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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biologia Aplicada, realizada sob a orientação científica do Doutor João Luís Oliveira Carvalho, Investigador do Departamento de Biologia da Universidade de Aveiro, e coorientação do Professor Doutor Luís Miguel do Carmo Rosalino, Professor Auxiliar da Faculdade de Ciências da Universidade de Lisboa.

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o júri

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Palavras-chave

Resumo

O Vale do Côa é um *hotspot* de biodiversidade, contudo, nas últimas décadas, o abandono rural, a renaturalização de algumas áreas e as políticas agroflorestais adotadas, tornaram a paisagem desta região particularmente vulnerável aos incêndios rurais. Desta forma, a introdução ou reintrodução de espécies de ungulados em regime semisselvagem tem sido testada como uma forma de controlar o fitovolume e biomassa vegetal, e assim diminuir a probabilidade de ocorrência de incêndios. Como espécies-chave do ecossistema Mediterrânico, os ungulados exercem um conjunto de impactos não apenas na vegetação, mas também noutras comunidades de vertebrados. Neste estudo, os micromamíferos foram definidos como grupo-modelo de forma a avaliar os impactos do pastoreio de ungulados a diferentes níveis de pressão na estrutura das comunidades. Os micromamíferos desempenham um papel fundamental nas cadeias alimentares, com efeitos "top-down" nas comunidades de plantas e invertebrados e "bottom-up" nos mamíferos e aves que destes se alimentam, sendo bioindicadores importantes com um tempo de resposta a perturbações relativamente rápido e mensurável. No âmbito deste trabalho, foram capturados micromamíferos em quatro áreas com diferentes níveis de pressão de ungulados (duas réplicas por parcela) na reserva da Faia Brava, Quinta do Sol e Quinta de São Paulo. Em cada ponto de amostragem foram registadas as espécies capturadas, métricas corporais e carga parasitária, procedendo-se, igualmente, à armadilhagem fotográfica de ungulados e mesocarnívoros de forma a perceber qual o uso efetivo que os ungulados e mesocarnívoros fazem das áreas amostradas. Ao longo das duas amostragens (outono 2020, primavera 2021), foram capturados indivíduos pertencentes a quatro espécies: os roedores *Apodemus sylvaticus*, *Mus spretus* e *Eliomys quercinus* e o insectívoro *Crocidura russula*. De forma a determinar quais variáveis que determinam a abundância e condição corporal dos micromamíferos, foram usados Modelos Lineares Generalizados Mistos (GLMM). A abundância de micromamíferos revelou ser superior na área de exclusão e reduzida na área com elevada densidade de ungulados. A área de pressão intermédia revelou também valores elevados na abundância de micromamíferos, para além de ter sido a área com maior riqueza específica de roedores, ungulados e mesocarnívoros. Os resultados demonstraram uma influência negativa do pastoreio a níveis elevados de densidade de ungulados sobre a abundância de micromamíferos. A exceção foi a espécie *A. sylvaticus* que revelou ser influenciada positivamente. No caso da condição corporal, esta revelou ser influenciada positivamente pela presença de ungulados no caso do *A. sylvaticus*. A espécie *C. russula* apresentou valores muito semelhantes em todas as áreas, com um ligeiro aumento na área de pressão intermédia, enquanto a espécie *M. spretus* sofreu um impacto negativo. Estes resultados revelaram que a reintrodução e manutenção das populações de ungulados a densidades intermédias não provoca uma perturbação acentuada e negativa na comunidade de micromamíferos, dado que proporciona uma heterogeneidade que favorece diferentes densidades de coberto arbustivo e arbóreo, sendo possível desta forma restaurar a funcionalidade e a resiliência dos ecossistemas Mediterrânicos.

Abstract   The Côa Valley is a biodiversity hotspot, however, in recent decades, the rural abandonment, the restoration of some areas and the agroforestry policies adopted have made the landscape of this region particularly vulnerable to rural fires. The introduction or reintroduction of ungulate species in a semi-wild regime has been tested as a way to control phytovolume and plant biomass, and thus decrease the probability of fire occurrence. As keystone species of the Mediterranean ecosystem, ungulates exert a range of impacts not only on vegetation, but also on other vertebrate communities. In this study, small mammals were defined as a model group in order to evaluate the impacts of ungulate grazing at different levels of pressure on wildlife communities. Small mammals play a fundamental role in food chains, with top-down effects on plant and invertebrate communities and bottom-up effects on the mammals and birds that feed on them, being important bioindicators with a relatively fast response time to disturbances. In this study, small mammals were captured in four areas with different levels of ungulate pressure (two replicates *per* plot) in the *Faia Brava* reserve, *Quinta do Sol* and *Quinta de São Paulo*. At each sampling point, the captured species, body metrics and parasite load were recorded. Camera trapping was used to determine the effective use that ungulates and mesocarnivores make of the sampled areas. During the two sampling periods (autumn 2020, spring 2021), individuals from four species were captured: the rodents *Apodemus sylvaticus*, *Mus spretus* and *Eliomys quercinus* and the insectivore *Crocidura russula*. Here, I used Generalized Linear Mixed Models (GLMM) to determine which variables determine the abundance and body condition of small mammals. The abundance of small mammals was higher in the exclusion area and reduced in the area with high density of ungulates. The intermediate-pressure area revealed high values in the abundance of small mammals and the highest richness of rodents, ungulates and mesocarnivores. The area of intermediate-pressure was characterised by the highest percentage of vegetation, shelter and food for small mammals. The results showed a negative influence of high grazing intensity on the abundance of small mammals, with the exception of *A. sylvaticus*. Body condition of *A. sylvaticus* was positively influenced by the presence of ungulates. The body condition of *C. russula* presented very similar values in all areas with a slight increase in the intermediate-pressure area, while *M. spretus* were negatively impacted. These results revealed that the reintroduction of ungulates at intermediate densities does not cause a marked disturbance in the small mammal community, as it contributes to maintain landscape heterogeneity and favours different densities of shrubs and tree cover. These results represent an important step towards the restoration of the functionality of Mediterranean ecosystems.

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Acronyms

ATN: Associação Transumância e Natureza

GLMM: Generalized Linear Mixed Models

H: High-pressure area

Ha: Grid a from the high-pressure area

Hb: Grid b from the high-pressure area

I: Intermediate-pressure area

Ia: Grid a from the intermediate-pressure area

Ib: Grid b from the intermediate-pressure area

L: Low-pressure area

La: Grid a from the low-pressure area

Lb: Grid b from the low-pressure area

N: Control area

Na: Grid a from the control area

Nb: Grid b from the control area

1. Introduction

Humans have a paramount influence on Earth ecosystems, from the bottom of the oceans to the highest mountains (Goudie, 2013). These influences can be either direct (e.g., fishery, harvest, land use change) and/or indirect (e.g., pollution of waterways, greenhouse gas emissions) (Rockström et al., 2009) and are expected to increase over the next 30 years (Gordon et al., 2016). The impact of anthropogenic action on nature is mainly due to overconsumption, faulty economic arrangements (e.g., the market prices of several resources are not reflective of the real social costs), and the development of misconceived environmental technologies (Dirzo and Raven 2003; Rockström et al., 2009). These social, economic and political drivers are responsible for several deleterious impacts on the Earth's systems, such as climate change, landscape fragmentation, biodiversity loss, among others (Dirzo and Raven 2003; Rockström et al., 2009).

Humans have been causing negative impacts on the planet since the Pleistocene Epoch (2.6 million to 11,700 years ago), leading to changes in the ecosystem level (75% of the land surface has been dramatically modified, more than 85% of the wetland has been lost, and 66% of the ocean area is facing increasing cumulative impacts) and at the species level (approximately 50% – 1 million – species are under the threat of extinction) (Díaz, 2019b). The degradation of nature is one of the most critical issues facing the planet. Its' reversal is only possible by establishing ambitious and well-defined common goals, as well as a credible set of actions to restore the functionality of ecosystems (Díaz et al., 2019a).

There is now a growing global recognition about the importance of wild landscapes, biodiversity and scenic values to human well-being (Gordon et al., 2021). Currently, several conservation approaches are being developed with the aim of reversing biodiversity loss. Some of these initiatives have been successful, namely the establishment of protected areas (PAs) (Pimm et al., 2001), ecological corridors (European Commission, 2020), or the introduction, reintroduction or restocking of wild species (Gordon et al., 2021).

1.1. Rewilding

Rewilding appeared in print for the first time in 1990, however its' popularity only began to gain momentum during the last decade. Rewilding is considered a recent and rapidly developing concept in ecosystem management, having developed from a theoretical concept into a practical idea (Jepson, 2016; Pettorelli et al., 2018; Pettorelli, 2019). It was originally defined as a conservation method based on the three C's approach (i.e., Cores areas, Carnivores and Corridors) (Soulé and Noss, 1998). This approach has been applied in North America and has been based on the reintroduction of native keystone species (mainly wolves *Canis lupus* Linnaeus, 1758) in wider territories and establishing large, well-connected core areas in order to actively promote the successful restoration of large wild vertebrates (e.g., elks *Cervus canadensis* Erxleben, 1777) (Donlan, 2005; Spiering, 2006; Lorimer et al., 2015; Carey, 2016).

There are three main perspectives in the current accepted definitions of "rewilding" (Pettorelli et al., 2018; Pettorelli, 2019). The first is related to resumption of wilderness, whereby degraded areas can recover their biodiversity and develop with no additional interference (Lorimer et al., 2015). The second is concerned with the reintroduction of extirpated species (i.e., local extinction) or their replacement by other with similar ecological functions aimed at restoring ecosystem function (Naundrup and Svenning, 2015; Prior and Brady, 2017). The third focuses on the self-sustaining functionality of an ecosystem, where managers can reorganise it to provide ecosystem services with

a minimum of intervention in the prevailing environmental conditions, without necessarily having to restore it to a previous state. This theme recognizes the fact that biodiversity in changing social-ecological systems, in which the perceived benefits and costs determine which parts of wilderness persist and which parts disappear (Pettorelli et al., 2018). Although these three perspectives have applications in different circumstances and regions, they are all based on a distinction between the concepts of rewilding and restoration (Pettorelli, 2019). They consider that rewilding involves the selection of new trajectories of change, directed to undefined future states, while restoration usually consists of a reversal of a trajectory of change to return to a previously defined state (Pettorelli, 2019). Whatever the perspectives being considered, the different rewilding processes can be grouped into four distinct frameworks: Pleistocene rewilding; ecological rewilding; passive rewilding; and trophic rewilding (Pettorelli, 2019). Pleistocene rewilding refers to the restoration of ecological interactions that were lost during the late-Pleistocene megafauna extinctions (Pettorelli et al., 2018; Pettorelli, 2019). Ecological rewilding involves limited active management towards the restoration of natural processes (Pettorelli et al., 2018; Pettorelli, 2019). Contrarily, passive rewilding refers to the abandonment of post-agricultural landscapes, which are no longer actively managed (i.e., without human interference) and can be seen as an alternative to classic environmental management, where nature management is replaced by management for nature. This framework is being adopted mainly in Europe. Finally, trophic rewilding requires the introduction of species to restore top-down trophic interactions (e.g., introduction of medium or large herbivores), so as to maintain or increase the biodiversity of the area (Pettorelli et al., 2018; Pettorelli, 2019). Regardless of the applied perspectives or frameworks, rewilding aims to ensure that the assigned areas will be able to support all native species, including top predators, wide-ranging species, and other keystone species, as well as preserve all ecosystem processes and types, resilience and disturbance regimes (Pettorelli, 2019).

The geographical diversity of rewilding initiatives has become increasingly relevant, as well as the variety of different approaches to this concept. Numerous rewilding projects have been implemented worldwide, all expected to have the potential to increase ecological resilience, local biodiversity, and the provision of ecosystem services (Ceaușu et al., 2015; Naundrup and Svenning, 2015; Gordon et al., 2021). Many are based on restoring natural ecological processes, instead of a particular species *per se*.

In recent years, the rewilding approach has been both promoted and criticized. As an emerging concept, rewilding raises several questions concerning its social acceptability and ecological sustainability (Perino et al., 2019). Some highlight its potential to exploit restoration opportunities while providing benefits to ecosystems and societies, while others emphasize the lack of sufficient knowledge about rewilding results and the lack of a definition that is consistent, in addition to the incorrect notion that rewilding actions are designed without considering the benefits and acceptability to society (Perino et al., 2019). For these reasons, rewilding has been one of the most strongly debated topics in conservation (Nogués-Bravo et al., 2016), raising several questions including: (I) is there a scale for rewilding? (Root-Bernstein et al., 2017); (II) when, where, how many and which species should be reintroduced? (Seddon et al., 2014; Root-Bernstein et al., 2017); (III) how does rewilding adjust to human activities? (Svenning et al., 2016); (IV) is trophic rewilding the optimal solution to restore large carnivore populations? (Root-Bernstein et al., 2017); and (V) how to estimate the impacts of rewilding? (Corlett, 2016). These important questions require further analysis, being dependent on data and rewilding baselines, which should be strategically chosen to involve all necessary stakeholders in order to create productive dialogues and facilitate particular actions (Root-Bernstein et al., 2017).

The concept of rewilding incites us to widen our perspective on how nature will respond to the human-induced changes and to reflect on how we should manage nature (Perino et al., 2019). As each ecosystem will have specific effects as a result of rewilding actions, it is extremely important to have a thorough knowledge and understanding of the processes that shape ecosystems in order to predict these effects, so that appropriate management actions can be implemented (Perino et al., 2019).

Several rewilding projects in Europe are focused on restoring abandoned agricultural landscapes and on promoting natural grazing by large herbivores (Agnoletti, 2014; Carey, 2016). The presence of large herbivores will contribute to maintain a mosaic of vegetation (Agnoletti, 2014; Carey, 2016), while promoting the restoration or the creation of complex, highly biodiverse ecosystems (Wieren, 1995; Sandom et al., 2014), in a passive management perspective (Ceaușu et al., 2015). Rewilding in Europe was inspired by Frans Vera's forest mosaic hypothesis, which suggested that European landscapes were characterized by a mosaic of forest grasslands that would be sustained by the grazing of large herbivores (Soga and Gaston, 2018). Rewilding in the European context is seen as a process rather than a state, as it aims to give ecosystems a functional "upgrade", regardless of their nature, location, or scale (Schepers and Jepson, 2016), focusing more on recently extinct animals instead of recreating Pleistocene megafauna, as in the North America approach (Carey, 2016).

In the Iberian Peninsula, rewilding is currently in the early stages of implementation, however, in the Côa Valley (eastern Portugal), the process of ecosystem restoration has been implemented in the Faia Brava reserve, where the reintroduction of semi-wild herbivores (i.e. horses and cows which are able to survive in the wild with minimal human intervention) is envisaged as a management measure to ensure the maintenance of mosaic and functional landscapes (Pereira and Navarro, 2015).

1.2. The Faia Brava reserve

Humans and wildlife have co-existed in the Côa Valley since the Paleolithic period, with evidence of their interaction dating back almost 30.000 years (engravings of horses and aurochs, with ibex and red deer in Côa Valley Archaeological Park; Zilhão et al., 1997). This valley presents a wide diversity of species and habitats, being a biodiversity hotspot, however, due to habitat loss and degradation, rural depopulation, wildlife persecution, and a lack of awareness about the importance of ecosystem services and natural heritage, its' structural and ecological functionality are at risk.

For centuries this area was used for extensive herding of sheep and goats and was intensively cultivated with interspersed wheat and rye fields, olive groves (*Olea europaea* Linnaeus, 1753), cork oaks (*Quercus suber* Linnaeus, 1753), and almond trees (*Prunus dulcis* Mill., 1967) (DeSilvey and Bartolini, 2018). The desertion of this landscape occurred in the second half of the 20th century, as young people left for the cities or abroad (DeSilvey and Bartolini, 2018). Rural abandonment resulted in increased shrub encroachment and fuel load composition, as domestic herbivores were no longer removing vegetation and wild herbivore populations were present in relatively low densities. Furthermore, monoculture plantations of pine and eucalyptus have made the landscape extremely vulnerable to large-scale rural fires, which has led to an increase in land abandonment in the Côa Valley (Moreira et al., 2011).

The Faia Brava reserve is the first Private Protected Area (APP) in Portugal and has been managed by the Associação Transumância e Natureza (ATN), a Non-Governmental Organization

(NGO), created in 2000 and based in Figueira de Castelo Rodrigo, district of Guarda (Northeast Portugal). By reintroducing semi-wild species, like Garrano horses *Equus ferus* subsp. *caballus* Linnaeus, 1758 and Maronesa cows *Bos taurus* Linnaeus, 1758, ATN aims to restore the natural mechanisms that sustain the landscape mosaic, to provide ecological benefits to a wide range of fauna and flora and to significantly reduce the risk of fire (Pereira and Navarro, 2015; DeSilvey et al., 2020).

Since the approach adopted in this reserve is not completely hands-off, it requires some level of management of the herds of cattle and horses so as to meet animal welfare requirements and landscape management goals (Gordon et al., 2021). As the area is basically devoid of predators, the reserve managers remove a defined number of horses and cows whenever the carrying capacity is reached (Gordon et al., 2021). These ungulates can access artificial water points in the reserve, and if necessary, can receive supplementary feeding, in particular during harsh conditions (Gordon et al., 2021).

The rWILD-COA - Ecological challenges and opportunities of trophic rewilding in Côa Valley is a project currently being developed in the Faia Brava reserve, aimed at testing and quantifying the potential role of semi-wild ungulates as "engineers" of the Mediterranean ecosystem. Given that ungulates have a key role in the trophic balance of ecosystems, one of the aims of this project is to understand how the activity of ungulates, (i.e., grazing, browsing, trampling and dropping) in a semi-natural regime, protects (i.e., buffer) or increases changes in species richness and diversity, particularly in small mammals. Small mammals were defined as the target group and model in this study to assess the impacts of grazing by ungulates in wildlife communities.

1.3. Herbivores impacts on vegetation and wildlife communities

Herbivores include approximately 4.000 species that inhabit all major terrestrial ecosystems, except Antarctica (Ripple et al., 2015). Ungulates, in particular, are characterized by having hooves, and are divided into two orders: Artiodactyla, which are ungulates with an even number of toes (e.g., fallow deer *Dama dama* Linnaeus, 1758; Bruinderink and Hazebroek, 1996; and moose *Alces alces* Linnaeus, 1758; Ramirez et al., 2018), and Perissodactyla, which have an odd number of toes (e.g., horse *Equus caballus* Linnaeus, 1758; and rhino *Rhinoceros unicornis* Linnaeus, 1758; Krausman and Bleich, 2013).

