



Universidade de Aveiro
2021

**Cátia Alexandra Silva
Lima**

**Interações tróficas entre mesocarnívoros em
ambientes Mediterrânicos: a raposa e a fuinha como
caso de estudo**

**Mesocarnivore trophic relationships in
Mediterranean landscapes: the case study of red fox
and stone marten**



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

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presidente

Prof. Doutora Maria Helena Abreu Silva
Professora Auxiliar, Departamento de Biologia, Universidade de Aveiro

arguente

Doutor Joaquim Pedro dos Santos das Mercês Ferreira
Colaborador, CESAM, Universidade de Aveiro

orientador

Doutor Luís Miguel do Carmo Rosalino
Professor Auxiliar, Faculdade de Ciências, Universidade de Lisboa

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palavras-chave

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resumo

A coexistência das espécies pode ser mediada por diversos mecanismos de partilha e segregação de recursos. Esta segregação pode ocorrer ao longo das várias dimensões do nicho, nomeadamente a dimensão espacial, temporal e trófica. Variações ao longo do nicho trófico ocorrem de acordo com a disponibilidade espacial e temporal dos recursos, com a estrutura da guilda de competidores, entre outros fatores. Muitos dos ecossistemas nativos mediterrânicos têm vindo a ser convertidos em ambientes antropogénicos como plantações de eucalipto na Península Ibérica. Considera-se que estas plantações apresentam uma baixa disponibilidade e diversidade de recursos, limitando consequentemente a dieta das espécies. No entanto, várias espécies coexistem nestes *habitats* através de alterações dos seus hábitos alimentares ou outros mecanismos de coexistência. A raposa e a fuinha são dos mesopredadores generalistas mais dispersos e comuns em Portugal, conhecidos por coocorrer nestes *habitats*. Na ausência de grandes predadores, estes carnívoros ocupam os níveis tróficos superiores, sendo responsáveis por regulações do tipo “top-down” no ecossistema. Desta forma, este estudo pretende: (i) avaliar o impacto das plantações de eucalipto nos comportamentos tróficos destes dois mesocarnívoros; (ii) analisar a importância de cada presa e variações sazonais nas suas dietas; (iii) e detetar interações interespecíficas na partilha do nicho trófico. Dejetos identificados morfológicamente como pertencentes a estas espécies foram recolhidos para análise da sua dieta. No entanto, a identificação de dejetos baseada apenas em aspetos morfológicos é, comprovadamente, propensa a erros que podem enviesar os resultados e análises futuras. Neste estudo, e de forma a prevenir este erro, os dejetos foram analisados molecularmente, uma prática que se tem tornado comum como método não invasivo e altamente fiável para a identificação de dejetos. De fevereiro de 2019 a setembro de 2020, oito áreas do centro de Portugal (seis em eucaliptais e duas em áreas nativas, como áreas de controlo) foram amostradas, resultando em 215 dejetos recolhidos. O sucesso da identificação molecular da espécie (taxa de sucesso de identificação da espécie em relação às amostras analisadas) foi baixo (33%), mas o sucesso da identificação morfológica (confirmação molecular da identificação morfológica) foi elevado (92% em *V. vulpes* e 71% em *M. foina*) em comparação com estudos anteriores. A dieta de ambos predadores provou-se ser maioritariamente composta por frutos e artrópodes (maioritariamente coleópteros). No entanto, foi detetado um padrão distinto no consumo de frutos: as ameixas foram principalmente consumidas por raposas, enquanto as amoras foram abundantemente consumidas pela fuinha. A sobreposição dos nichos variou de acordo com a estação e *habitat* e a amplitude do nicho foi altamente dinâmica, destacando o comportamento oportunista de ambas as espécies. A sobreposição dos nichos foi menor em situações em que os recursos eram menos abundantes (eucaliptais e estação seca) provavelmente relacionado com mecanismos de repartição de recursos. Por outro lado, a sobreposição dos nichos foi maior em ambientes nativos e na estação chuvosa, destacando um potencial mecanismo de partilha de recursos, permitido pelo aumento na disponibilidade de recursos. No geral, os resultados realçam o comportamento oportunista e generalista de ambas as espécies, assim como os seus mecanismos de partilha e divisão de recursos que promovem a coexistência de acordo com as diferentes condições do meio.

