increasing trend in free-ranging horse consumption by wolves over time, with studies conducted from 1990 to 2000 showing a lower relevance of horses in wolf diet than the studies conducted since 2010 (e.g. Cruz Vermelha 2010-2017, Vez/Soajo 2008-2010 and Arga 2018). Considering the three studied packs with more than one sampling period, horse consumption remained relatively constant in the Arga pack during consecutive years, decreased slightly by 13% in the Gerês pack also during consecutive years, and increased by 31% in the Vez/Soajo pack between late 1990s and 2010 (Fig. 7).

Regarding seasonal variation, horse consumption by wolves was generally higher during the colder seasons (Autumn/Winter), except for the Gerês pack in 1997-1998 and the Vez/Soajo pack in 1999-2000, where this trend was reversed. This pattern was less evident when considering the four seasons separately, but nonetheless was still present (see Appendix III, Fig. S1). However, the intensity of seasonal variation in horse consumption was limited and similar between different packs, with the smallest variation observed in the Pitões pack (difference of 11% in F.O. between the colder and warmer seasons) while the more accentuated variation was observed in the Vez/Soajo pack during 2008-2010 (difference of 18% in F.O.) (Fig. 8; Appendix III, Fig. S1).

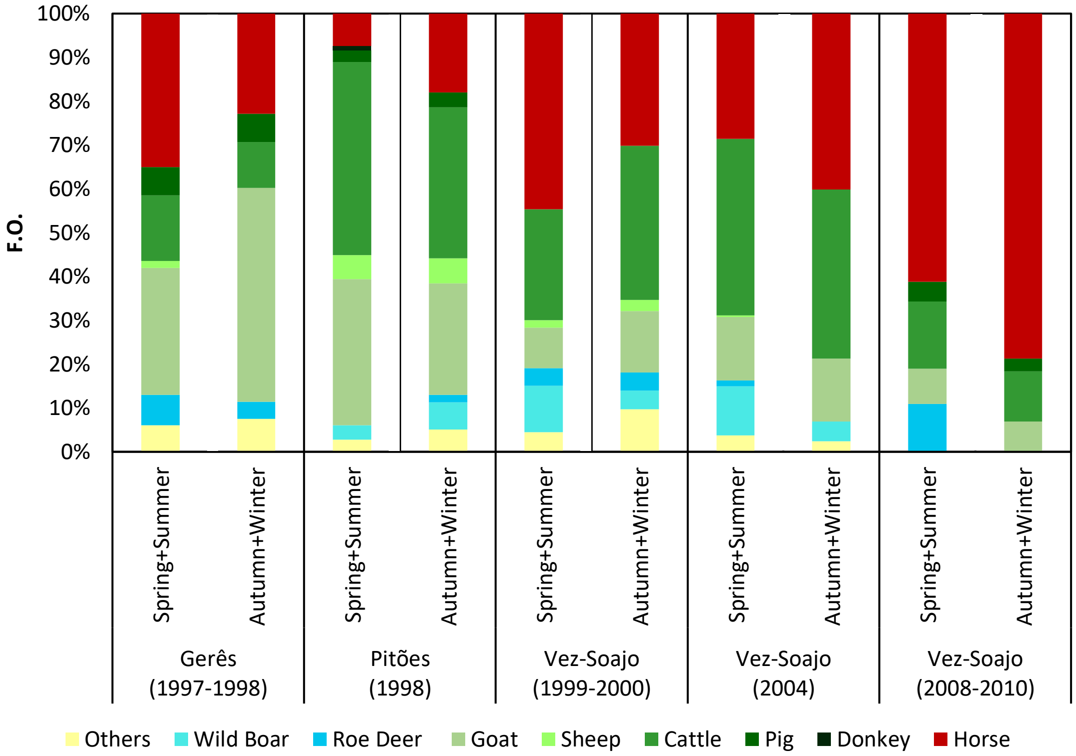

Figure 8. Seasonal variation of wolf diet in five packs/sampling periods from northwest Portugal, considering the magnitude of prey consumption (expressed as F.O.) for free-ranging horses (in red), livestock species (in green) wild ungulates (in blue) and other prey (in yellow). See Appendix III, Table S3 for detailed information for each dietary study and Figure S1 for variation by each season (Spring, Summer, Autmn and Winter).

Finally, as mentioned above, the magnitude of horse consumption in the Vez/Soajo pack has increased over the last years, with free-ranging horses comprising less than half of the packs' diet in 1999-2000 (F.O.= 45% in Spring/Summer; F.O.=30% in Autumn/Winter) but representing the majority of the packs' diet during 2008-2010 (F.O.= 62% in Spring/Summer; F.O.=80% in Autumn/Winter) (Fig. 8).

3.2.3. Determinants for horse consumption by wolves

From the 13 ecological and human-related variables considered relevant to horse consumption, 5 variables showed no influence on magnitude of horse consumption. Namely no positive nor negative relation was observed between the magnitude of horse consumption and the variables representing: 1) trophic niche breadth, 2) cattle density, 3) wolf pack size, 4) proportion on forest cover and 5) number of declared wolf attacks to horses (Fig. 13, Appendix III, Figs. S2-S5). The remaining variables showed a possible relation with the magnitude of horse consumption (Figs. 9-13), and 5 GLMs were constructed based on the variables included in the respective linear regression graphs (Tables S4-S8).

The intensity of consumption of both domestic and wild ungulates seemed negatively related to horse consumption levels, with horse consumption decreasing with higher consumption of either domestic or wild prey (Fig. 9). However, the GLM analysis showed no significant effects for these variables (Table S4; p>0.05).

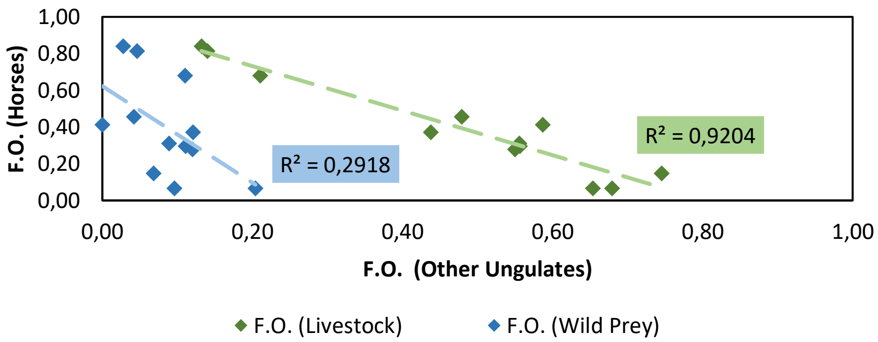

Figure 9. Linear regression between magnitude of horse consumption (F.O.) and consumption of other ungulates, considering either livestock (in green) or wild prey (in blue) species, based in 12 studies on wolf diet. See Appendix III, Table S4 for respective GLM analysis.

Similar to alternative prey consumption (Fig. 9), the diversity of alternative prey in wolf diet seemed to be negatively related to the intensity of horse predation by wolves, with lower levels of horse consumption found in wolf packs with a larger number of prey classes in their diet (Fig. 10, Fig. 11). This was particularly observed in packs that consumed more domestic prey species, such as the Pitões pack (Fig. 10; see Appendix III, Table S3). However, none of the variables had a significant effect on the variation of horse consumption (Tables S5-S6; p>0.05)

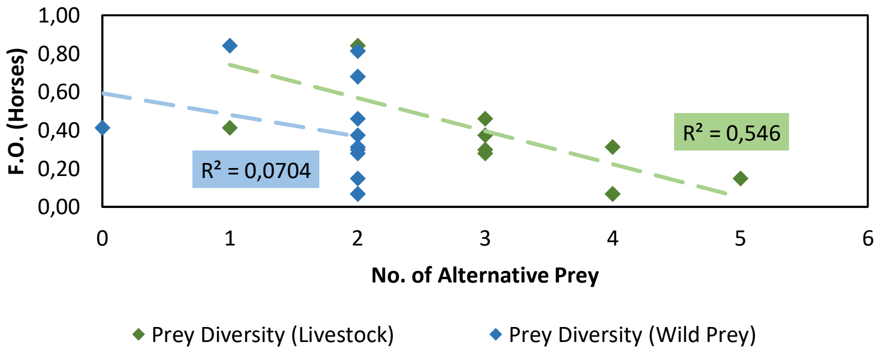

Figure 10. Linear regression between magnitude of horse consumption (F.O.) and total number of other prey species (Alternative Prey), considering either livestock (in green) or wild prey (in blue) species, based in 12 studies on wolf diet. See Appendix III, Table S5 for respective GLM analysis.

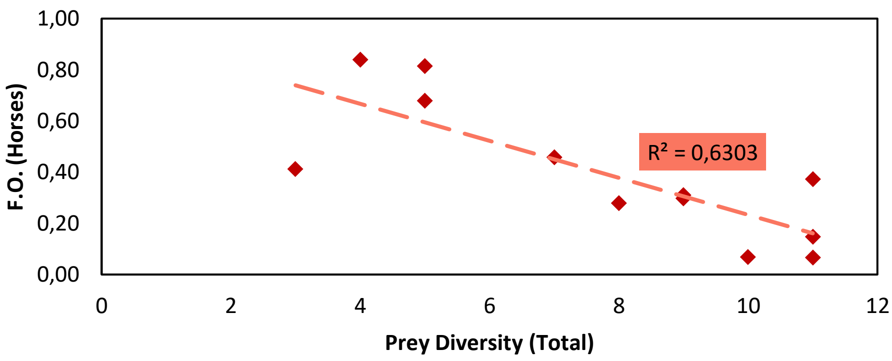

Figure 11. Linear regression between magnitude of horse consumption (F.O.) and total number of prey species (Prey Diversity), based in 12 studies on wolf diet[2]. See Appendix III, Table S6 for respective GLM analysis.

Free-ranging horses showed a strong positive selection by wolves in all dietary studies reviewed (Ivlev's Index, D > 0.5), and higher selectivity values seemed to be related to increasing intensity of horse consumption (Fig. 12; see Appendix III, Table S3). However, similarly to previous variables, no significant effects were found (Table S7, p>0.05).

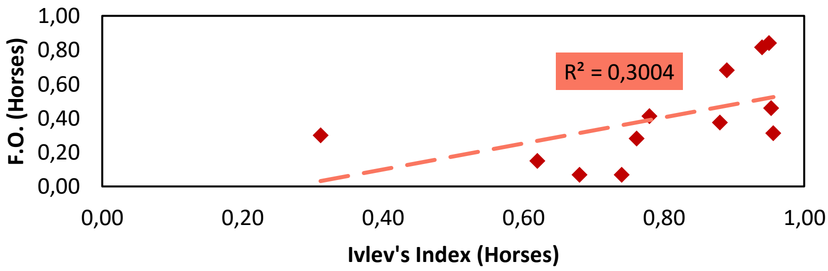

Figure 12. Linear regression between magnitude of horse consumption (F.O.) and value of Ivlev's Index for horses (e.g. measure of prey selection), based in 12 studies on wolf diet. See Appendix III, Table S7 for respective GLM analysis.

Regarding the effect of prey availability, higher densities of free-ranging horses seemed to be related to higher levels of horse predation, while higher densities of cattle and other smaller domestic prey (e.g. sheep and goats), seemed to have little effect on horse consumption (Fig. 13). But none of the variables included in the GLM analysis significantly affected the magnitude of horse consumption by wolves (Table S8, p>0.05).

---

[2]Occasional prey with minimal presence on wolf diet are considered here, in opposition to Figure S-3 (see below) which only includes the main prey classes consumed by wolves.

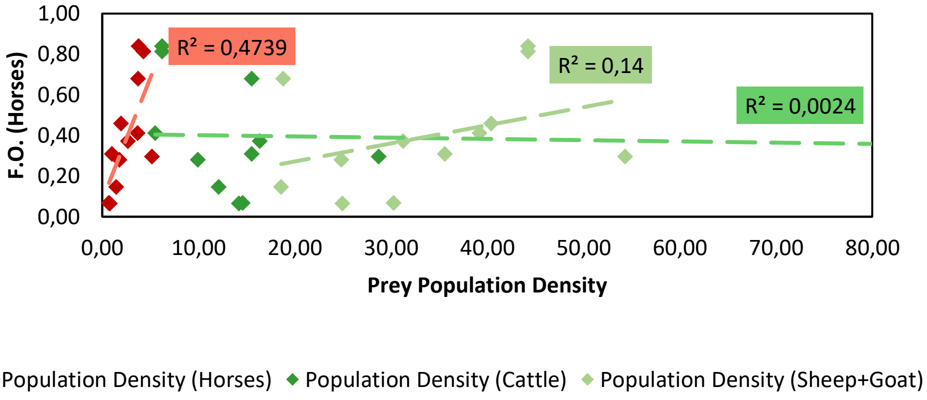

Figure 13. Linear regression between magnitude of horse consumption (F.O.) and local densities of horses (in red), cattle (in darker green) and small ruminants (sheep+goat, in lighter green), based in 12 studies on wolf diet. See Appendix III, Table S8 for respective GLM analysis.

3.3. Discussion

This study provides valuable information regarding the spatiotemporal patterns of free-ranging horse consumption by wolves in northwest Portugal, and the influence of relevant ecological and human-related variables on the magnitude of horse consumption by Iberian wolves. Current knowledge focusing the factors that determine horse consumption by wolves is limited, due to the absence of predators in most horse ranges and the limited importance of horses in wolf diet worldwide (Álvares, 2011; Freitas *et al.*, *in press;* Lagos & Bárcena, 2020; Oom & Reis, 1986). Therefore, this study provides valuable knowledge not only for free-ranging horses of Iberian Peninsula, but also for other endangered wild equid populations (e.g. Przewalskii horses or Khulans) that suffer strong predation pressure from wolves (Feh *et al.*, 1994; Van Duyne *et al.*, 2009).

When analysing the dietary information at a regional scale, the diet of the Iberian wolf in northwest Portugal was mostly comprised by livestock, and free-ranging horses were one of the main prey classes for more than half of the packs. Furthermore, horse consumption varied across the region, with horses being more consumed on packs located in the western limit of the study area, although in these packs dietary information was also collected during most recent years. Although wolf diet is not identical across the Iberian Peninsula (Barja, 2009; Llaneza *et al.*, 1996), prevalence of domestic species in the diet has been documented for northwest Portugal (Álvares, 2011; Álvares *et al.*, 2019; Casimiro, 2017; Salvador & Abad, 1987) and south of Douro (Torres *et al.*, 2015) in Portugal, and León (Salvador & Abad, 1987), Asturias (Llaneza *et al.*, 1996) and Galicia (Lagos & Bárcena, 2018) in Spain. This type of diet is commonly attributed to the combination of low densities of wild prey and high availability of extensively grazed livestock or dumpsites, typical of anthropogenic habitats (Newsome *et al.*, 2016; Zlatanova *et al.*, 2014). Additionally, the importance of horses on the diet of wolves from northwest Portugal has been widely reported (Álvares *et al.*, 2019; Casimiro, 2017; Freitas *et al.*, *in press*). Given the dietary information available for each wolf pack, the variation in horse consumption intensity across the study area can be related to both regional and temporal patterns, and result of differences in the local abundances of potential prey over time (Álvares, 2020; Llaneza *et al.*, 1996). When analysing the dietary information at a temporal scale, free-ranging horses seemed to become a more relevant wolf prey in recent decades. Some packs with studies from consecutive years (Arga and Gerês) showed little yearly variation. However, the Vez/Soajo pack showed an increase in horse consumption intensity across years, despite the increase of wild prey density in the region in recent decades (Casimiro, 2017). This increase in horse consumption seems to be associated with a decrease in both number of consumed prey species and availability of small ruminants (goats and sheep) on the pack territory, resulting in horses being more consumed to compensate for the lack of alternative prey (Casimiro, 2017). When analysing the dietary variation at a seasonal scale, free-ranging horse consumption showed limited variation between colder and warmer seasons, but the seasonal tendency to consume more horses varied according to location or collection year. The higher consumption of horses on colder months (autumn and winter) can have several explanations: 1) alternative prey is less available (e.g. cattle and small ruminants are kept in stables during the colder months), which forces wolves to consume easily accessible prey such as unattended horses (Álvares, 2011, 2020; Lagos & Bárcena, 2015); 2) free-ranging horses have poorer body condition and higher mortality rates due to a decrease in forage abundance and harsh environmental conditions which, in turn, increases their vulnerability to predation and the quantity of carcasses available for scavenging (Freitas *et al.*, *in press;* Gomes, 1996; Hovens & Tungalaktuja, 2005; Rio-Maior *et al.*, 2006); 3) in some areas (e.g. PNPG) wolves occupy lower altitudes during winter as protection against cold temperatures, which increases the probability of encountering horses (Oom & Reis, 1986). The higher consumption of free-ranging horses on warmer months in Gerês (1997-1998) and Vez/Soajo (1999-2000) can be related to the increase in new-borns during March-July (Gomes, 1996), which are highly vulnerable to predation during the first months of age, making them easy to prey (Álvares, 2020; Bárcena, 2012; Costa, 2000; Llaneza *et al.*, 1996). Due to the low number of reviewed studies addressing seasonal patterns in wolf diet (n=5, for 3 packs), it is not possible to conclude if seasonal differences in horse consumption are determined by local or regional conditions. Therefore, the analysis of wolf diet discriminated by season in future studies is highly recommended.

Regarding the effect of ecological and human-related variables on horse consumption by wolves, none of the factors could be confirmed as an evident determinant of horse consumption intensity in northwest Portugal. Nonetheless, wolf predation on free-ranging horses seemed to be higher in areas with higher horse density, which might indicate a potential influence on horse consumption. Horse density has been reported to influence the probability of horse predation in northwest Portugal (Pimenta *et al.*, 2018). Additionally, previous studies in Europe and North America concluded that prey density and vulnerability can influence its consumption by wolves (Llaneza *et al.*, 1996; Mech & Peterson, 2010; Meriggi & Lovari, 1996). At the same time, diversity and consumption of alternative prey generally suggested a negative relation with horse consumption by wolves. Higher diversity and availability of prey, particularly of wild prey, have been reported to decrease predation on livestock worldwide (Newsome *et al.*, 2016; Werhahn *et al.*, 2019). Likewise, higher predation levels of free-ranging horses in northwest Portugal have been observed for the packs with a less diverse diet, possibly to compensate for the lack of alternative prey (Casimiro, 2017; Freitas, 2019). It was not possible to assess the effect of availability of wild prey on horse consumption intensity due to a lack of updated densities of wild ungulates in northwest Portugal (Ferreira, 2003; Freitas, 2019; Terras de Sicó, 2017), but this variable was expected to have influence on horse consumption by wolves since wild ungulates are scarce in the area (Álvares, 2011; Vos, 2000). However, availability of alternative livestock (e.g. cattle, small ruminants) did not show any significant effect on horse consumption intensity. This pattern goes against the initial hypothesis that lower availability of alternative prey is related to higher horse consumption (Álvares, 2020). The lack of influence of alternative domestic prey abundance has been previously reported for northwest Portugal (Pimenta *et al.*, 2018) and might be related to the positive selection of horses by Iberian wolves. Iberian wolves have been widely reported to have a strong preference towards free-ranging horses (Álvares, 2011; Freitas, 2019; Lagos & Bárcena, 2018), which has been attributed to several factors: 1) the traditional free-ranging system in northwest Portugal, which makes horses especially accessible to predation by wolves (Casimiro, 2017; Freitas, 2019; Freitas *et al.*, *in press;* Serôdio, 1992; Vos, 2000); 2) a specialization of Iberian wolves towards free-ranging horses as a result of their ancient predator-prey relationship (Bárcena, 2012; Freitas *et al.*, *in press*); 3) the high biomass and nutritional value per killed horse (Costa, 2000); 4) the absence of anti-predatory behaviours on horses in poor condition or occupying territories recently recolonized by wolves (Álvares, 2020; Freitas, 2019). In fact, most packs showed a strong preference towards horses and the predictive model suggested a non-significant positive relation between horse consumption intensity and horse selectivity. Moreover, trophic niche breadth values highlighted a moderately specialized diet in most studied packs. Therefore, densities of available alternative prey might not be sufficient to minimize the effect of horse selectivity on wolf diet. The variables chosen as a proxy for predation risk (wolf pack size and proportion of forest cover inside the pack territory) did not show any clear relation to horse consumption variation. Previous studies on livestock from Europe and Asia have reported higher predation risk in areas with higher forest cover (Davie *et al.*, 2014; Dondina *et al.*, 2015; Rio-Maior *et al.*, 2006) and bigger wolf pack size (Imbert *et al.*, 2016). Additionally, wild-living Przewalski's horses were reported to be more predated in areas closer to forests (Van Duyne *et al.*, 2009). This can indicate that the variables chosen for this study can be poor indicators of predation risk for free-ranging horses in northwest Portugal, given the particular ecological conditions in this region. In fact, Pimenta *et al.* (2018) report that higher predation probability on livestock (including free-ranging horses) was significantly supported by decreasing distance to nearest pack, higher altitudes, and high proportion of shrubland cover. Therefore, the use of additional proxy variables for predation risk on future models is recommended.