Ungulates, both wild or domestic, are responsible for causing changes in ecosystems due to their activities, including browsing, grazing, trampling, stripping, uprooting, feeding and dispersal of seeds and fruits, and defecation (Bruinderink and Hazebroek, 1996; Reimoser, 2003; Pastor et al., 2006), being these interactions fundamental determinants of the dynamics and structure of ecosystems (Ramirez and Poorter, 2018). For thousands of years, ungulates have had a significant impact on ecosystems, as they have the ability to modify almost every factor related to the local environment, having shaped the structure and function of landscapes, modified community composition, as well as nurtured and maintained biologically rich and productive ecosystems where they occur (Franklin et al., 1981; Goderie et al., 2013; Ripple et al., 2015; Navarro-Castilla et al., 2017).

Previous studies have shown that ungulates can create open spaces that promote germination, which is expected to produce significant effects on the ecosystem by affecting the composition and dynamics of plant communities, plant traits, richness, vertical profiles, water infiltration, soil compaction and erosion, among other factors (Milchunas et al., 1988; Noy-Meir et al., 1989;

Biondini et al., 1998; Korpimäki and Norrdahl, 1998; McIntyre and Lavorel, 2001; Rodríguez et al., 2003; Torre et al., 2007).

They have the potential to influence animal species diversity by causing changes in animal communities, directly and indirectly, through the food web (Ripple et al., 2015; Navarro-Castilla et al., 2017). Some of the species that are affected include mammals (e.g., wood mouse *Apodemus sylvaticus* Linnaeus, 1758; Grant et al., 1982; Foster et al., 2014), birds (e.g., tawny owl *Strix aluco* Linnaeus, 1758; Bock et al., 1984; Foster et al., 2014) and insects (e.g., white wood butterfly *Leptidea sinapis* Linnaeus, 1758; Warren and Key, 1991). Ungulates also have the capacity to alter abiotic processes, including soil properties, nutrient cycles and fire regimes (Ripple et al., 2015). Thus, a reduction in the population of large herbivores can lead to changes in ecosystems, namely the loss of ecological interactions as well as key ecosystem services (Ripple et al., 2015).

According to Clutton-Brock and Harvey (1983), there is a strong relationship between the body dimensions of ungulates and their behavior and ecology (Clutton-Brock and Harvey, 1983). Conservation measures adopted for grazing ecosystems generally involve the introduction of herbivores as substitutes for naturally extinct herbivores, which were considered essential for the preservation of these ecosystems (Pettorelli, 2019). When a particular species is extinct, the restoration of its ecological functions can only be reached by ecological replacement, which consists in the introduction of a species relative, that is functionally similar (Pettorelli, 2019). Some important criteria in the evaluation of a potential ecological surrogate are the functional equivalence and the taxonomic relationship with the original herbivore species, although the main difficulty for its generalized use in conservation is the difficulty in predicting its effects on the recipient ecosystems (Pettorelli, 2019). Despite this, one way around the taxonomic relationship issue is to introduce livestock as a replacement for extinct wild herbivores, since domestic breeds are descended from wild herbivore ancestors, which are taxonomically and theoretically functionally similar to wild herbivores (Pettorelli, 2019). An illustrative example is the introduction of domestic herbivores or native species, such as bison, to achieve grassland restoration (Towne et al., 2005). However, introducing livestock species on the same area with wild co-evolved native herbivores, can induce competition between them to the point that the wild native herbivores are excluded (Mishra et al., 2002).

Anthropogenic activities and environmental changes that have occurred since the local extinction of a particular herbivore might produce new and different communities and, consequently, a new ecological equilibrium (Smith, 2005). Therefore, the way communities respond to the introduction or reintroduction of large herbivores will be driven by the intensity by which the recipient ecosystem has been changed, as the reintroduction of extirpated species results in complex effects on plant communities and may in turn lead to mixed management outcomes (Pettorelli, 2019).

The effects of trophic rewilding can be translated into biodiversity outcomes and/or functional ecosystem impacts. Some possible biodiversity outcomes are an increment in diversity and abundance of organisms with strong interactions with megafauna, through disturbance and bottom-up as well as top-down trophic effects, at both local and landscape scales (Pykälä, 2000; Pettorelli, 2019; van Klink et al., 2020). Possible functional impacts on ecosystems are: the higher rates of long-distance dispersal of nutrients and propagules; positive effects on landscape heterogeneity, which will increase with increased functional diversity of herbivore species; increased heterogeneity that tends to be more pronounced when the topographic-edaphic conditions and initial vegetation structure are heterogeneous; increased vegetation diversity and environmental heterogeneity at the local and landscape scale (Pettorelli, 2019). The impacts are context-

dependent, but are expected to be particularly noted in moderately humid and relatively warm climates, where vegetation is tending towards stability between the herbaceous and woody domain (Pettorelli, 2019).

There is a growing number of exclosure experiments that have demonstrated the strong impacts of ungulate grazing on small mammal composition, with most of these studies finding lower abundance and species richness in grazing areas when compared to the exclosure compartments (i.e., areas without ungulates). The explanation for these results was based on the negative effect of grazing on the quantity and/or quality of the vegetation and resources present in the area (Keesing, 1998).

A large number of rodents consume vegetation and thus have the potential to compete with other herbivores, in particular ungulates (Keesing, 1998; Eccard et al., 2000). If there are ungulates in a certain area and their foraging reduces the quantity (Schmidt et al., 2005) and/or quality (Keesing, 1998) of food resources available for rodents, it will cause changes in the rodent populations, consequently decreasing the number of individuals (Keesing, 1998).

In Portugal, one of the trophic rewilding measures that has been implemented is the reintroduction of semi-domestic livestock, in particular modern European wild horse breeds (e.g., Garrano and Sorraia horses) in locations where they had become extinct, since they perform an important role in European ecosystems similar to their ancestors (i.e., original European wild horses) (Linnartz and Meissner, 2014). Maronesa cows are also being reintroduced as a replacement for their important extinct ancestor, the aurochs *Bos primigenius* Bojanus, 1827, a wild cow that played a vital role in the maintenance of biodiversity and became extinct in Europe in 1627 (Navarro and Pereira, 2012; Pereira and Navarro, 2015). Regarding the wild native ungulate species, there are only four native species which appear in mainland Portugal, the wild boar *Sus scrofa* Linnaeus, 1758; the red deer *Cervus elaphus* Linnaeus, 1758; the roe deer *Capreolus capreolus* Linnaeus, 1758; and the Iberian wild goat *Capra pyrenaica* Schinz, 1838 (Figueiredo et al., 2020). The European fallow deer *Dama dama* and the European mouflon *Ovis aries* subsp. *musimon* (Pallas, 1811) can also be found, ungulate species which were introduced into Portugal (Bencatel et al., 2017).

1.4. Small mammals as a study model

The abundance and distribution of small mammals (Mammalia: orders Rodentia and Insectivora) is extremely important in ecosystems at both functional and ecological levels, since they are the main prey of higher trophic levels, namely the mammalian carnivores (in particular mesocarnivores; e.g., least weasel *Mustela nivalis* Linnaeus, 1766; Sheffield and King, 1994), reptiles (e.g., Lataste's viper *Vipera latastei* Boscà, 1878; Pleguezuelos et al., 2007) and birds of prey (e.g., Barn owl *Tyto alba* Scopoli, 1769; Rosalino et al., 2011a; Rosalino et al., 2011b; Milchev, 2015).

The diet of small mammals is comprised of plants (including fruits and seeds), lichen, fungi, and invertebrates (Sunyer et al., 2013). Small mammals are seed dispersers which contribute to the natural recovery of habitats (Sunyer et al., 2013) and important disseminators of the symbiotic ectomycorrhizal fungi that facilitate the absorption of water and nutrients by woody plants (Carey and Johnson, 1995). Furthermore, the droppings of domestic livestock are extremely important in the diet of several species, including a large number of mammals, in particular shrews (e.g., species of *Crocidura*) (Simões, 2009). Small mammals are key elements in an ecosystem, due to their position in the trophic chain (Torre et al., 2007) and for presenting a rapid response to disturbances

(Cabral et al., 2005), which allows the detection of changes in the quality and suitability of the habitats being studied (Simões, 2009). Thus, it is expected that their response to management practices and habitat structure reflects the condition of other species (e.g., mesocarnivores) (Simões, 2009). For these reasons and due to their high abundance and easy capture, small mammals are a good model to study the impacts of grazing by ungulates, being important indicators of ecosystem functionality (Sieg, 1988; Ma, 1989; Rhim et al., 2014).

Studies have shown that areas with high grazing and low shrub and herbaceous vegetation present a reduced population of small mammals (Putman et al., 1989; Dennis et al., 2002). The decrease in abundance of small mammal populations causes significant modifications in the structure and composition of their consumers (meso and top predators) (Moreira-Arce et al., 2015). In contrast, a higher abundance and diversity of small mammals is associated to areas with abundant herbaceous and shrub vegetation (Petty, 1998); this occurs for example in semi-natural habitats (i.e., ecosystems influenced by human activities on a limited extent) (Van Meerbeek et al., 2019) with reduced grazing intensity, which provide suitable habitats for a wide variety of small mammals and their predators (Simões, 2009; Guilherme, 2010).

1.5. Objectives

The present study aims to increase knowledge in the possible impacts of ungulate activity on the richness, diversity, parasite load and body condition of small mammal species. For this purpose, we compared small mammals captured at Faia Brava reserve and Quinta do Sol with three different levels of ungulate pressure and at Quinta de São Paulo, here used as a control area. I intend to test the following hypotheses (table 1):

Table 1. Hypothesis proposed in this study, its' reasoning and supporting references.

Hypotheses	Reasoning	Supporting references
The species richness and abundance of small mammals in the control area are higher when compared to areas with the presence of ungulates.	The areas without grazing are structurally more complex, as they have a higher diversity of vegetation and cover, and consequently more food and shelter are available, resulting in a higher diversity and abundance of populations of small mammals.	Putman et al., 1989.
The body condition of small mammals is lower in the high-pressure area in both seasons, due to the lower food availability.	The reduced availability of food resources, frequently associated with grazed areas, can negatively influence the body condition of small mammals.	Keesing, 1998.
The parasite load of small mammals is higher in the control, area with an increase parasite activity during spring.	The presence of a high percentage of shrub cover can promote a higher abundance of parasites in small mammals and the increase of temperature in spring induces an increase in the activity of ectoparasites	Estrada-Peña et al., 2004; Ribeiro, H. 2007.

2. Materials and methods

2.1. Study area

The study area is located in the northwest of Portugal (40º53'N, 07º04'W), in the municipality of Vila Nova de Foz Côa, more specifically in the Faia Brava reserve, Quinta do Sol and Quinta de São Paulo (figure 1).

Faia Brava is a private farmstead covering about 860 ha and is included in the Special Protection Area (SPA) of the Côa Valley (PTZPE0039; Natura 2000 network), in the Important Bird and Biodiversity Area (IBA Côa valley PT006) (Rufino and Neves, 2000), in the Forest Intervention Zone (ZIF) of Algodres / Vale de Afonsinho (ATN, 2011) (figure 2), and in the Côa Valley Archaeological Park (PAVC), designated by UNESCO as World Heritage (DeSilvey and Bartolini, 2018).
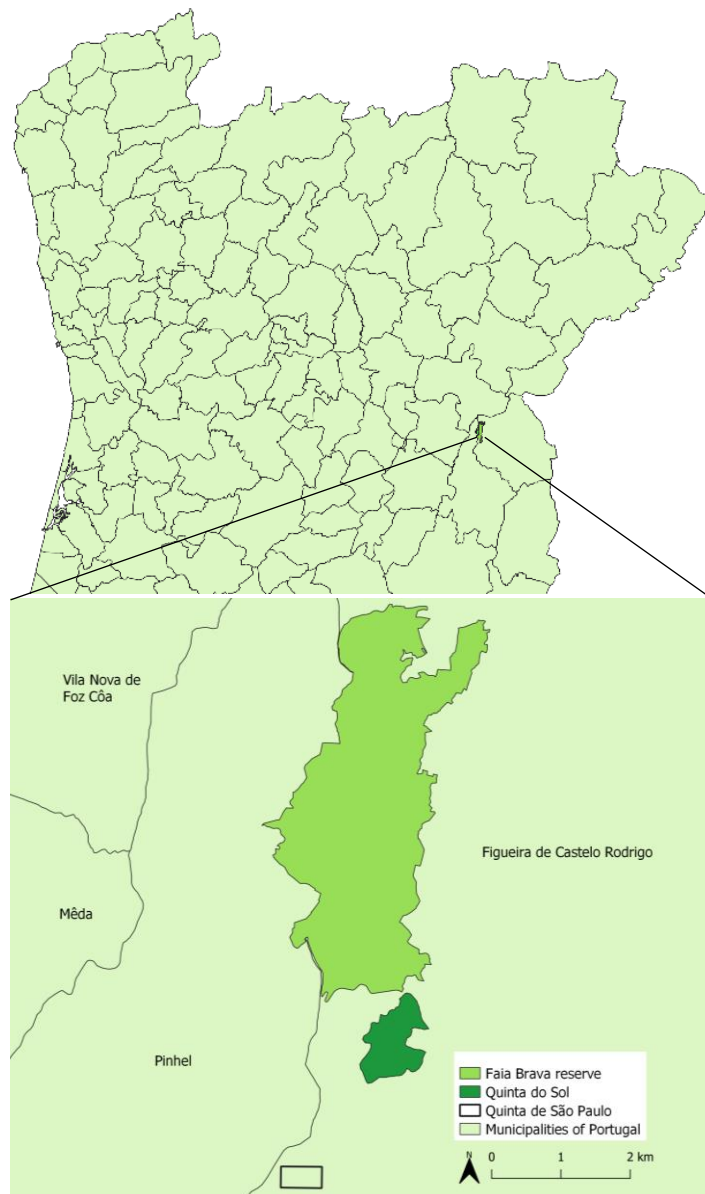
Figure 1. Location of the study area.
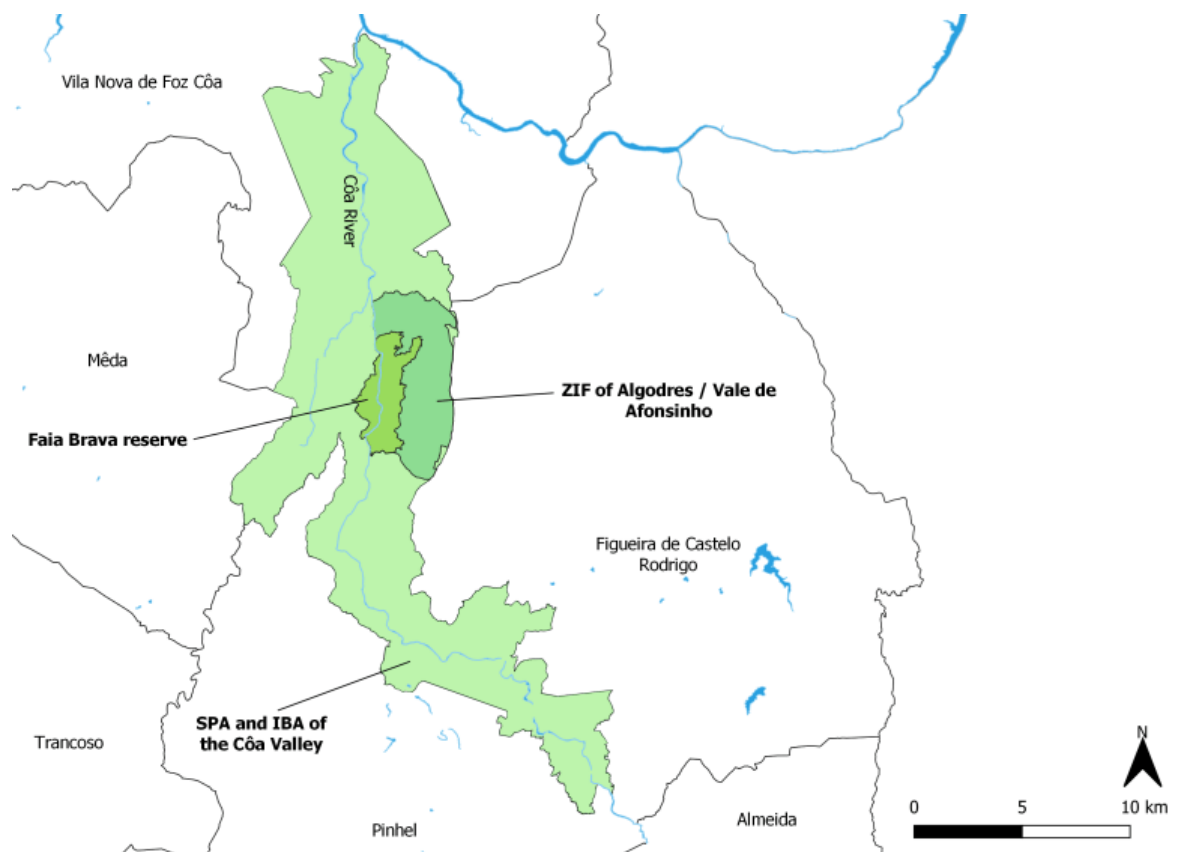
Figure 2. Geographic location of the Faia Brava reserve, the Forest Intervention Zone (ZIF) of Algodres / Vale de Afonsinho, the Special Protection Area (SPA) and Important Bird and Biodiversity Area (IBA) of the Côa Valley.

This area sits on a deep and steep valley that has resulted from the erosive action of the Côa river (ATN, 2011) (figure 3). The Côa riverbed has an average altitude of 200 m, with a minimum altitude of 170 m and a maximum altitude of 509 m (ATN, 2010). In relation to lithology, there is a predominance of medium-grained and granitic porphyroid rocks which are characterized by low impermeability (ATN, 2011). The Côa river presents steep slopes, formed by granite cliffs, which are the origin of the reserve designation - "faia" meaning cliff - often used by rupicolous birds for nidification (e.g., Egyptian Vulture *Neophron percnopterus* Linnaeus, 1766 and Griffon Vulture *Gyps fulvus* Hablizl, 1783) (ATN, 2010; ATN, 2011).

Figure 3. Image of the Côa river with steep slopes formed by cliffs (© S. Sabino).

It presents a dry Mediterranean climate, with continental influences, characterized by low average rainfall and wide temperature ranges, with hot dry summers and cold winters with occasional snowfalls, resulting in poor soils with little vegetation cover (ATN, 2011).