keywords

diet, coexistence, eucalyptus plantations, Mediterranean ecosystems, mesopredators, non-invasive sampling, trophic niche

abstract

Species coexistence can be mediated by several mechanisms of resource sharing and partitioning. Segregation may occur along several niche dimensions, namely spatial, temporal and trophic. Trophic niche is known to vary according to local and temporal resource availability, and competitor's guild structure, among other factors. In Mediterranean ecosystems, many native environments have been converted into anthropic systems, such as eucalyptus plantation in Iberia. These plantations are considered to have a low resource availability and diversity, thus constraining species' diet. Nonetheless, several species can coexist in these environments through changes in their feeding behaviour or other coexistence mechanisms. Red fox and stone marten are among the most widespread and common generalist mesopredators in Portugal that are known to co-occur within this habitat. In the absence of large predators, these carnivores occupy the highest trophic levels, being responsible for top-down regulation of the ecosystem. Therefore, this study aims to: (i) evaluate the impact of eucalyptus plantations on the trophic behaviour of these two mesocarnivores; (ii) assess the importance of each prey taxa and seasonal changes in their diet and (iii) uncover interspecific interactions in trophic niche partitioning. In the field, scats morphologically assigned to these species were collected for diet assessment. However, scat identification based only on morphological identification has proved to be prone to errors that may bias the results and further analyses. To prevent this error, molecular scatology was applied in this research and has become a common practice as a non-invasive and highly reliable method for scat identification. From February 2019 to September 2020 eight areas in central Portugal (six eucalyptus plantations and two native forests, that acted as control sites) were sampled, and 215 scats were collected. The success of molecular confirmation of species identification (rate of successful identification of species, relatively to analysed samples) was low (33%) but the success of morphological identification (molecular confirmation of morphological identification) was high (92% for *V. vulpes* and 71% for *M. foina*) when compared with previous studies. The diet of both mesopredators were mainly composed by fruits and adult arthropods (mainly coleopterans). Nevertheless, a distinctive pattern of fruit consumption was detected: plums were mainly eaten by red fox, whereas blackberries were largely consumed by stone marten. Trophic niche breadth and overlap varied between seasons and habitats, and species niche breadth proved to be dynamic, highlighting the opportunistic behaviour of both species. Trophic niche overlap was lower in situation of low resource abundance (eucalyptus plantations and dry season) probably linked to a resource partitioning mechanism. On the other hand, species niche overlap was higher in native habitats and in the rainy season, highlighting a potential resource sharing mechanism, allowed by an increase in resource availability. Overall, the results outline the generalist and opportunistic behaviour of both species, as well as their mechanisms of resource sharing and partitioning to promote coexistence according to different conditions.

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1 Introduction

1.1 *Species coexistence*

The ecological niche of a population describes its distribution along a set of dimensions characterized by a gradient of environmental conditions (biotic and abiotic) and resources needed for the organism's survival (Elton, 1927; Schoener, 1974). In fact, there are an undefined number of dimensions (or niche axes) that can limit the distribution of populations and it is impossible to identify all the relevant ones (Gordon, 2000; Schoener, 1974). Schoener (1974) states that, ideally, these dimensions should be few and independent, but in reality, environmental variables are often closely intertwined (Elton, 1927). The solution proposed by Schoener (1974) was to group closely related dimensions in broad categories of food, space, and time, thus reducing the effect of dependence. However, organisms may not locally explore the full range of their fundamental ecological niche due to interspecific interactions or other locally implemented pressures (i.e. realized niche), leading to niche partitioning (Elton, 1927; Monterroso, 2013; Schoener, 1974). Furthermore, complete competitors cannot coexist and they must segregate along one or more dimensions of their ecological niche to minimize competition (Elton, 1927; Hardin, 1960; MacArthur & Levins, 1967). Segregation appears to be generally multidimensional and in the universe of the three niche dimensions previously stated, two is the most common number of dimensions in which species differ (Schoener, 1974). This means that even functionally similar species must segregate along one of the niches (at least) in order to coexist. Segregation along the spatial (e.g. habitat) dimension is often identified as the most important mechanism allowing co-existence, followed by food-type and finally, the temporal dimension (Schoener, 1974). However, this general pattern has several nuances and Schoener (1974) highlighted that for terrestrial mammals, spatial segregation linked to habitat use patterns is more pronounced than in other groups, and that among predators, segregation occurs along the time dimension. However, several mammalian carnivores still show a clear spatial segregation.