The variable reflecting the level of horse vigilance and management per study pack did not show any significant relation to horse consumption intensity as well. However, free-ranging husbandry systems have been reported to influence risk of wolf predation on horses both in Portugal (Pimenta *et al.*, 2018) and Mongolia (Namgail *et al.*, 2007; Van Duyne *et al.*, 2009). Likewise, the use of damage prevention measures has been reported to minimize predation risk on livestock (Musiani *et al.*, 2003; Pimenta *et al.*, 2017; Shivik *et al.*, 2003). The lack of relation between the horse attacks declared by owners to ICNF and horse consumption intensity is possibly related to the fact that the current compensation system is not compatible with the traditional free-ranging husbandry regime practiced in northwest Portugal, leading many horse owners to choose not declaring horses killed by wolves. In particular: 1) required measures by the compensation system, such as the sanitary surveys (e.g. microchip, control for diseases such as brucellosis) and damage prevention measures (e.g. shepherds, livestock guarding dogs, fences), are difficult to implement in free-ranging husbandry systems (Álvares *et al.*, 2019; Freitas & Álvares, *in press;* Freitas *et al.*, *in press*); 2) carcasses of attacked horses can be fully consumed by wolves before being found and reported by the owners or examined by ICNF technicians (Costa, 2000; Lançós, 1999; Leite, 2012); 3) foals younger than a month old are excluded from receiving compensations (Freitas

& Álvares, *in press*); and 4) after January 2022, current legislation determines that free-ranging livestock without a shepherd or guarding dog will be excluded from compensation rights (Álvares *et al.*, 2019). Additionally, the bureaucratic process is slow and the compensation values insufficient, which limits the confidence from owners on the current compensation scheme (Freitas & Álvares, *in press;* Milheiras & Hodge, 2011). This can lead owners to be either reluctant or unable to apply for compensation (Freitas *et al.*, *in press;* Milheiras & Hodge, 2011), resulting in discrepancies between wolf dietary information and official data on reported wolf attacks, as already documented in several of the reviewed studies (Casimiro, 2017; Costa, 2000; Guerra, 2004). Ultimately, this human-related variable might have provided an inaccurate assessment of the predation risk for free-ranging horses in the study area. The use of alternative sources of information, directly gathered by questionnaires to horse owners, could provide other variables more relevant to determine the effect of the free-ranging husbandry systems on horse consumption intensity.

In conclusion, this chapter highlighted the importance of free-ranging horses in wolf diet, with some variation between neighbouring packs. Regarding temporal variation, horse consumption seems to have increased over the years, particularly in Vez/Soajo pack, although no apparent trend was observed across seasons. There were no clear determinants that significantly influenced horse consumption intensity in northwest Portugal, although consumption seems to be higher in areas of high availability of free-ranging horses, while availability of alternative prey and wolf abundance seemed to have limited influence on horse consumption. Finally, the human-related factors used as a proxy for the level of horse management showed no relation to horse consumption intensity, despite husbandry practices being an important determinant for livestock depredations in Iberia (Lagos & Bárcena, 2018; Pimenta *et al.*, 2017; Vos, 2000) and worldwide (Dondina *et al.*, 2015; Meriggi & Lovari, 1996; Namgail *et al.*, 2007).

4. STUDY II: Spatial ecology of *Garrano* horses in Serra de Santa Luzia

4.1. Methodology

4.1.1. Data collection and treatment

To study patterns on the spatial ecology of free-ranging *Garrano* horses in northwest Portugal, two adult mares[3], belonging to the same owner, were followed by GPS telemetry between 18th April and 13th June 2020 in Serra de Sta. Luzia (Viana do Castelo, Portugal) using GPS tail tags, which were firstly developed by ELECTRICBLUE (CIBIO-InBIO) for this study. The GPS tags were from the model 'WildTrack' and communicated location data via satellite (namely, via the Globalstar satellite system), which allowed the retrieval of location data without having to be on the field. Horse I was tagged with the smaller version of the tag, weighting 130 g; Horse II was tagged with the larger version, which weighed 225 g (ELECTRICBLUE, 2020). Both tags were braided onto the horses' tails (Fig. 14) during their brief confinement, followed by release into a free-ranging husbandry system. The tracking period ended when the tail-tags were retrieved from the animals. The two tags were pre-programmed with different reception schedules: one collected GPS-locations every two hours, while the other collected locations every thirty minutes. The evaluation in the performance of the GPS-tags considering the two reception schedules is explored in **Study III**. In order to produce a statistically and biologically relevant dataset for spatial analyses, missing locations on both datasets were estimated using the GPS coordinates from sampled locations (Mitas & Mitasova, 1999). The interpolation of missing data was done using the package *zoo* (Zeileis & Grothendieck, 2005) and considering the tag schedule for each tagged horse.

[3] Additional eight horses from a different geographical area (i.e. Serra D'Arga) were GPS tagged in summer 2021, but the collected data was not timely enough to be included in this dissertation.

Figure 14. GPS tail tag, deployed on a tail braid of one of the studied *Garrano* horses in Serra de Sta. Luzia during the spring of 2020.

In order to analyse habitat use and selection patterns of the tagged horses, landscape-related features such as habitat types, altitude, and slope were obtained from geo-referenced datasets from Serra de Sta. Luzia. Habitat types were obtained from the official Land Cover and Land Use 2018 Map from the Portuguese General Territorial Directorate (DGT 2018), which is the most detailed and up-to-date land cover cartography map for the study area. The elevation-related variables (i.e. altitude and slope) were collected from a DEM (Digital Elevation Model), calculated using a hypsometric map developed by LEAF (University of Lisbon) and the R packages *sf* and *raster* (Hijmans & van Etten, 2012; Pebesma, 2018).

4.1.2. Data analysis

Spatial analyses focused mostly on providing basic information of horse movements and space use, such as home range and core areas size, as well as their temporal variation across a circadian cycle (namely diurnal and nocturnal) and the tagging period (at weekly intervals of 7 days each). Home ranges, defined as the area used for grazing, water, shelter, and other activities (Keiper, 1986; Linklater *et al.*, 2000), and core areas, defined as the main areas of activity within the home range (Samuel *et al.*, 1985), were obtained for the two tagged mares using kernel density methods considering 95% and 50% of the GPS locations, respectively (Worton, 1989). This analysis was performed by using the package *adehabitatHR* (Calenge, 2006). The habitat selection analysis was performed by using data from the two tagged horses combined, given their similar home ranges (see **sections 4.2.1** and **4.2.2**). Habitat selection was calculated for the three land cover types available in the study area: “Eucalyptus Forest”, “Pine Forest” and “Shrubland”, as defined by the DGT land cover map for the study area. To characterize horse use of different types of land cover in relation to their overall availability in the study area, habitat selection was calculated using Ivlev's Electivity Index (Ivlev, 1961):

$$EI_{veg\ type\ "x"} = \frac{(\%\ horse\ use\ in\ "x" - \%\ of\ \ "x"\ available)}{(\%\ horse\ use\ in\ "x" + \%\ of\ \ "x"\ available)}$$

with "x" representing a habitat type, and considering the proportion of a given habitat in horse home ranges in comparison with its proportion within the whole study area. This index varies from -1 to 1 and determines if a given habitat is avoided (negative values), preferred (positive values), or used in accordance to their availability (0). To determine the range of altitude and slope values used by the tagged horses in Serra de Sta. Luzia, box-plot graphs with summary statistics (i.e. average and standard deviation) were used.

Furthermore, horse space use and habitat selection were analysed along different timescales. Specifically, data from horse home ranges, core areas and habitat selection were categorized into day (locations collected between 07:00-18:59) and night (locations collected between 19:00-06:59) to analyse variations related to circadian factors. Additionally, space use data was divided into 8 weekly intervals with 7 days each (i.e. 18-24 April; 25 April-1 May; 2-8 May; 9-15 May; 16-22 May; 23-29 May; 30 May-5 June and 6-13 June), to evaluate movement reoccurrence and spatial-temporal variations in home range and core areas across the tracking period.

All spatial analysis were performed on ArcGIS 10.8 (ESRI 2019), on R 4.0.4 (R Core Team, 2021) and on RStudio 1.4.1106 (RStudio Team, 2021).

4.2. Results

4.2.1. Overall patterns of space use and habitat selection

The two free-ranging *Garrano* horses monitored with GPS tags occupied the northern section of Serra de Sta. Luzia. Both horses largely occupied the same geographical range, with home ranges and core areas of the two individuals overlapping almost completely (Fig. 15). Horse I occupied a home range of 1.5 Km^2 while Horse II occupied a slightly larger home range, with 2.1 Km^2. In terms of core areas, Horse I had only one main activity centre with 0.5 Km^2, located approximately at the centre of its home range; Horse II had two core areas comprising a total of 0.5 Km^2, with one (0.3km^2) located at the centre of its home range (overlapping with Horse's I core area) and another (0.2km^2) located at the easternmost limit of Horse's II home range (Fig. 15; see Appendix IV, Table S9, Fig. S6).

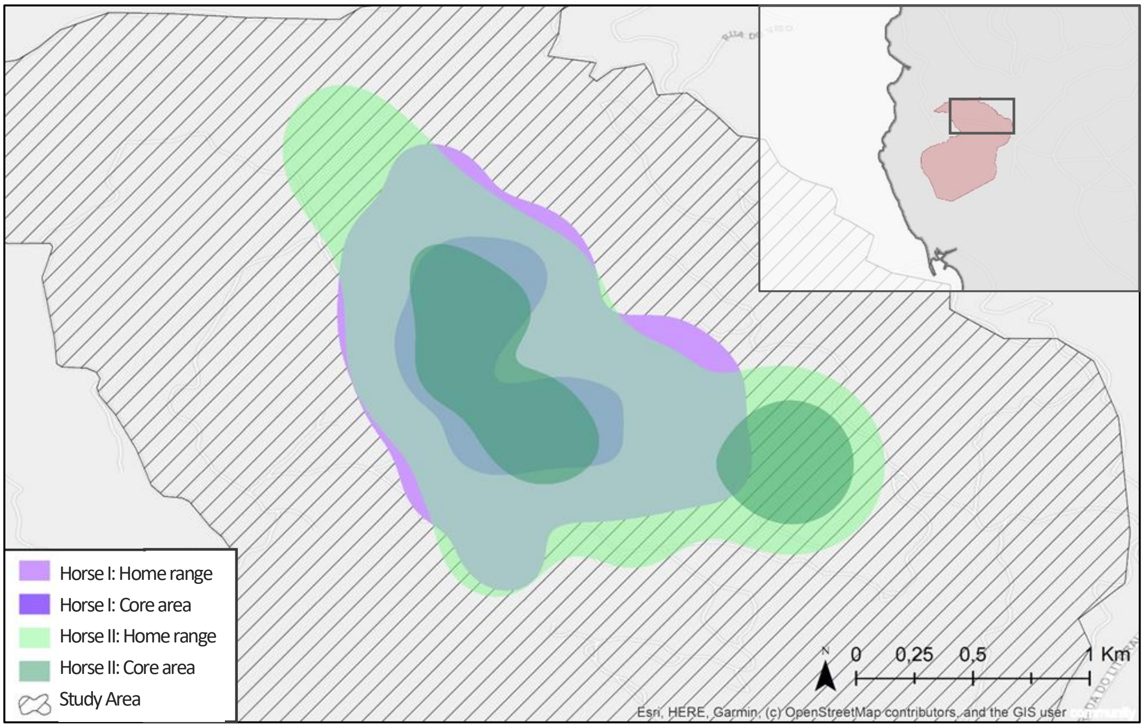

Figure 15. Home ranges (lighter colours) and Core areas (darker colours) of two free-ranging horses in Serra de Sta. Luzia, estimated using GPS locations obtained between April 18 and June 13, 2020. Tagged horse I is represented in purple; Tagged horse II is represented in green. See Appendix IV, Fig. S6 for space use of each horse separately.

Since the home ranges of both tagged horses were very similar and overlapped extensively (74%), the data from both individuals was grouped for the subsequent analyses of habitat use and selection. Habitat selection patterns were similar for the home ranges and core areas of the tagged horses. Inside home ranges, eucalyptus forests were negatively selected (-0.8), pine forests were used according to their availability (-0.03) and shrublands were slightly positively selected (0.2). Inside core areas, eucalyptus forests were even more negatively selected (-1.0) while shrublands (0.2) and pine forests (0.5) were positively selected (Fig. 16). Considering the availability of these three habitat types inside the horse's home range and in the total study area, it becomes evident that eucalyptus forests are strongly avoided in the main areas used by both tagged horses, while shrublands and pine forests (only in core areas) are preferably used (Appendix IV, Fig. S7).

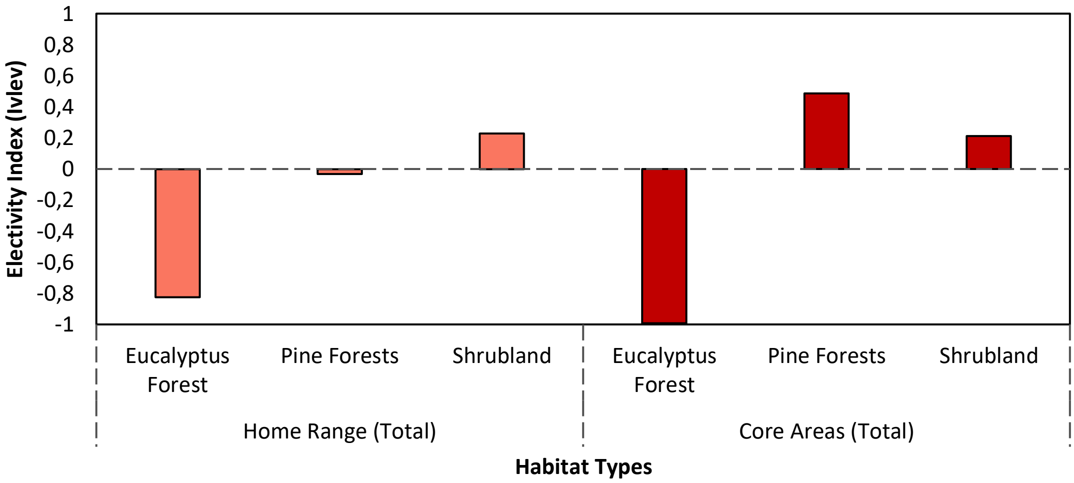

Figure 16. Habitat selection (estimated using Ivlev's Electivity Index) for different habitat types (Eucalyptus forests; Pine forests; Shrubland) inside the Home Ranges and Core Areas of two free-ranging horses in Serra de Sta. Luzia, monitored by GPS telemetry.

Regarding topographic features, the tagged horses occupied areas with a mean altitude of 439m (sd=24.5) and comprising flatter regions with a mean slope of 16% (sd=10%) (Fig. 17).

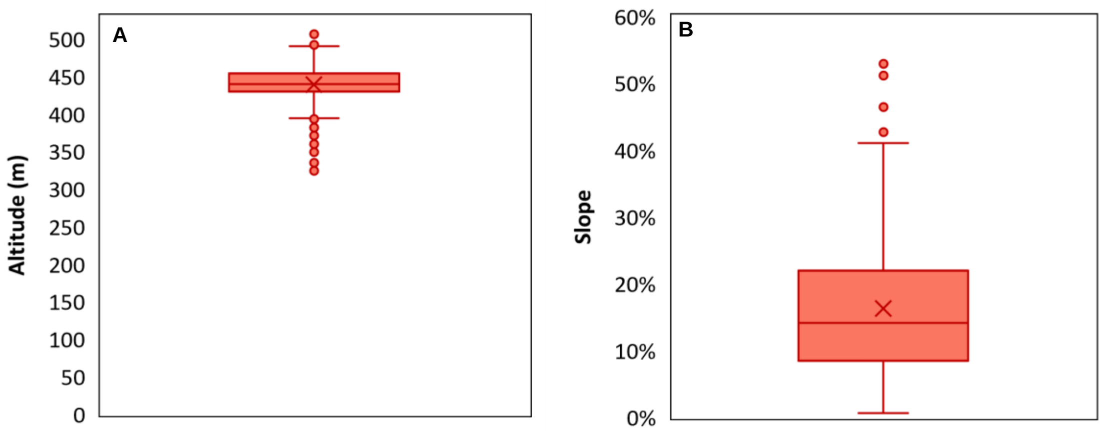

Figure 17. Average values and standard deviation of A) altitude and B) slope used by two free-ranging horses in Serra de Sta. Luzia, monitored by GPS telemetry.

4.2.2. Circadian patterns in space use and habitat selection

The areas occupied by the tagged horses during day and night periods were, in general, similar in terms of size and shape. For Horse I, diurnal and nocturnal home ranges (Diurnal: 1.7 Km^2; Nocturnal: 1.6 Km^2) and core areas (Diurnal: 0.5 Km^2; Nocturnal: 0.4 Km^2) extensively overlapped. However, core areas were composed of two small areas during the day which merged into a single core area during the night. For Horse II, home ranges (***Diurnal:*** 2.2 Km^2; ***Nocturnal:*** 2.2 Km^2) and core areas (***Diurnal:*** 0.6 Km^2; ***Nocturnal:*** 0.6 Km^2) were almost identical across the two circadian periods. Horse II presented two core areas, where one was slightly smaller during night while the other was slightly larger (Fig. 18; see Appendix IV, Table S9, Fig. S8).

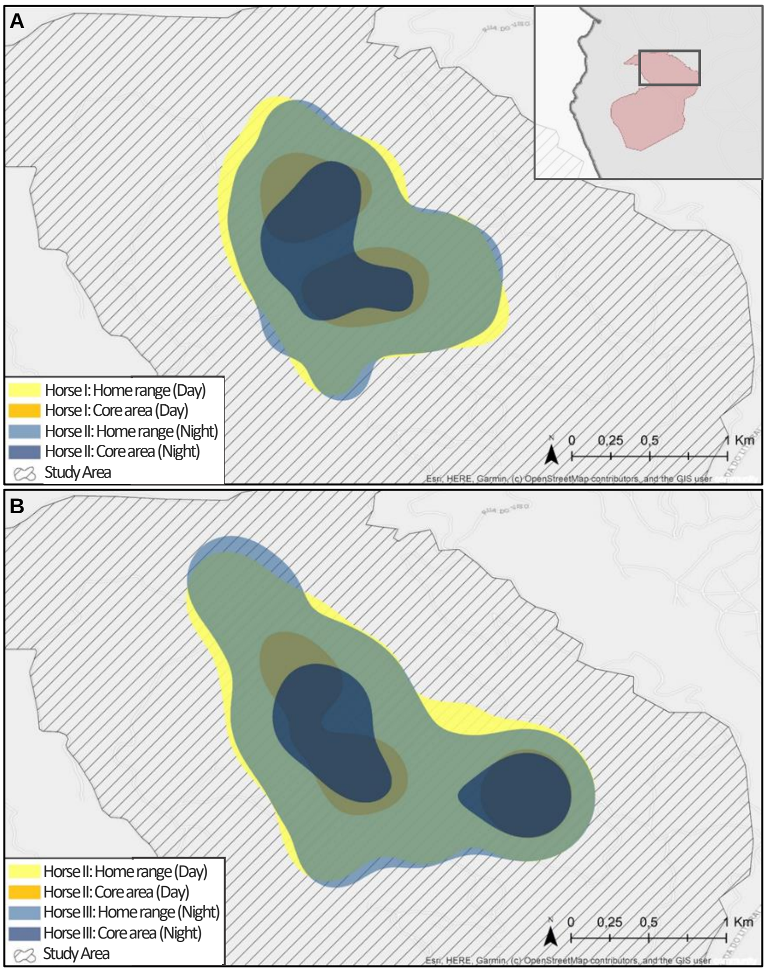

Figure 18. Home ranges and Core areas of two free-ranging horses in Serra de Sta. Luzia during Day (in yellow) and Night (in blue), estimated using GPS locations obtained between April 18 and June 13, 2020: A) Tagged horse I; B) Tagged horse II. See Appendix IV, Fig. S8 for space use of each horse separately.

The diurnal and nocturnal habitat use and selection patterns of both horses (with their data pooled together) follow the general patterns observed for the overall tracking period (see Figs. 16-17). Habitat selection is similar for both the home ranges and core areas

(Fig. 19). The most evident circadian variation was observed for the selection of pine forests in the core areas, which were used according to their availability during the day (0.09) but were positively selected during the night (0.5). Contrastingly, selection patterns for eucalyptus forests (negative) and shrublands (slightly positive) remained similar across the two circadian periods (Fig. 19; Appendix IV, Fig. S9).

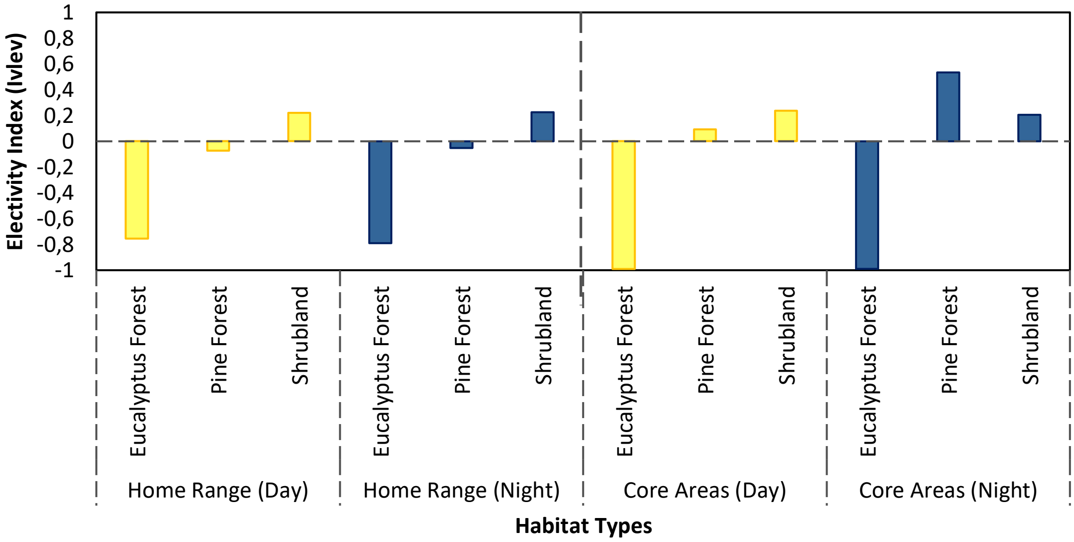

Figure 19. Habitat selection (estimated using Ivlev's Electivity Index) for different habitat types (Eucalyptus forests; Pine forests; Shrubland) inside the Home Ranges and Core Areas of two free-ranging horses in Serra de Sta. Luzia, considering day (07:00-18:59) and night (19:00-06:59) periods.

Regarding topographic features, the tagged horses occupied the same altitude range during both circadian periods (Day: 438.1m, sd=22.8; Night: 440.0m, sd=26.1). The same pattern was observed for slope, with horses occupying areas with lower slopes during both circadian periods (Day: 16%, sd=10%; Night: 16%, sd=10%) (Fig. 20).