The Côa river regime is characterized by two distinctly marked seasons with contrasting characteristics (ATN, 2010). Winter is the period with the highest levels of precipitation, and in contrast, during the summer, there is practically no precipitation, presenting a scarcity of water during these warmer months (ATN, 2010). The decrease in precipitation combined with the high increase in evapotranspiration is sufficient to eliminate the existing flow, since the groundwater is limited due to the low permeability of the basin's constituent rocks (e.g., granites and schists) (ATN, 2010). As the availability of water in summer is currently very low, there are some small artificial ponds that have recently been created in the reserve (figure 4), which are areas of water accumulation in sandy soils, associated with natural springs or water lines (ATN, 2010; Conceição, 2019).

Figure 4. Artificial pond in the Faia Brava reserve during the winter period (© S. Sabino).

The area supports some anthropogenic activities, such as cattle breeding, extensive herding of sheep (*Ovis aries* Linnaeus, 1758), which is very localized and scarcely representative (figure 5) of traditional agriculture (Pellis, 2019). Agricultural practices include the maintenance of traditional olive groves for olive oil production, vineyards and orchards with almond trees (ATN, 2010). Traditional rural constructions are scattered throughout the landscape, which constitute elements of high importance for local biodiversity, that also confer significant heritage value to this area (ATN, 2010). Some traditional constructions of the Côa Valley include corrals, haylofts, dovecotes and stone walls (figure 6) (ATN, 2010).

Figure 5. Herding sheep in the Faia Brava reserve (© UVS).

Figure 6. Traditional buildings of the Côa Valley. a- corral; b- stone wall (murete) (© S. Sabino).

The most common habitats are dominated by: holm oak (*Quercus ilex* Linnaeus, 1753) and cork oak (*Quercus suber*) forests, with scattered *Celtis australis* (Linnaeus, 1753) and *Quercus faginea* (Lam., 1785) trees; evergreen holm oak forests dominated by *Quercus ilex*; riparian shrublands with *Flueggea tinctoria* (Linnaeus, 1984); evergreen shrubland; and rupicolous formations encompassing *Rhamnus lycioides* subsp. *oleoides* (Linnaeus, 1932), *Pistacia terebinthus* (Linnaeus, 1753), *Phillyrea angustifolia* (Linnaeus, 1753), among other species (ATN, 2010). Successional degraded structures constitute the vegetation with the highest territorial representation, which include brambles dominated by *Rosa canina* (Linnaeus, 1753), *Clematis*

campaniflora (Brot., 1804), and *Rubus ulmifolius* (Schott, 1818); the shrublands composed by *Cytisus multiflorus* (L'Hér., 1826); the sub-stepical formations of annuals and grasses; and the perennial shrub composed by *Pistacia terebinthus*, *Rhamnus lycioides* subsp. *oleoides* (Linnaeus, 1762), *Juniperus oxycedrus* subsp. *oxycedrus* (Linnaeus, 1753), *Arbutus unedo* (Linnaeus, 1753), and *Phillyrea angustifolia* (Linnaeus, 1753) (figure 7) (ATN, 2010).

Figure 7. Predominant vegetation formations in the Faia Brava reserve (a- holm oak *Quercus ilex*; b- terebinth *Pistacia terebinthus*; c- grasses; d- shrublands of *Cytisus multiflorus* and French lavender *Lavandula pedunculata*) (© S. Sabino).

The knowledge about the diversity of species belonging to the groups of fungi, lichens and bryophytes that can be found in the reserve is very limited, considering that not many studies have been performed in this region (figure 8) (ATN, 2010).

Figure 8. Macro-fungal specimens that can be found in the Faia Brava reserve (© S. Sabino).

Vertebrate biodiversity in the Côa Valley is highly diversified, being composed of a total of 149 vertebrate species (ATN, 2010). According to data from ATN and STRIX (pers. Comm.), it consists of 25 mammals (including Garrano horses and Maronesa cows), 100 birds, 6 fishes, 14 amphibians, and 19 reptiles (figure 9) (ATN, 2010). Birds are the taxa that stand out the most, due to their diversity and the occurrence of species of high conservation value, with rupicolous birds being the subgroup with a higher number of species with a higher threat status (e.g., *Aquila chrysaetos* Linnaeus, 1758 and *Aquila fasciata* Vieillot, 1822 (Cabral et al., 2005; ATN, 2010). For this reason, Faia Brava is a very important area for rupicolous avifauna at a national level (SPEA).

Figure 9. Reptile specimens present in the Faia Brava reserve (© S. Sabino).

Based on a rewilding perspective, several projects are being implemented in this area to restore ancient ecosystems and communities and restore the landscape mosaic (DeSilvey et al., 2020). Since 2005, ATN has been reintroducing a small extensive livestock farm with Garrano horses and Maronesa cows in the reserve (figure 10) (ATN, 2010; Helmer et al., 2015). These ungulates have been introduced within enclosures in a semi-wild state, so that their browsing and grazing activities can maintain the ruderal species (i.e., plant communities that develop in environments strongly disturbed by human action) under control and in turn make the landscape less vulnerable to fires (ATN, 2011; DeSilvey et al., 2020). Initially, these animals were confined within an enclosure of approximately 11 hectares, however, in the last few years this area has been increased (ATN, 2011), with livestock being present in practically the entire reserve. Between 2020 and 2021, the herd was composed of 46 Garrano horses and 35 Maronesa cows.

Figure 10. Ungulate species that were reintroduced in the Faia Brava reserve (a- Garrano horses; b- Maronesa cows) (© UVS).

Quinta de São Paulo was defined in this study as a control area, since no ungulates were reintroduced in this area (i.e., exclusion area).

At an environmental level, this area can be considered similar in several aspects to the Faia Brava reserve, although the vegetation is more developed due to the inexistence of large herbivores (i.e., ungulates) (figure 11), while there are no climate or meteorological differences. It is also possible to detect a few loose stone structures in this area (figure 12).

Figure 11. a-b- Habitat structure present in the Quinta de São Paulo area (© S. Sabino).

Figure 12. One of the stone structures present in this area, which were used for sheltering in the rocks (© S. Sabino).

2.2. Field strategy

2.2.1. Sampling design

To evaluate the influence of grazing on small mammals, four sampling areas were selected according to the ungulate pressure gradient and the representativeness of the main habitats. Two areas were defined in the Faia Brava reserve, one area in Quinta do Sol (adjacent to the Faia Brava reserve, but located outside it), and one area in Quinta de São Paulo (outside the Faia Brava reserve) (figure 13). In each area, we monitored the small mammals' communities based on two grids to increase the robustness of the experiment. The Faia Brava reserve was divided into two areas, between which ungulates do not move, as they were fenced off: the Ha and Hb (high ungulate pressure) in the southern region, and Ia and Ib (intermediate ungulate pressure) in the northern region of the reserve. The La and Lb (low ungulate pressure) grids were located at Quinta do Sol, which are also fenced. One sampling area was defined in the Quinta de São Paulo - the Na and Nb - that acted as a control area (i.e., without ungulates) and was fenced off by an exclusion fence.
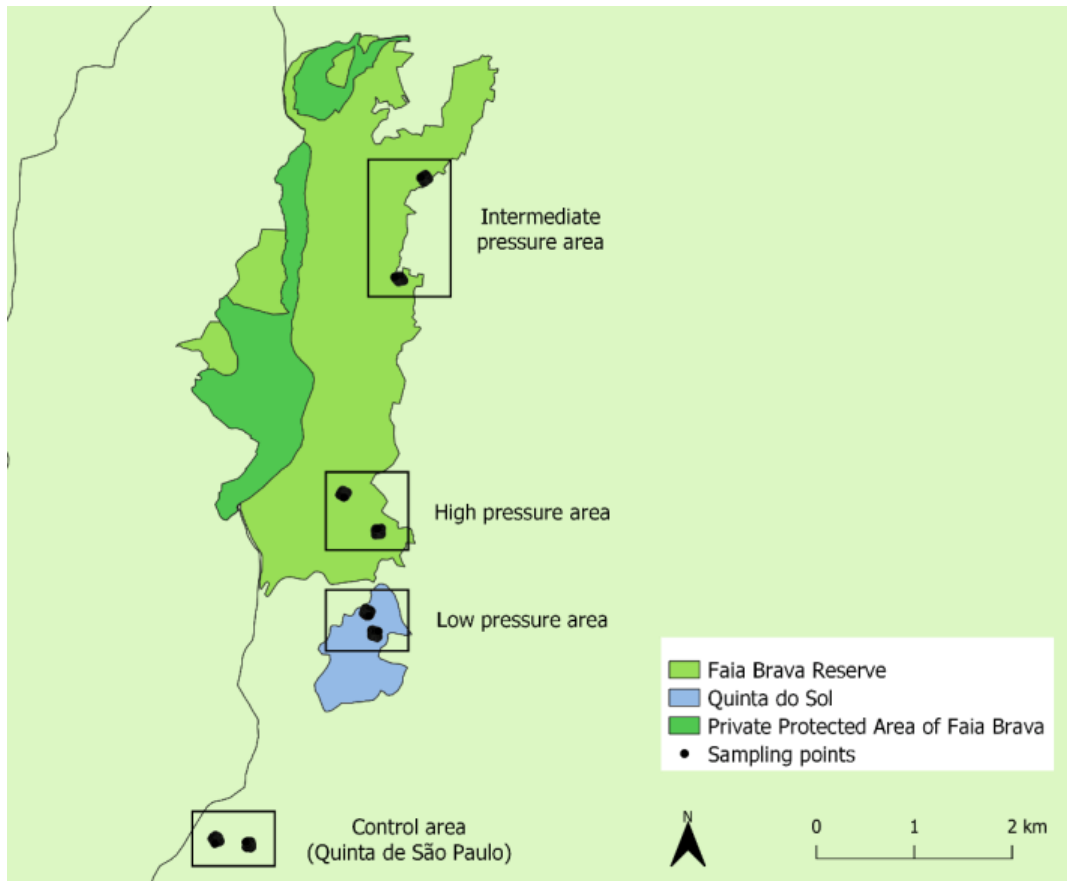
Figure 13. Location of the four sampled areas.

The higher density area is located in the south of the reserve. The habitats present are Thermo-Mediterranean and pre-desert shrub, and European dry heaths (figure 14).

The intermediate density area, located north of Faia Brava, has an intermediate density and grazing pressure of ungulates, that explore landscapes dominated by sparse vegetation of cork oaks forests, with a monumental centennial cork oak tree over 500 years old being found in this area, which is one of the symbols of the reserve. In this area the habitats of European dry heaths are present, evergreen *Quercus* spp. forests, *Quercus rotundifolia* and *ilex* forests, and sub-steppe grasses of the Thero-Brachypodietea, being the study area with the highest diversity of habitats.

In Quinta do Sol the ungulate density and grazing pressure is lower, hosting only a small herd of 6 horses. This region corresponds to an area with a higher development of shrubs, containing many widely distributed low stone walls (muretes). This area is defined by a very diverse habitat compared to the rest of the reserve, with the presence of evergreen *Quercus* spp. forests, sub-steppe grasses of the Thero-Brachypodietea, and European dry heaths.

Finally, the Quinta de São Paulo is an ungulate exclusion area that does not host any ungulate. Quinta de São Paulo is located to the south of the Faia Brava reserve and has an identical habitat structure as the one present in the other areas.
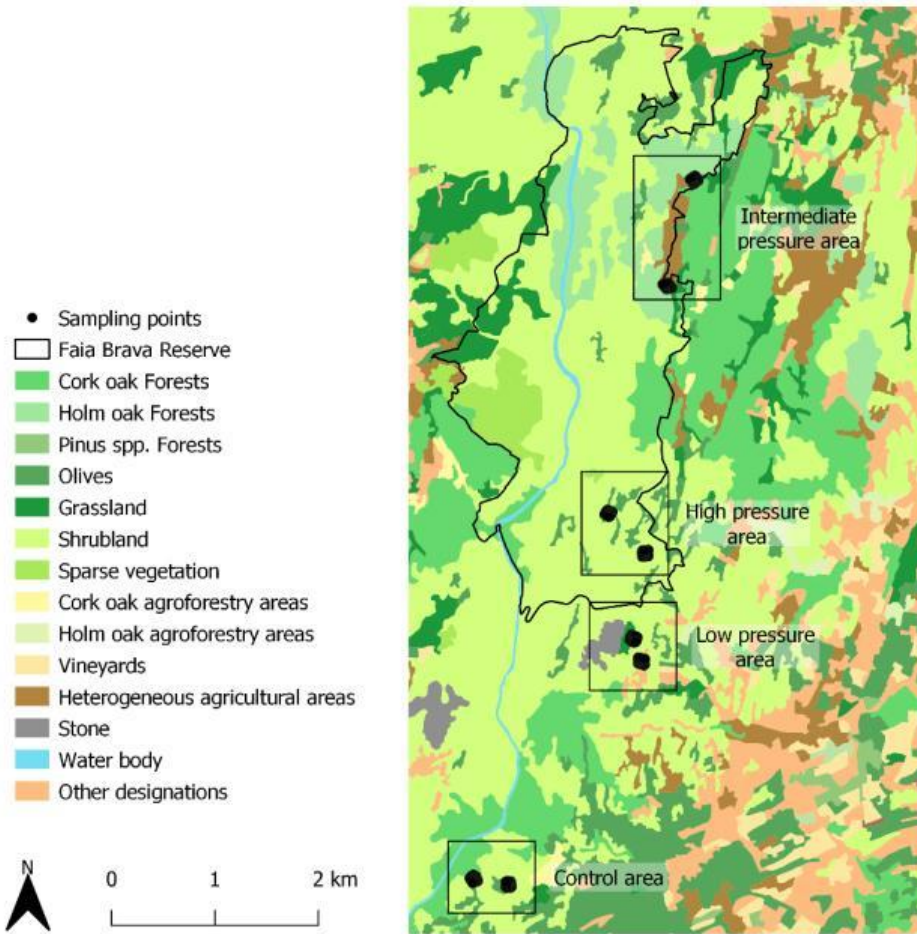
Figure 14. Representation of the land use and occupation present in the sampled areas.

The sampled areas, corresponding to plots with higher, intermediate and lower abundance of large herbivores, and without ungulates, were spaced at approximately 2 km distance from each other, and, in each area, two sampling grids were established (i.e., replicas) with a distance of at least 150 m between them, since this distance ensures the spatial independence of the records (Schemnitz et al., 2012). Each grid was composed of 49 trapping points (7×7), with points spaced 10 m from each other, covering a total area of 3.600 m² for each grid. Small mammal traps with dimensions 23 x 8 x 9 cm were used, which allowed the capture-trap-recapture method to be applied, since this does not involve the death of the captured individuals. These traps were set in each trapping point (figure 15), totaling 98 trapping points per sampling area and a total of 392 trapping points sampled.
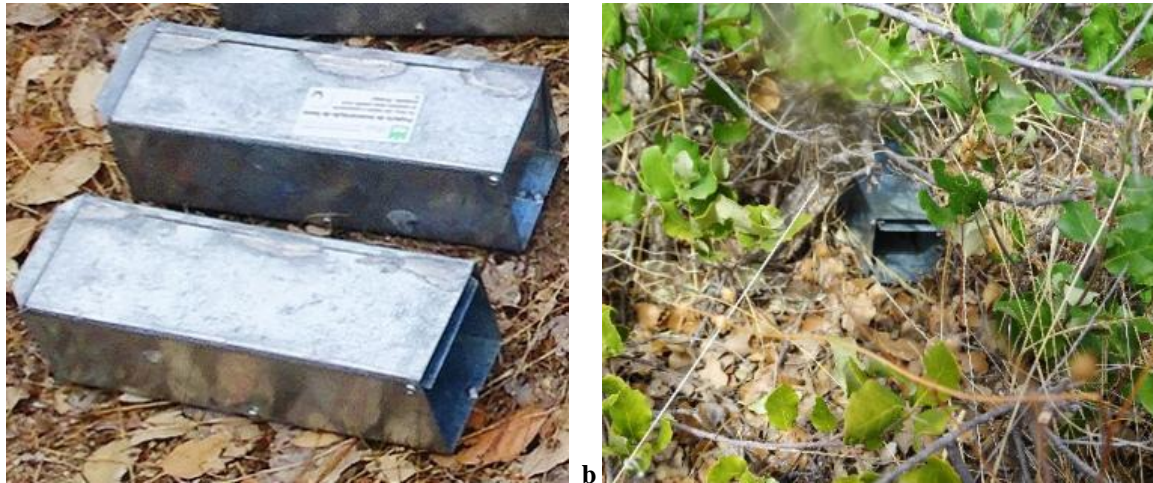
Figure 15. Small mammal trapping (a- two small mammal traps; b- a small mammal trap placed in the sampling point) (© S. Sabino).

Small mammal trapping was conducted in the autumn (October 2020) and in the spring (May 2021) to represent two important life-cycle periods: the pre- and post-reproduction period, respectively (Rosário and Mathias, 2004). The traps were aligned to ground level and covered with vegetation to avoid direct sunlight exposure and to be hidden. These traps were baited with a mixture of oatmeal and canned sardine in oil, and carded cotton was added to provide a better thermal comfort, reducing the stress and protecting the captured animals from hypothermia (Gurnell and Flowerdew, 2019).

All traps were subject to a pre-trapping period, where traps were deactivated (closed) for one night to allow the small mammals to become accustomed to the traps and reduce trap avoidance. Then, after the acclimation period, traps were set active for four consecutive nights and checked each morning. Bait and bedding were checked at every trap visit, and in the case where the bait had disappeared, this was replaced. Each sampling period had a duration of four consecutive days, in order to respect the period considered minimum for obtaining a representative sample, while preventing the traps from becoming a point of habituation or attraction of certain specimens (Gurnell and Flowerdew, 2019).

2.2.2. Small mammals capture and handling

All captured animals were analysed at the capture site and then transferred to a bag with cotton soaked in diethyl ether in order to sedate them for easier handling. Trapped animals were identified to the species level by observing morphological characteristics (Macdonald and Barrett, 2002), and their age class (juvenile, adult) and gender were determined using the criteria according to Gurnell and Flowerdew (2006). Several body metrics were recorded (figure 16), including body mass (using a precision scale with an accuracy of 0.1 g) and head-body, tail and hind foot length (using a ruler with an accuracy of 0.1 cm) (figure 17). These body metrics were used in species diagnosis (e.g., hind foot length; Macdonald and Barrett, 2002) and for the calculation of individual body condition indices (e.g., body weight and length).