1.2 *Spatial dimension in coexistence*

Mesocarnivore communities vary in composition along the range of the multiple species distribution and thus spatial relations among species are also not constant

(Monterroso, 2013; Monterroso et al., 2016). For example, seasonal variations in space occupancy between co-occurring species were detected in Liguria (Italy), namely among red fox (*Vulpes vulpes*) and European badger (*Meles meles*) (Torretta et al., 2016) and in Bulgaria, between red fox and two martens (*Martes martes* and *M. foina*) (Petrov et al., 2016). This pattern was mainly driven by the abundance of shared food resources - in seasons where this resource is scarce, competitive stress increases, and individuals tend to segregate along the space axis to avoid encounters (Petrov et al., 2016; Torretta et al., 2016). Moreover, still in Bulgaria, when comparing the two martens' realized niches across two sampled sites, the authors found a discrepancy, revealing how inconstant and context-dependent the spatial co-occurrence of two species are (Petrov et al., 2016).

Studies between closely related species that are ecologically similar were not able to find a global and general pattern as well. While in Zimbabwe, a constant spatial segregation among closely related jackals (*Canis mesomelas* and *C. adustus*) was detected, pointing out the importance of avoidance to prevent aggressive interspecific interactions and the mechanism of shared preference organization (Loveridge & Macdonald, 2003); in Portugal, two martens live in sympatry (*M. martes* and *M. foina*), co-occurring at the expenses of adjustments in other niche dimension (Monterroso et al., 2016). Scale effect is also important. For example, a study on mesocarnivore coexistence in Iberian Peninsula did not reveal any evidence of competitive exclusion between any pair of the studied species, namely between red fox and stone marten (*M. foina*; Monterroso, 2013), however, a finer analysis on the relations between the same species also in Portugal revealed spatial segregation at the individual level as the mechanism behind coexistence (Pereira et al., 2012). It seems that spatial segregation remains as one of the most important mechanisms mediating coexistence, probably more frequent in asymmetrical interactions (Monterroso, 2013). Additionally, a change in spatial occupancy can result in divergent diets (Torretta et al., 2016).

Besides the horizontal spatial segregation, sympatric species can also organize themselves over the vertical space dimension. Species morphologically adapted to arboreal niches, like the stone marten and the common genet (*Genetta genetta*), are more prone to use resources available above the ground, while terrestrial ones, like red fox and Egyptian mongoose (*Herpestes ichneumon*), can more easily take advantage of terrestrial resources such as reptiles and dwelling coleopterans, although may also consume fallen fruits (Santos

et al., 2007). This is a general pattern, common throughout Mediterranean environments. In Central Greece and Central Italy, apples and pears fall on the ground during summer and become available for foxes that use them as food resources, while sorb-apples and wild plums are still only available on trees, and consequently, only martens have access to them (Papakosta et al., 2010; Serafini & Lovari, 1993).

1.3 Temporal dimension in coexistence

The segregation along the temporal dimension can be of great importance when encounters can lead to lethal consequences (Harrington et al., 2009; Schoener, 1974; Torretta et al., 2017). In fact, predators will more likely be active when their prey show peaks of activity, thus partitioning resources by temporal segregation (Schoener, 1974). Several studies report the importance of sequential use of the diel cycle describing three separate classes according to the evidenced behaviour: diurnal, facultative nocturnal and strictly nocturnal (Curveira-Santos et al., 2017; Monterroso et al., 2014). Curiously, the three most dominant co-occurring mesopredators fall in distinctive categories – Egyptian mongoose is diurnal, red fox is facultative nocturnal and badger is strictly nocturnal (Curveira-Santos et al., 2017; Monterroso et al., 2014). These differences in activity patterns are probably associated with the reduction of direct interference within carnivore assemblages (Curveira-Santos et al., 2017; Torretta et al., 2016). Harrington *et al.* (2009) tracked the actual changes in the ecological niche of a species, exhibiting the actual mechanism of segregation along the different axis to promote coexistence. In order to avoid agonistic encounters after the reintroduction of native otters and polecats in southern England, the mink, an invader species inhabiting this area, shifted its activity pattern and became predominantly diurnal (Harrington et al., 2009).

Similarly to the space, temporal dimension can also be analysed at several scales, from daily, to seasonal and even yearly, but the most studied temporal scale is the diel activity pattern (Halle & Stenseth, 2000). Studies on seasonal differentiation normally dwell on food resources availability and abundance. In Spain, fig consumption by genets and stone martens uncovered a seasonal resource partitioning mechanism – both species consume this resource at large proportions but alternately, while figs are an important part of the diet of stone martens in the autumn, in the summer their interest on this fruit decreases and genets

become its main consumer (Barrientos & Virgós, 2006). Apart from the stated examples, temporal segregation is considered relatively rare (Schoener, 1974).