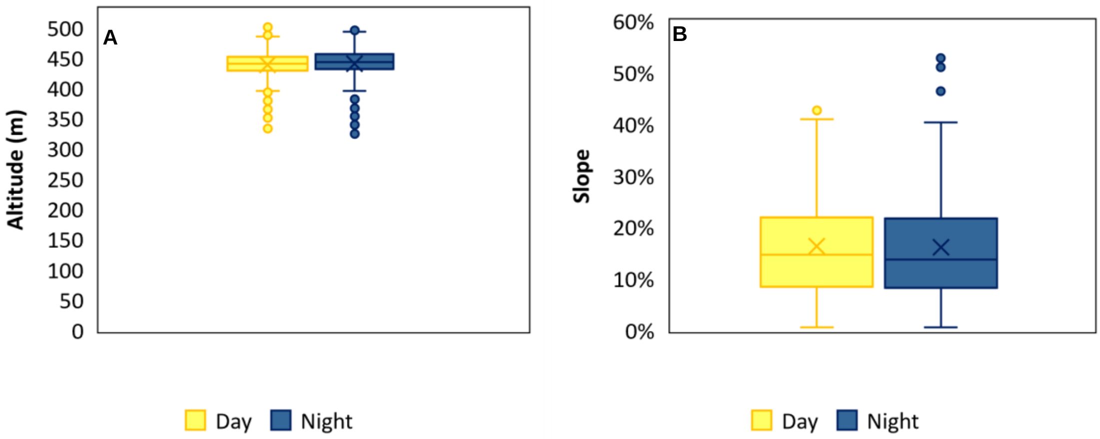

Figure 20. Average values and standard deviation of A) altitude and B) slope used by two free-ranging horses in Serra de Sta. Luzia, during day (07:00-18:59) and night (19:00-06:59) periods.

4.2.3. Temporal reoccurrence in space use

Both tagged horses showed different movement patterns across the tracking period, as revealed by the analysis of their space use during consecutive weekly periods (Fig. 21; Appendix IV, Figs. S10-11). Horse I generally occupied the same area over the eight weeks of tracking, although with a slight variation in the size of its home range and core areas. During week 2 this horse occupied the largest area (Home Range: 2.0 Km^2; Core Areas: 0.5 Km^2), while in week 6 this horse used a smaller home range and core area (Home Range: 1.1 Km^2; Core Areas: 0.3 Km^2) (Fig. 21; see Appendix IV, Fig. S10).

Contrastingly, Horse II showed a wider variation in space use across the tracking period, which was reflected in the size of both its home range and core areas. On the first two weeks of tracking, Horse II occupied a small patch on the study area (Home Range: 0.03 Km^2; Core Areas: 0.006 Km^2), which increased substantially by week 3 (Home Range: Horse II: 1.8 Km^2; Core Areas: Horse II: 0.4 Km^2), reaching similar sizes compared to Horse I. On the following two weeks the home range of Horse II slightly decreases in size (although maintaining the size of its core area) and increases once again after week 6, reaching its largest home range size on week 8 (***Home Range:*** 2.1Km^2; ***Core Areas:*** 0.7 Km^2) (Fig. 21; see Appendix IV, Fig. S11).

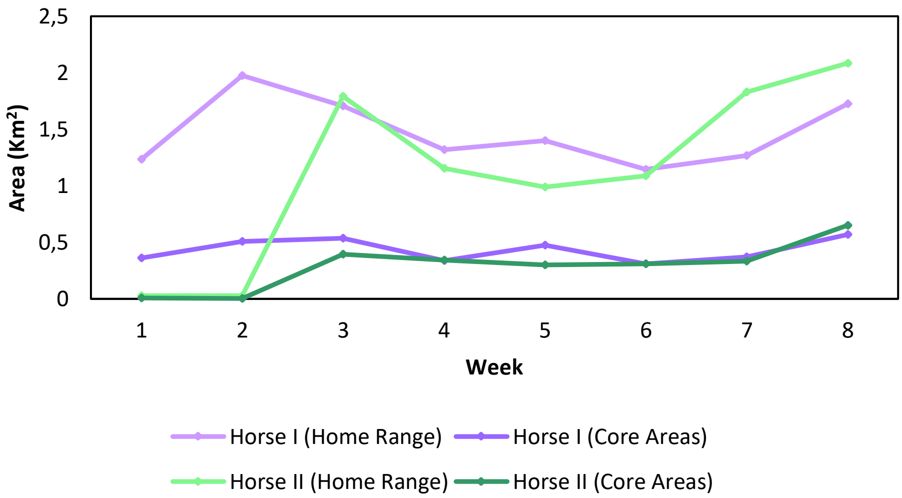

Figure 21. Home range and Core area sizes of two free-ranging horses in Serra de Sta. Luzia during consecutive weekly periods, estimated using GPS locations obtained between April 18 and June 13, 2020. Tagged Horse I is represented in purple shades; Tagged Horse II is represented in green shades.

4.3. Discussion

Despite the limited sample size regarding number of tagged horses and tracking period, our findings provide valuable and innovative information on the spatial ecology of free-ranging *Garrano* horses, by using GPS tail-tags for the first time in Portugal. Currently, there are no previous studies specifically addressing the spatial ecology of the *Garrano* breed, and most of the information on its movements and space use is derived from general descriptions of the breed (e.g. Bárcena, 2012; D'Andrade, 1938; Leite, 2012; Lima, 1872; Portas, 2020) or as a product of indirect evidences in other ecological studies (e.g. Gomes, 1996; Oom & Reis, 1986). Thus, the characterization of general spatial ecology of *Garrano* horses using telemetry conducted in this chapter provides valuable insight into some basic patterns in terms of space use (e.g., home range, core areas and movement reoccurrence) and habitat selection, particularly during mid-late spring. Taking into consideration the small sample size that was used in this study (2 tagged horses) as well as the limited tracking period (approx. 2 months), the analysis developed was primarily descriptive in nature, instead of making statistical inferences that would most likely be biased.

The tagged *Garrano* horses occupied small home ranges of approximately 1.5-2 Km^2, with core areas of 0.45-0.53 Km^2. There is no previous information on the home range sizes for *Garrano* horses, but when comparing with values reported for other free-ranging horse populations it is possible to observe that *Garranos* use a much smaller territory than feral or wild horses. In fact, reported home range sizes for feral horse populations in North America and New Zealand are 73-303 Km^2 (Miller, 1983b), 12 Km^2 (Ganskopp & Vavra, 1986), 1-17 Km^2 (Linklater *et al.*, 2000), 12-90 Km^2 (Girard *et al.*, 2013a) and 40 Km^2 (Hennig *et al.*, 2018), while for wild horse populations (e.g. Przewalski's horses) in Central Asia reported home range values are 1-24 Km^2 (King & Gurnell, 2005) and 471 Km^2 (Kaczensky *et al.*, 2008). These large differences in home range sizes between free-ranging horse populations are expected, given the fact that they can be substantially affected by the amount of available forage and water, herd and population sizes, or exposure to human-related disturbance (Ganskopp & Vavra, 1986; Girard *et al.*, 2013a; King, 2002; McCort, 1984; McInnis, 1984). Concerning the home range sizes obtained for *Garrano* horses in our study area, several factors might justify the observed low values: 1) the short tracking period comprising only two months during mid-late spring, coinciding with foaling season when movements are more limited (Gomes, 1996); 2) the level of husbandry management and vigilance by owners, which can greatly limit horse movements (Serôdio, 1992); 3) the limited area with mountain pastures available in Serra de Sta. Luzia, which only encompasses approximately 20 Km^2; and 4) the fact that horses might occupy a small area due to high resource availability in the area, which minimizes the need to make wide movements in search of food or water (King, 2002; Linklater *et al.*, 2000). Future studies should address the effect of these factors in home range and core area sizes by assuring a longer tracking period (at least a yearly cycle) as well as collecting information regarding horse management by owners, herd size of tagged horses, resources availability and population sizes inside study areas. The home ranges of the two tagged horses in Serra de Sta. Luzia overlapped almost completely, corroborating the initial hypothesis that *Garrano* horses occupy non-exclusive home ranges, similar to other horse populations (Keiper, 1986). Different degrees of overlap between tagged individuals have been reported on other feral and wild horse populations, ranging from limited overlap (Girard *et al.*, 2013a; King, 2002) to almost complete intersection (Ganskopp & Vavra, 1986; Linklater *et al.*, 2000; McInnis, 1984; Miller, 1983b), corroborating our results. However, it is not possible to use this finding alone to confirm if the horses belong to the same herd, due to the fact that territoriality in horses is expressed as the defence of the social group instead of the defence of a territory (Keiper, 1986; Leite, 2012). For instance, Oom & Reis (1986) have previously described that *Garrano* herds in Peneda-Gerês National Park move and do their daily activities within large overlapping home ranges, with harems in close proximity delimiting their space with faecal piles or aggressive behaviour.

Within home ranges, free-ranging *Garrano* horses selected available habitats in a different manner. More specifically, eucalyptus forests were strongly avoided, especially within the core areas, pine forests were mostly used according to their availability but seemed to be positively selected inside core areas during night-time, and shrublands were slightly positively selected in general. *Garrano* horses occupy a human-dominated landscape with characteristics that are not common to locations from previous studies on horse habitat selection, which curtails the comparability between results. In fact, the geographic range of *Garrano* horses is not composed by grassy plains or steppes, as in most other populations of feral horses (Ransom & Kaczensky, 2016), but by mountainous habitats with more closed vegetation, such as shrublands and forest plantations (Bárcena, 2012; Perez, 2012). Furthermore, *Garrano* horses are heavily predated, a scenario not observed in most of the previous studies focusing horse spatial ecology (Bárcena, 2020; Freitas, 2019; Perez, 2012). Nonetheless, some general patterns of habitat selection from our tagged *Garrano* horses are similar to other feral horse populations, as *Garrano* horses in Serra de Sta. Luzia seem to prefer open habitats (in this case, shrublands) and avoid forested areas (in this case, eucalyptus forests), similar to previous findings for other free-roaming horse breeds (Bhattacharyya, 2012; Girard *et al.*, 2013a; Linklater *et al.*, 2000). This pattern in *Garrano* horses might be explained by two main factors. Firstly, forage quality and abundance is one of the primary determinants for habitat selection on horses, which makes open areas and shrublands typically richer in terms of grasses and small shrubs that are more preferable for horses in general (Crane *et al.*, 1997; Duncan, 1983; Salter & Hudson, 1979), and for *Garranos,* in particular. In fact, *Garrano* horses feed mostly on native spiny shrubs (*Ulex* sp., *Genista* sp.) (Portas, 2020) that dominate mountain shrublands in NW Iberia but tend to be less available inside forest plantations, particularly of Eucalyptus. Secondly, free-ranging horses, including in NW Portugal, have been found to be exposed to a higher predation closer to or inside forests and tend to avoid these forested habitats to reduce predation risk (Girard *et al.*, 2013b; Rio-Maior *et al.*, 2006; Van Duyne *et al.*, 2009). This anti-predatory strategy reflected on habitat selection is also reported for other domestic and wild ungulates (Creel *et al.*, 2005; Meriggi & Pagnin, 1994; Muhly *et al.*, 2010). The preference for pine forests in core areas during night-time by our tagged *Garrano* horses is, however, an unexpected finding, considering that this also constitutes a forested area with potential risk of predation and that conifer forests have been previously reported to be specially avoided during spring in Girard *et al.* (2013a), due to low forage availability after winter in comparison to other habitats (e.g. grasslands). However, other studies have reported pine forests to be positively selected by free-ranging horses, particularly at night for shelter (Jodkowska *et al.*, 2015), or as an extra source of forage (e.g. pine needles) (Bhattacharyya, 2012). Therefore, further studies are necessary to ascertain if pine forest selection is observed for the whole *Garrano* breed or if it is particular to the horses in Serra de Sta. Luzia.

In terms of topography, the tagged horses in our study area occupied areas at high altitudes (an average of 440m, in a mountain with 550m as the highest peak) and with low slopes (averaging around 16%), both during their diurnal and nocturnal activities. Considering the period in which this study took place – i.e. mid to late Spring – the elevation value corroborated previous reports for the *Garrano* horses in NW Iberia, as they move up the mountains during spring (namely starting on March/April) and stay at higher elevations throughout the summer season, after which they move to lower altitudes to withstand the harsh winter conditions in more sheltered sites (Leite, 2012; Portas, 2020). Therefore, *Garrano* horses were expected to be found on the higher areas of the mountains they inhabit, as observed. Contrastingly, Oom & Reis (1986) reported an opposite pattern, in which free-ranging *Garrano* horses in Serra do Gerês were found at higher elevations only during winter due to water scarcity at those high altitudes on other seasons, but this is not expected to be an issue on Serra de Sta. Luzia, in which permanent humid pastures and streams are frequent at higher altitudes (CM Viana do Castelo 2017). The low slope values observed in our study followed the pattern documented in feral horse populations worldwide, in which horses prefer flat or slightly sloping areas (Girard *et al.*, 2013b; Vavra & Ganskopp, 1987). However, since *Garrano* horses are known to be more adapted to mountainous and rugged terrain than their feral counterparts worldwide (Bárcena, 2020; Gomes, 1996), it is possible that a higher range of slope values can be used by this horse breed, but was not detected due to the limited tracking period.

Almost no relevant variation was found on the size of home ranges and core areas across time periods, either between day/night or consecutive weeks. Regarding the variation on home ranges between day/night periods, it seems that *Garrano* horses use the available space equally throughout a circadian cycle, which is further confirmed when considering the similarity between the use of habitat types, altitude, and slope for the diurnal and nocturnal periods. The most curious finding was the positive selection of pine forests at night, which requires further testing to form a robust conclusion. All previous spatial data from *Garrano* horses was collected by direct observation, which took place during the day (Oom & Reis, 1986), and horse circadian patterns are rarely reported in the literature and are not consistent across the whole range. In fact, King (2002) reports that Przewalski horses in Mongolia occupied low valleys during mornings and evenings and forests or rocky areas at higher altitudes in the middle of the day, a pattern that changed seasonally. Furthermore, Jodkowska *et al.* (2015) report that horses in Poland occupied meadows during the day, and forested areas at night. Therefore, there was no specific *a priori* expectation regarding the nocturnal activity of *Garrano* horses and our data provides valuable insight over this topic. Regarding the weekly variation between the home ranges of the two tagged mares, they appear to be quite mobile within their home ranges. However, there was no evidence of a migration during our study, with the horses staying within the northern limit of Serra de Sta. Luzia during the entire tracking period. For instance, the sudden increase in home range and core area sizes of Horse II in week 3 was not related to an ecological event but reflected the release of this mare from confinement the week prior, which had to stay confined longer due to birthing a foal. Temporal variations in horse movement and space use are generally related to the time of day (Jodkowska *et al.*, 2015; King, 2002) or different seasons (Girard *et al.*, 2013a; Leite, 2012; Linklater *et al.*, 2000; Portas, 2020; Salter & Hudson, 1980), so no significant changes were expected considering our short tracking period.

In conclusion, GPS tracking devices can improve our knowledge on spatial ecology of free-ranging livestock as well as provide managers and owners with scientific-based information for an adequate herd management, due to: 1) collecting robust data independently climatic and landscape constraints or the visual detection of individuals in the field (Cagnacci *et al.*, 2010; Frair *et al.*, 2010; Millspaugh & Marzluff, 2001) and 2) providing information that is not obtainable by direct observation, such precise home range sizes or night-time space use patterns (Hebblewhite & Haydon, 2010; Oom & Reis, 1986). In fact, our GPS tracking provided some basic metrics, innovative insights, and updated information regarding the spatial ecology of free-ranging *Garrano* horses during spring. Despite the limited sample size, free-ranging horses in Serra de Sta. Luzia seem to occupy small, non-exclusive home ranges composed mostly by shrublands located at high altitudes and flat slopes. In terms of habitat selection, eucalyptus forests seem to be highly avoided while shrublands are slightly selected and pine forests are positively selected at night inside core areas. Finally, no major changes in space use across time periods were observed. Notwithstanding, this analysis was exploratory in nature and should be regarded as preliminary data for several reasons. Firstly, the used datasets from the GPS tail-tags specially developed for this study had a low fix success rate which, although apparently not related to landscapes features (see **Study III**), can introduce bias in the obtained results, especially for habitat selection (Frair *et al.*, 2010; Parraga Aguado *et al.*, 2017). Secondly, although we decided to use the whole dataset in this analysis in order to have enough information to yield representative results, it is likely that the GPS locations from both horses have some degree of autocorrelation (Frair *et al.*, 2010; Girard *et al.*, 2013a). Lastly, the small sample size of tagged horses (n=2) and short tracking period (n=57 days) of this study did not allow us to take strong conclusions from our findings and explore some spatial patterns at a larger scale. Additionally, it is important to note that direct observation still provides important qualitative data relevant to spatial ecology analyses, such as animal behaviour or social organization (Hebblewhite & Haydon, 2010), despite the advantages of telemetry. For instance, telemetry data alone was not enough to conclude if the tagged horses belonged to the same herd.

5. STUDY III: Assessing the performance of tail tags for equid studies

5.1. Methodology

5.1.1. Data collection

To evaluate the field efficiency of the new GPS tail-tags firstly developed to study the spatial ecology of free-ranging *Garrano* horses in northwest Portugal, the original telemetry datasets collected in scope of **Study II** were used to perform a methodological assessment regarding the durability and fix success frequencies (defined in section **5.1.2**) of the tail-tags under different reception schedules, daily periods, and eco-climatic conditions. The tail-tags collected locations between 18th April and 13th June 2020 in Serra de Sta. Luzia (Viana do Castelo, Portugal), before being retrieved from the animals. Each tag had a different reception schedule: one collected GPS-locations every two hours, while the other collected locations every thirty minutes. The datasets were adapted to include all the expected entries (i.e. GPS locations) considering the tracking period (18th April-13th June) and reception schedules (2hr and 30 min). The entries were then recategorized into sampled/unsampled locations: entries with sampled locations (also called fixes) were represented as 1, while entries without sampled locations were represented as 0.

In order to determine the effect of eco-climatic conditions on tag performance, information on seven climate variables (namely temperature, relative humidity, solar radiation, atmospheric pressure, wind velocity and direction, and total precipitation) were obtained from the meteorological station of the Monitoring and Environmental Interpretation Centre of Viana do Castelo (41.696779 N -8.818148 W) (CMIA 2020). Furthermore, landscape-related features such as forest canopy, altitude and slope, were considered relevant to evaluate the reception of locations of the tail tags but were ultimately excluded due to the fact that data from the georeferenced datasets (described in section **4.1.1**) could not be collected for unsampled (0) entries.

5.1.2. Data analysis

The performance of the GPS tail-tags was evaluated according to durability and fix success frequency. Following the approach in Kluever *et al.* (2012), durability was defined by the lifespan and integrity of the tail-tags. Lifespan was determined as the total number of days a tag stayed attached to the animal. Integrity was determined as the total number of days a tag collected locations before becoming compromised: if a tag stopped collecting locations indefinitely, it was considered compromised. Fix success frequency was defined as the proportion of fixes (1) in relation to the total number of expected locations (0+1). Daily, weekly, and monthly fix success frequencies for each tag were calculated to 1) determine if integrity was compromised during the study; 2) assess how fix success frequencies varied over the tracking period. Daily frequencies encompassed 57 days (18 April-13 June); weekly frequencies encompassed the 8 weekly intervals of 7 days (i.e. 18-24 April; 25 April-1 May; 2-8 May; 9-15 May; 16-22 May; 23-29 May; 30 May-5 June and 6-13 June); monthly frequencies encompassed 3 months (April, May and June). To assess the effect of reception schedules (2hr or 30 min) on reception of locations by the tags, the variation between the daily fix success frequencies of each tag was evaluated using box-plots graphs with summary statistics (average and standard deviation) and the Wilcoxon test, a non-parametric significance test equivalent to the t-test (Woolson, 2007).

The effect of daily periods and eco-climatic factors on the reception of locations was evaluated separately for each tag, given that the two tags showed significant differences in fix success frequency considering reception schedules (see **sections 5.2** and **5.3**). To evaluate the effect of daily periods, the daily fix success frequencies for diurnal (collected from 07:00-18:59) and nocturnal (collected from 19:00-06:59) locations were evaluated using box-plots graphs with summary statistics and the Wilcoxon test. To evaluate the effect of different eco-climatic conditions, density plots were used to observe at which climatic values there was a higher density of sampled (1) and unsampled (0) locations. Each climate variable considered for this study (temperature, relative humidity, solar radiation, atmospheric pressure, wind velocity and direction, and total precipitation) was analysed separately.

All the analyses were performed on R 4.0.4 (R Core Team, 2021) and RStudio 1.4.1106 (RStudio Team, 2021).

5.2. Results

The lifespan and integrity values for both tail-tags were of 57 days, corresponding to the duration of tracking period. Regarding lifespan, both GPS tags remained attached to the horses for the whole duration of the tracking period. Regarding integrity, none of the tags showed a compromise in integrity, i.e. a fix frequency success of 0% for an indefinite time interval, at any point of the study (Appendix V, Figs. S12-14).

Reception schedule (2hr or 30min) significantly affected the reception of locations (Wilcoxon: W=2692.5, p<0.0001). The tail-tag with a location periodicity of two hours had a better performance, receiving, on average, 41% of expected locations (sd=0.1). The tag with a location periodicity of thirty minutes received, on average, 27% of expected locations (sd=0.08) (Fig. 22).

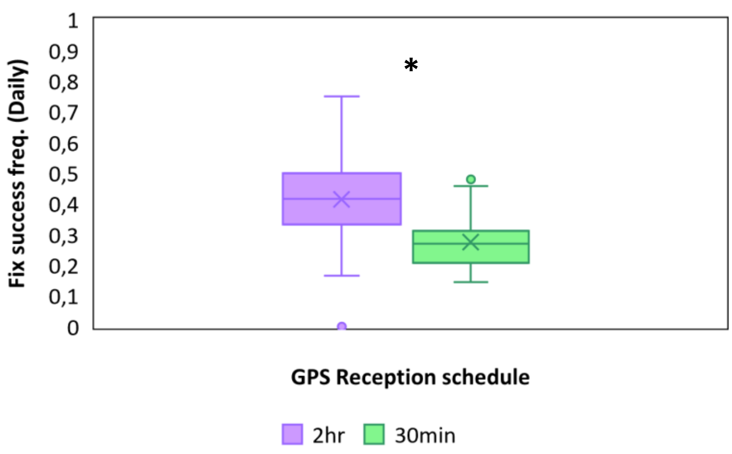

Figure 22. Average values and standard deviation of daily rates of received locations (fix success frequency), for two GPS reception schedules: periodicity of 2 hours (in purple) and 30 minutes (in green). Significant results marked with *.