Figure 16. Different procedures on small mammals (a- weighing - body mass; b- tail measurement; c- hair marking; d- identification of sex) (© S. Sabino).
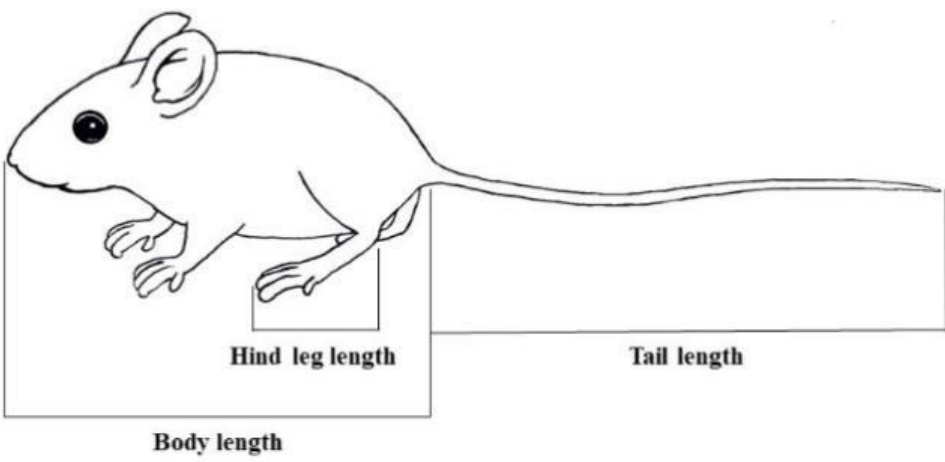
Figure 17. Scheme of body metrics in small mammals (drawing © S. Sabino).

All captured animals were individually marked with a unique combination of haircuts in predefined areas of the animal's body, as these marks would allow each individual to be easily identified in future recapture events (Gurnell and Flowerdew, 2019).

After the handling procedure, each small mammal was released near the trapping point where it was captured. Capture and handling processes were conducted according to national and international standards (according to Gannon et al., 2007) as well as under capture permits 802_2020_PERTURB_signed; 803_2020_PERTURB_signed; 804_2020_PERTURB_signed; 896 / 2021 / CAPT /PERTURB; 897 / 2021 / CAPT /PERTURB; and 898 / 2021 / CAPT /PERTURB (ICNF - Nature Conservation Institute and Forests).

During each visit, if there were traps that were closed but empty or contained individuals other than small mammals (e.g., insects) they were counted as inactive, while traps that were open but with evidence of the presence of small mammals were recorded as traps visited but not counted for abundance calculations.

2.2.3. Landscape and vegetation structure and composition

A description of the vegetation on each sampling grid was performed at the level of each trap and at the grid level. At each trapping point (i.e., 49 points per sampling grid) the understory vegetation was evaluated in order to analyse the variations at the understory level between the different sampling points and to test the effects of this variation in relation to the parameters under analysis. For this purpose, a trap-level circle with a 1 m radius around the trap was defined and a visual evaluation of the vegetation structure and diversity was carried out using the point-intercept method, by estimating the current percentage of understory cover. The percentage of vegetation was evaluated and separated according to vegetation type (i.e., herbaceous, shrubs and trees), additionally the percentage of rocks and outcrops, the presence of shelter/refuge (e.g., shrub cover) and the main species present were also recorded. To describe the vegetation and the other components previously described at each grid level, the arithmetic mean of the percentages of each component was calculated, providing an average value of the 49 trapping locations for each one of the 8 sampling grids, thus allowing their comparison. The shrubs and trees were subdivided according to the type of fruit they produce, since each type may have a different influence on the small mammals' diet. The fruit was classified into four types, dry (leguminous), fleshy, acorns and other dry fruits.

2.2.4. Meso and large mammal presence at the landscape level

Camera trapping is a powerful and non-invasive methodology compared to other techniques (e.g., capture-mark-recapture and radio telemetry) and reduces the chances of stressing or injuring the target animals (Locke et al., 2012), along with having the ability to collect information on elusive species, namely the carnivores that are usually scarce, nocturnal, and difficult to detect (Ferreras et al., 2017). Camera traps are automatic cameras with infrared sensors that can be triggered whenever they sense movement in the landscape around them, being used to collect photographic or video evidence of the presence of animals in field research (McCallum, 2013), representing a method of indirect observation that allows the study of animal behaviour (Young, 2012). Another advantage of this method is the fact that the cameras can collect large amounts of data and can be left in the field for a very long period of time, which reduces the risk of human disturbance in the study area and has a minor influence on animal behaviour (McCallum, 2013;

Wearn and Glover-Kapfer, 2017). Data collection through camera traps depends mainly on the quality of the equipment (e.g., quality of picture resolution, data storage capacity and battery life) and the ability of the operator(s) (McCallum, 2013). When estimating species richness through camera trapping, it is highly dependent on the survey effort (i.e., total number of days of camera trapping), although different combinations of the number of cameras and their time of activity may lead to different results (Mugerwa et al., 2013; Si et al., 2014).

Camera trapping allows monitoring, estimating numbers, recording the distribution and observing the hours of activity of each species, providing an essential tool for monitoring and obtaining critical data of wildlife and their habitats, being extremely relevant for the management and conservation of biodiversity (Gotelli and Colwell, 2001; Ferreras et al., 2017).

The camera sampling was only carried out for the Faia Brava reserve and Quinta do Sol, having not been performed for the control area as it was an exclusion area of ungulates. To evaluate the presence of ungulates and mesocarnivores in the Faia Brava reserve and Quinta do Sol, we installed cameras in the areas of high-, intermediate- and low-pressure in order to quantify the variation in (I) species richness, (II) occupation patterns (presence/absence data) and (III) habitat use intensity, with an aim to understanding how the reintroduction of semi-wild animals such as Garrano horses and Maronesa cows impacts the biodiversity in this area (figure 18).

Figure 18. a- Camera trap; b- a fixed camera trap at one of the sampling sites (© S. Sabino).

Using this sampling method, the aim was to record the ungulates and mesocarnivores present in the study areas, making it possible to find five species of ungulates, two being semi-wild species (*Equus ferus caballus*, *Bos Taurus*), two wild species (*Capreolus capreolus*; and *Sus scrofa*), and one domestic species (*Ovis aries*), while there are eight species of mesocarnivores (*Felis silvestris* Schreber, 1775; *Genetta genetta* Linnaeus, 1758; *Lutra lutra Linnaeus, 1758*; *Martes foina* Erxleben, 1777; *Mustela nivalis*; *Meles meles* Linnaeus, 1758; *Herpestes ichneumon* Linnaeus, 1758; and *Vulpes vulpes* Linnaeus, 1758) (ATN, 2010).

Ungulates and mesocarnivores monitoring was conducted from October 2020 to January 2021, using a camera trapping protocol (figures 19-20). Three cameras were placed in each of the

sampling grids, accounting for 18 cameras in total, and were fixed on a wooden stake and placed at a height of 30-40 cm above the ground in an open location that was likely to be commonly used by the target species, in order to be able to clearly record the presence of the target animals. No bait was used, and all cameras were programmed to capture a set of three photographs per trigger in each event with each set being 1 second apart. The cameras were positioned facing the area with less vegetation in order to avoid accidental activation of the cameras caused by the movement of said vegetation. The operating time was set to be 24 hours per day, being reviewed at the end of the active period (30 days). Occasionally the animals (mostly horses and cows) interacted with the camera trap, leaving it tilted or falling to the ground. Consequently, there were periods when the cameras did not have the initial landscape range, which may have resulted in fewer records of the activity of ungulates and mesocarnivores.

Figure 19. Ungulates recorded by camera traps. a- Garrano horse *Equus ferus caballus*; b- Maronesa cow Bos Taurus; c- roe deer *Capreolus capreolus*; d- wild boar *Sus scrofa* (© UVS).

Figure 20. Mesocarnivores recorded by camera traps. a- Red fox *Vulpes vulpes*; b- Wildcat *Felis silvestris*; c- Egyptian mongoose *Herpestes ichneumon*; d- European badger *Meles meles*; e- Beech Marten *Martes foina* (© UVS).

2.3. Data analysis

2.3.1. Estimation of population abundance and species diversity

For the analysis of the collected data, parametric and non-parametric statistical tests were used. Non-parametric tests were adopted when the assumptions of normality and/or homogeneity of variance were not observed. All statistical analyses were performed using R statistical *software* (R Code Team, 2018), with the packages or extensions mentioned specifically for each analysis.

All areas and trapping points were georeferenced and their respective geographical coordinates were transferred to Quantum GIS (QGIS) *software*, version 3.22.5 (Quantum GIS Development Team, 2020) in order to produce the map of the study area.

For each sampling point in the Faia Brava reserve, Quinta do Sol and in the Quinta de São Paulo, we calculated the number of small mammal individuals captured, species richness (i.e., number of different species captured) and diversity, using the Shannon-Wiener (H') index (Zar, 2010). Species diversity was calculated according to the equation 1:

$$H' = -\sum_{i=1}^{S} \left(* \frac{n_i}{N} * \ln \left(\frac{n_i}{N} \right) \right)$$

eqn 1

where n_i is the number of individuals of species i captured; N is the total number of individuals captured; and S is the number of species captured.

The normality assumptions of the number of individuals, species richness and diversity were verified with the Shapiro-Wilk normality test (Zar, 2010), and depending on whether the data respected or not the assumptions of parametric statistics, the t-test or the non-parametric Wilcoxon test (Zar, 2010) was used, to verify the existence of statistical differences in the number of individuals, species richness and diversity through the comparison of each any two areas at the same time.

We calculated the relative abundance of each small mammal species, order (Rodentia and Insectivora) and for the small mammal group (i.e., including all individuals from all species). This analysis was performed at the level of each sampling grid and sampling area for each sampling period, according to equation 2: Pounds relative abundance index (Pounds, 1981):

$$I_i = \frac{N_i}{T * R - (\sum C - r)} * 1000$$

eqn 2

where N_i is the number of captured individuals of the species I; T the number of available traps; R the number of daily trap inspections during the sampling period; C the number of captures and recaptures of other species; and r the number of recaptures of species i.

The equitability of species was calculated for each sampling grid using Shannon's equitability (E_H), which is a measure to estimate how similar the abundance of different species is within a population (Magurran, 1998). Thus, a population with high equitability (i.e., when the proportion of all species is close to 1) will present all species showing approximately the same abundance, while a population with low equitability (i.e., when the proportion of all species is close to 0) will present one or a few dominant species and several rare species (Kricher, 1972). The equitability of species was calculated according to equation 3:

$$E_H = H/\ln(S)$$

eqn 3

where H is diversity by the Shannon-Wiener index; and S is the number of species captured.

We also assessed the sex-ratio of species in all sampled areas to verify the existence of differences using a proportional test (Armitage, 1966). To perform the proportional test in R we used the functions binom.test() and prop.test(), where the binom.test() function was applied when the sample size was small (n < 30), while the prop.test() function was used when the sample size was large (n > 30) (Eberhart-Phillips et al., 2017).

2.3.2. Body condition and parasite load

A body condition index and parasite abundance were calculated for each small mammal captured in both seasons (i.e., autumn and spring). The presence of ectoparasites (i.e., fleas, ticks and mites) was verified to evaluate the parasite load of each animal, by collecting each external parasite using a tweezer and preserving it inside of an Eppendorf tube (figure 21) (Estrada-Peña et al., 2004). For this purpose, the abundance of parasites in each small mammal was calculated according to the area and period of sampling, the separation of the age class of small mammals given the low number of individuals, not having been made.

Figure 21. Ectoparasite sampling in small mammals in an Eppendorf tube (© S. Sabino).

Juvenile individuals, identified through body measurements and mass, as well as pregnant females were not included in the body condition analysis in order to minimize variation due to age and breeding effects, but were included in the parasite load analysis. To calculate the body condition of the individuals, we used the Scaled Mass Index (SMI), being considered an efficient indicator. It is considered robust for the effect of the growth on body size, as well as the scaling relationship between body length (L) and body mass (M) (Peig and Green, 2009), namely the effects of ontogenic variation in body size and sexual dimorphism (Peig and Green, 2010). The Scaled mass index (SMI) was estimated according to equation 4:

$$SMI = M_i \left[\frac{L_0}{L_i} \right]^{b_{SMA}} \qquad\qquad \text{eqn 4}$$

where M_i and L_i are the body mass and body length of individual i respectively; L_0 is the arithmetic mean of the body length of all individuals belonging to the same species as individual i; b_{SMA} is a scaling exponent regression derived from the standardized major-axis (SMA) of the body mass on body length (value obtained from the reduced straight line equation produced) (Peig and Green, 2010).

This index standardises all individuals, in this case individuals belonging to the same species, to the same L_0 value and adjusts their body mass values according to what they would have in the new L value, according to the scaling trend between M and L, which means that this index standardises all individuals to the same growth phase (Peig and Green, 2010).

Body condition is intrinsically related to the health and fitness of an animal (Peig and Green, 2009), and has been widely recognized as an important determinant of fitness (Peig and Green, 2010). Only data from the species *Apodemus sylvaticus* (Linnaeus, 1758), *Mus spretus* (Lataste,

1883) and *Crocidura russula* (Hermann, 1780) were considered in this analysis, due to the limited number of *Eliomys quercinus* (Linnaeus, 1766) sampled.

2.3.3. Effect of environmental parameters

In order to test the influence of the independent variables (percentage of ungulate traces - i.e., footprints and droppings; percentage of camera records of horses and cows; percentage of camera records of ungulates excluding horses and cows; percentage of camera records of mesocarnivores, species richness of edible vegetation, percentage of shelter, and parasite load) that may be influencing small mammal abundance and body condition), a Generalized Linear Mixed Models (GLMM) approach (Zuur et al., 2009) was applied using the LME4 package (Bates et al., 2022) for R (version 4.1.2) (R Core Team, 2020), with a Gaussian distribution. To incorporate spatial correlation into the analysis, we used the trapping grid as a random variable.

Mixed effects models comprise several models that have a central feature of both fixed and random effects (Starkweather, 2015). In a multilevel model, we define fixed effects if the effects are constant, i.e., if they are identical for all the groups in a population, and if the effects differ between groups they are defined as random (Gelman, 2005).

The analysis of potential factors influencing abundance was based on the species *Apodemus sylvaticus*, *Mus spretus* and *Crocidura russula*, as they were the most representative, with the individuals being separated only according to species and sex, since they could not be separated according to age class as the juveniles were not very representative and thus their separation would not allow a robust analysis. In the procedure of the factors that influence the body condition of small mammals, the same three species were also included in this analysis, however juveniles and pregnant females were excluded since their presence would cause variations in the index values. In the case of individuals without information about their gender, they were also excluded from both procedures. A model with all independent variables combined was produced for each analysis, and then the p-values produced were verified in order to identify which of the independent variables had an influence on the dependent variables (i.e. p-values with statistically significant differences). Thus, for each species, we produced a model for all individuals of that species, one for females and one for males (i.e. 3 subgroups), and in the case of individuals without information about their gender they were also excluded from both procedures.

The value of each independent variable in each sampling grid resulted from merging the data from the two sampling periods.

3. Results

3.1. Landscape and vegetation structure and composition

In order to obtain an average value to analyse the variation between the vegetation of the sampled areas, the arithmetic mean of the values obtained in the 49 trapping points was calculated for each sampling grid (table 2).

Regarding the percentage of vegetation present in the sampled areas, the high-pressure area was the area with the lowest percentage of herbaceous plants and the control area had the highest percentage, while the shrub percentage was lower in the control area and in the intermediate-pressure area it was higher, the high- and low-pressure areas were the areas with the lowest presence of trees, and the control area had the highest abundance. Thus, it was possible to verify that the control area was the area that presented the highest percentage of vegetation (83%), on the other hand, the high pressure area presented only 41% of vegetation.

The majority of rocks and outcrops were present in the low-pressure area, while the lowest percentage was located in the control area.

In the case of the presence of traces of horse and cow activity, the control area was the area with the lowest percentage, as opposed to the high-pressure area which had the highest percentage.

Table 2. Description of the percentage of vegetation (i.e., herbaceous plants, shrubs, and trees), rocks, outcrops, and ungulates (i.e., traces of horse and cow activity) present in each sample grid and in each sample area. Ha (grid a from the high-pressure area), Hb (grid b from the high-pressure area), H (high-pressure area), Ia (grid a from the intermediate-pressure area), Ib (grid b from the intermediate-pressure area), I (intermediate-pressure area), La (grid a from the low-pressure area), Lb (grid b from the low-pressure area), L (low-pressure are), Na (grid a from the control area), Nb (grid b from the control area), N (control area).

Grids	% Herbaceous	% Shrubs	% Trees	% Rocks	% Outcrops	% Ungulates
Ha	13.0%	23.0%	4.0%	18.0%	10.2%	77.6%
Hb	11.0%	29.0%	2.0%	15.0%	6.0%	93.9%
H	12.0%	26.0%	3.0%	16.5%	8.1%	85.8%
Ia	15.0%	33.0%	4.0%	25.0%	10.2%	34.7%
Ib	16.0%	24.0%	16.0%	12.0%	10.2%	47.0%
I	15.5%	28.5%	10.0%	18.5%	10.2%	40.9%
La	23.0%	34.0%	5.0%	29.0%	26.5%	55.1%
Lb	18.0%	20.0%	1.0%	21.0%	10.2%	77.6%
L	20.5%	27.0%	3.0%	25.0%	18.4%	66.4%
Na	51.0%	19.0%	8.0%	15.0%	6.0%	0.0%
Nb	48.0%	22.0%	18.0%	8.0%	4.0%	6.0%
N	49.5%	20.5%	13.0%	11.5%	5.0%	3.0%

In the sampled areas the following fruit species were found: two species with acorns (*Quercus ilex* and *Quercus suber*), two species with other types of dry fruits (*Lavandula pedunculata* and *Fraxinus angustifolia*), four species with fleshy fruits (*Daphne gnidium*, *Rubus ulmifolius*, *Olea europaea* and *Pistacia terebinthus*), and one species with dry fruits (*Cytisus multiflorus* and *Acacia*) (table 3).

Table 3. Classification of vegetation by type of fruit in each sample grid and in each sample area. Ha (grid a from the high-pressure area), Hb (grid b from the high-pressure area), H (high-pressure area), Ia (grid a from the intermediate-pressure area), Ib (grid b from the intermediate-pressure area), I (intermediate-pressure area), La (grid a from the low-pressure area), Lb (grid b from the low-pressure area), L (low-pressure are), Na (grid a from the control area), Nb (grid b from the control area), N (control area).