1.4 Trophic dimension in coexistence

Seasonal variation in the diet of Mediterranean species is very common and almost inevitable due to the natural variance in the availability of food resources (Santos et al., 2007). Mesocarnivores often show a marked seasonal niche breadth difference. In Spain, the genet and the stone marten have broader niches in the summer and narrower in the winter (Barrientos & Virgós, 2006). In Italy, the seasonal variation exhibited by badger populations was not statistically relevant, whereas the red fox had a larger trophic niche than badger in the spring, decreasing in the autumn (Ciampalini & Lovari, 1985). However, an overall pattern cannot be established, as often is linked to the landscape context and its ability to provide food resources. Also in Italy, the red fox was described as having a broader trophic niche in the summer and in the autumn, comparing to other seasons and contrasting with the previous study (Patalano & Lovari, 1993). A contrasting pattern was found in Greece, where the red fox and stone marten exhibited a narrow niche in the summer (Papakosta et al., 2010). These differences in trophic niche breath can be related with the co-occurring species, habitat type, availability of particular food resource or even the mechanisms behind coexistence. In Bulgaria, a study about niche partitioning among red fox and two martens in two mountains where these species coexist revealed seasonal differences in food diversity and food consumption, but also differences between locations (Petrov et al., 2016).

In fact, understanding the plasticity of a species' feeding behaviour through the changes in their trophic niche can help understand coexistence by trophic niche segregation. As an example, in Italy, during the autumn – when the niche breadth is the lowest – an increase in the trophic niche overlap between the red fox and the badger was reported, allowed by an increase in shared food resource availability that promoted co-occurrence (Ciampalini & Lovari, 1985). Coexistence is also promoted when the co-occurring species have trophic segregation at a finer scale. For example, also in Italy, the red fox and the wolf preyed both on the same species, but the wolf targeted larger individuals and the red fox preyed upon the smallest and in a small quantity, complementing its diet with other food resources not shared with wolf (Bassi et al., 2012). Stone marten and red fox are known to

have an high degree of diet overlap in Portugal, Italy and Greece, but in order to coexist, these species found a way to segregate along this axis (Papakosta et al., 2010; Santos et al., 2007; Serafini & Lovari, 1993). In northern Italy, before the introduction of an exotic and competitive species, stone martens' diet was based mainly on fruits, birds, lagomorphs and small rodents (Balestrieri et al., 2013). In Central Spain, stone marten also exhibits a dependence on lagomorphs, especially in spring (Barrientos & Virgós, 2006). In comparison, in Portugal, where stone martens share the niche with three generalist species, including red fox, its diet is more frugivorous, avoiding lagomorphs and rats but frequently consuming small rodents (Santos et al., 2007). Moreover, in Central Italy, where stone marten and red fox live in sympatry, the latter rely their diet on rodents and ungulates during the winter and spring, leaving aside fruits while stone marten increases the consumption of fruits and beetles in these seasons to reduce the predation on mice, consumed during the summer and the autumn (Serafini & Lovari, 1993).

In general, it is expected that a niche overlap between sympatric species would be greater when resources are abundant and smaller when resources are scarce, although this does not mean that competition is stronger in any of the time-frames (Wiens, 1993). Furthermore, the relative dominance position between similar sized competitors is not constant. For example, stone marten is dominant over the pine marten in Iberia, but not in other areas where the same species co-occurred (Monterroso, 2013). Also, sometimes it is possible that species of the same guild frequently predate on each other: in Sweden, due to a change in population densities of red foxes and pine martens, a case of intra-guild predation was reported, where foxes actively pursue and kill martens (Lindstrom et al., 1995).

Study species coexistence is of great complexity and may probably be sustained only through ecological divergence, as a consequence of individuals acting based on several simultaneous trade-off that shape the ecological niche in order to survive and maximize its reproductive success (Futuyma, 1973; Hardin, 1960; Schoener, 1974). Thus, the coexistence of the same species in different communities may be promoted by different mechanisms (Kotler & Brown, 1988).