The daily fix success frequency was highly variable for both tags: the 2hr tag received between 0% (31st May) and 75% (3rd May) of expected locations per day; the 30min tag received between 14% (25th April) and 47% (18th April) of the expected locations per day (Appendix V, Fig. S12). The weekly and monthly fix success frequencies were more constant over the tracking period, indicating that efficiency in receiving locations did not evidently increase or decrease over time. May was the month with the best performance for both tags (2hr: 44%; 30-min: 29%), particularly between 16th and 22nd May (week 5; 2hr: 52%; 30min: 31%) (Appendix V, Figs. S13-S14).

Time of day did not significantly affect the reception of locations for any of the tags (2hr: Wilcoxon: W=1613.5, p>0.05; 30min: Wilcoxon: W=1496, p>0.05). Additionally, the average diurnal and nocturnal performance rates were similar to the general performance rates (see Fig. 22). The 2hr tag received an average of 41% locations by day (sd=0.2) and 42% locations by night (sd=0.2); the 30min tag received, on average, 27% locations by day (sd=0.1) and 27% locations by night (sd=0.09) (Fig. 23).

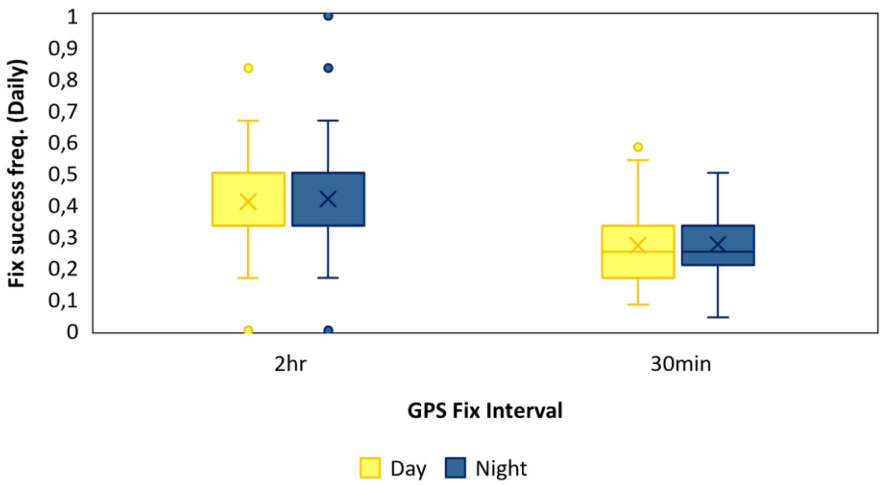

Figure 23. Average values and standard deviation of daily rates of received locations (fix success frequency), during day (07:00-18:59) and night (19:00-06:59) periods, for two GPS reception schedules (periodicity of 2 hours and 30 minutes).

Eco-climatic conditions had no effect on the reception of locations of both tail-tags, with the distributions of sampled and unsampled locations overlapping almost completely (Fig. 24). No variation between distribution curves was observed for temperature (Fig. 24A), wind velocity (Fig. 24E) and total precipitation (Fig. 24G). Limited variation was observed on the relative humidity (Fig.24B), radiation (Fig. 24C), pressure (Fig. 24D) and wind direction (Fig. 24F): for both tags, a slightly higher density of unsampled locations is observed for higher humidity values as well as lower radiation and pressure values; for the 30-min tag, a slightly higher density of unsampled locations is observed for higher wind direction values (Fig. 24).

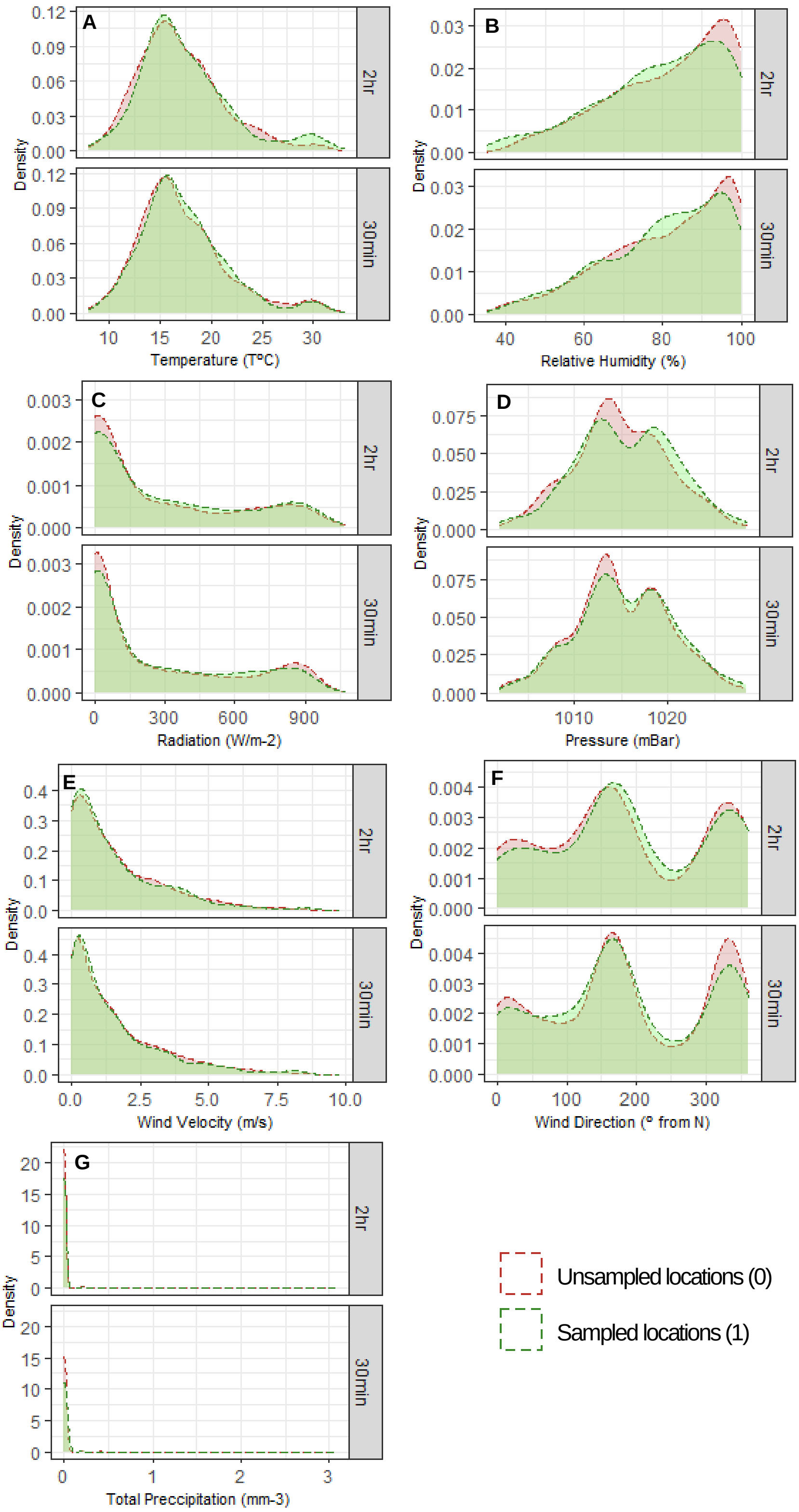

Figure 24. Differences in eco-climatic values for unsampled (in red) or sampled (in green) locations, for two GPS reception schedules (periodicity of 2 hours and 30 minutes). The climatic conditions considered were: A) Temperature; B) Relative Humidity; C) Radiation; D) Atmospheric Pressure; E) Wind Velocity; F) Wind direction; G) Total Precipitation.

5.3. Discussion

Despite the limited number of tags (2 tagged horses) and tracking period (57 days) of this study, this chapter provided relevant information regarding the durability and performance of the innovative GPS tail-tags developed for this study. This work was the first instance in which tail tags were used to study spatial ecology patterns of free-ranging horses (**Study II**), and the second time tail-tags were used in horses in general, as Schoenecker *et al.* (2020) firstly used tail-tags in a welfare/technical study including captive horses. Due to their novelty, the findings obtained in this study are highly relevant to understand the current feasibility of using tail-tags to perform biological assessments and assure further optimization for future studies.

The tags deployed on the horses seem durable, remaining attached to the animals until the end of the tracking period without any compromise of integrity, as expected. The observed lifespan was smaller than the value reported by Schoenecker *et al.* (2020), where tail-tags remained attached to the horses an average of 221 days. Likewise, studies on free-ranging horses using different tracking devices reported higher average lifespans, such as 139 days for ear tags (Kluever *et al.*, 2012; Lagos & Bárcena, 2010) and 188 days for collars (Collins *et al.*, 2014). The tail-tags used in this study were not retrieved because of technical issues (e.g. battery life, detachment from the tail), and it is possible that the observed lifespan does not reflect the true durability of the device. In terms of integrity, only one study performed on free-ranging horses with VHF ear-tags (Kluever *et al.*, 2012; Lagos & Bárcena, 2010) reported a loss of emission signal caused by antenna damage (after 71 days), which might indicate that this is not a common issue on studies focusing free-ranging horses. Considering the average lifespan reported on other publications and the durability of the tags in this study, the GPS tail-tags seem fit to conduct short-term studies (with the duration of up to two months). However, their durability over the two-month tracking period is currently unknown.

In terms of successfully receiving locations, both tags performed unsatisfactorily, receiving only an average of 27% or 41% of all expected locations. Despite the lack of reported fix success frequencies on previous literature, a comprehensive study on Przewalski's horses and other large mammals in Mongolia documented reception rates of 79-97% for GPS collars (Kaczensky *et al.*, 2010), which are noticeably higher than the rates obtained in our GPS-tags. Besides, an average fix success of 75% has been reported for studies on terrestrial mammals in general (Hofman *et al.*, 2019). Although the time of day has been previously observed to have effects on GPS performance on other mammals (Frair *et al.*, 2010; Parraga Aguado *et al.*, 2017), the average diurnal and nocturnal success frequencies for the tail-tags showed no variation between both circadian periods. Likewise, the selected eco-climatic factors showed no influence on reception of locations. In fact, the climatic conditions associated with sampled and unsampled locations were almost identical, confirming that climate has not affected the performance of GPS-tags. Climate has been reported to have some indirect effect on GPS performance, because certain animal behaviours associated with climate (e.g. rest periods, search for shelter) can cause shifts in antennae orientation or block satellite reception (e.g. on ibex; Parraga Aguado *et al.*, 2017). However, its effect has generally been observed at the seasonal scale and it would be unlikely to see any effects on our study. Three hypotheses that could not be tested for this study can provide a plausible explanation for the low success of the GPS-tail tags. Poor tag performance can be the result of unknown technical issues affecting the collection and/or transmission of location data, possibly prior to tag deployment since there was no sudden decrease in performance throughout the study. Contrastingly, the reception and/or communication of location data may have been affected by the different weights of each tag or due to landscape features, such as canopy cover or complex topography. Available literature on GPS telemetry indicates canopy closure and topographic complexity as the predominant factors obstructing GPS satellite reception (Cain *et al.*, 2005; Di Orio *et al.*, 2003; Girard *et al.*, 2013a; Janeau *et al.*, 2004; Lewis *et al.*, 2007 and others). Besides, these hypotheses provide a more logical explanation for the huge discrepancy between the daily frequency values. Considering that the availability of pine or eucalyptus forests on the tagged horses' home ranges is minimal (see **Study II**), canopy density seems unlikely to significantly affect the reception success of the tail tags in this particular study. However, further testing is necessary to accurately assess the impacts of canopy and topography on tag reception.

Finally, the success frequencies for the two reception schedules were significantly different, with the 30min tag receiving, on average, 12% less locations than the 2hr tag. This result contrasts with previous literature from terrestrial animals, where tags with shorter location periodicity (e.g. reception every 30 minutes) tend to have higher success frequencies (Cain *et al.*, 2005; Janeau *et al.*, 2004; Lewis *et al.*, 2007; McGregor *et al.*, 2016). Tags have a time limit to receive a location, and tags with longer schedules cannot use previous data to receive information quicker, receiving less locations overall (Cain *et al.*, 2005). The variation observed in this study was not related to either the time of day or the eco-climatic conditions tested. Since the home ranges of the two horses largely overlapped (see **Study II**), topography and canopy do not seem to be related to this variation as well. Additionally, due to the small sample size, the variation may be related to other coincidental factors. For example, the 30-min tag could have a malfunction affecting the reception or transmission of locations; or the horse could have spent more time laying down, incidentally covering the tag more often. Thus, caution is needed to make assumptions regarding this variation before increasing sampling effort.

In conclusion, tail tags could be a good alternative to other traditional tracking devices as they seem to be a durable device that provides a solution to the common problems observed on previous tags used on horses, such as 1) the inability to use collar tags on certain individuals (specifically male horses) to avoid damage to the tags caused by fighting or grooming behaviours (Collins *et al.*, 2014; Girard *et al.*, 2013a); 2) the potential for ear tags and collars to be invasive by involving the piercing of the ear and/or the possibility to cause injuries (e.g. chaffing, hair loss) to horses, specifically in juveniles whose neck is still developing (Collins *et al.*, 2014; Lagos & Bárcena, 2010; Schoenecker *et al.*, 2020); and 3) the frequent detachment of ear tags that become entangled in the vegetation (Lagos & Bárcena, 2010). At the same time, the positioning on the tail makes tail tags more susceptible to shifts in tag orientation or obstruction in satellite reception while horses are in a resting position (D'eon & Delparte, 2005; Jiang *et al.*, 2008; Parraga Aguado *et al.*, 2017) which can potentially decrease fix success frequencies. Additionally, our analyses have shown that they need further optimization before being deployed on large-scale experiments. The tags collected less locations than expected and no clear explanations for this finding have been revealed so far, which can lead to an incomplete picture of the spatial ecology of the tagged horses. For instance, low fix success rates may result in under-sampling of habitats that are less used or have less satellite reception, constraining results of habitat use and selection (Cain *et al.*, 2005; Di Orio *et al.*, 2003). Furthermore, when the probability of obtaining fixes is broad (e.g. 50% to 100%), estimates of home range areas can be biased (Frair *et al.*, 2010; Parraga Aguado *et al.*, 2017).

6. General conclusions and future research

This work increased our understanding on the ecology of free-ranging horses in northwest Portugal, by providing valuable information on the factors influencing the predator-prey relationship between free-ranging horses and the Iberian wolf and the basic space use patterns of *Garrano* horses. Additionally, the GPS tail-tag specially developed for this study provides an innovative approach to increase our understanding of the spatial ecology of other horse populations worldwide.

Similar to previous studies from Europe and Central Asia (Freitas *et al.*, *in press;* Gula, 2008; Hovens & Tungalaktuja, 2005; Imbert *et al.*, 2016; Meriggi & Lovari, 1996; Pimenta *et al.*, 2018), **Study I** highlighted the relevance of free-ranging horses on wolf diet in areas seemingly with low diversity and consumption of alternative prey and high horse availability, resulting in a strong wolf preference towards this animal. However, none of the variables included in this study significantly affected the intensity of free-ranging horse consumption. In fact, some of the selected variables, such as the level of herd management by horse owners and wolf abundance, showed no influence on horse consumption despite being frequently mentioned in previous literature as a relevant factor for horses (Álvares, 2020; Freitas, 2019; Namgail *et al.*, 2007; Pimenta *et al.*, 2018) and for other livestock species (Davie *et al.*, 2014; Dondina *et al.*, 2015; Pimenta *et al.*, 2017). Therefore, the inclusion of additional study areas (e.g. other regions in the Iberian Peninsula and worldwide) and different variables (e.g. quantifying predation risk or level of horse vigilance and management) in future studies is recommended. In particular, we highly suggest the use of alternative variables to assess the effect of husbandry systems on horse consumption, given that the current compensation scheme inadequately compensates attacks on free-ranging horses (Freitas, 2019; Milheiras & Hodge, 2011). For example, questionnaires conducted on horse owners regarding local management practices, horse productivity and herd social structure could provide specific data on damage prevention measures previously reported to influence livestock predation rates, such as: 1) use of guarding animals (Newsome *et al.*, 2016; Petrucci-Fonseca *et al.*, 2000); 2) use of fences (Pimenta *et al.*, 2017); 3) frequency of vigilance and removal of animals from the population (Álvares, 2011; Lagos, 2020); 4) quality of sanitary conditions (Álvares, 2020; Guerra, 2004); 5) age and sex of animals (Gomes, 1996; Guerra, 2004; Lagos, 2013); or 6) medium band size (Gomes, 1996; Mech *et al.*, 2000; Meriggi & Lovari, 1996).

The use of telemetry in **Study II** provided innovative information on spatial ecology patterns of *Garrano* horses that could not be obtained by direct observation, such as home range and core area sizes and nocturnal space use patterns. *Garrano* horses share similar space use and habitat selection patterns with other feral horse populations worldwide (Ganskopp & Vavra, 1986; Girard *et al.*, 2013a; Keiper, 1986; Linklater *et al.*, 2000; Ransom & Kaczensky, 2016; Vavra & Ganskopp, 1987), as initially hypothesised. For instance, *Garrano* horses occupy small non-exclusive home ranges at high altitudes and flat slopes and prefer open habitats while avoiding forested areas. However, unique and unexpected space use patterns, namely the use of small home ranges and the positive selection of pine forests in core areas during night-time, were revealed and require further testing. Also, the small sample size and short duration of this study prevented the inference of robust conclusions regarding seasonal spatial patterns. Therefore, future studies should focus on tagging a larger number of horses from different sites across northwest Portugal and for a longer period, in order to allow a more meaningful and complete picture on the spatial ecology of the *Garrano* breed in their mountainous habitats. Namely, tagging horses from different ages, sexes, socials status or landscapes features can be relevant to explore intraspecific variation in space use patterns. Longer tracking periods can unveil seasonal or annual patterns regarding home range and core area sizes and habitat preferences, or potential migrations within home ranges. Additionally, future studies should simultaneously tag free-ranging horse and wolves to further explore the spatial relationship between these two animals and assess the influence of wolf movements in the space use of *Garrano* horses and their anti-predator strategies.

In comparison to other tags previously used in horses (Collins *et al.*, 2014; Kaczensky *et al.*, 2010; Kluever *et al.*, 2012; Lagos & Bárcena, 2010; Schoenecker *et al.*, 2020), the methodological assessment performed on **Study III** showed that GPS tail-tags can be a promising tool for horse spatial ecology, proving to be durable for short-term and, potentially, long-term studies. However, the tags performed unsatisfactorily in terms of receiving locations and need to be further optimised before being widely used in field studies. Future tagging of a larger sample size, over longer periods of time and under different environmental conditions is thus recommended, in other to: 1) assess tag suitability, in terms of welfare as well as reception of locations, for individuals of different ages, sexes and social status; 2) further characterize tag durability, namely over long tracking periods; and 3) explore which problems are affecting fix success frequencies and how these can be optimized before further deployment. Tail-tag performance could be further evaluated by performing stationary tests under semi-controlled conditions, by installing tags on several selected locations encompassing a wide range of known canopy and topography characteristics and comparing fix success rates according to canopy closure, slope, terrain ruggedness, and other environmental variables. Additionally, tagging horses with different tag types (e.g. collars or ear tags) could provide valuable information regarding the performance of different tracking devices under the same ecological settings.

Finally, it is important to communicate the results obtained from this and other similar studies to stakeholders directly involved in the management of the *Garrano* breed, such as horse owners, local and regional horse owner organizations or governmental offices. Negative attitudes from livestock owners towards wolves can make owners reluctant to participate in ecological studies, hindering research efforts (Álvares, 2020; Breck *et al.*, 2012). Therefore, sharing these findings outside the scientific community can build trust on the research being done and improve tolerance regarding future experiments. Additionally, the knowledge regarding the spatial ecology of the *Garrano* breed and its relationship with the Iberian wolf provided by this study allows scientists, managers and horse owners to make more informed decisions regarding the adequate management of this endangered autochthonous horse breed.

7. References

Aldezabal, A., Mandaluniz, N., & Laskurain, N. (2013). Gorse (ulex spp.) use by ponies in winter: Is the spatial pattern of browsing independent of the neighbouring vegetation? *Grass and Forage Science, 68*(1), 49-58.

Álvares, F. (2011). *Ecologia e conservação do lobo (canis lupus, l.) no noroeste de portugal* [PhD Dissertation, University of Lisbon]. Lisbon.

Álvares, F. (2020). *O garrano e o lobo-ibérico: Contexto ecológico e gestão do conflito.* Percursos do Homem e do Garrano - Actas dos Seminários do Projecto, Viana do Castelo.

Álvares, F., Freitas, J. M. A. C. F. d., Abrantes, J., Casimiro, J., & Godinho, R. (2021, 1-3 July 2021). Horse-predator interactions: Ecological settings, human constraints and methodological advances. 1º Congresso Internacional de Equinologia e Turismo Equestre, Viana do Castelo.

Álvares, F., Sá, I., Rodrigues, J. B., & Casimiro, J. (2019). *Manual de convivência com o lobo* [Manual].

Bárcena, F. (2012). Garranos: Os póneis selvagens (equus ferus sp.) do norte da península ibérica. Livro de Atas, I Congresso Internacional do Garrano, Arcos de Valdevez,

Bárcena, F. (2020). *La importancia de los garranos en el mantenimiento de los ecosistemas naturales de montaña: Su interés en las áreas protegidas.* Percursos do Homem e do Garrano - Actas dos Seminários do Projecto, Viana do Castelo.

Barja, I. (2009). Prey and prey-age preference by the iberian wolf canis lupus signatus in a multiple-prey ecosystem. *Wildlife Biology, 15*(2), 147-154.

Bas-López, S. (2017). A dieta das greas de garranos salvaxes (*equus ferus atlanticus*) da parte norte da serra do seixo (pontevedra). *BRAÑA, 15*, 5-11.