Location	Type of fruit			
	Acorns	**Other dry fruits**	**Fleshy**	**Dry (legumes)**
Ha	*Quercus ilex* *Quercus suber*	*Lavandula pedunculata*	*Daphne gnidium* *Rubus ulmifolius* *Olea europaea*	*Cytisus multiflorus*
Hb	*Quercus ilex*	*Lavandula pedunculata* *Fraxinus angustifolia*	*Daphne gnidium* *Rubus ulmifolius*	*Cytisus multiflorus*
H	*Quercus ilex* *Quercus suber*	*Lavandula pedunculata* *Fraxinus angustifolia*	*Daphne gnidium* *Rubus ulmifolius* *Olea europaea*	*Cytisus multiflorus*
Ia	*Quercus ilex* *Quercus suber*		*Daphne gnidium* *Rubus ulmifolius*	*Cytisus multiflorus*
Ib	*Quercus ilex* *Quercus suber*	*Lavandula pedunculata*	*Rubus ulmifolius*	*Cytisus multiflorus*
I	*Quercus ilex* *Quercus suber*	*Lavandula pedunculata*	*Daphne gnidium* *Rubus ulmifolius*	*Cytisus multiflorus*
La	*Quercus ilex* *Quercus suber*	*Lavandula pedunculata*	*Daphne gnidium* *Rubus ulmifolius* *Olea europaea*	*Cytisus multiflorus*
Lb	*Quercus ilex*	*Lavandula pedunculata*	*Rubus ulmifolius*	*Cytisus multiflorus*
L	*Quercus ilex* *Quercus suber*	*Lavandula pedunculata*	*Daphne gnidium* *Rubus ulmifolius* *Olea europaea*	*Cytisus multiflorus*
Na	*Quercus ilex*	*Lavandula pedunculata*	*Pistacia terebinthus*	*Cytisus multiflorus*
Nb	*Quercus ilex*	*Lavandula pedunculata*	*Daphne gnidium* *Rubus ulmifolius* *Olea europaea* *Pistacia terebinthus*	*Cytisus multiflorus*
N	*Quercus ilex*	*Lavandula pedunculata*	*Daphne gnidium* *Rubus ulmifolius* *Olea europaea* *Pistacia terebinthus*	*Cytisus multiflorus*

The species richness of fruits presents in the different sampling areas varied between 6 and 8, with the maximum value obtained in the high-pressure and control area and the minimum value in the intermediate-pressure area (table 4).

Table 4. Species richness of fruits in each sample grid and in each sample area. Ha (grid a from the high-pressure area), Hb (grid b from the high-pressure area), H (high-pressure area), Ia (grid a from the intermediate-pressure area), Ib (grid b from the intermediate-pressure area), I (intermediate-pressure area), La (grid a from the low-pressure area), Lb (grid b from the low-pressure area), L (low-pressure are), Na (grid a from the control area), Nb (grid b from the control area), N (control area).

Location	Species richness
Ha	7
Hb	6
H	8
Ia	5
Ib	5
I	6
La	7
Lb	4
L	7
Na	5
Nb	8
N	8

3.2. Camera-trapping of ungulates and mesocarnivores

3.2.1. Camera-trapping of ungulates

Camera-trapping effort resulted in a total of 5251 records of 5 ungulates species which 62.14% (N= 3263) occurred in the high-pressure area, 26.39% (N= 1386) in the intermediate-pressure area, and 11.46% (N= 602) in the low-pressure area. These individuals belong to Garrano horse *Equus ferus caballus* (N = 2194), Maronesa cow *Bos taurus* (N = 2169), sheep *Ovis aries* (N = 81), roe deer *Capreolus capreolus* (N = 11), and wild boar *Sus scrofa* (N = 796) (table 5).

All the five species of ungulates present in Faia Brava reserve were recorded in the present study (ATN, 2010).

The location with the highest number of ungulates was the high-pressure area, while the low-pressure area was the area with the fewest specimens.

Table 5. Number of ungulates of each species captured by camera sampling in each sampling area. H (high-pressure area), I (intermediate-pressure area), L (low-pressure area).

Area	*Equus ferus caballus*	*Bos taurus*	*Ovis aries*	*Capreolus capreolus*	*Sus scrofa*	**Total**
H	1261	1685	36	0	281	3263
I	398	484	45	8	451	1386
L	535	0	0	3	64	602
Total	2194	2169	81	11	796	**5251**

The high-pressure area captured *Equus ferus* subsp. *caballus*, *Bos taurus*, *Ovis aries* and *Sus scrofa*, in the intermediate-pressure area *Equus ferus caballus*, *Bos taurus*, *Ovis aries*, *Capreolus capreolus* and *Sus scrofa* were sampled, while in the low-pressure area *Equus ferus caballus*, *Capreolus c*apreolus and *Sus scrofa* were observed (table 6). The intermediate-pressure area was the area that presented the camera record of all 5 species of ungulates, while the low-pressure area presented the lowest species richness with only 3 species recorded. *Equus ferus caballus* and *Sus*

scrofa were the only species to be recorded in the three sampled areas, meanwhile the rarest species recorded was *C. capreolus*.

Table 6. Species richness of ungulates by sampling area. H (high-pressure area), I (intermediate-pressure area), L (low-pressure are).

Area	Species richness
H	4
I	5
L	3

3.2.2. Camera-trapping of mesocarnivores

Camera-trapping effort resulted in a total of 536 records of 6 mesocarnivores species which 40.86% (N= 219) occurred in the high-pressure area, 32.65% (N= 175) in the intermediate-pressure area, and 26.49% (N= 142) in the low-pressure area. These individuals belong to Wildcat *Felis silvestris* (N = 3), Common genet *Genetta genetta* (N = 1), Beech Marten *Martes foina* (N = 6), Red fox *Vulpes vulpes* (N = 446), European badger *Meles meles* (N = 73), and Egyptian mongoose *Herpestes ichneumon* (N = 7) (table 7).

Among the mesocarnivore species that can be found in the Faia Brava reserve, only the Eurasian otter *Lutra lutra* (Linnaeus, 1758) and least weasel *Mustela nivalis* (Linnaeus, 1766) were the only species that were not recorded in this study (ATN, 2010).

The highest number of specimens was observed in the high-pressure area, while the low-pressure area had the lowest number of individuals.

Table 7. Number of mesocarnivores of each species captured by camera sampling in each sampling area. H (high-pressure area), I (intermediate-pressure area), L (low-pressure area).

Area	*Felis silvestris*	*Genetta genetta*	*Martes foina*	*Vulpes vulpes*	*Herpestes ichneumon*	*Meles meles*	**Total**
H	0	0	0	203	2	14	219
I	3	1	1	146	5	19	175
L	0	0	5	97	0	40	142
Total	3	1	6	446	7	73	**536**

In the high-pressure area the species *Vulpes vulpes*, *Herpestes ichneumon* and *Meles meles* were observed, in the intermediate-pressure area *Felis silvestris*, *Genetta genetta*, *Martes foina*, *Vulpes vulpes*, *Herpestes ichneumon* and *Meles meles* were observed, and in the low-pressure area *Martes foina*, *Vulpes vulpes* and *Meles meles* were recorded (table 8). In the three sampled areas, only two of the six mesocarnivore species (*Vulpes vulpes* and *Meles meles*) were captured by camera traps, meanwhile the rarest species were *Genetta genetta* and *Felis silvestris*.

Table 8. Species richness of mesocarnivores by sampling area. H (high-pressure area), I (intermediate-pressure area), L (low-pressure are).

Area	Species richness
H	3
I	6
L	3

3.3. Diversity and abundance of small mammals

In the total of the four sampling areas (392 sampling points), and during the two sampling seasons, 208 individuals from four species were captured in a total of 273 capture/recapture events, which 16.3% (N= 34) occurred in the high-pressure area, 31.3% (N= 65) in the intermediate-pressure area, 19.2% (N= 40) in the low-pressure area and 33.2% (N= 69) in the control area (figure 22). These individuals belong to three rodent species [wood mouse *Apodemus sylvaticus* (N = 85), Algerian mouse *Mus spretus* (N = 20) and garden dormouse *Eliomys quercinus* (N = 2)], and one insectivore species [greater white-toothed shrew *Crocidura russula* (N = 101)] (figure 23). In the first sampling period 84 individuals were captured, while in the second sampling period 124 specimens were sampled, with the last sampling period having the highest number of captures. In all four sampled areas three of these species (*Apodemus sylvaticus*, *Mus spretus* and *Crocidura russula*) were captured, while all four species were present only in the intermediate-pressure area. Only species with a significant number of captures were considered in the data analysis: *Apodemus sylvaticus*, *Mus spretus* and *Crocidura russula*.

Specific richness and total captures

	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring
	Apodemus sylvaticus		*Mus spretus*		*Eliomys quercinus*		*Crocidura russula*	
H	8	14	0	1	0	0	7	4
I	10	29	1	0	2	0	14	9
L	0	11	3	0	0	0	6	20
N	1	12	11	4	0	0	21	20
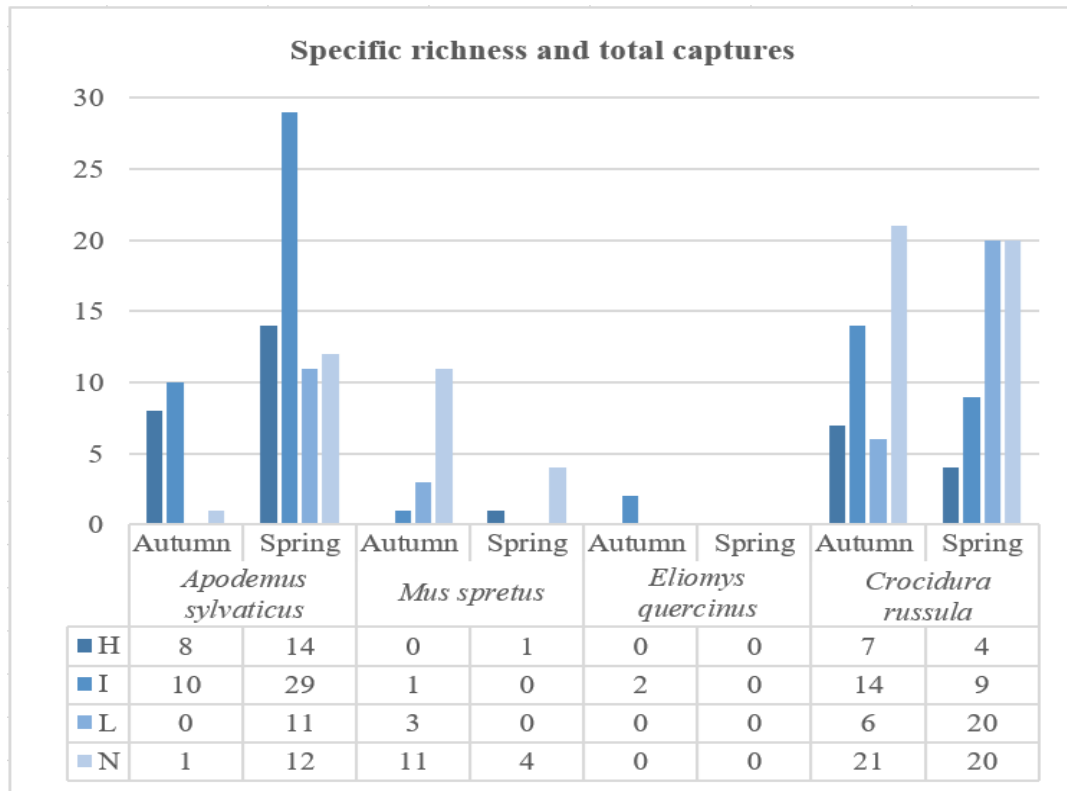
Figure 22. Small mammal captures by species, sampled area and sampling season. H (high-pressure area), I (intermediate-pressure area), L (low-pressure area), N (control area).
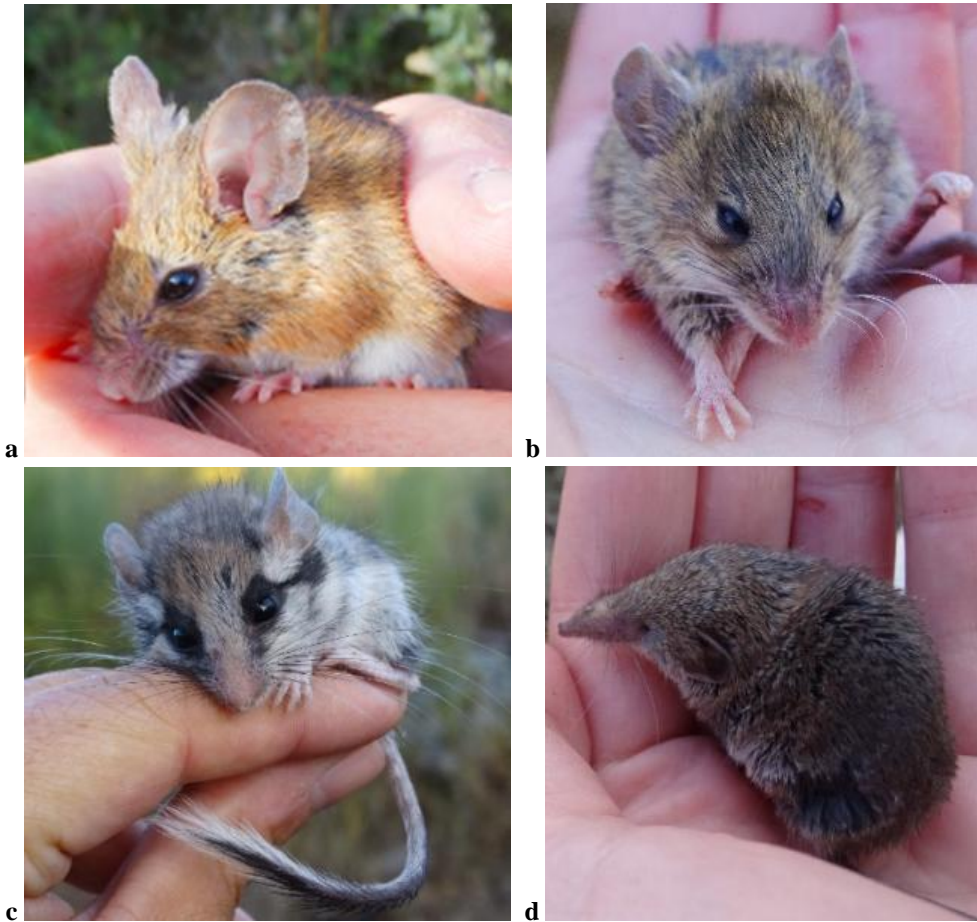
Figure 23. Small mammal species captured in the present study. A- wood mouse *Apodemus sylvaticus*; b- Algerian mouse *Mus spretus*; c- garden dormouse *Eliomys quercinus*; d- greater white-toothed shrew *Crocidura russula* (© S. Sabino).

Among the 16 species of small mammals present in the Faia Brava reserve, we targeted 7 species of rodents and 3 species of insectivores. In this study, the rodent species southwestern water vole *Arvicola sapidus* (Miller, 1908), Cabrera's vole *Microtus cabrerae* (Thomas, 1906), Lusitanian pine vole *Microtus lusitanicus* (Gerbe, 1879) and black rat *Rattus rattus* (Linnaeus, 1758) and the insectivorous species European hedgehog *Erinaceus europaeus* (Linnaeus, 1758) and Spanish mole *Talpa occidentalis* (Cabrera, 1907) were not captured (ATN, 2010).

There was a predominance of two species sampling, *Crocidura russula* and *Apodemus sylvaticus*. The shrew was the dominant species in all sampling areas, with the highest number of captures in the control area during autumn, while the wood mouse was the second most captured species, with only no records in the low-pressure area during the first sampling. The Algerian mouse was captured occasionally, particularly in the control area, whereas the garden dormouse was recorded only in the intermediate-pressure area during sampling in autumn.

In general, most of the sampled grids showed higher captures in the second sampling, with the exception of the Nb grid that had higher values in the autumn sampling (figure 24). Grid Ia presented the highest number of individuals observed, while grid Ib had the lowest number of individuals, being the area (i.e., intermediate-pressure area) with the highest oscillation between values. In the sampling performed in the autumn, the Ia and Nb grids had the highest number of individuals, while the Ib and La grids had the lowest. During the second sampling, the highest

number of individuals of small mammals was observed in grids Ia and Na, with Ha and Ib having the lowest.
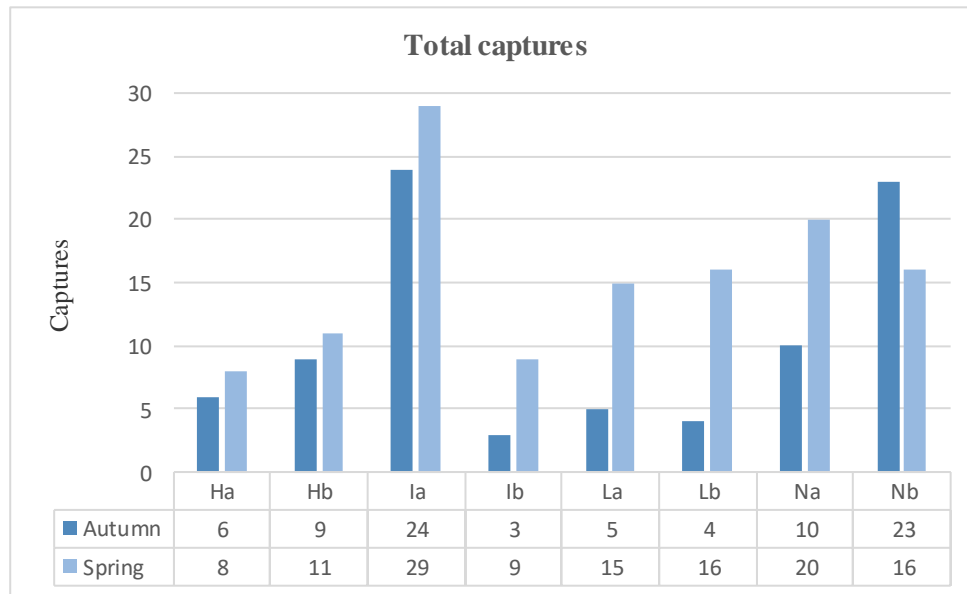
Figure 24. Values of the total captures of small mammals in each sampling grid and sampling season. Ha (grid a from the high-pressure area), Hb (grid b from the high-pressure area), Ia (grid a from the intermediate-pressure area), Ib (grid b from the intermediate-pressure area), La (grid a from the low-pressure area), Lb (grid B from the low-pressure area), Na (grid a from the control area), Nb (grid b from the control area).