1.5 Ecologically poor environments and coexistence

When studying coexistence patterns it is also useful to analyse some factors that interfere with local species diversity (and consequently, resources), since these large scale patterns and processes help contextualize coexistence (Gordon, 2000). In fact, the mechanisms previously described are a link between an individual behaviour and community structure (Kotler & Brown, 1988). Individuals need food and refuge to survive or, in other words, each individual requires a certain amount of resources to thrive (Gordon, 2000). A more complex habitat can provide an amplitude of new niches, such as a complex vegetation structure, high diversity and multidimensional structure that often correlates with an abundance of food resources and hiding places (Cruz et al., 2015; Pereira et al., 2012). On the other hand, ecologically poor environment are commonly associated with monocultures, with open, simple and homogeneous structures (Cruz et al., 2015). This type of habitat is also associated with an increase in foraging time and, consequently, great predation risk, but also lower abundance of species (Cruz et al., 2015; Heithaus, 2001). In addition, several studies already proved the importance of heterogeneity in landscapes in order to promote diversity and coexistence (Cruz et al., 2015; Palmer, 2003; Pereira et al., 2012; Ramírez & Simonetti, 2011).

Therefore, productivity and habitat complexity, commonly referred to as local factors, are intimately connected with coexistence, for a more complex habitat can create new niche axes that can harbour more species and decrease the probability of interspecific encounters and competition, promoting coexistence (Abramsky, 1988; Gordon, 2000; Kotler & Brown, 1988; Palmer, 2003). When the amount of available resources is scarce, resource partitioning becomes even more important to maintain species diversity (Gordon, 2000). Reinforcing the above mentioned, a higher overlap of species niche normally occurs in the most productive season due to the abundance of available resources, and higher segregation is observed in season with low abundance of resources (especially food), highlighting the importance of assessing resource partitioning mechanisms in poor environments, to understanding co-existence in such challenging environments.

1.6 Eucalyptus plantations and biodiversity change

Eucalyptus stands are anthropogenic environments, usually characterized by open, simple and homogeneous habitats, with 10 years-cycle of harvesting (in Europe) and intense management regimes that can significantly change the landscape in short time frames, thus considered as low-quality habitats (Alves et al., 2012; Mangas et al., 2008). Production forests already account for 1.15 billion ha, about 30% of all forest globally (FAO, 2020). In Portugal, by 2015, Eucalyptus plantation represented about 26% of the continental forest, with a tendency to increase (ICNF, 2016). By 2018 a report on the use of soil accounted for 928 thousand ha of Eucalyptus monoculture, which represented an increase of 83 thousand hectares in three years (DGT, 2020). The observed increase of Eucalyptus plantations' cover over the years was achieved at the expense of the transformation of native habitats in economically rentable production forestry (Campinhos, 1999; DGT, 2020). In fact, Eucalyptus production is profitable due to its high yield, rapid growth rate, ability to adapt to different conditions, thriving even in poor soils, and high demand of their products, in particular to supply wood, pulp and paper industry (Campinhos, 1999). Despite being a highly productive system, with fast return of investment, Eucalyptus plantation have generated an intense debate over the years and the main cons focus on the reduction of water availability and the lack of biodiversity, reason why these plantations are called “green deserts” (Campinhos, 1999). Additionally, some Eucalyptus species already started to show a clear invasive behaviour that, in the absence of appropriate management and control, can lead to considerable and long lasting negative ecological effects (Badalamenti et al., 2018).

All considered, their impact on biodiversity remains a hot topic among scientists. In fact, when compared to native environments, they present lower abundance and diversity of basic food resources of Mediterranean trophic webs, like arthropods, fleshy fruits and rodents, probably as a consequence of low understory cover (Calviño-Cancela et al., 2012; Rosalino & Santos-Reis, 2009; Teixeira et al., 2017; Zahn et al., 2010). Furthermore, management practices shape the trees, by trimming all the secondary branches, simplifying the vertical habitat structure, which can impose challenges for arboreal species (Piña et al., 2019; Teixeira et al., 2017). Also, one of the managing practices includes fertilization that has a major negative impact on animal diversity (Vanbeveren & Ceulemans, 2019). Understory clearcutting is also often used as a management measure in plantations (although the scale varies with the management regimes), and can be a limiting driver of wildlife

abundance (Carrilho et al., 2017). Studies targeting *Eucalyptus* plantations biodiversity showed that this habitat can host significant richness and abundance of wildlife in mature stands where understory was not removed (e.g. Carrilho et al 2017). Furthermore, the natural evolution of plantations from open shrubland-like stands (i.e. young plantation stages) to shade tolerant species typical of native forests favours biodiversity (Calviño-Cancela et al., 2012). Nevertheless, diversity in *Eucalyptus* plantations, is always lower than in native forests and rare species are seldom present (Calviño-Cancela et al., 2012; Carrilho et al., 2017). Also, Piña et al. (2019) argued that *Eucalyptus* plantations biodiversity may depend on the surrounding characteristics and that stands may only act as corridors or complementary habitats. Overall, the quality of *Eucalyptus* plantations for wildlife depends strongly on the management regimes implemented and the heterogeneity of the surrounding environment. Nevertheless, these are generally poor habitats. Furthermore, interspecific competition due to resource shortage (in the less productive season for example), tends to be more intense in anthropogenic and ecologically poor habitats, such as plantations (Teixeira et al., 2020).