Baskaran, N., Ramkumaran, K., & Karthikeyan, G. (2016). Spatial and dietary overlap between blackbuck (antilope cervicapra) and feral horse (equus caballus) at point calimere wildlife sanctuary, southern india: Competition between native versus introduced species. *Mammalian Biology, 81*(3), 295-302.

Begon, M., Howarth, R. W., & Townsend, C. R. (2014). *Essentials of ecology*. Wiley New York (USA).

Begon, M., Townsend, C. R., & Harper, J. L. (2006). *Ecology: From individuals to ecosystems* (Fourth ed.). Blackwell Publishing.

Berger, J. (1977). Organizational systems and dominance in feral horses in the grand canyon. *Behavioral Ecology and Sociobiology*, 131-146.

Bhattacharyya, J. (2012). *Knowing naŝlhiny (horse), understanding the land: Free-roaming horses in the culture and ecology of the brittany triangle and nemiah valley* [PhD Dissertation, University of Waterloo]. Waterloo, Ontario.

Bouman, J. (1986). Particulars about the przewalski horse. *For the Foundation for Preservation and Protection of the Przewalski Horse*, 33.

Boyce, P., Hennig, J., Brook, R., & McLoughlin, P. (2021). Causes and consequences of lags in basic and applied research into feral wildlife ecology: The case for feral horses. *Basic and Applied Ecology*.

Breck, S., Clark, P., Howery, L., Johnson, D., Kluever, B., Smallidge, S., & Cibils, A. (2012). A perspective on livestock–wolf interactions on western rangelands. *Rangelands, 34*(5), 6-11.

Burns, A. V. (2014). *Determining home range and preferred habitat of feral horses on the nevada national security site using geographic information systems* [Report, University of Denver]. https://digitalcommons.du.edu/geog_ms_capstone/50

Cagnacci, F., Boitani, L., Powell, R. A., & Boyce, M. S. (2010). Animal ecology meets gps-based radiotelemetry: A perfect storm of opportunities and challenges. *365*(1550), 2157-2162. doi.org/10.1098/rstb.2010.0107

Cain, J. W., Krausman, P. R., Jansen, B. D., & Morgart, J. R. (2005). Influence of topography and gps fix interval on gps collar performance. *Wildlife Society Bulletin, 33*(3), 926-934.

Calenge, C. (2006). The package “adehabitat” for the r software: A tool for the analysis of space and habitat use by animals. *Ecological modelling, 197*(3-4), 516-519.

Câmara Municipal de Viana do Castelo. (2017). *Escola da natureza. Rede natura 2000 de viana do castelo* [Didactic Catalog].

Campos, R. d. F. (2018). *Survival and population dynamics of the iberian wolf in portugal* [MSc Dissertation, University of Porto]. Porto.

Casimiro, V. J. G. (2017). Wolf predation on livestock in relation to husbandry practices and wild prey availability: Regional and temporal patterns.

Centro de Monitorização e Interpretação Ambiental. (2020). *Dados da estação metereológica do cmia (viana do castelo) para o ano de 2020* [Metereological Dataset].

Chapple, R. (2005). The politics of feral horse management in guy fawkes river national park, nsw. *Australian Zoologist, 33*(2), 233-246.

Childress, M. J., & Lung, M. A. (2003). Predation risk, gender and the group size effect: Does elk vigilance depend upon the behaviour of conspecifics? *Animal behaviour, 66*(2), 389-398.

Ciucci, P., Boitani, L., Pelliccioni, E. R., Rocco, M., & Guy, I. (1996). A comparison of scat-analysis methods to assess the diet of the wolf canis lupus. *Wildlife biology, 2*(3), 37-48.

Collins, G. H., Petersen, S. L., Carr, C. A., & Pielstick, L. (2014). Testing vhf/gps collar design and safety in the study of free-roaming horses. *PloS one, 9*(9), e103189.

Costa, G. N. S. F. d. (2000). *Situação populacional e ecologia trófica do lobo-ibérico (canis lupus signatus cabrera, 1907) na serra do soajo* [Internship report, University of Lisbon]. Lisbon.

Costa, J. C., Aguiar, C., Capelo, J., Lousã, M., & Neto, C. (1998). Biogeografia de portugal continental.

Costa, L. F. (2001). *Control of water use in northwest portugal* (Publication Number 1-205) [PhD Dissertation, The University of Arizona]. Arizona.

Cozza, K., Fico, R., Battistini, M.-L., & Rogers, E. (1996). The damage-conservation interface illustrated by predation on domestic livestock in central italy. *Biological Conservation, 78*(3), 329-336.

Crane, K. K., Smith, M. A., & Reynolds, D. (1997). Habitat selection patterns of feral horses in southcentral wyoming. *Rangeland Ecology & Management/Journal of Range Management Archives, 50*(4), 374-380.

Creel, S., Winnie Jr, J., Maxwell, B., Hamlin, K., & Creel, M. (2005). Elk alter habitat selection as an antipredator response to wolves. *Ecology, 86*(12), 3387-3397.

Creel, S., & Winnie Jr, J. A. (2005). Responses of elk herd size to fine-scale spatial and temporal variation in the risk of predation by wolves. *Animal Behaviour, 69*(5), 1181-1189.

D'Andrade, R. (1938). *Garranos* (Separata do Boletim Pecuário, Ano VI, nº 2, Issue.

D'eon, R. G., & Delparte, D. (2005). Effects of radio‐collar position and orientation on gps radio‐collar performance, and the implications of pdop in data screening. *Journal of Applied Ecology, 42*(2), 383-388.

Davie, H. S., Murdoch, J. D., Lhagvasuren, A., & Reading, R. P. (2014). Measuring and mapping the influence of landscape factors on livestock predation by wolves in mongolia. *Journal of Arid Environments, 103*, 85-91.

Denny, E. A., & Dickman, C. (2010). *Review of cat ecology and management strategies in australia*.

DeSilvey, C., & Bartolini, N. (2019). Where horses run free? Autonomy, temporality and rewilding in the côa valley, portugal. *Transactions of the Institute of British Geographers, 44*(1), 94-109.

Destoumieux-Garzón, D., Bonnet, P., Teplitsky, C., Criscuolo, F., Henry, P.-Y., Mazurais, D., Prunet, P., Salvat, G., Usseglio-Polatera, P., & Verrier, E. (2020). Animal board invited review: Oneark: Strengthening the links between animal production science and animal ecology. *animal*, 100053.

Di Orio, A. P., Callas, R., & Schaefer, R. J. (2003). Performance of two gps telemetry collars under different habitat conditions. *Wildlife Society Bulletin*, 372-379.

Direção-Geral do Território. (2018). *Carta de uso e ocupação do solo - 2018* [Land Cover Map].

Dondina, O., Meriggi, A., Dagradi, V., Perversi, M., & Milanesi, P. (2015). Wolf predation on livestock in an area of northern italy and prediction of damage risk. *Ethology Ecology & Evolution, 27*(2), 200-219.

Duncan, P. (1983). Determinants of the use of habitat by horses in a mediterranean wetland. *The Journal of Animal Ecology*, 93-109.

Dupuis, A. (2017). *What factors influence horse distribution patterns on sable island?* [Bachelor Degree Final Thesis, Saint Mary's University]. Halifax, Nova Scotia.

ELECTRICBLUE. (2020). *Electricblue. Tools for environmental monitoring and biologging.* https://electricblue.eu/

Environmental Systems Research Institute. (2019). *Arcgis desktop.* In https://www.esri.com/

European Environment Agency. (2000). *Copernicus land monitoring service 2000* [Land cover Map].

European Environment Agency. (2006). *Copernicus land monitoring service 2006* [Land Cover Map].

European Environment Agency. (2018). *Copernicus land monitoring service 2018* [Land Cover Map].

Fagúndez, J. (2016). Grazing effects on plant diversity in the endemic erica mackayana heathland community of north-west spain. *Plant Ecology & Diversity, 9*(2), 207-217.

Fagúndez, J., Hermida, R. J., & Abarzuza, L. L. (2017). Brezales, lobos y caballos salvajes en galicia. *Quercus*(377), 20-28.

Faria, M. (2012). *Valorização do garrano.* Livro de Atas, I Congresso Internacional do Garrano, Arcos de Valdevez.

Feh, C., Boldsukh, T., & Tourenq, C. (1994). Are family groups in equids a response to cooperative hunting by predators? The case of mongolian kulans (equus hemionus luteus matschie). *Revue d'écologie*(49), 11-20.

Ferreira, J. (2003). *Estudo e monitorização de cervídeos no parque nacional da peneda-gerês* [Technical Report](Projeto de Gestão dos Habitats do Lobo-Ibérico no Parque Nacional da Peneda-Gerês, Issue.

Ferreira, J. P., Leitão, I., Santos-Reis, M., & Revilla, E. (2011). Human-related factors regulate the spatial ecology of domestic cats in sensitive areas for conservation. *PLoS One, 6*(10), e25970.

Fico, R., Morosetti, G., & Giovannini, A. (1993). The impact of predators on livestock in the abruzzo region of italy [brown bear, wolf]. *Revue Scientifique et Technique de l'OIE (France)*.

Figueiredo, A. M., Valente, A. M., Barros, T., Carvalho, J., Silva, D. A., Fonseca, C., Carvalho, L. M. d., & Torres, R. T. (2020). What does the wolf eat? Assessing the diet of the endangered iberian wolf (canis lupus signatus) in northeast portugal. *Plos one, 15*(3), e0230433.

Fletcher, R., & Fortin, M.-J. (2018). Introduction to spatial ecology and its relevance for conservation. In *Spatial ecology and conservation modeling* (pp. 1-13). Springer.

Frair, J. L., Fieberg, J., Hebblewhite, M., Cagnacci, F., DeCesare, N. J., & Pedrotti, L. (2010). Resolving issues of imprecise and habitat-biased locations in ecological analyses using gps telemetry data. *Philosophical Transactions of the Royal Society B: Biological Sciences, 365*(1550), 2187-2200.

Fraser, D. (2010). Toward a synthesis of conservation and animal welfare science.

Freitas, J. M. A. C. F. d. (2019). *Patterns and behavioral determinants related to wolf predation on free-ranging horses* [MSc Dissertation, University of Porto].

Freitas, J. M. A. C. F. d., & Álvares, F. (*in press*). Economic impact of wolf predation on free-ranging horses in nw portugal: Management and socio-cultural implications. *Carnivore Damage Prevention News*.

Freitas, J. M. A. C. F. d., Lagos Abarzuza, L., & Álvares, F. (*in press*). Geographical patterns on consumption of horses by wolves: A worldwide review. *Carnivore Damage Prevention News*.

Fundação Francisco Manuel dos Santos. (2021). *Pordata: Base de dados de portugal contemporâneo*. Fundação Francisco Manuel dos Santos. Retrieved September 2021 from https://www.pordata.pt/

Ganskopp, D., & Vavra, M. (1986). Habitat use by federal horses in the northern sagebrush steppe.

Ganskopp, D. C. (1984). *Habitat use and spatial interactions of cattle, wild horses, mule deer, and california bighorn sheep in the owyhee breaks of southeast oregon* [PhD Dissertation, Oregon State University]. Oregon.

Gazzola, A., Bertelli, I., Avanzinelli, E., Tolosano, A., Bertotto, P., & Apollonio, M. (2005). Predation by wolves (canis lupus) on wild and domestic ungulates of the western alps, italy. *Journal of Zoology, 266*(2), 205-213.

Giesteira, P. (1999). *O garrano do gerês: Um cavalo português.*

Girard, T. L., Bork, E. W., Neilsen, S. E., & Alexander, M. (2013a). Seasonal variation in habitat selection by free-ranging feral horses within alberta's forest reserve. *Rangeland Ecology & Management, 66*(4), 428-437.

Girard, T. L., Bork, E. W., Neilsen, S. E., & Alexander, M. J. (2013b). Landscape-scale factors affecting feral horse habitat use during summer within the rocky mountain foothills. *Environmental management, 51*(2), 435-447.

Gomes, J. C. S. M. (1996). *Bases para a conservação e gestão do garrano (equus caballus, l., 1758) no parque nacional da peneda-gerês* [Internship Report, University of Lisbon]. Lisbon.

Gooch, A. M., Petersen, S. L., Collins, G. H., Smith, T. S., McMillan, B. R., & Eggett, D. L. (2017). The impact of feral horses on pronghorn behavior at water sources. *Journal of Arid Environments, 138*, 38-43.

Gordon, I. J., Hester, A. J., & FESTA ‐ BIANCHET, M. (2004). The management of wild large herbivores to meet economic, conservation and environmental objectives. *Journal of Applied Ecology, 41*(6), 1021-1031.

Graves, H. (1984). Behavior and ecology of wild and feral swine (sus scrofa). *Journal of animal science, 58*(2), 482-492.

Guerra, A. M. M. (2004). *Estudo das relações ecológicas entre o lobo-ibérico e equinos e bovinos no alto minho: Propostas para a minimização do impacto predatório* [Internship report, University of Lisbon]. Lisbon.

Gula, R. (2008). Wolf depredation on domestic animals in the polish carpathian mountains. *The Journal of wildlife management, 72*(1), 283-289.

Hackmann, T., & Spain, J. (2010). Invited review: Ruminant ecology and evolution: Perspectives useful to ruminant livestock research and production. *Journal of dairy science, 93*(4), 1320-1334.

Hampson, B., De Laat, M., Mills, P., & Pollitt, C. (2010). Distances travelled by feral horses in 'outback'australia. *Equine Veterinary Journal, 42*, 582-586.

Hebblewhite, M., & Haydon, D. T. (2010). Distinguishing technology from biology: A critical review of the use of gps telemetry data in ecology. *Philosophical Transactions of the Royal Society B: Biological Sciences, 365*(1550), 2303-2312.

Hebblewhite, M., & Pletscher, D. H. (2002). Effects of elk group size on predation by wolves. *Canadian Journal of Zoology, 80*(5), 800-809.

Hennig, J. D. (2021). *Feral horse movement, habitat selection, and effects on pronghorn and greater sage-grouse habitat in cold-arid-steppe* [PhD Dissertation, University of Wyoming].

Hennig, J. D., Beck, J. L., & Scasta, J. D. (2018). Spatial ecology observations from feral horses equipped with global positioning system transmitters. *Human–Wildlife Interactions, 12*(1), 75-84.

Hijmans, R. J., & van Etten, J. (2012). Raster: Geographic analysis and modeling with raster data. R package version 2.0-12.

Hofman, M. P., Hayward, M. W., Heim, M., Marchand, P., Rolandsen, C. M., Mattisson, J., Urbano, F., Heurich, M., Mysterud, A., & Melzheimer, J. (2019). Right on track? Performance of satellite telemetry in terrestrial wildlife research. *PloS one, 14*(5), e0216223.

Hovens, H., & Rijkers, T. (2013). On the origins of the exmoor pony: Did the wild horse survive in britain? *Lutra, 56*(2), 129-136.

Hovens, J., & Tungalaktuja, K. (2005). Seasonal fluctuations of the wolf diet in the hustai national park (mongolia). *Mammalian Biology, 70*(4), 210-217.

Howery, L. D., & DeLiberto, T. J. (2004). Indirect effects of carnivores on livestock foraging behavior and production.

Hughes, J., & Macdonald, D. W. (2013). A review of the interactions between free-roaming domestic dogs and wildlife. *Biological Conservation, 157*, 341-351.

Imbert, C., Caniglia, R., Fabbri, E., Milanesi, P., Randi, E., Serafini, M., Torretta, E., & Meriggi, A. (2016). Why do wolves eat livestock?: Factors influencing wolf diet in northern italy. *Biological Conservation, 195*, 156-168.

Inoue, S., Yamamoto, S., Ringhofer, M., Mendonça, R. S., & Hirata, S. (2020). Lateral position preference in grazing feral horses. *Ethology, 126*(1), 111-119.

Inoue, S., Yamamoto, S., Ringhofer, M., Mendonça, R. S., Pereira, C., & Hirata, S. (2019). Spatial positioning of individuals in a group of feral horses: A case study using drone technology. *Mammal Research, 64*(2), 249-259.

Instituto de Conservação da Natureza e das Florestas. (2021). *Sítios designados no âmbito da directiva habitats - rn2000/sic-zec-lista nacional* [Protected Areas (Territory) Map]. ICNF.

Instituto Nacional de Estatística. (1999). *Recenseamento agrícola 1999* [Animal population numbers per parish]. INE.

Instituto Nacional de Estatística. (2019). *Recenseamento agrícola 2019* [Animal population numbers per parish]. INE.

Ivlev, V. (1961). *Experimental ecology of the feeding of fishes. New haven, ct: Yale univ. Press. 302 p*.

Jacobs, J. (1974). Quantitative measurement of food selection. *Oecologia, 14*(4), 413-417.

Janeau, G., Adrados, C., Joachim, J., Gendner, J.-P., & Pépin, D. (2004). Performance of differential gps collars in temperate mountain forest. *Comptes Rendus Biologies, 327*(12), 1143-1149.

Jiang, Z., Sugita, M., Kitahara, M., Takatsuki, S., Goto, T., & Yoshida, Y. (2008). Effects of habitat feature, antenna position, movement, and fix interval on gps radio collar performance in mount fuji, central japan. *Ecological Research, 23*(3), 581-588.

Jodkowska, E., Kuźniak, A., Pikuła, R., & Smugała, M. (2015). Analysis of polish primitive ponies migration under registration of the global positioning system. *GSTF Journal of Veterinary Science (JVet), 1*(2), 1-5.

Kaczensky, P., Ganbaatar, O., Von Wehrden, H., & Walzer, C. (2008). Resource selection by sympatric wild equids in the mongolian gobi. *Journal of Applied Ecology, 45*(6), 1762-1769.

Kaczensky, P., Ito, T. Y., & Walzer, C. (2010). Satellite telemetry of large mammals in mongolia: What expectations should we have for collar function? *Wildlife biology in practice (Online), 6*(2), 108.

Kaczensky, P., & Walzer, C. (2003). Przewalski horses, wolves and khulans in mongolia. *International Takhi Group Research Report*.

Keiper, R. R. (1986). Social structure. *Veterinary Clinics of North America: Equine Practice, 2*(3), 465-484.

King, S. (2002). Home range and habitat use of free-ranging przewalski horses at hustai national park, mongolia. *Applied Animal Behaviour Science, 78*(2-4), 103-113.

King, S. R., & Gurnell, J. (2005). Habitat use and spatial dynamics of takhi introduced to hustai national park, mongolia. *Biological Conservation, 124*(2), 277-290.

King, S. R., Schoenecker, K. A., Fike, J. A., & Oyler ‐ McCance, S. J. (2021). Feral horse space use and genetic characteristics from fecal DNA. *The Journal of Wildlife Management*.

Kluever, B. M., Howery, L. D., Breck, S. W., & Bergman, D. L. (2009). Predator and heterospecific stimuli alter behaviour in cattle. *Behavioural Processes, 81*(1), 85-91.

Kluever, B. M., Lagos, L., Breck, S. W., Howery, L. D., Sanmartín, M. L., Bergman, D. L., & Bárcena, F. (2012). Integrity and retention of ear ‐ tag radiotransmitters in domestic cattle and feral horses. *Wildlife Society Bulletin, 36*(1), 189-193.

Koene, P., & Ipema, B. (2014). Social networks and welfare in future animal management. *Animals, 4*(1), 93-118.

Krebs, C. (1989). *Ecological methodology.− harper collins publishers* (Vol. 654).

Krueger, K. (2008). Social ecology of horses. In *Ecology of social evolution* (pp. 195-206). Springer.

Lacerda, A. C., Tomas, W., & Marinho ‐ Filho, J. (2009). Domestic dogs as an edge effect in the brasília national park, brazil: Interactions with native mammals. *Animal Conservation, 12*(5), 477-487.

Lagos, L. (2013). *Ecología del lobo (canis lupus), del poni salvaje (equus ferus atlanticus) y del ganado vacuno semiextensivo (bos taurus) en galicia: Interacciones depredador-presa* [PhD Dissertation, University of Santiago de Compostela]. Santiago de Compostela.

Lagos, L. (2020). *O sistema tradicional de gestão dos póneis atlânticos selvagens na galiza: Entre a memória e os desafios de futuro* Percursos do Homem e do Garrano - Actas dos Seminários do Projecto, Viana do Castelo.

Lagos, L., & Bárcena, F. (2010). Valoración de los radiotransmisores sobre crotal en el seguimiento de ponis salvajes. *Galemys, 22 (Special Number)*, 103-122.

Lagos, L., & Bárcena, F. (2015). Eu sanitary regulation on livestock disposal: Implications for the diet of wolves. *Environmental Management, 56*(4), 890-902.

Lagos, L., & Bárcena, F. (2018). Spatial variability in wolf diet and prey selection in galicia (nw spain). *Mammal research, 63*(2), 125-139.

Lagos, L., & Bárcena, F. (2020). *O garrano e o lobo ibérico: Caminhos que se cruzam.* Percursos do Homem e do Garrano - Actas dos Seminários do Projecto, Viana do Castelo.

Lagos, L., Muñoz-Barcia, C. V., & Fagúndez, J. (2019). Manejo, problemática y oportunidades de los caballos salvajes de galicia como herramienta de conservación de hábitats prioritarios en la red natura 2000/management, challenges and opportunities for the galician wild ponies as a priority habitats conservation tool in the natura 2000 network. *Galemys, 31*, 35-45.

Laliberte, A. S., & Ripple, W. J. (2004). Range contractions of north american carnivores and ungulates. *BioScience, 54*(2), 123-138.

Lançós, J. P. C. d. O. d. M. (1999). *Contribuição para o conhecimento da ecologia do lobo no parque nacional da peneda-gerês* [Internship Report, University of Porto]. Porto.

Leite, J. A. M. V. (2012, June 2012). *A raça equina garrana.* I Congresso Internacional do Garrano - Livro de Atas, Arcos de Valdevez.

Levin, S. A. (2013). *Encyclopedia of biodiversity*. Elsevier Inc.

Lewis, J. S., Rachlow, J. L., Garton, E. O., & Vierling, L. A. (2007). Effects of habitat on gps collar performance: Using data screening to reduce location error. *Journal of applied ecology, 44*(3), 663-671.