Species richness according to grid and sampling period varied between 2 and 3 species, however in grid Ia during the first sampling period the 4 total species were sampled with *Eliomys quercinus* being captured, hence it was the area with the highest species richness (table 9). Despite this, the area of intermediate-pressure was the area that presented the highest variation in the number of species, since in the second sampling only the species *A. sylvaticus* and *C. russula* were captured.

Table 9. Species richness per season (autumn and spring) and per sampling grid. Ha (grid a from the high-pressure area), Hb (grid b from the high-pressure area), Ia (grid a from the intermediate-pressure area), Ib (grid b from the intermediate-pressure area), La (grid a from the low-pressure area), Lb (grid b from the low-pressure area), Na (grid a from the control area), Nb (grid b from the control area).

	Species richness	
	Autumn	Spring
Ha	2	2
Hb	2	3
Ia	4	2
Ib	2	2
La	2	2
Lb	2	2
Na	3	3
Nb	2	3

The Shapiro-Wilk normality tests allow us to reject the hypothesis of normality of the data of the total number of individuals, species richness and diversity, given that the p-values were lower than 0.05 (appendix 1).

As the data on number of individuals, species richness and diversity do not follow a normal distribution, the Wilcoxon test was applied to verify if there are statistical differences comparing two areas at a time. There were significant statistical differences between areas H and N, I and N, and L and N regarding the number of individuals and species richness (p-value < 0.05; table 10).

Table 10. Values obtained by the Wilcoxon test for the number of individuals and species richness and diversity through comparison of two sampling areas simultaneously. H (high-pressure area), I (intermediate-pressure area), L (low-pressure area), N (control area).

	Areas	W	P-value
Number of individuals	H and I	4268	0.085
	H and L	4475	0.284
	H and N	3434	p < 0.001
	I and L	5041	0.460
	I and N	4103	0.047
	L and N	3757	0.003
Species richness	H and I	4341	0.136
	H and L	4491	0.307
	H and N	3435	p < 0.001
	I and L	4970	0.602
	I and N	3942	0.014
	L and N	3721	0.002
Diversity	H and I	4568	0.227
	H and L	4855	0.743
	H and N	4657	0.430
	I and L	5093	0.124
	I and N	4888	0.684
	L and N	4603	0.263

Regarding the relative abundance, we can verify that the values obtained for *A. sylvaticus* and for rodents is, in general, very similar in the two sampling periods (tables 11-12). The relative abundance values of *C. russula* were slightly higher in the first sample when compared to the rodent values, however in the second sample the rodent group showed higher relative abundance values in the high- and intermediate-pressure areas and the shrew showed higher levels in the low-pressure and control areas.

Table 11. Relative abundance in each sampling grid and in each sampling area from the first sampling. Ha (grid a from the high-pressure area), Hb (grid b from the high-pressure area), H (high-pressure area), Ia (grid a from the intermediate-pressure area), Ib (grid b from the intermediate-pressure area), I (intermediate-pressure area), La (grid a from the low-pressure area), Lb (grid b from the low-pressure area), L (low-pressure are), Na (grid a from the control area), Nb (grid b from the control area), N (control area).

	Ha	Hb	H	Ia	Ib	I	La	Lb	L	Na	Nb	N
Apodemus sylvaticus	0.010	0.031	0.021	0.035	0.008	0.021	0.000	0.000	0.000	0.004	0.000	0.002
Mus spretus	0.000	0.000	0.000	0.005	0.000	0.002	0.008	0.004	0.006	0.008	0.039	0.024
Eliomys quercinus	0.000	0.000	0.000	0.009	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.000
Crocidura russula	0.021	0.016	0.018	0.058	0.004	0.030	0.013	0.012	0.012	0.029	0.059	0.044
Rodentia	0.010	0.031	0.021	0.046	0.008	0.027	0.008	0.004	0.006	0.013	0.039	0.026
Insectivora	0.021	0.016	0.018	0.058	0.004	0.030	0.013	0.012	0.012	0.029	0.059	0.044
Small mammals	0.031	0.046	0.038	0.093	0.012	0.054	0.020	0.016	0.018	0.040	0.091	0.065

Table 12. Relative abundance in each sampling grid and in each sampling area from the second sampling. Ha (grid a from the high-pressure area), Hb (grid b from the high-pressure area), H (high-pressure area), Ia (grid a from the intermediate-pressure area), Ib (grid b from the intermediate-pressure area), I (intermediate-pressure area), La (grid a from the low-pressure area), Lb (grid b from the low-pressure area), L (low-pressure are), Na (grid a from the control area), Nb (grid b from the control area), N (control area).

	Ha	Hb	H	Ia	Ib	I	La	Lb	L	Na	Nb	N
Apodemus sylvaticus	0.024	0.033	0.029	0.082	0.036	0.059	0.022	0.037	0.029	0.043	0.022	0.033
Mus spretus	0.000	0.004	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.016	0.011
Eliomys quercinus	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Crocidura russula	0.008	0.009	0.009	0.041	0.000	0.020	0.058	0.048	0.053	0.059	0.048	0.053
Rodentia	0.024	0.037	0.031	0.082	0.036	0.059	0.022	0.037	0.029	0.049	0.037	0.043
Insectivora	0.008	0.009	0.009	0.041	0.000	0.020	0.058	0.048	0.053	0.059	0.048	0.053
Small mammals	0.032	0.044	0.038	0.113	0.036	0.075	0.076	0.079	0.078	0.099	0.079	0.089

In general, grids revealed equitability between the species present, with the arithmetic mean of the grids being 0.731 (table 13). We can verify that grid Ha was the local with the highest equitability as it presented two species (*Apodemus sylvaticus* and *Crocidura russula*) with very similar abundances. On the other hand, grid Ib was the grid with the lowest equitability, with the dominance of *A. sylvaticus* and *C. russula* being a rare species.

Table 13. Equitability of species for each sampling grid. Ha (grid a from the high-pressure area), Hb (grid b from the high-pressure area), Ia (grid a from the intermediate-pressure area), Ib (grid b from the intermediate-pressure area), La (grid a from the low-pressure area), Lb (grid b from the low-pressure area), Na (grid a from the control area), Nb (grid b from the control area).

Grid	E_H
Ha	0,985
Hb	0,679
Ia	0,650
Ib	0,414
La	0,730
Lb	0,750
Na	0,817
Nb	0,826

During the two sampling seasons, from the 85 individuals of *Apodemus sylvaticus*, 36 females and 44 males, 75 adults and only 1 juvenile were identified. For *Mus spretus*, we identified 11 males and 6 females, 2 juveniles and 15 adults from the 20 individuals sampled. *Eliomys quercinus* had only 1 adult female and 1 juvenile male. And from the 101 *Crocidura russula* captured, it was possible to identify 40 males and 48 females, 8 juveniles and 89 adults.

No statistically significant differences in sex-ratio were found at species level (table 14) as well as at area level (table 15), except for the species *A. sylvaticus* which in the control area (N) showed statistical differences (p-value < 0.05) indicating a dominance of males. There were also no statistically significant differences in sex-ratio when the sex-ratio of each species was compared by each two sampling areas (table 16).

Table 14. Statistical values of sex-ratio per species.

Species	Ratio		P-value
	Females	Males	
Apodemus sylvaticus	0.45	0.55	0.434
Mus spretus	0.35	0.65	0.332
Crocidura russula	0.55	0.45	0.456

Table 15. Statistical values of sex-ratio per species and per sampling area. H (high-pressure area), I (intermediate-pressure area), L (low-pressure area), N (control area).

Area	Species	Ratio		P-value
		Females	Males	
H	*Apodemus sylvaticus*	0.45	0.55	0.824
	Crocidura russula	0.64	0.36	0.549
I	*Apodemus sylvaticus*	0.51	0.49	1
	Crocidura russula	0.48	0.52	1
L	*Apodemus sylvaticus*	0.58	0.42	0.774
	Mus spretus	0.33	0.67	1
	Crocidura russula	0.41	0.59	0.629
N	*Apodemus sylvaticus*	0.15	0.85	0.022
	Mus spretus	0.36	0.64	0.424
	Crocidura russula	0.62	0.38	0.188

Table 16. Statistical values of the sex-ratio of each species through comparison of two sampling areas simultaneously. H (high-pressure area), I (intermediate-pressure area), L (low-pressure area), N (control area).

Species	Areas	P-value	χ^2
Apodemus sylvaticus	H and I	0.858	0.032
	H and L	0.715	0.133
	H and N	0.166	1.920
	I and L	0.938	0.006
	I and N	0.055	3.692
	L and N	0.069	3.306
Mus spretus	L and N	1	$p < 0.001$
Crocidura russula	H and I	0.619	0.247
	H and L	0.439	0.599
	H and N	1	$p < 0.001$
	I and L	0.923	0.009
	I and N	0.411	0.675
	L and N	0.252	1.315

3.3.1. Total captures of *Apodemus sylvaticus*

The wood mouse had a high number of captures, with 85 individuals sampled in total from both sampling seasons in the 8 sampling grids (figure 25).

All sampled grids showed a higher number of observations in the second sampling, since *A. sylvaticus* was not observed in the La, Lb and Nb grids during the first sampling. The highest sampling of this species was detected in grid Ia, while the lowest was observed in grids La and Nb. The La, Lb and Nb grids were the grids that had the lowest numbers of captures in the autumn sampling, in contrast, the Ia grid had the highest number of individuals. In addition, the La and Nb grids once again had the lowest capture of specimens in the spring sampling, just as the Ia grid once again had the highest number of samples.

The low-pressure area had the lowest number of individuals sampled, while the intermediate-pressure area had the highest number.

The low-pressure area had no *Apodemus* recorded, being the area with the lowest captures in autumn, while the intermediate-pressure area had the most observations of the target species. The same pattern is recorded in the spring sampling, the low-pressure area being the area with the lowest number of captures, and the intermediate-pressure area being the area with most observations.
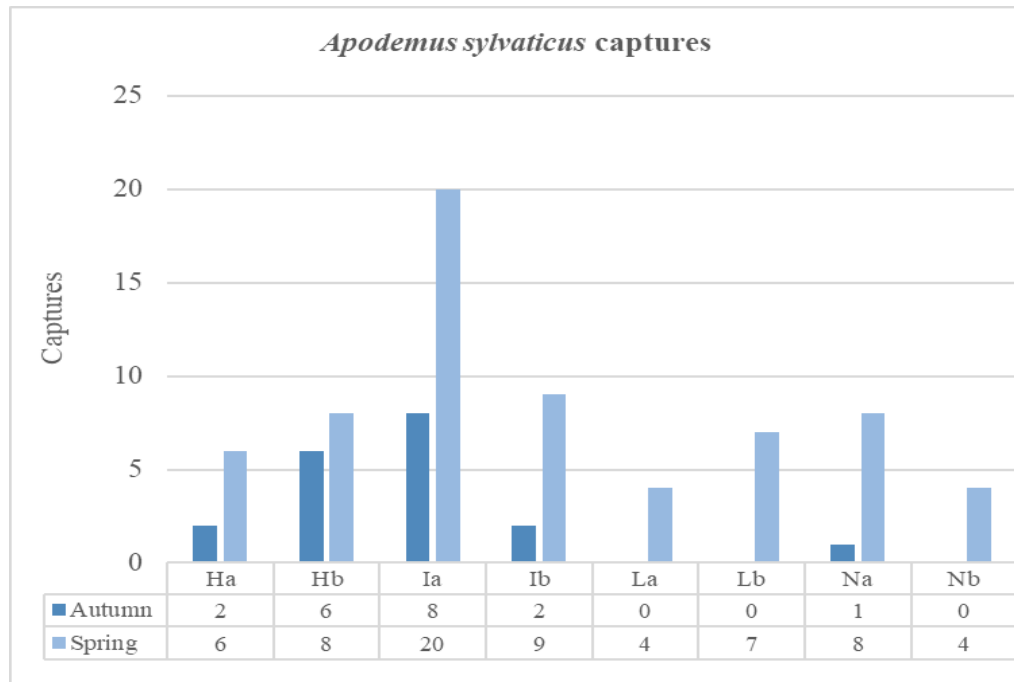
Figure 25. Captures of *Apodemus sylvaticus* in each sampling grid and sampling seasons. Ha (grid a from the high-pressure area), Hb (grid b from the high-pressure area), Ia (grid a from the intermediate-pressure area), Ib (grid b from the intermediate-pressure area), La (grid a from the low-pressure area), Lb (grid b from the low-pressure area), Na (grid a from the control area), Nb (grid b from the control area).

3.3.2. Total captures of *Mus spretus*

During the two sampling periods 20 *Mus spretus* were captured (figure 26), with the number of individuals captured varying significantly according to the grid and the sampling period, ranging from 0 to 9 individuals sampled during the first sampling period, while during the spring sampling period the numbers ranged only from 0 to 3.

In the first sampling, the Ha, Hb and Ib grids were those with the lowest number of observations of the target species since there were no records, while the Nb grid had the highest number of captures. In the second sampling, only the Hb, Na and Nb grids had records, with the last one having the highest number of individuals.

The Nb grid was the only one that presented several individuals, while the other grids had some records that varied only between 1 and 3. The control area had the highest number of *Mus* observations, while the areas of high- and intermediate-pressure had the lowest number.

	Ha	Hb	Ia	Ib	La	Lb	Na	Nb
■ Autumn	0	0	1	0	2	1	2	9
■ Spring	0	1	0	0	0	0	1	3
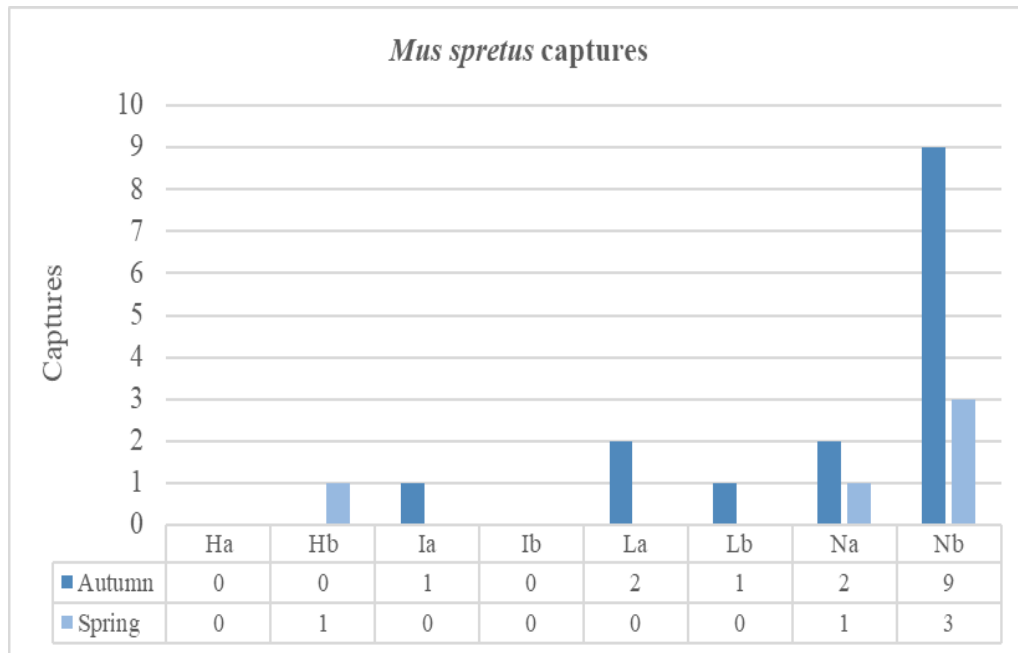
Figure 26. Captures of *Mus spretus* in each sampling grid and sampling seasons. Ha (grid a from the high-pressure area), Hb (grid b from the high-pressure area), Ia (grid a from the intermediate-pressure area), Ib (grid b from the intermediate-pressure area), La (grid a from the low-pressure area), Lb (grid b from the low-pressure area), Na (grid a from the control area), Nb (grid b from the control area).

3.3.3. Total captures of *Crocidura russula*

A total of 101 records of *C. russula* were gathered during the two sampling periods (figure 27). The number of observations of this species differed substantially between the various grids and sampling periods, with values ranging from 1 to 14 individuals in autumn and 0 to 11 in spring.

The Ha, Hb, Ia, Ib and Nb grids were those with the highest number of captures in the first sampling period, in contrast to the La, Lb and Na grids, which had the highest number of observations in the second sampling period. Grid Ib had the fewest shrew observations, while grid Nb had the most. During the first sampling period, grid Ib presented the lowest number of samples, while grid Ia presented the highest, and the number of observations in this area oscillated significantly. In the second sampling period, the same pattern of captures occurred again, grid Ib being the grid with the fewest observations and grid Ia with the most animals sampled.

The high-pressure area had the lowest number of records of this species, while the control area had the highest number of records.

In the autumn sampling, the low-pressure area had the lowest catches of individuals, while the intermediate-pressure area had the highest number of individuals. In spring sampling, the high-pressure area had the lowest number of observations, while the low-pressure and control area had the highest number of individuals.
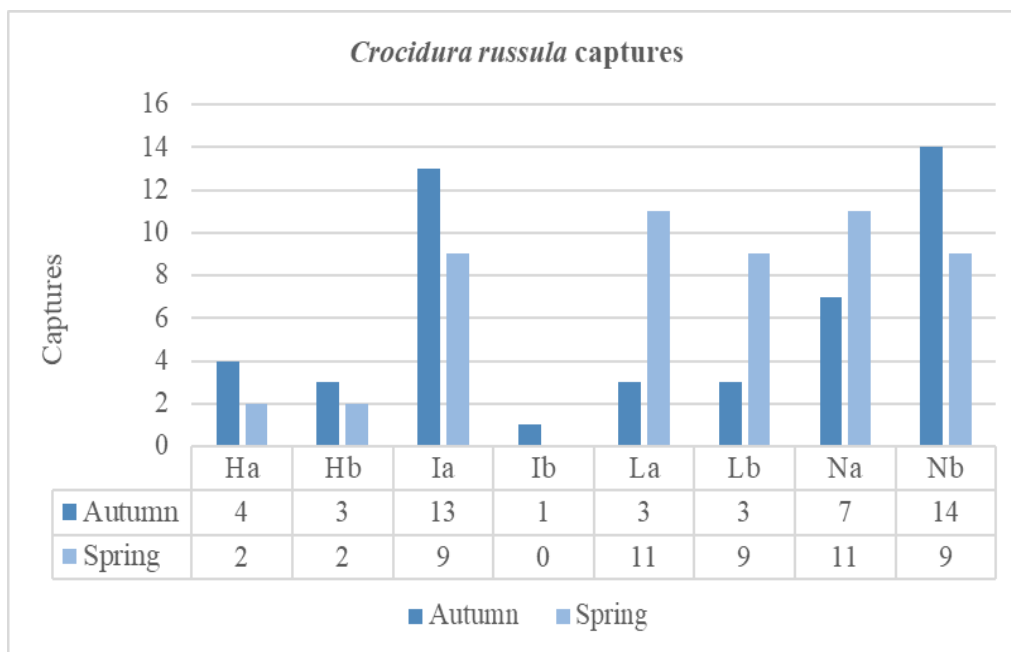
Figure 27. Captures of *Crocidura russula* in each sampling grid and sampling seasons. Ha (grid a from the high-pressure area), Hb (grid b from the high-pressure area), Ia (grid a from the intermediate-pressure area), Ib (grid b from the intermediate-pressure area), La (grid a from the low-pressure area), Lb (grid b from the low-pressure area), Na (grid a from the control area), Nb (grid b from the control area).