Several studies have reported that habitat fragmentation due to human activities have a significant impact on wildlife, from genetic diversity to species richness (Jankielsohn et al., 2001; Lino et al., 2019; Virgós & García, 2002). Although many mammals appear to be adapting and overcoming habitat alterations due to their ability to explore novel available resources (Hipólito et al., 2016; Rosalino et al., 2005), only generalist species appear to thrive in *Eucalyptus* plantations, due to resource instability and human interference, decreasing the overall species richness (Mangas et al., 2008; Teixeira et al., 2020). Furthermore, most of carnivores that are able to use plantations are not threatened species (Ferreira et al., 2018). This is especially concerning when it has been detected an increase in the number of threatened species worldwide, with terrestrial systems being the most affected by habitat change and loss (IUCN, 2021).

1.7 *Mesocarnivores as models*

It is long known that carnivores are important components of the ecosystem, with some being considered as umbrella or indicator species, although all are crucial to maintain a functional ecosystem (Noss et al., 1996; Roemer et al., 2009). Among them, mesocarnivores are much more speciose, usually more abundant and widely dispersed than

the most charismatic and flagship species of large carnivores such as the wolf, *Canus lupus*, or the Iberian lynx, *Lynx pardinus* (Roemer et al., 2009). Their range of behaviour and ecology is also very broad: from habitat specialists to generalists; solitary to highly social species; or even from frugivorous to hypercarnivorous species (Roemer et al., 2009). In the absence of apex predator, these species can become the regulating force of community structure through prey control, seed dispersal or even waste removal and possibly facilitate nutrient flows between adjacent ecosystems (Ćirović et al., 2016; Roemer et al., 2009; Rosalino & Santos-Reis, 2009). Although their ecological role is the result of complex interactions between biotic and abiotic components of the environment, by being less vulnerable to extinction than larger carnivores, they can assume the role as apex predators in communities more often (Roemer et al., 2009).

Despite their proved importance for the ecosystem, there are several documented conflicts with human activities. Anthropogenic habitat transformation are a direct threat to mesocarnivores, but mesocarnivores can also take advantage of some human associated resources (e.g. food or shelter), leading sometimes to economic damage and retaliation by the owners (Sillero et al., 2007). For instance, European badger diet in an agroforestry area in Portugal showed a high consumption of wheat, a resource used by humans to feed game species (Hipólito et al., 2016). Moreover, mesocarnivore population control is a common game management practice in Portugal, as these predators are seen as important game species consumers that may be the cause for a decrease in small game species abundance. Thus, the Portuguese law allows the active hunt of red foxes and Egyptian mongooses, without empirical and scientific support of data that clearly show the mentioned relations (Rosalino et al., 2009; Santos et al., 2007).

Their important functional role, wide distribution and relation with human activities, make mesocarnivores an excellent model to assess the impacts of landscape changes on the community interactions (Teixeira et al., 2020), reinforced by the complex intra-guild coexistence mechanisms that depend on the habitat characteristics and resource availability (Cruz et al., 2015; Monterroso, 2013).

Two of the most widespread and common Portuguese mesocarnivores that were already detected co-occurring within Eucalyptus plantations are the red fox and the stone marten (Bencatel et al., 2018; Cruz et al., 2015). These species are generalist feeders that feed

upon a wide range of food items according to their availability (Bakaloudis et al., 2012; Díaz-Ruiz et al., 2013). In Iberia, red fox has a preference for lagomorphs increasing the consumption of small mammals and fruits where and when they are scarce (Díaz-Ruiz et al., 2013). Stone marten as a tendency for fruit consumption, but also exploiting mainly insects (adults and larvae) in seasons with less fruit availability (Bakaloudis et al., 2012).