Lima, S. B. (1872). Os escriptos de silvestre bernardo lima - equinos. *Bol. Dir. G. Agric, 10*.

Linklater, W. L., Cameron, E. Z., Stafford, K. J., & Veltman, C. J. (2000). Social and spatial structure and range use by kaimanawa wild horses (equus caballus: Equidae). *New Zealand Journal of Ecology*, 139-152.

Linnartz, L., & Meissner, R. (2014). *Rewilding horses in europe: Background and guidelines - a living document*. Rewilding Europe.

Llaneza, L., Fernández, A., & Nores, C. (1996). Dieta del lobo en dos zonas de asturias (españa) que difieren en carga ganadera. *Doñana, Acta Vertebrata, 23*(2), 201-213.

Llaneza, L., & López-Bao, J. V. (2015). Indirect effects of changes in environmental and agricultural policies on the diet of wolves. *European Journal of Wildlife Research, 61*(6), 895-902.

Lopéz-Bao, J. V., Sazatornil, V., Llaneza, L., & Rodríguez, A. (2013). Indirect effects on heathland conservation and wolf persistence of contradictory policies that threaten traditional free ‐ ranging horse husbandry. *Conservation letters, 6*(6), 448-455.

Marlow, C., Gagnon, L., Hovland, E., & Irby, L. (1990). Feral horse distribution, habitat use and population dynamics in theodore roosevelt national park. *The UW National Parks Service Research Station Annual Reports, 14*, 123-125.

Martinson, K. L., Siciliano, P. D., Sheaffer, C. C., McIntosh, B. J., Swinker, A. M., & Williams, C. A. (2017). A review of equine grazing research methodologies. *Journal of Equine Veterinary Science, 51*, 92-104.

McCort, W. D. (1984). Behavior of feral horses and ponies. *Journal of Animal Science, 58*(2), 493-499.

McGregor, H., Legge, S., Jones, M., & Johnson, C. (2016). Gps collars are more efficient when collecting high-frequency data. *Australian Mammalogy, 38*(2), 237-240.

McInnis, M. L. (1984). *Ecological relationships among feral horses, cattle, and pronghorn in southeastern oregon* [PhD dissertation, Oregon State University]. Oregon.

Mech, L. D., Harper, E. K., Meier, T. J., & Paul, W. J. (2000). Assessing factors that may predispose minnesota farms to wolf depredations on cattle. *Wildlife Society Bulletin*, 623-629.

Mech, L. D., & Peterson, R. O. (2010). Wolf-prey relations. In L. D. Mech & L. Boitani (Eds.), *Wolves: Behaviour, ecology and conservation* (pp. 131-160). University of Chicago Press.

Mendonça, R. S., Ringhofer, M., Pinto, P., Inoue, S., & Hirata, S. (2020). Feral horses'(equus ferus caballus) behavior toward dying and dead conspecifics. *Primates, 61*(1), 49-54.

Meriggi, A., & Lovari, S. (1996). A review of wolf predation in southern europe: Does the wolf prefer wild prey to livestock? *Journal of applied ecology*, 1561-1571.

Meriggi, A., & Pagnin, E. (1994). Oral communications: Influence of wolves (canis lupus) on social behaviour and habitat selection of free - grazing cows (bos taurus). 40.

Milheiras, S., & Hodge, I. (2011). Attitudes towards compensation for wolf damage to livestock in viana do castelo, north of portugal. *Innovation: The European Journal of Social Science Research, 24*(3), 333-351.

Miller, R. (1983a). Habitat use of feral horses and cattle in wyoming's red desert competition for forage. *Rangeland Ecology & Management/Journal of Range Management Archives, 36*(2), 195-199.

Miller, R. (1983b). Seasonal movements and home ranges of feral horse bands in wyoming's red desert. *Rangeland Ecology & Management/Journal of Range Management Archives, 36*(2), 199-201.

Millspaugh, J., & Marzluff, J. M. (2001). *Radio tracking and animal populations*. Academic Press.

Mitas, L., & Mitasova, H. (1999). Spatial interpolation. In *Geographical information systems: Principles, techniques, management and applications* (Vol. 1).

Muhly, T. B., Alexander, M., Boyce, M. S., Creasey, R., Hebblewhite, M., Paton, D., Pitt, J. A., & Musiani, M. (2010). Differential risk effects of wolves on wild versus domestic prey have consequences for conservation. *Oikos, 119*(8), 1243-1254.

Musiani, M., Mamo, C., Boitani, L., Callaghan, C., Gates, C. C., Mattei, L., Visalberghi, E., Breck, S., & Volpi, G. (2003). Wolf depredation trends and the use of fladry barriers to protect livestock in western north america. *Conservation Biology, 17*(6), 1538-1547.

Nakamura, M., Maior, H. R., Godinho, R., & Álvares, F. (2019). *Investigação aplicada à conservação do lobo no noroeste de portugal: Plano de monitorização do lobo no âmbito do sobreequipamento dos parques eólicos do alto minho i e de arga (abril 2018 - março 2019)* [Technical Report]. CIBIO/InBIO.

Nakamura, M., Rio-Maior, H., Godinho, R., Petrucci-Fonseca, F., & Álvares, F. (2021). Source-sink dynamics promote wolf persistence in human-modified landscapes: Insights from long-term monitoring. *Biological Conservation, 256*, 109075.

Namgail, T., Fox, J. L., & Bhatnagar, Y. V. (2007). Carnivore-caused livestock mortality in trans-himalaya. *Environmental management, 39*(4), 490-496.

Nelder, J. A., & Wedderburn, R. W. (1972). Generalized linear models. *Journal of the Royal Statistical Society: Series A (General), 135*(3), 370-384.

Newman, E. (2000). *Applied ecology and environmental management* (Second ed.). Blackwell Publishing.

Newsome, T. M., Boitani, L., Chapron, G., Ciucci, P., Dickman, C. R., Dellinger, J. A., López‐Bao, J. V., Peterson, R. O., Shores, C. R., & Wirsing, A. J. (2016). Food habits of the world's grey wolves. *Mammal Review, 46*(4), 255-269.

Nimmo, D. G., & Miller, K. K. (2007). Ecological and human dimensions of management of feral horses in australia: A review. *Wildlife Research, 34*(5), 408-417.

Nimmo, D. G., Miller, K. K., & Adams, R. (2007). Managing feral horses in victoria: A study of community attitudes and perceptions. *Ecological Management & Restoration, 8*(3), 237-241.

Oom, M. d. M., & Reis, M. S. (1986). Ecology, social organization and behaviour of the feral horses in the peneda-geres national park. *Arquivos do Museu Bocage serie A, 3*, 169-195.

Parraga Aguado, M. A., Sturaro, E., & Ramanzin, M. (2017). Individual activity interacts with climate and habitat features in influencing gps telemetry performance in an alpine herbivore. *Hystrix, 28*(1).

Pebesma, E. J. (2018). Simple features for r: Standardized support for spatial vector data. *R J., 10*(1), 439.

Perez, X. (2012). O cabalo pura raza galega. Livro de Atas, I Congresso Internacional do Garrano, Arcos de Valdevez.

Perry, N. D., Morey, P., & San Miguel, G. (2015). Dominance of a natural water source by feral horses. *The Southwestern Naturalist*, 390-393.

Petrucci-Fonseca, F., Pires, A., Ribeiro, S., Almendra, L., Clemente, A., Collaço, M., Matos, J., & Simoes, F. (2000). Cães de gado na conservação do lobo em portugal. *Galemys, 12*, 135-148.

Pimenta, V., Barroso, I., Boitani, L., & Beja, P. (2017). Wolf predation on cattle in portugal: Assessing the effects of husbandry systems. *Biological Conservation, 207*, 17-26.

Pimenta, V., Barroso, I., Boitani, L., & Beja, P. (2018). Risks a la carte: Modelling the occurrence and intensity of wolf predation on multiple livestock species. *Biological conservation, 228*, 331-342.

Portas, M. (2020). *Desafios da conservação da raça equina garrana.* Percursos do Homem e do Garrano - Actas dos Seminários do Projecto, Viana do Castelo.

R Core Team. (2021). *R: A language and environment for statistical computing.* In R Foundation for Statistical Computing. https://www.R-project.org/

Ransom, J. I., & Kaczensky, P. (2016). *Wild equids: Ecology, management, and conservation*. JHU Press.

Rees, P. A. (2015). *Studying captive animals: A workbook of methods in behaviour, welfare and ecology*. John Wiley & Sons.

Rheinhardt, R. D., & Rheinhardt, M. C. (2004). Feral horse seasonal habitat use on a coastal barrier spit. *Rangeland Ecology and Management, 57*(3), 253-258.

Ringhofer, M., Go, C. K., Inoue, S., Mendonça, R. S., Hirata, S., Kubo, T., Ikeda, K., & Yamamoto, S. (2020). Herding mechanisms to maintain the cohesion of a harem group: Two interaction phases during herding. *Journal of Ethology, 38*(1), 71-77.

Ringhofer, M., Inoue, S., Mendonça, R. S., Pereira, C., Matsuzawa, T., Hirata, S., & Yamamoto, S. (2017). Comparison of the social systems of primates and feral horses: Data from a newly established horse research site on serra d'arga, northern portugal. *Primates, 58*(4), 479-484.

Rio-Maior, H., Malveiro, E., Álvares, F., & Pettruci-Fonseca, F. (2006). *O lobo e o gado extensivo no noroeste de portugal - um estudo das relações ecológicas.* [Final Report do the project POCTI (BSE/72039/2001).].

Rozen‐Rechels, D., van Beest, F. M., Richard, E., Uzal, A., Medill, S. A., & McLoughlin, P. D. (2015). Density‐dependent, central‐place foraging in a grazing herbivore: Competition and tradeoffs in time allocation near water. *Oikos, 124*(9), 1142-1150.

RStudio Team. (2021). *Rstudio: Integrated development environment for r.* In RStudio, Inc. http://www.rstudio.com/

Saastamoinen, M., Fradinho, M. J., Santos, A. S., & Miraglia, N. (2012). *Forages and grazing in horse nutrition* (Vol. 132). Springer Science & Business Media.

Salter, R., & Hudson, R. (1979). Feeding ecology of feral horses in western alberta. *Rangeland Ecology & Management/Journal of Range Management Archives, 32*(3), 221-225.

Salter, R., & Hudson, R. (1980). Range relationships of feral horses with wild ungulates and cattle in western alberta.

Salter, R. E. (1978). *Ecology of feral horses in western alberta* [MSc Dissertation, University of Alberta]. Edmonton, Alberta.

Salvador, A., & Abad, P. (1987). Food habits of a wolf population (canis lupus) in león province, spain.

Samuel, M. D., Pierce, D., & Garton, E. O. (1985). Identifying areas of concentrated use within the home range. *The Journal of Animal Ecology*, 711-719.

Schoenecker, K. A., King, S. R., & Collins, G. H. (2020). Evaluation of the impacts of radio-marking devices on feral horses and burros in a captive setting. *Human–Wildlife Interactions, 14*(1), 73-86.

Serôdio, C. (1992). *Estudo do sistema de criação do garrano no concelho de terras de bouro* [Internship Report, Universidade de Trás-os-Montes e Alto Douro]. Vila Real.

Shivik, J. A., Treves, A., & Callahan, P. (2003). Nonlethal techniques for managing predation: Primary and secondary repellents. *Conservation Biology, 17*(6), 1531-1537.

Stevens, E. F. (1988). Contests between bands of feral horses for access to fresh water: The resident wins. *Animal Behaviour, 36*(6), 1851-1853.

Terras de Sicó. (2017). *Fauna, javali*. http://www.terrasdesico.pt/turismo-fauna/javali

Torres, R., Silva, N., Brotas, G., & Fonseca, C. (2015). To eat or not to eat? The diet of the endangered iberian wolf (canis lupus signatus) in a human-dominated landscape in central portugal. *PloS one, 10*(6), e0129379.

Turner Jr, J. W. (2015). Environmental influences on movements and distribution of a wild horse (equus caballus) population in western nevada, USA: A 25-year study. *Journal of Natural History, 49*(39-40), 2437-2464.

Turner Jr, J. W., & Morrison, M. L. (2001). Influence of predation by mountain lions on numbers and survivorship of a feral horse population. *The Southwestern Naturalist*, 183-190.

Van Duyne, C., Ras, E., De Vos, A. E., De Boer, W. F., Henkens, R. J., & Usukhjargal, D. (2009). Wolf predation among reintroduced przewalski horses in hustai national park, mongolia. *The Journal of Wildlife Management, 73*(6), 836-843.

Vavra, M., & Ganskopp, D. (1987). Slope use by cattle, feral horses, deer, and bighorn sheep. *Northwest Science, 61*(2), 74-81.

Vieira e Brito, N., & Silva, C. (2020). *O garrano: Candidatura a património nacional* Percursos do Homem e do Garrano - Actas dos Seminários do Projecto, Viana do Castelo.

Vos, J. (2000). Food habits and livestock depredation of two iberian wolf packs (canis lupus signatus) in the north of portugal. *Journal of zoology, 251*(4), 457-462.

Werhahn, G., Kusi, N., Li, X., Chen, C., Zhi, L., Martín, R. L., Sillero-Zubiri, C., & Macdonald, D. W. (2019). Himalayan wolf foraging ecology and the importance of wild prey. *Global Ecology and Conservation, 20*, e00780.

Wockner, G., Singer, F. J., & Schoenecker, K. A. (2004). *An animal location-based habitat suitability model for bighorn sheep and wild horses in bighorn canyon national recreation area and the pryor mountain wild horse range, montana, and wyoming*.

Woolson, R. (2007). Wilcoxon signed‐rank test. *Wiley encyclopedia of clinical trials*, 1-3.

Worton, B. J. (1989). Kernel methods for estimating the utilization distribution in home‐range studies. *Ecology, 70*(1), 164-168.

Zeileis, A., & Grothendieck, G. (2005). Zoo: S3 infrastructure for regular and irregular time series. *arXiv preprint math/0505527*.

Zhang, Y., Cao, Q. S., Rubenstein, D. I., Zang, S., Songer, M., Leimgruber, P., Chu, H., Cao, J., Li, K., & Hu, D. (2015). Water use patterns of sympatric przewalski's horse and khulan: Interspecific comparison reveals niche differences. *PloS one, 10*(7), e0132094.

Zlatanova, D., Ahmed, A., Valasseva, A., & Genov, P. (2014). Adaptive diet strategy of the wolf (canis lupus l.) in europe: A review. *Acta zoologica bulgarica, 66*(4), 439-452.

8. Supplementary material

Appendix I: literature review

Table S1. Compilation of available studies addressing the spatial ecology of horses (namely home ranges, space use or movement), including Bibliographic reference, Year of data collection, Country, Horse type, Methodological approach and Presence of natural predators.

Bibliographic reference	*Data collection Year(s)*	*Country*	*Horse type*	*Methodological approach*	*Presence of predators?*
Berger (1977)	1974	USA	Feral	Observation	N
Duncan (1983)	1975-1976	France	Free-Ranging	Observation	N
Salter & Hudson (1980); Salter (1978)	1976	Canada	Feral	Observation	N
Miller (1983b)	1976-1979	USA	Feral	Observation	N
Miller (1983a)	1977-1979	USA	Feral	Observation	N
McInnis (1984)	1979-1981	USA	Feral	Observation + Telemetry	N
Ganskopp & Vavra (1986); Ganskopp (1984)	1979-1981	USA	Feral	Observation + Telemetry	N
Vavra & Ganskopp (1987)	1979-1981	USA	Feral	Observation	N
Oom & Reis (1986)	1986	Portugal	Free-Ranging	Observation	Y
Turner Jr (2015)	1987-2012	USA	Feral	Observation	Y
Marlow *et al.* (1990)	1989-1990	USA	Feral	Observation	N
Crane *et al.* (1997)	1992-1993	USA	Feral	Observation	N
Linklater *et al.* (2000)	1994-1997	New Zealand	Feral	Observation	N
King & Gurnell (2005)	1995-1997	Mongolia	Wild	Observation	Y
Rheinhardt & Rheinhardt (2004)	1997	USA	Feral	Observation	N
King (2002)	1998	Mongolia	Wild	Observation	Y
Kaczensky *et al.* (2008)	2001-2005	Mongolia	Wild	Observation + Telemetry	Y
Aldezabal *et al.* (2013)	2006-2007	Spain	Free-Ranging	Observation	N
Bhattacharyya (2012)	2007-2009	Canada	Feral	Interviews + Observation + Genetics	Y
Dupuis (2017)	2008-2010	Canada	Feral	Observation	N
Girard *et al.* (2013a)	2008-2010	Canada	Feral	Telemetry	Y
Girard *et al.* (2013b)	2009-2010	Canada	Feral	Observation	Y
Hampson *et al.* (2010)	2010	Australia	Feral	Telemetry	N
Zhang *et al.* (2015)	2010-2011	China	Wild	Camera Trapping	Y
Jodkowska *et al.* (2015)	2011-2012	Poland	Free-Ranging	Telemetry	N
Rozen-Rechels *et al.* (2015)	2008-2013	Canada	Feral	Observation	N
Baskaran *et al.* (2016)	2012-2013	India	Feral	Observation	N
Burns (2014)	2014	USA	Feral	Observation	N
King *et al.* (2021)	2014	USA	Feral	Genetics	N
Hennig *et al.* (2018)	2017	USA	Feral	Telemetry	N
Hennig (2021)	2017-2021	USA	Feral	Telemetry	N

Table S2. Compilation of available studies reporting the use of telemetry on horses, including Bibliographic reference, Year of data collection, Country, Horse type, Transmitter type and location, Presence of natural predators and addressed research topic(s).

Bibliographic reference	*Data collection Year(s)*	*Country*	*Horse type*	*Transmitter type*	*Transmitter location*	*Presence of predator?*	*Research topic(s)*
McInnis (1984)	1979-1981	USA	Feral	VHF	Collar	N	Spatial Ecology; Intersp. Interaction
Ganskopp & Vavra (1986); Ganskopp (1984)	1979-1981	USA	Feral	VHF	Collar	N	Spatial Ecology
Kaczensky *et al.* (2010)	2001-2008	Mongolia	Wild	VHF	Collar	Y	Spatial Ecology; Intersp. Interaction; Technical procedures
Kluever *et al.* (2012); Lagos & Bárcena (2010)	2007-2009	Spain	Free-ranging	VHF	Ear Tag	Y	Technical procedures
Girard *et al.* (2013a)	2008-2010	Canada	Feral	GPS	Collar	N	Spatial Ecology
Collins *et al.* (2014)	2009-2010	USA	Domestic; Feral	Both	Collar	N	Welfare; Technical procedures
Hampson *et al.* (2010)	2010	Australia	Feral	Both	Collar	N	Spatial Ecology
Jodkowska *et al.* (2015)	2011-2012	Poland	Free-Ranging	GPS	Collar	N	Spatial Ecology
Schoenecker *et al.* (2020)	2015	USA	Feral	Both	Collar; Mane Tag; Tail Tag	N	Welfare
Hennig *et al.* (2018)	2017	USA	Feral	GPS	Collar	N	Spatial Ecology
Hennig (2021)	2017-2021	USA	Feral	GPS	Collar	N	Spatial Ecology; Intersp. Interaction

Appendix II: questionnaire for horse owners[4]

Date: ________________ Interviewer: ___________________________________

QUESTIONNAIRE: GARRANO-RELATED HUSBANDRY PRACTICES
INFORMATION | OWNERS

1. Name (optional):
2. Age:
3. Gender: F ☐ M ☐
4. Education Level:
5. Occupation:
6. For how many years have you been a garrano horse' breeder?
7. How many hours, per month, do you dedicate to your prospection?
8. County of Residence:
9. Site/Parish/County of garrano horses' grazing site:
10. Which is the equid management strategy do you usually use? (Mark it in the table below)

			Grazing regime			
			Fenced pastures / stable (intensive)	Semi-fenced (mixed)	Free-ranging (extensive)*	Other (specify)
Time spent by horses in pastures **	All Year	F				
		M				
		J				
	Summer	F				
		M				
		J				
	Winter	F				
		M				
		J				
Pasture site	Same pasture site year-round					
	Regular change of pasture site					

* Free-ranging – Grazing regime in which horses graze at communal lands without supervision and any type of confinement.; ** Summer (May-October); Winter (November-April).; F – Adult Female Horse (>3 years); M – Adult Male Horse (>4 years); J – Foal/Juvenile (0-3 years if female; 0-4 years if male).

11. Does it correspond to the traditional techniques used in the region? Yes ☐ No ☐.
12. If not, what are the techniques traditionally used in the region?
13. Reason(s) for being a garrano owner: a. Familiar Tradition ☐ b. Sale of Animals ☐ c. Tourism ☐ d. Recreation ☐ e. Other(s) ☐
14. From one to five, how important are, for you, garrano horses?

Not Important — Very Important

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5

15. What does it mean, for you, to have garrano horses?
16. What is, in your perspective, the role that garrano horses play in the landscape? a. Aesthetic role ☐ b. Protection of the natural habitat ☐ c. Vegetation control ☐ d. Preservation of regional traditions ☐ e. Other(s) ☐

INFORMATION | GARRANOS

17. Information regarding your owned horses:

	Herd	Sex* (**F/M**)	Social Position** (**S/A/J**)	Age	Age it started accompanying the herd	Registered in the Garrano studbook? (**Yes/No**)	Has microchip? (**Yes/No**)	No. of pregnancies (if applicable)	Kinship relations with other horses (if applicable)***
1									Parent(s) – Foal(s) –

* F – Female; M – Male ; ** S - Stallion; A - Adult (>3 years if female; >4 years if male); J – Foal/Juvenile (0-3 years if female; 0-4 years if male).; *** Write number of the horse to which you are referring to.

18. The horses are divided in how many herds?
19. During the 2018-2020 period, did any of your horses die / disappear / get wounded / sell any of your horses? Yes, in the year(s) __ ☐ No ☐ . If yes:

[4] A Portuguese version of this questionnaire was also developed for the interviews with horse owners.