3.4. Physical condition of small mammals

To analyse which factors influence the fitness of small mammals, two different but complementary indicators were used: body condition and parasite load.

3.4.1. Body condition

The body condition index of small mammals presented variations among species, sampling periods and sampling areas, with, in general, the lowest body condition values obtained in the high-pressure area during the sampling performed in spring, while in the case of the highest values were observed in the intermediate-pressure area during autumn for *A. sylvaticus* and during spring for *C. russula*.

3.4.1.1. Body condition of *Apodemus sylvaticus*

The body condition of *A. sylvaticus* showed a slight variation between the different areas and the two sampling periods, reaching higher values in autumn, revealing a better body condition in the intermediate- and low-pressure areas during the first sampling, while the lowest values were observed in the high- and intermediate-pressure areas during spring (figure 28).

45
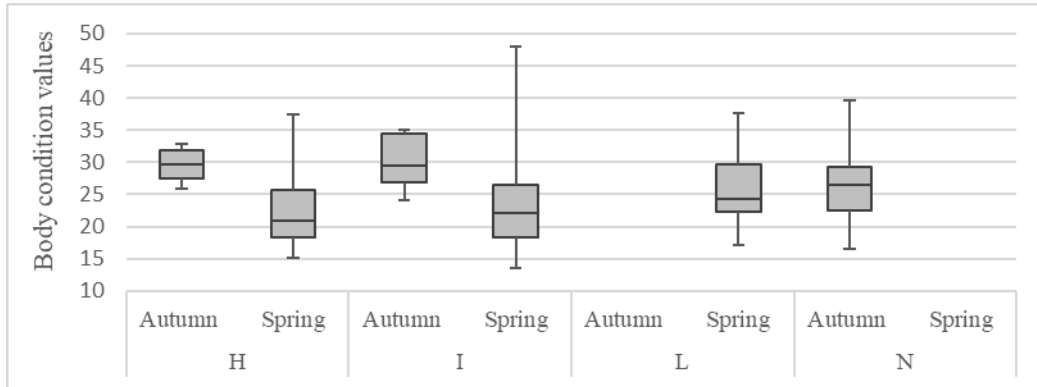
Figure 28. Body condition values of *Apodemus sylvaticus* by sampling season (autumn and spring) in each sampling areas. H (high-pressure area), I (intermediate-pressure area), L (low-pressure area), N (control area).

Regarding the body condition among the different sexes of this species, it demonstrated to be very similar, although it was slightly high in males (figure 29).
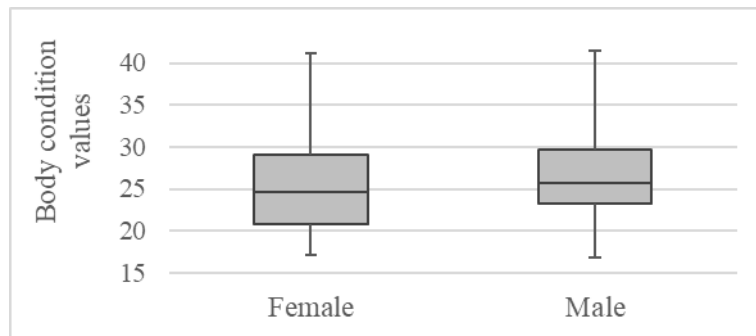
Figure 29. Body condition values of *Apodemus sylvaticus* by sex.

3.4.1.2. Body condition of *Mus spretus*

Since the Algerian mouse was only sampled in the control area during the two sampling periods and in the intermediate-pressure area during autumn, its' representation was quite limited preventing an accurate comparison of the differences in body condition values obtained in the different areas and sampling periods (figure 30). Despite this, it was possible to observe that the control area showed higher values of body condition during autumn.
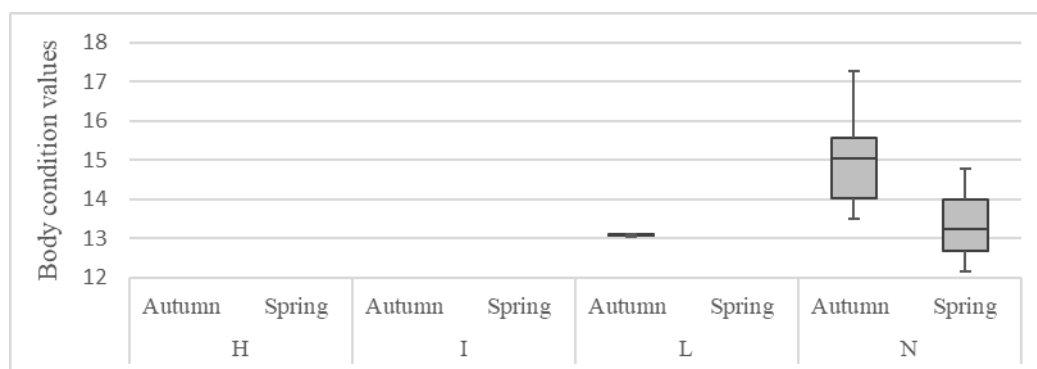
Figure 30. Body condition values of *Mus spretus* by sampling season (autumn and spring) in each sampling areas. H (high-pressure area), I (intermediate-pressure area), L (low-pressure area), N (control area).

When comparing body condition between sexes, it was slightly higher in females (figure 31).
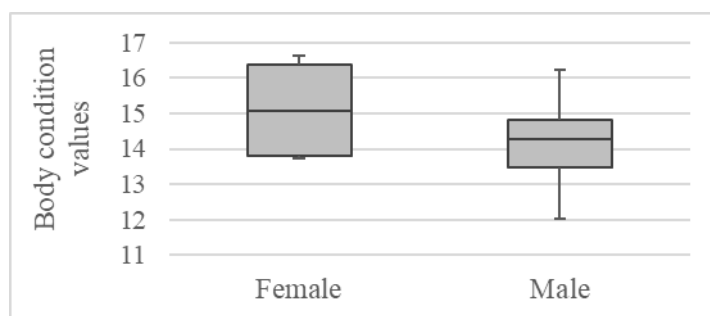
Figure 31. Body condition values of *Mus spretus* by sex.

3.4.1.3. Body condition of *Crocidura russula*

The body condition of *C. russula* reached higher values in the intermediate-pressure area during spring sampling, and the lowest value was found in the high-pressure area during the second sampling period (figure 32).
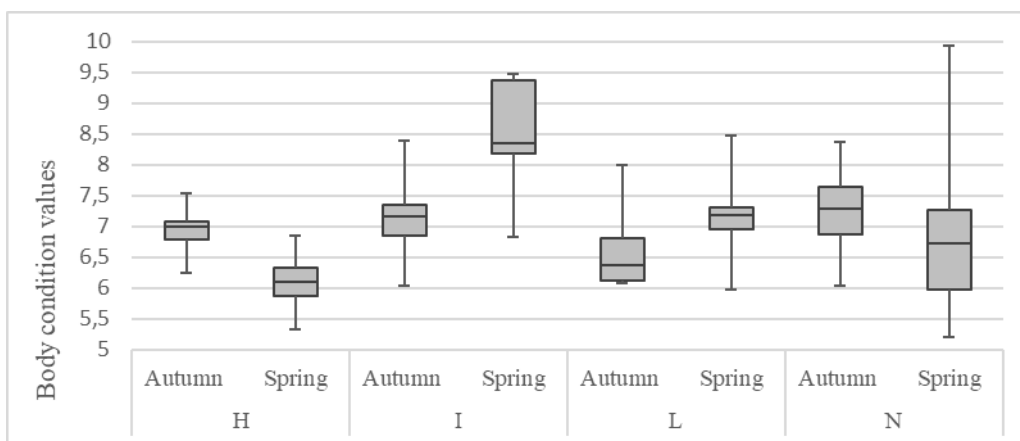
Figure 32. Body condition values of *Crocidura russula* by sampling season (autumn and spring) in each sampling areas. H (high-pressure area), I (intermediate-pressure area), L (low-pressure area), N (control area).

The body condition between the different sexes of this species presented very similar values (figure 33).
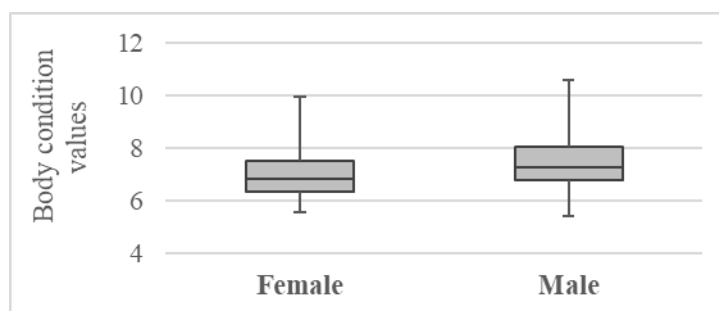
Figure 33. Body condition values of *Crocidura russula* by sex.

3.4.2. Parasite load

A total of 275 ectoparasites were collected in the two samplings, with 6 collected during autumn and 269 during spring, with the parasite load being substantially higher during the second sampling (figure 34). *A. sylvaticus* was the small mammal species that presented the major parasite load compared to the other species, especially in the low-pressure and control area during the second sampling period. In the case of *M. spretus* and *C. russula*, among the parasites collected, most were sampled in the control area.

Total parasites

	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring
	H		I		L		N	
■ *Apodemus sylvaticus*	0	2	0	20	0	180	0	65
■ *Mus spretus*	0	0	0	0	0	0	1	0
■ *Crocidura russula*	0	0	0	0	0	1	5	1
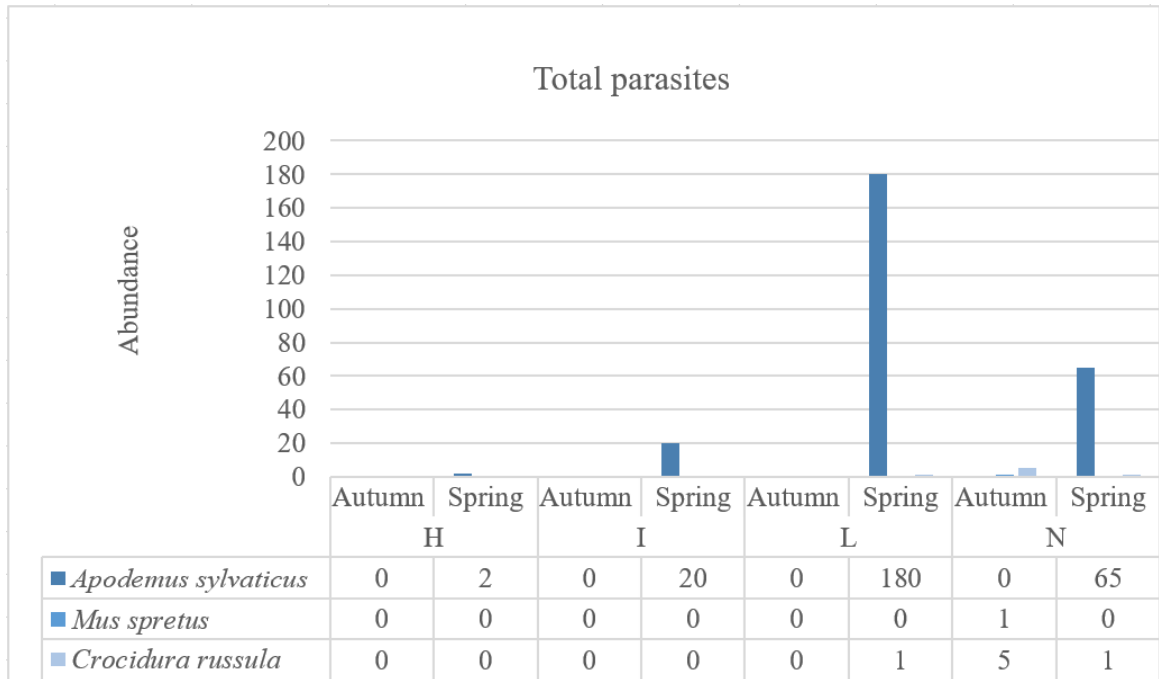
Figure 34. Total number of ectoparasites collected per species, per sampled area and per sampling season. H (high-pressure area), I (intermediate-pressure area), L (low-pressure area), N (control area).

3.5. Effect of environmental parameters

The Generalized Linear Mixed Models developed to analyse the variability in abundance and body condition of the three species are presented in table 17. The values of each independent variable correspond to the arithmetic means of the 49 sampling points of each sampling grid.

Table 17. Descriptive values of the independent variables for each sampling location. Traces - Percentage of ungulate traces; Horses and Cows - Percentage of camera records of horses and cows; Ungulates - Percentage of camera records of ungulates excluding horses and cows; Mesocarnivores - Percentage of camera records of mesocarnivores; Edible vegetation - Species richness of edible vegetation; Shelter - Percentage of shelter; Parasites - Parasite load. Ha (grid a from the high-pressure area), Hb (grid b from the high-pressure area), Ia (grid a from the intermediate-pressure area), Ib (grid b from the intermediate-pressure area), La (grid a from the low-pressure area), Lb (grid b from the low-pressure area), Na (grid a from the control area), Nb (grid b from the control area).

Location	Traces	Horses and Cows	Ungulates	Mesocarnivores	Food	Shelter	Parasites
Ha	77.60	14.89	3.64	21.27	7	27.00	0
Hb	93.90	41.21	2.40	19.59	6	31.00	2
Ia	34.70	11.56	6.00	25.93	5	37.00	20
Ib	47.00	5.24	3.60	6.72	5	40.00	0
La	55.10	10.19	1.28	26.49	7	39.00	161
Lb	77.60	0.00	0.00	0.00	4	21.00	20
Na	0.00	0.00	0.00	0.00	5	27.00	66
Nb	6.00	0.00	0.00	0.00	8	40.00	6

3.5.1. Factors influencing variation in abundance of small mammals

3.5.1.1. Factors influencing variation in abundance of *Apodemus sylvaticus*

Production of the combined model for the abundance of each subgroup of wood mouse resulted in statistically significant differences for all independent variables (p-value < 0.05; tables 18-20).

Table 18. Results of p-values for each independent variable for the subgroup that included all individuals of *Apodemus sylvaticus*.

Independent variable	p-value
Traces	$p < 0.001$
Cattle	$p < 0.001$
Ungulates	$p < 0.001$
Mesocarnivores	$p < 0.001$
Food	$p < 0.001$
Shelter	$p < 0.001$
Parasites	$p < 0.001$

Table 19. Results of p-values for each independent variable for the subgroup that included females of *Apodemus sylvaticus*.

Independent variable	p-value
Traces	p < 0.001
Cattle	p < 0.001
Ungulates	p < 0.001
Mesocarnivores	p < 0.001
Food	p < 0.001
Shelter	p < 0.001
Parasites	p < 0.001

Table 20. Results of p-values for each independent variable for the subgroup that included males of *Apodemus sylvaticus*.

Independent variable	p-value
Traces	p < 0.001
Cattle	p < 0.001
Ungulates	p < 0.001
Mesocarnivores	p < 0.001
Food	p < 0.001
Shelter	p < 0.001
Parasites	p < 0.001

3.5.1.2. Factors influencing variation in abundance of *Mus spretus*

Similarly to the other species, *M. spretus* subgroups showed significant statistics for all independent variables in the combined models (tables 21-23).

Table 21. Results of p-values for each independent variable for the subgroup that included all individuals of *Mus spretus*.

Independent variable	p-value
Traces	p < 0.001
Cattle	p < 0.001
Ungulates	p < 0.001
Mesocarnivores	p < 0.001
Food	p < 0.001
Shelter	p < 0.001
Parasites	p < 0.001

Table 22. Results of p-values for each independent variable for the subgroup that included females of *Mus spretus*.

Independent variable	p-value
Traces	$p < 0.001$
Cattle	$p < 0.001$
Ungulates	$p < 0.001$
Mesocarnivores	$p < 0.001$
Food	$p < 0.001$
Shelter	$p < 0.001$
Parasites	$p < 0.001$

Table 23. Results of p-values for each independent variable for the subgroup that included males of *Mus spretus*.

Independent variable	p-value
Traces	$p < 0.001$
Cattle	$p < 0.001$
Ungulates	$p < 0.001$
Mesocarnivores	$p < 0.001$
Food	$p < 0.001$
Shelter	$p < 0.001$
Parasites	$p < 0.001$

3.5.1.3. Factors influencing variation in abundance of *Crocidura russula*

The combined models for *C. russula* subgroups also showed statistically significant differences for all independent variables (tables 24-26).

Table 24. Results of p-values for each independent variable for the subgroup that included all individuals of *Crocidura russula*.

Independent variable	p-value
Traces	$p < 0.001$
Cattle	$p < 0.001$
Ungulates	$p < 0.001$
Mesocarnivores	$p < 0.001$
Food	$p < 0.001$
Shelter	$p < 0.001$
Parasites	$p < 0.001$

Table 25. Results of p-values for each independent variable for the subgroup that included females of *Crocidura russula*.

Independent variable	p-value
Traces	p < 0.001
Cattle	p < 0.001
Ungulates	p < 0.001
Mesocarnivores	p < 0.001
Food	p < 0.001
Shelter	p < 0.001
Parasites	p < 0.001

Table 26. Results of p-values for each independent variable for the subgroup that included males of *Crocidura russula*.

Independent variable	p-value
Traces	p < 0.001
Cattle	p < 0.001
Ungulates	p < 0.001
Mesocarnivores	p < 0.001
Food	p < 0.001
Shelter	p < 0.001
Parasites	p < 0.001

3.5.2. Factors influencing variation in body condition of small mammals

3.5.2.1. Factors influencing variation in body condition of *Apodemus sylvaticus*

The variable of ungulate traces exhibited statistically significant differences in the combined model for the subgroup of all *Apodemus sylvaticus* individuals (table 27), while, when the individuals were separated by gender, both males and females showed no statistically significant differences for the independent variables (tables 28-29). In the female subgroup, since ectoparasites were collected from only two females of this species, it was not possible to calculate the p-value of the parasite load.