1.8 Methods used in mesocarnivore studies

Direct methods can be quite challenging when studying mesocarnivores because they usually live in low densities, with wide home ranges, and are nocturnal, cryptic and highly sensitive to disturbances (Gros et al., 1996; Wilson & Delahay, 2001). Invasive techniques are typically used in mesocarnivore ecological studies (e.g., trapping, radio-tracking; Sadlier et al., 2004), but several concerns in the application of such methodological approaches have been highlighted: impracticality to apply in larger spatial scales, ethical questions, local norms and regulations, costs and logistics, risk of harming the animal, among others (Gompper et al., 2006). For these reasons, non-invasive and indirect methods are widely used and recommended (Barea-Azcón et al., 2007; Hoffmann et al., 2010). No single technique is ideal for all carnivore species neither a method for every question, thus the method of choice should take in consideration the behavioural ecology of the target species, size of the sampling area, the aim of the study and the available resources (Gompper et al., 2006; Majdi et al., 2018; Wilson & Delahay, 2001). Surveying field signs are the most classic and efficient method for carnivore monitoring, since tracks and scats can be easily recorded and identified due to their characteristic appearance (Barea-Azcón et al., 2007; Hoffmann et al., 2010; Wilson & Delahay, 2001; but see Alexandre et al., 2020). Furthermore, scats are used by carnivores as territorial marks across their home range and may be regularly deposited at predictable locations (Wilson & Delahay, 2001).

Scats can truly be an invaluable source of information (Putman, 1984). Besides being used for presence-absence studies, as the remaining majority of the non-invasive methods, they can provide a wide range of information (Gompper et al., 2006; Putman, 1984). Scats are instruments of intra and interspecific communication from which species can be identified through the analysis of their morphology (Putman, 1984). Home range limits and patterns of habitat usage can also be inferred through the observation of scat distribution over the territory (Putman, 1984). Furthermore, the collection of a faecal sample allows for

dietary composition studies with approaches more or less complex - from simple species identification to more complete assessment of the proportion or relative contribution of each food item (Putman, 1984).

Nowadays, scat surveys are usually paired with DNA analyses (Gompper et al., 2006). In fact, scats can only be useful upon correct species identification. Evaluation of scat morphology alone is prone to misidentifications among sympatric species, and the season, the studied area and the relative abundance of the species are known factors that influence error rates (Monterroso et al., 2013; Wilson & Delahay, 2001). Thus, when a species is at low densities in the study site, not only it is more difficult to find scats, as the judgement of the surveyor is worse (Davison et al., 2002). Also, scats of closely related species, like stone and pine marten, that often live in sympatry, cannot be morphologically distinguished, so they are frequently handled as a group (Petrov et al., 2016). In addition, higher similarity between scats of different generalist species should be expected during seasons of low resource availability due to high dietary niche overlap (Monterroso et al., 2013).

From the necessity to accurately identify the scats' "owner", molecular scatology emerged and become a common practice as a complementary method (Davison et al., 2002; Kohn & Wayne, 1997; Monterroso et al., 2013). This analysis is based on the principle that faeces contain cells shed from the intestinal lining and their DNA can be isolated and amplified (Kohn & Wayne, 1997). This is a rapid and widely applicable method that produces a definitive and reliable result that can even be used in relatively old and rain-washed samples (Davison et al., 2002; Foran et al., 1997; Kohn & Wayne, 1997). Besides allowing a more accurate estimation of population densities, individual core area, home range, and effective population size or the study of diet and diseases, a more detailed genetic information can enhance our knowledge of communities structure and dynamics, through studies targeting paternity relationships, genetic variation and phylogeography (Kohn & Wayne, 1997). Therefore, combining the conventional data from scats with this DNA technique provides a more comprehensive and accurate picture on carnivore ecology.

1.9 Objectives

Considering all the ecological constraints associated with Eucalyptus plantations, and the wide debate regarding this exotic system effect on wildlife, this study aims to evaluate the impact of anthropogenic landscapes on the trophic behaviour of two mesocarnivores, red fox (*Vulpes vulpes*) and stone marten (*Martes foina*). Therefore, through the characterization of their diet composition in two different landscapes (native forests and Eucalyptus plantations) the goal is to assess the importance of each prey taxa for both carnivores in both ecosystems, describe seasonal changes related to variations in resource availability, and understand the interspecific relation between predators. Based on all the bibliographical information gathered it is expected that trophic niche overlap would be: 1) overall low due to the different feeding strategies evidenced by the model species (stone marten: more arboreal; red fox: ground feeding) (Santos et al., 2007); 2) lower in seasons with higher availability of food resources (i.e. in the dry season; Barrientos & Virgós, 2006); and 3) higher in Eucalyptus plantations due to low understory complexity of these habitats, that will induce lower food resources availability (Cruz et al., 2015).