		2018*					2019*					2020*				
		S	AM	F	J	**T**	S	AM	F	J	**T**	S	AM	F	J	**T**
Not caused by predation (**disease; accidents; others**)	Injured															
	Missing															
	Dead															
Caused by **predation**	Injured															
	Missing															
	Dead															
	Sold															

* S – Stallion; AM – Adult Male (excluding stallions; >4 years); F – Mares/Adult Female (>3 years); J – Foal/Juvenile (0-3 years if female; 0-4 years if male); T – Total.

a. Main reason(s) for injury(ies):
b. Main reason(s) for disappearance(s):
c. Main reason(s) for death(s):

20. During the 2018-2020 period, did the number of foals change? Yes ☐ No ☐ . If yes:

	Births *		Disappearances *		Deaths*		Sales*	
	F	M	F	M	F	M	F	M
2018								
2019								
2020								

*F – Female; M – Male.

a. Are mares collected during pregnancy/birth? Yes ☐ No ☐.
b. Where are foals kept after birth? i. Stables ☐ ii. Fenced pastures ☐ iii. Free-ranging regime (open pastures) ☐ iv. Other ☐.
c. Do foals stay near their mothers after birth? Yes ☐ No ☐.
d. With what age do foals start to accompany the herds? i. 0-15 days ☐ ii. 15-30 days ☐ iii. 1-3 months ☐ iv. 3-6 months ☐ v. 6 months - 1 year ☐ vi. 1-2 years ☐ vii. 2-4 years ☐ viii. After reaching adulthood ☐

INFORMATION | HUSBANDRY PRACTICES

21. No. of visits/month to the horses:
a. Reasons for visit(s):
22. In case the horses remain in a free-ranging regime:
a. Do the garrano horses remain in a free-ranging regime throughout the whole year or are they collected for a period? Free-ranging ☐ Collected ☐ .
b. If they are collected, when and for how much time are they collected?

January	**February**	**Marçh**	**April**	**May**	**June**
____ day(s)	____ day(s)	____ day(s)	____ day(s)	____ day(s)	____ day(s)
July	**August**	**September**	**October**	**November**	**December**
____ day(s)	____ day(s)	____ day(s)	____ day(s)	____ day(s)	____ day(s)

c. Are all the horses collected? Yes ☐ No ☐ . If not, specify:
d. Reasons for collection:
23. Are some individuals removed from their herds (to sell, due to regional management practices, etc.)? Yes ☐ No ☐ . If yes: a. Stallions ☐ / Other Males ☐ / Females ☐ b. Adults ☐ / Foals ☐
c. Frequency of removal:
d. Reasons for removal:
24. Are the garrano' horses identified with a microchip? Yes ☐ No ☐ .
25. Are horses subjected to frequent sanitary control? Yes ☐ No ☐ .
26. If yes,
a. Which types of controls are done: i. Internal deworming ☐ ii. External deworming ☐ iii. Vaccination (African horse sickness ☐; Equine encephalitis virus ☐; Anthrax ☐; Rabies ☐; Other(s)__) iv. Equine Influenza ☐ v. Equine infectious anemia ☐ vi. Covering sickness ☐ vii. Glanders ☐ viii. Vesicular stomatitis ☐ ix. Contagious equine metritis ☐ x. Equine viral arteritis ☐ xi. Other(s) ☐
b. Which is the frequency (per year) of this control?
27. Do you currently use any measure to minimize wolf predation on the horse? a. Yes, hedges/fences ☐ b. Yes, shepherd dogs ☐ c. Yes, collection of the horses at night ☐ d. Yes, collection of the most vulnerable animals (pregnant mares, foals) ☐ e. Yes, other(s) ☐ f. No ☐
28. Do you think that the most common protection measures are effective in protecting garrano horses, having in mind that such animals tend to live in a free-ranging regime? Yes ☐ No ☐.
29. Which measures do you think are the most adequate to reduce predation on free-ranging garrano horses?

INFORMATION | PROSPECTION PROFITABILITY

30. Which is the approximate annual cost related to your prospection?
31. In your opinion, which are the main areas in terms of costs (please do an estimate for the years 2019 and 2020):

Expenses	2019 (estimate in euros (€))	2020 (estimate in euros (€))
Individual registration (i.e., microchip identification, enrolment on the Studbook)		
Sanitary Control		
Medical expenses not related with sanitary control (injuries, pregnancies, etc.)		
Horse visits		
Temporary horse collection		
Removal of horses from the population (to sell, due to regional practices)		
Damage caused by wolves		
Loss of horses (because of diseases, accidents, etc.)		
Replacement of horses		
Other(s)		

32. Which do you consider to be the group with more costs per individual? Adult Males ☐ Adult Mares ☐ Foals ☐.
33. At the moment, do you use any strategy to profit out of your prospection? Yes ☐ No ☐ .
a. If yes, which strategy(ies) do you use/plan to use to profit from your prospection? i. Selling horses ☐ ii. Selling horse meat ☐ iii. Equestrianism ☐ iv. Ecotourism v. Equine tourism ☐ vi. Other(s) ☐
b. Within the chosen strategy(ies), which ones are the most profitable? (please do an estimate for the years 2019 and 2020):

Strategy	2019 (estimate in euros (€))	2020 (estimate in euros (€))
Selling horses		
Selling meat for consumption		
Equestrianism		
Ecotourism		
Equine Tourism		
Other(s)		

34. Are the profits enough to cover all the expenses related to the prospection? Yes ☐ No ☐ .
35. In your opinion, are there any other strategies that could be used to promote the breeding and rentability of garrano horses?
36. Which are the main challenges associated to the prospection of garrano horses? (mark all options that apply): a. Low profitability/High costs associated to the exploitation and maintenance of horses ☐ b. Lack of support (financial) ☐ c. Lack of support (non-financial) ☐ d. Decrease and aging of owners ☐ e. Lack of promotion of this practice ☐ f. Damage caused by wolves ☐ g. Other(s) ☐ h. No associated challenges ☐
37. Which actions do you think could be taken to approach and/or decrease such problems?
38. In your opinion, how will the future of garrano horse’s prospections be?

INFORMATION | AID FOR GARRANO HORSE’S PROSPECTIONS

39. Do you receive any financial aid (tax benefits/subsidies) for your prospection? a. Yes, municipal subsidies ☐ b. Yes, aid from associations related to garranos ☐ (specify:) c. Yes, subsidies from the portuguese government/European Union ☐ d. Yes, tax benefits provided by the portuguese government/ European Union☐ e. Yes, other(s) ☐ f. No ☐
40. From one to five, how pleased are you with the financial aid (tax benefits/subsidies) given currently to the garrano breeders?

Financial aid	Completely displeased Completely pleased
Municipal subsidies	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐
Garrano horses’ associations	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐
Subsidies of the portuguese government/European Union	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐
Tax benefits provided to breeders	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐
Other(s) ______________________	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐

41. If you are not currently pleased, which are the main problems associated to these supports? a. Quantity of available aid ☐ b. Available information about the different aids / transparency during the application process ☐ c. Difficulty of accessing aid ☐ d. Sum of the subsidy(ies) ☐ e. Reply time for aid’s application ☐ f. Other(s) ☐

42. Do you think that the financial aid available to date are enough to cover the essential costs related to the prospection? Yes ☐ No ☐.
43. In your opinion, in what way such aid could be improved?
44. Do you receive any other types of support for your prospection? a. Yes, at the medical level (veterinary help) ☐ b. Yes, at the technical control level (microchip setup, health control, etc.) ☐ c. Yes, regarding the registration in the Garrano Studbook ☐ d. Yes, regarding the preservation and improvement of the garrano race ☐ e. Yes, at the technical advice level (advice for the increase of productivity/profit and decrease of costs, subsidies' application, etc.) ☐ f. Yes, regarding protection against predation (implementation of protection measures, reward system, etc.) ☐ g. Yes, other(s) ☐ h. No ☐.
45. If yes, who is providing this type of support (mark all that apply): a. Municipal bodies ☐ b. Garrano horses' associations ☐ (specify:) c. Portuguese government bodies (ICNF, IFAP, etc.) ☐ (specify:) d. European Union bodies ☐ e. Other(s) ☐.
46. Do you think this type of support is useful and suited to the needs of the breeders? Yes ☐ No ☐.
a. If not, which problems do you associate to this type of support?
b. In your opinion, in what way could this support be improved?
47. Have you ever applied to/know the compensation system for damages caused by the iberian-wolf of ICNF? Yes ☐ No ☐.
48. If yes, do you agree with the currently used system?

Do not agree Completely agree
1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐

49. From one to five, how satisfied are you with the following aspects of the current compensation system:

	Totally unsatisfied Totally satisfied
Easiness to declare wolf attacks	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐
Number of necessary requirements to obtain compensation	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐
Accuracy in the evaluation of the damage	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐
Waiting time until obtaining the final result	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐
Support provided by ICNF throughout the whole process	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐
Support provided by other associations throughout the whole process	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐
Sum of compensation obtained	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐
Waiting time until obtaining the compensation	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐
Maximum compensation limit per year and per breeder	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐
Degree of suitability of the system to the garranos	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐
Other(s) ______________________	1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐

50. Do you think the obtained compensations are enough to cover all the caused damage? Yes ☐ No ☐.
51. Do you identify any problem in the current compensation system of damages from ICNF?
52. If yes, how do you think such system could be improved?
53. Are you aware that, as of 2022, compensations for damages caused by wolves will no longer be awarded? Yes ☐ No ☐.
54. Will such change have any impact on your exploitation?

Negative impact No impact Positive Impact
1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐

55. In your opinion, which measure(s) do you think should be taken to compensate the damages caused by wolves in the future? a. Compensation system equal to the current one ☐ b. Improved compensation system ☐ c. Other measure(s) ☐ d. No measure(s) ☐

NOTES:

Appendix III: wolf dietary studies and determinants for horse consumption by wolves

Table S3. Information regarding wolf dietary studies reporting horse consumption, including name of wolf packs, year of data collection (with corresponding bibliographic reference) and respective variables considered for the analysis of determinants for horse consumption by wolves. The variables included in this analysis were as follows: Frequency of occurrence (of horses, domestic ungulates and wild ungulates), Prey diversity (of domestic and wild ungulates), Trophic niche breadth, Population density (of horses, cattle and sheep+goats), Ivlev's index (for horses), Wolf pack size (per year), Proportion of forest cover (per pack territory) and Number of horse attacks declared to ICNF for compensation (per year).

Pack (Collection Years)	*F.O. (Horses)*	*F.O. (Livestock)*	*F.O. (Wild Prey)*	*Prey diversity (Livestock)*	*Prey diversity (Wild Prey)*	*Prey diversity (Total)*	*Trophic niche breadth*	*Ivlev's index (Horse)*	*Population density (Horses)*	*Population density (Cattle)*	*Population density (Sheep+Goat)*	*Wolf pack size*	*Proportion of forest cover*	*No. of declared horse attacks*
Arga (2017)[1]	0.81	0.14	0.05	2	2	5	0.37	0.94	4.31	6.26	44.26	7	0.02	0
Arga (2018)[1]	0.84	0.13	0.03	2	1	4	0.46	0.95	3.80	6.26	44.26	7	0.02	2
Gerês (1996)[2]	0.41	0.59	0.00	1	0	3	0.97	0.78	3.70	5.50	39.20	5	0.28	16
Gerês (1997-1998)[3]	0.28	0.55	0.12	3	2	8	0.30	0.76	1.82	9.98	24.84	6	0.28	24
Vez/Soajo (1999-2000)[4]	0.37	0.44	0.12	3	2	11	0.30	0.88	2.70	16.40	31.30	13	0.19	168
Vez/Soajo (2004)[5]	0.30	0.56	0.11	3	2	9	0.32	0.31	5.18	28.74	54.34	18	0.16	167
Vez/Soajo (2008-2010)[6]	0.68	0.21	0.11	2	2	5	0.51	0.89	3.76	15.54	18.84	13	0.16	107
Larouco (1998)[7]	0.07	0.65	0.10	4	2	11	0.53	0.68	0.80	14.20	25.00	8	0.09	1
Pitões (1998)[7]	0.15	0.75	0.07	5	2	11	0.29	0.62	1.50	12.10	18.60	8	0.05	28
Leiranco (1999)[7]	0.07	0.68	0.20	4	2	10	0.47	0.74	0.70	14.60	30.30	5	0.09	0
Boulhosa (2011-2017)[8]	0.31	0.56	0.09	4	2	9	0.47	0.96	1.04	15.58	35.63	3	0.17	2
Cruz Vermelha (2010-2017)[8]	0.46	0.48	0.04	3	2	7	0.50	0.95	2.00	83.24	40.46	5	0.26	22

References of wolf dietary studies reviewed:

[1] Freitas (2019); [2] Vos (2000); [3] Lançós (1999); [4] (Álvares, 2011); Costa (2000); [5] Guerra (2004); [6] Casimiro (2017); [7] Álvares (2011); [8] Álvares *et al.* (2019)

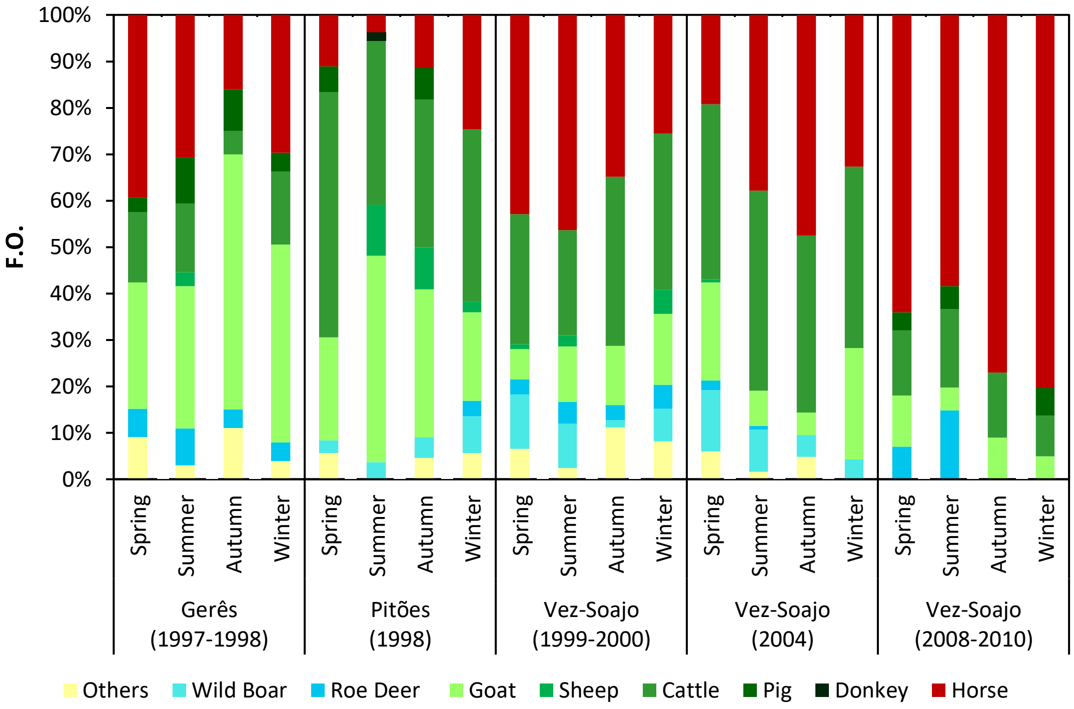

Figure S1. Seasonal variation of wolf diet (for each season separately) in five packs/sampling periods from northwest Portugal, considering the magnitude of prey consumption (expressed as F.O.) for free-ranging horses (in red), livestock species (in green) wild ungulates (in blue) and other prey (in yellow).

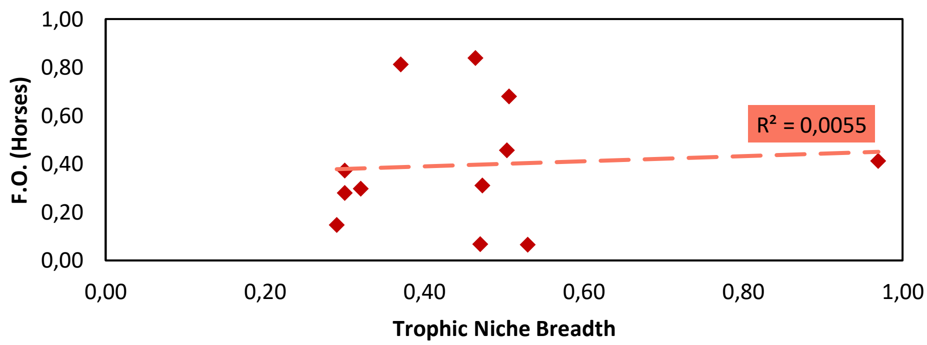

Figure S2. Linear regression between magnitude of horse consumption (F.O.) and value of trophic niche breadth (measured using Levin's Index), based in 12 studies on wolf diet.

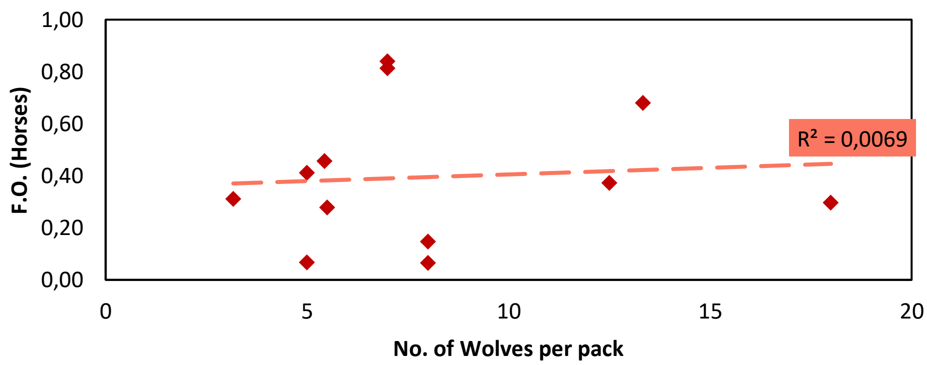

Figure S3. Linear regression between magnitude of horse consumption (F.O.) and number of wolves per pack (average pack size per year), based in 12 studies on wolf diet.

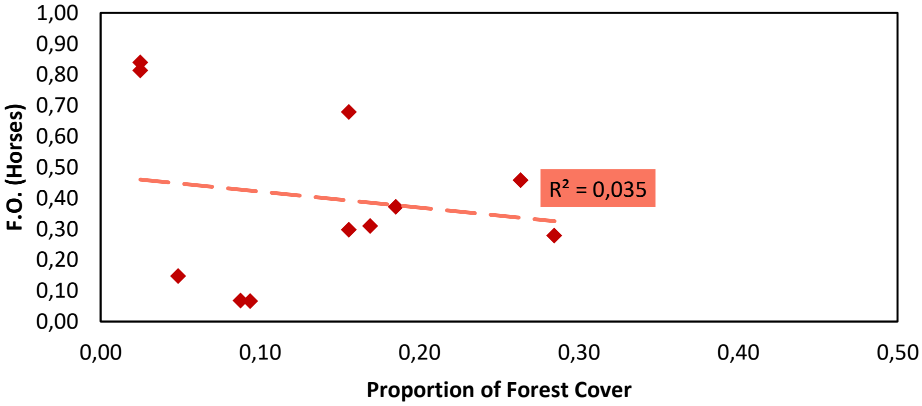

Figure S4. Linear regression between magnitude of horse consumption (F.O.) and the proportion of forest cover (inside each pack territory), based in 12 studies on wolf diet.

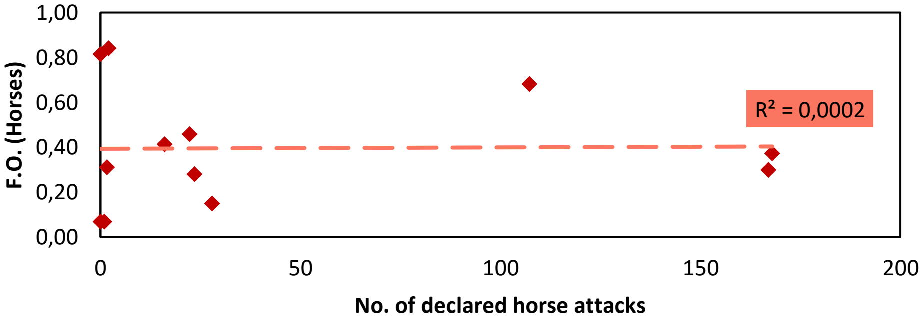

Figure S5. Linear regression between magnitude of horse consumption (F.O.) and the number of wolf attacks on horses declared by owners to ICNF, based in 12 studies on wolf diet.

Table S4. Results from GLM analysis representing the effect of domestic and wild prey consumption on the magnitude of horse consumption by wolves.

Parameter	*Estimate*	*Std. Error*	*Z value*	*Pr(>|z|) (p-value)*
(Intercept)	2.671	2.129	1.254	0.210
F.O. (Livestock)	-5.248	3.800	-1.381	0.167
F.O. (Wild Prey)	-7.630	14.944	-0.511	0.610

Table S5. Results from GLM analysis representing the effect of the number of domestic and wild ungulate species present in wolf diet on the magnitude of horse consumption by wolves**.**

Parameter	*Estimate*	*Std. Error*	*Z value*	*Pr(>|z|) (p-value)*
(Intercept)	1.513	2.046	0.739	0.460
Prey Diversity (Livestock)	-1.187	0.960	-1.236	0.216
Prey Diversity (Wild Prey)	0.857	1.417	0.605	0.545

Table S6. Results from GLM analysis representing the effect of alternative prey diversity intensity on the magnitude of horse consumption by wolves[5].

Parameter	*Estimate*	*Std. Error*	*Z value*	*Pr(>|z|) (p-value)*
(Intercept)	2.026	1.905	1.064	0.287
Prey Diversity (Total)	-0.325	0.242	-1.339	0.181

Table S7. Results from GLM analysis representing the effect horse selectivity by wolves (measured using Ivlev's Index) on the magnitude of horse consumption by wolves.

Parameter	*Estimate*	*Std. Error*	*Z value*	*Pr(>|z|) (p-value)*
(Intercept)	-3.626	3.655	-0.992	0.321
Ivlev's Index (Horses)	3.969	4.359	0.910	0.363

Table S8. Results from GLM analysis representing the effect of horse, cattle, and small ruminants (sheep and goat) density on the magnitude of horse consumption by wolves.