Table 27. Results of p-values for each independent variable for the subgroup that included all individuals of *Apodemus sylvaticus*.

Independent variable	p-value
Traces	0.018
Horses and Cows	0.068
Ungulates	0.793
Mesocarnivores	0.759
Food	0.370
Shelter	0.445
Parasites	0.481

Table 28. Results of p-values for each independent variable for the subgroup that included females of *Apodemus sylvaticus*.

Independent variable	p-value
Traces	0.474
Horses and Cows	0.277
Ungulates	0.624
Mesocarnivores	0.231
Food	0.394
Shelter	0.673
Parasites	-

Table 29. Results of p-values for each independent variable for the subgroup that included males of *Apodemus sylvaticus*.

Independent variable	p-value
Traces	0.080
Horses and Cows	0.485
Ungulates	0.221
Mesocarnivores	0.254
Food	0.114
Shelter	0.773
Parasites	0.883

3.5.2.2. Factors influencing variation in body condition of *Mus spretus*

In the combined model for the Algerian mouse, it was not possible to obtain p-values for the majority of the variables, due to the low number of captures of this species in the study areas and when it was sampled it was mainly in the control area (i.e., without camera records of horses and cows, ungulates and mesocarnivores) (tables 30-32). However, there were only statistically significant differences in the percentage of camera records of horses and cows and percentage of ungulate traces variables in the female subgroup.

Table 30. Results of p-values for each independent variable for the subgroup that included all individuals of *Mus spretus*.

Independent variable	p-value
Traces	0.588
Horses and Cows	0.843
Ungulates	-
Mesocarnivores	-
Food	0.335
Shelter	-
Parasites	-

Table 31. Results of p-values for each independent for the subgroup that included females of *Mus spretus*.

Independent variable	p-value
Traces	0.001
Horses and Cows	0.001
Ungulates	-
Mesocarnivores	-
Food	-
Shelter	-
Parasites	-

Table 32. Results of p-values for each independent variable for the subgroup that included males of *Mus spretus*.

Independent variable	p-value
Traces	0.116
Horses and Cows	-
Ungulates	-
Mesocarnivores	-
Food	0.553
Shelter	-
Parasites	-

3.5.2.3. Factors influencing variation in body condition of *Crocidura russula*

In the combined model for the subgroup that included all *Crocidura* individuals statistically significant differences in all variables were found with the exception of shelter (table 33). In the case of the male subgroup, it presented statistically significant differences in the same variables with the exception of percentage of camera records of horses and cows, while the female subgroup did not present statistically significant differences in any of the independent variables (table 34-35). Furthermore, the female subgroup was unable to produce a p-value for the parasite load variable.

Table 33. Results of p-values for each independent variable for the subgroup that included all individuals of *Crocidura russula*.

Independent variable	p-value
Traces	0.018
Horses and Cows	0.033
Ungulates	0.036
Mesocarnivores	0.024
Food	0.018
Shelter	0.103
Parasites	0.029

Table 34. Results of p-values for each independent variable for the subgroup that included females of *Crocidura russula*.

Independent variable	p-value
Traces	0.927
Horses and Cows	0.272
Ungulates	0.642
Mesocarnivores	0.425
Food	0.296
Shelter	0.230
Parasites	-

Table 35. Results of p-values for each independent variable for the subgroup that included males of *Crocidura russula*.

Independent variable	p-value
Traces	0.009
Horses and Cows	0.128
Ungulates	0.033
Mesocarnivores	0.020
Food	0.024
Shelter	0.154
Parasites	0.021

4. Discussion

4.1. Diversity and abundance

Here, I confirm the presence of three species of rodents (*Apodemus sylvaticus*, *Eliomys quercinus* and *Mus spretus*) and an insectivore (*Crocidura russula*) in the Faia Brava reserve, representing only 25% of the recorded diversity (i.e., from the total of 16 documented species; ATN, 2010), while in Quinta do Sol and Quinta de São Paulo the same species were detected, with the exception of *Eliomys quercinus*, representing 18.75% of the recorded diversity.

Considering that the variables associated with the vegetation of an area can affect the presence of small mammal species, one may argue that they also affect their abundance (MacArthur, 1984; Bowman et al., 2001), and thus it is expected that the small mammal species that are able to colonise these areas, are also able to reach a higher population growth (Hobbs et al., 2003; Rosalino et al., 2014). Consequently, the existence of a more developed understory had a positive influence on the distribution, species richness and abundance of small mammals (MacArthur, 1984; Stallings, 1990; Martin et al., 2012).

The prediction that the control area would be the area with the highest species richness of small mammals was not confirmed. In general, the number of species was similar between the areas with presence of horses and cows and in the areas without them, in both life-cycle periods of small mammals, although in the intermediate-pressure area it was slightly higher in autumn (only one more species), while in spring only two species were captured. A possible explanation for small mammal species richness being slightly higher in the intermediate-pressure area as opposed to the control area, may relate to the intermediate disturbance hypothesis (IDH) which suggests that species diversity tends to reach a peak in areas with an intermediate scale of disturbance (Moi et al., 2020). Generally, the highest abundance of small mammals occurred during the spring sampling period. This can be attributed to the increase in temperature during this season, which promotes an increase in the vegetation cover, providing an abundance of food and shelter (Ribeiro, 2007). The percentage of vegetation of each sampled area was strictly related to the abundance of small mammals, with the area without grazing reaching the maximum value (83%) being in turn the area with the highest number of small mammals. On the other hand, the area of high-pressure presented only 41% of vegetation and in turn was the area with fewer small mammals. Our results support the prediction that the abundance of small mammals in the control area is positively influenced by a more complex structural understory, since it reached higher values when compared to areas with the presence of horses and cows. The selection of these sites by small mammals seems to be associated with a greater capacity of protection provided by this stratum (i.e., shelter), resulting in a reduced probability of being detected during their daily activities by terrestrial carnivores' predators and birds of prey (Rosalino et al., 2012). Similar studies have shown a positive influence of understory on the abundance of small mammals. For example, Putman et al. (1989), indicate the existence of a higher structural complexity in non-grazed areas compared to grazed areas, since it has a higher diversity of vegetation and cover, and consequently more food is available, resulting in a higher abundance of small mammal populations. Another study demonstrated that a higher diversity of plant communities enhances the richness of insect communities by providing a wider range of plant resources and a more complex vegetation structure that can provide protection, hence a more diverse insect community can in turn support larger populations of insectivorous mammals (e.g., shrews) (Haddad et al., 2001; Johnson, 2017).

The insectivore *C. russula* was the most representative species in the sampled areas, since the coverage of shrub understory positively influenced the abundance of this species, due to the fact that it occupies mainly open habitats with good vegetation cover (Araújo et al., 2012). On the other hand, the *Apodemus sylvaticus* was the second most representative species, essentially because it is a generalist species with an adaptability to the available food resources (e.g., seeds, fruits, and berries) (Macdonald and Barrett, 2002), frequently associated to areas with more developed shrub understory (Jubete, 2007). Although *Mus spretus* is a generalist species, it has shown to be not particularly abundant, being concentrated in areas with a higher percentage of shrub vegetation (Palomo, 2007). The *Eliomys quercinus* is a species with a conservation status of DD (Data Deficient) according to the Red Book of Vertebrates of Portugal, since the existing information is insufficient to evaluate and categorise it (Cabral et al., 2005). The species occurred in the Faia Brava APP, however, the absence of captures of this species is probably associated with its natural low abundance in the study areas (ATN, 2010).

Considering the fact that small mammals were more abundant in the control area and less predominant in the high-pressure area, it is possible to emphasise a potential negative impact of ungulates when present in high densities, as they cause alterations in vegetation composition and structure. The number of individuals of *A. sylvaticus* was higher in areas of intermediate- and high-pressure, whereas the abundance of *M. spretus* and *C. russula* tended to be inversely proportional to the density of ungulates, as these small mammals were more numerous in control areas and, in turn, less abundant in areas with high livestock pressure.

Small mammals are extremely important due to their bottom-up effects on their predators (mammals and medium-sized birds) and their top-down effects on plant and arthropod communities (Torre et al., 2007). This group normally affected by ungulate grazing, since the trampling of livestock causes the compaction of the soil leading to a decrease in the suitability of the soil for the development of burrows, and their consumption of vegetation leads to a decrease in the availability or quality of food resources and causes changes in the percentage and height of the vegetation cover, leading to an increased risk of predation on small mammals in structurally simpler areas (Torre et al., 2007).

The availability of resources for small mammals in each sampled area was calculated, in particular food and shelter, since the latter allows us to understand the potential that the habitat has for the construction of burrows and nests in the breeding season and to enable the small mammals to escape from predators. Food availability for rodents (i.e., *A. sylvaticus*, *M. spretus* and *E. quercinus*) was represented by the abundance of potentially edible vegetation and the species richness of fruits present in each area, while the availability of shelter was represented by the abundance of shrub vegetation and trees that were present. The results of this study show that although the availability of shelter does not differ significantly between areas, the areas with an intermediate density of livestock have a higher availability of shelter, however, in the area excluded from grazing the availability value of this resource was quite similar. Similarly, food availability showed no differences between grazing exclusion and non-exclusion sites, suggesting that horses and cows do not significantly influence the abundance of small mammals.

The results of the GLMM approach for the abundance of *Apodemus sylvaticus*, *Crocidura russula* and *Mus spretus* revealed that there are significant effects on all independent variables combined, as they presented p-values below 0.05.

The variable that had a significant positive influence on the total abundance of *C. russula* was the percentage of shelter, i.e., the total abundance of this species is higher where the percentage of shrub understory is higher, since it provides a higher availability of food as well as shelter for *C.*

russula and protection from predators (see Eccard et al., 2000; Schmidt et al., 2005; Brahmi et al., 2012).

In the case of *A. sylvaticus*, the percentage of ungulate traces, the percentage of camera records of horses and cows, the percentage of camera records of ungulates excluding horses and cows and the parasite load were the variables that showed a significant positive influence on the total abundance of this species. The results of the abundance of wood mouse are opposite to the results obtained in other similar studies, since the abundance of this species tends to be higher in non-grazed areas (Putman et al., 1989). However, Gelling et al. (2007) indicate that this species presents lower abundances in areas of high grass cover, due to lower seed productivity and the higher levels of disturbance to which these areas are commonly exposed.

Regarding *M. spretus*, the abundance was negatively influenced by the percentage of camera records of horses and cows, percentage of camera records of ungulates excluding horses and cows and percentage of camera records of mesocarnivores, as it reached higher values in the control area, given that grazing areas tend to have less structural diversity within the vegetation and reduced cover when compared to areas without ungulates, and in turn have a lower availability of food, resulting in a lower abundance of small mammals (Putman et al., 1989).

4.2. Physical condition

The results of the present study demonstrate that the presence of ungulates in an intensive regime produces a significant negative effect on the body condition of small mammals during spring. Since *C. russula* and *A. sylvaticus* were the only species with higher representativity in almost all sampling areas and trapping seasons, their body condition indices revealed that they tended to have higher values during autumn, with the exception of *A. sylvaticus* in the intermediate- and low-pressure areas which were higher during the second sampling. The body condition between the different sexes of *Apodemus* and *Crocidura* was very similar, meanwhile the females of *M. spretus* seemed to have a superior body condition than the males, which can be related to the territorial behaviour of males which leads them to move more frequently and over longer distances when compared to females, thus expending more energy and in turn having a lower body condition index (Fernandez et al., 1996).

These results lead to an acceptance of part of the initial hypothesis that the body condition of small mammals is lower in the high-pressure area, but only in spring, as the results of the autumn sampling contradicted what was expected. The results of the autumn sampling can be related to the fact that this season is a period with more abundant precipitation and humidity, and even in areas with higher grazing density it is possible to provide food (e.g., acorns) for small mammals without decreasing the body condition. Regarding the results of the second sampling, these can be explained by the fact that in areas with intensive grazing there is reduced availability of food resources due to the increased competition with ungulates. The negative influence of ungulate pressure on the body condition of small mammals may lead to long-term consequences in individual fitness.

Parasite load data indicates that there is a higher prevalence of parasites in the spring season than in autumn. These results may be related to the increase in temperature in spring, as it induces an increase in ectoparasite activity (Estrada-Penã et al., 2004; Ribeiro, 2007). However, contrary to my expectations, the highest number of ectoparasites was collected in the low-pressure area instead of the control area. This may be related to the fact that most parasites that use small mammals as hosts (e.g., *Hyalomma* and *Ixodes* species) usually have a two- or three-host life cycle, in which

larvae and nymphs may feed on small mammals (Szabó et al., 2013; Sponchiado et al., 2015), but also adults may feed on larger hosts, such as cattle, horses, wild boar and deer (Guglielmone et al., 2003; Estrada-Peña et al., 2004). Given that the number of ungulates in the low-pressure area is relatively low (i.e., has mostly horses) and the number of small mammals is also lower than in other areas (except the high-pressure area), parasites tend to parasitize more frequently on small mammals, and in turn as there are no cattle in the control area, the parasite species that need more hosts are more absent.

A. sylvaticus was the small mammal species that presented the highest number of parasites, since this species when compared to the other species is the one that presents the largest body dimensions and has a more extensive territoriality and vital area (Fernandez et al., 1996; Macdonald and Barrett, 2002), being factors that contribute to a major mobility of the species and, as a result, a larger probability of being parasitized by ectoparasites that occur in shrub vegetation (Fernandez et al., 1996). This species showed the highest parasite load in Quinta de São Paulo and in the low-pressure area during the spring sampling, which can be explained by the increase in temperature during spring, which promotes an increase in ectoparasite activity, which combined with a high percentage of vegetation promotes the abundance of parasites (Estrada-Peña et al., 2004; Ribeiro, 2007). *C. russula* was the second species with more parasites, while *M. spretus* had only one individual with one parasite.

The GLMM model for body condition of *Apodemus* individuals revealed the existence of significant effects within the variable of percentage of ungulate traces, given that the body condition was slightly higher in males and it was only possible to calculate the body condition for this species from the grids of high- and intermediate-pressure areas, and from one grid of each of the remaining areas, and in turn body the condition was higher in areas with a higher percentage of ungulate traces.

The body condition of *Crocidura russula* demonstrated to be influenced by species richness of edible vegetation, percentage of ungulate traces, percentage of camera records of ungulates excluding horses and cows, percentage of camera records of mesocarnivores, and parasite load, having the most independent variables with statistical differences.

M. spretus, due to its reduced representation in the body condition, as the body condition was calculated only for adult individuals, and the fact that they were mainly captured in the control area grids (i.e., without records of horses and cows, ungulates and mesocarnivores), it was not possible to calculate p-values for most variables for the three subgroups. Despite this, in the female subgroup there was a positive effect on the percentage of ungulate traces and on the percentage of camera records of horses and cows, given that in the control area, the body condition exhibited higher values.

5. Conclusion

With this study it was possible to verify that the areas with different ungulate pressures support a very similar diversity of small mammal species. In general, there was a negative impact of the presence of ungulates at high densities, since this area showed a lower population abundance of small mammals, while the ungulate control area showed a higher abundance of small mammals. However, in the case of *Apodemus sylvaticus*, it showed higher abundance in areas with high and intermediate livestock density, reaching maximum abundance in the last one, with body condition being also higher especially in areas of high- and intermediate-pressure. The abundance of *C. russula* was inversely proportional to the pressure level of ungulates, reaching its maximum value in the control area, while the body condition was quite similar in all areas with a slight increase in the intermediate-pressure area. *Mus spretus* seems to be particularly sensitive to any of the grazing levels, being practically excluded from grazing areas with higher pressure levels since the higher abundance of ungulates the lower their abundance, with the same pattern having been detected in its body condition. This negative response to the presence of high densities of ungulates is due to the strong direct influence that horses and cows have on the composition of the plant community, by affecting, directly or indirectly, the availability of food and shelter for *M. spretus* and *C. russula* (Eccard et al., 2000; Schmidt et al., 2005; Brahmi et al., 2012). The area of intermediate-pressure of ungulates also revealed a very high abundance of small mammals, together with the highest richness of small mammals, wild ungulates and mesocarnivores, and the highest percentage of vegetation with potential as shelter and food. The small mammal community would be improved by an adequate livestock management, which should include a moderation of the grazing intensity to an intermediate level. Natural grazing of semi-wild large herbivores reintroduced at intermediate pressures would provide a denser shrub cover, favouring not only small mammals but also other species that depend on shrub vegetation (Mangas et al., 2008). Such strategy will benefit the restoration of Mediterranean habitats as well as promote biodiversity and trophic complexity, facilitating in the future the return of other species such as the Iberian lynx *Lynx pardinus* (Temminck, 1827). Intermediate levels of grazing do not lead to such a marked disturbance of small mammal abundance, although *M. spretus* abundance tends to decrease. This pattern reveals that grazing pressure at intermediate levels induce lower levels of disturbance, as the shrub vegetation remains dense enough for protection of small mammals, while creating some level of habitat heterogeneity that promotes biodiversity (Moi et al., 2020). The disturbance induced by cows and horses and the consequent destruction of vegetation seem not to significantly also disturb the community of wild ungulates and mesocarnivores.

The results of this study represent a significant contribution to the knowledge of the impact that reintroduced large herbivores have on the community of small mammals present in the Côa Valley. On the other hand, this is an original study where new knowledge indicates possible research directions. Furthermore, it provides crucial insights that may guide rewilding processes in sustainable ways. Future studies in the Côa Valley should improve sampling by using more replicates from different areas with intermediate ungulate pressure in order to achieve a deeper understanding of how the pressure associated with these large herbivores presence influences the small mammal community, individuals body condition and performance.

6. References

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7. Appendix

Appendix 1. Values obtained by the Shapiro-Wilk test for the number of individuals, species richness and diversity. H (high-pressure area), I (intermediate-pressure area), L (low-pressure area), N (control area).

	Areas	W	P-value
Number of individuals	H	0.527	$p < 0.001$
	I	0.615	$p < 0.001$
	L	0.612	$p < 0.001$
	N	0.768	$p < 0.001$
Species richness	H	0.542	$p < 0.001$
	I	0.644	$p < 0.001$
	L	0.616	$p < 0.001$
	N	0.753	$p < 0.001$
Diversity	H	0.257	$p < 0.001$
	I	0.380	$p < 0.001$
	L	0.230	$p < 0.001$
	N	0.330	$p < 0.001$