Parameter	*Estimate*	*Std. Error*	*Z value*	*Pr(>|z|) (p-value)*
(Intercept)	-1.716	2.133	-0.805	0.421
Population Density (Horse)	0.594	0.604	0.983	0.326
Population Density (Cattle)	0.004	0.033	0.108	0.914
Population Density (Sheep+Goat)	-0.011	0.078	-0.144	0.885

[5] Occasional prey with minimal presence on wolf diet are considered, in opposition to Table 3 which only includes the main prey classes consumed by wolves.

Appendix IV: home ranges and core areas of Garrano horses in Serra de Sta. Luzia

Table S9. Home ranges and Core areas sizes (in Km^2) during the overall tracking period (Total), Day (07:00-18:59) and Night (19:00-06:59) periods of two free-ranging horses in Serra de Sta. Luzia, estimated using GPS locations obtained between April 18 and June 13, 2020.

Tagged horse	Total		Day		Night	
	Home ranges	***Core areas***	***Home Ranges***	***Core Areas***	***Home Ranges***	***Core Areas***
Horse I	1.5 Km^2	0.4 Km^2	1.7 Km^2	0.5 Km^2	1.6 Km^2	0.4 Km^2
Horse II	2.1 Km^2	0.5 Km^2	2.2 Km^2	0.5 Km^2	2.2 Km^2	0.6 Km^2

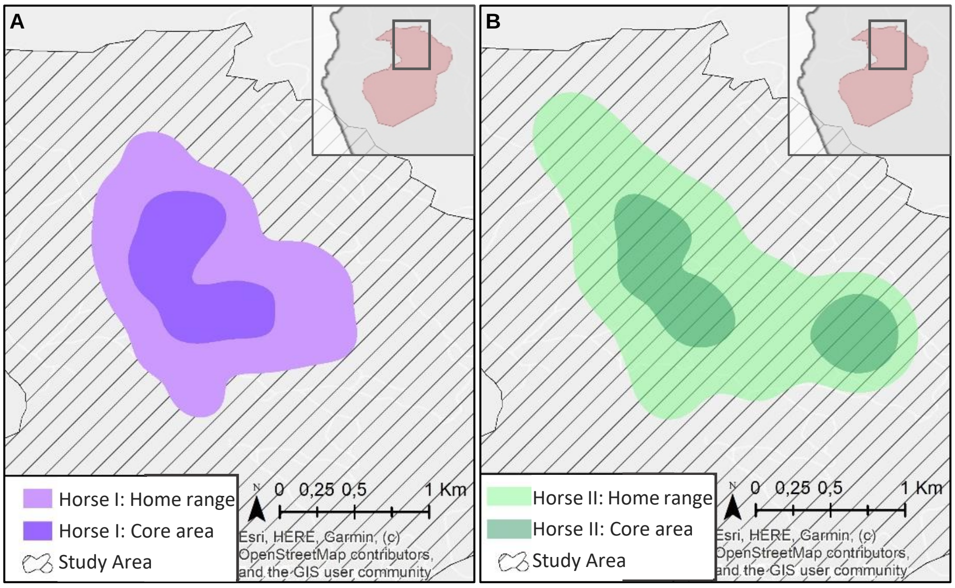

Figure S6. Home ranges (lighter colour) and Core areas (darker colour) of two free-ranging horses in Serra de Sta. Luzia, estimated using GPS locations obtained between April 18 and June 13 2020: A) Tagged horse I; B) Tagged horse II.

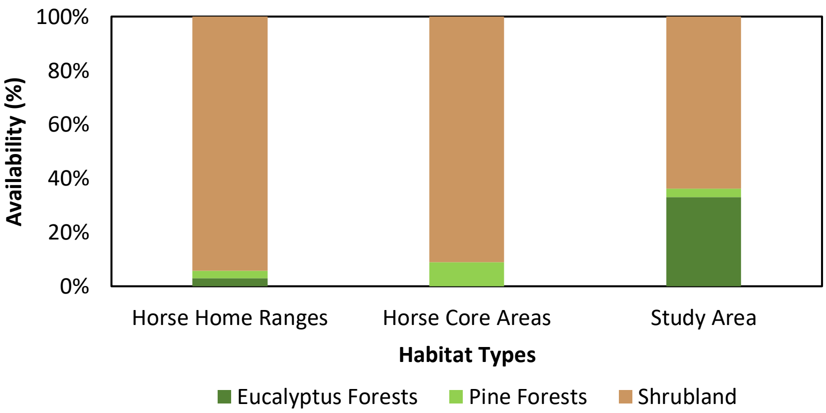

Figure S7. Availability of Eucalyptus Forest (in dark green), Pine Forests (in light green) and Shrubland (in brown) in the home ranges of the two tagged horses and in the Study Area.

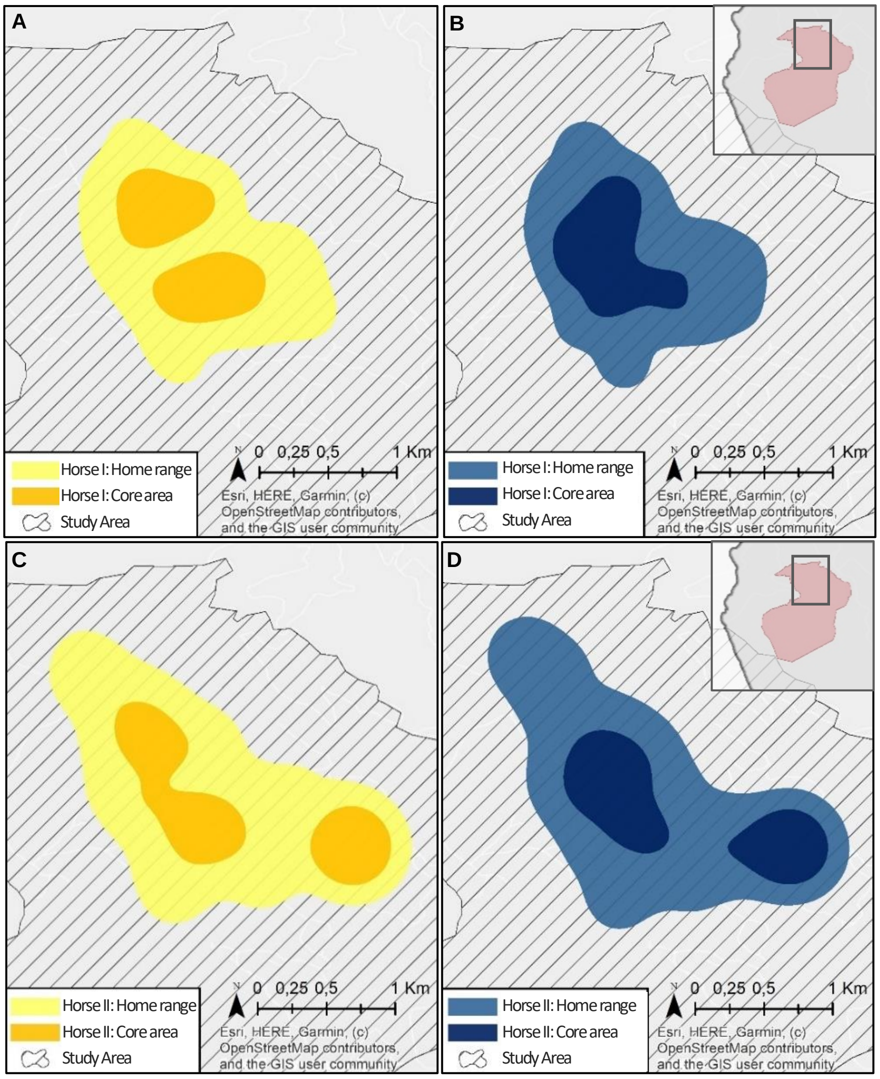

Figure S8. Home ranges (lighter colour) and Core areas (darker colour) during Day (in yellow) and Night (in blue) of two free-ranging horses in Serra de Sta. Luzia, estimated using from GPS locations obtained between April 18 and June 13 2020: A) Tagged horse I (Day); B) Tagged horse I (Night); C) Tagged horse II (Day); D) Tagged horse II (Night).

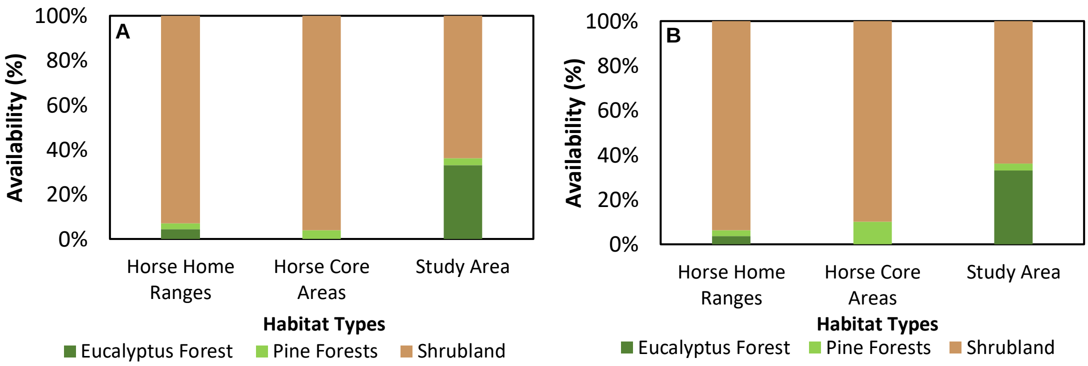

Figure S9. Availability of Eucalyptus Forest (in dark green), Pine Forests (in light green) and Shrubland (in brown) in the home ranges of the two tagged horses and in the Study Area, during A) Day (07:00-18:59) and B) Night (19:00-06:59).

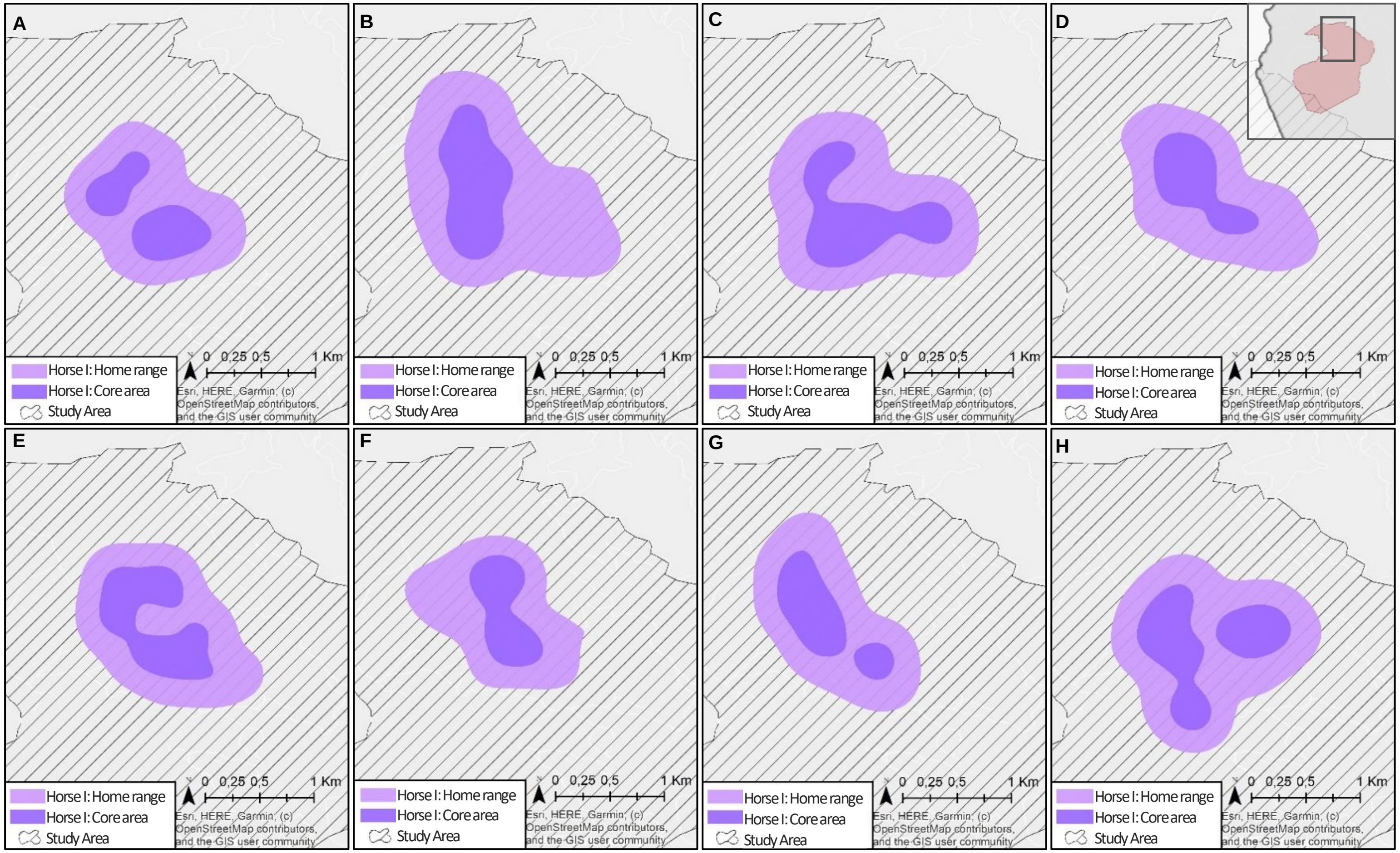

Figure S10. Home ranges (lighter colour) and Core areas (darker colour) of tagged horse I during 8 consecutive periods of one week – 7 days - (A to H), estimated using GPS locations obtained between April 18 and June 13, 2020.

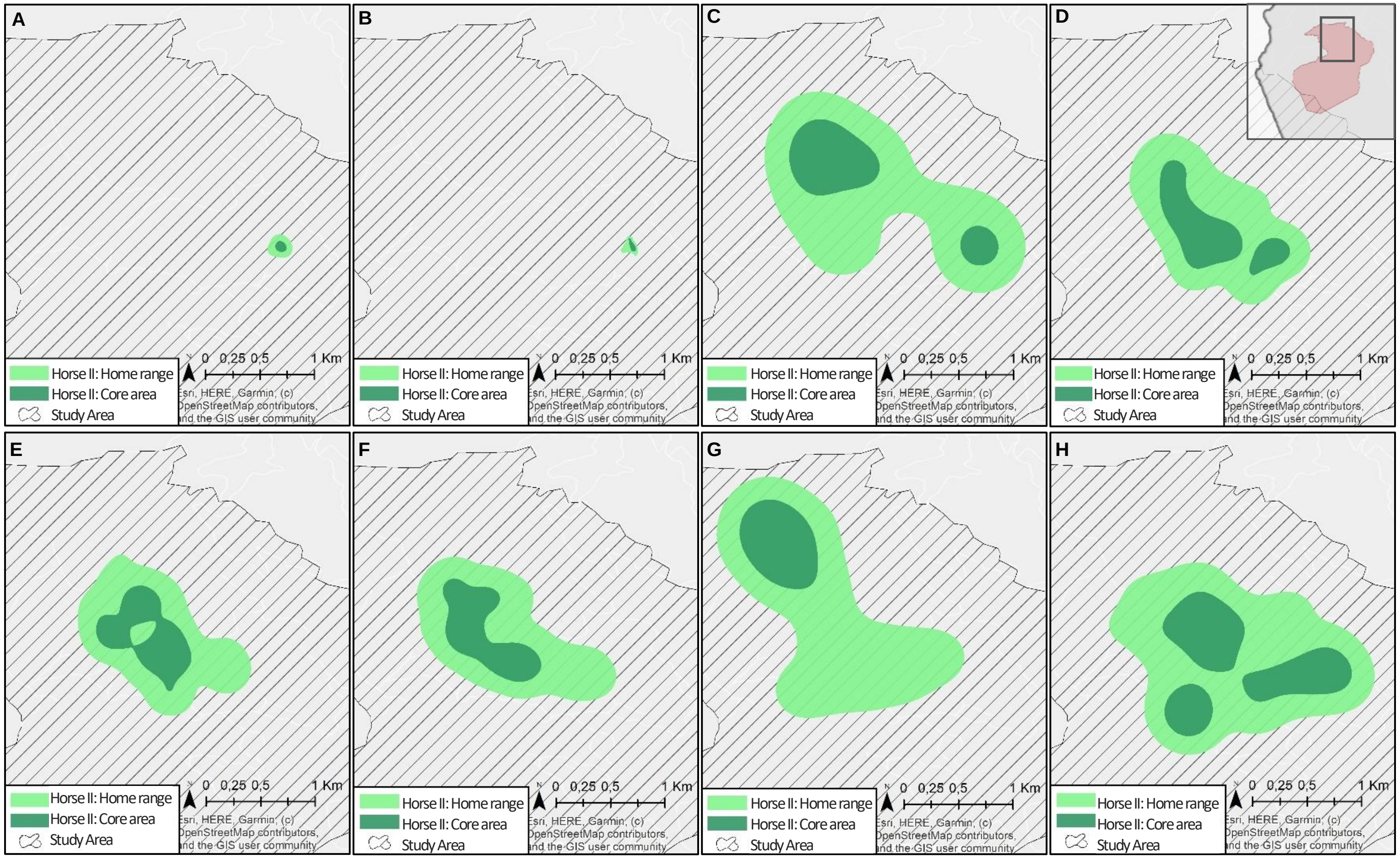

Figure S11. Home ranges (lighter colour) and Core areas (darker colour) of tagged horse II during 8 consecutive periods of one week – 7 days - (A to H), estimated using from GPS locations obtained between April 18 and June 13, 2020.

.

Appendix V: success rates for GPS tail tags used in free-ranging horses

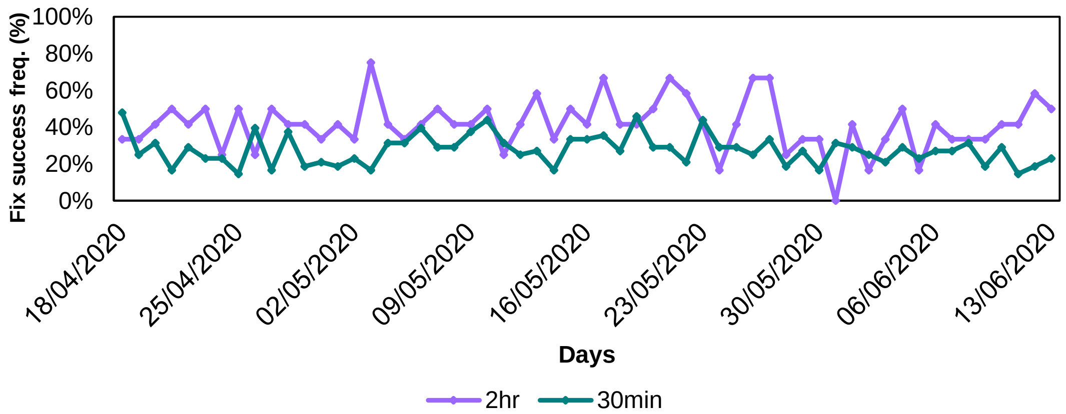

Figure S12. Daily rates of received locations (fix success frequency) for two GPS reception schedules: locations periodicity of 2 hours (in purple) and 30 minutes (in green).

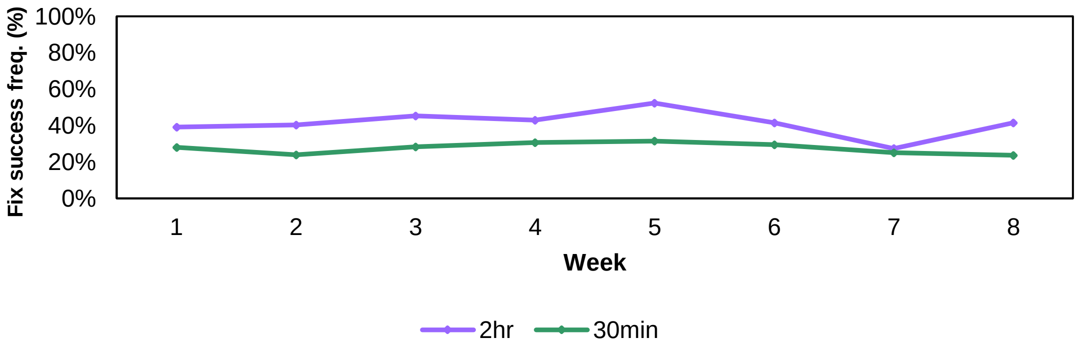

Figure S13. Weekly rates of received locations (fix success frequency) for two GPS reception schedules: locations periodicity of 2 hours (in purple) and 30 minutes (in green).

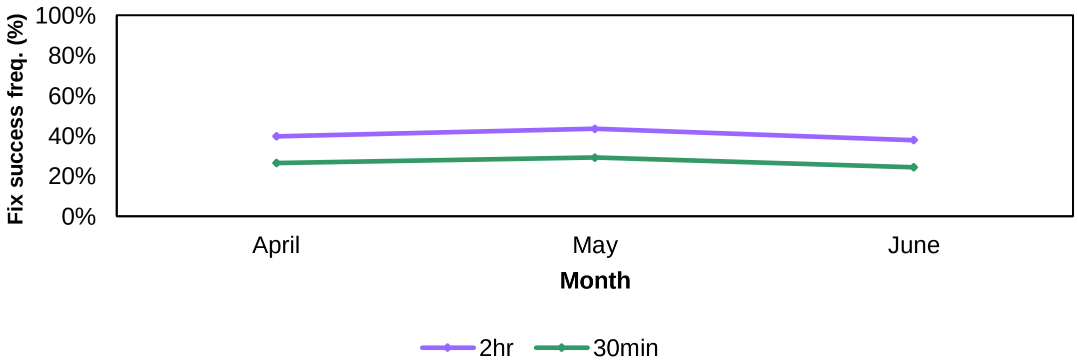

Figure S14. Monthly rates of received locations (fix success frequency) for two GPS reception schedules: locations periodicity of 2 hours (in purple) and 30 minutes (in green).