Understanding species coexistence at the ecological scale is a challenging quest, but this research aims to unveil some of the underlying mechanisms. Through the identification of the main key resources for both species, advises can be made regarding management regimes in plantations in order to increase the abundance of the identified resources and thus, promote a wildlife-friendly environment and species coexistence.

2 Materials and methods

2.1 Study area

This study was carried out in eight areas throughout Central Portugal, split into two regions: the eastern study areas located in Castelo Branco region (Fundão, Penha Garcia, Penamacor and Malcata sampling sites) and the western areas situated in Coimbra (Lousã, Góis and Pampilhosa da Serra sampling sites) and Viseu (Mortágua sampling site) (Fig. 1) regions. In both regions, three study areas were located within *Eucalyptus* plantations and one acted as a control group, being predominantly dominated by native species: Lousã and Malcata, in the western and eastern regions, respectively.

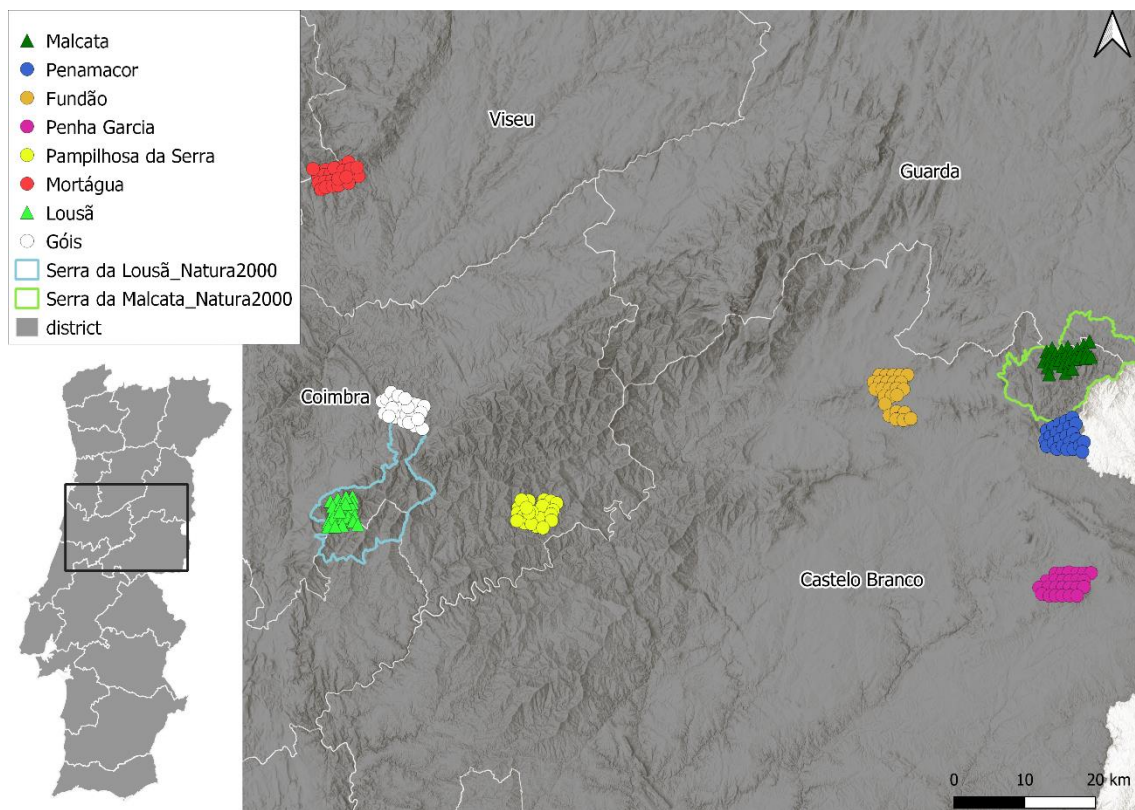


Fig. 1 | Study areas distribution by district. Circles indicate the locations in plantation forests and triangles indicate the sampled sites in native forests. Contours of the Natura 2000 sites are highlighted. Altitudinal variation is depicted through shading, with darker tones highlighting the slopes and valleys.

The sample sites are located in the Mediterranean Biogeographical Region, therefore are characterized by a Mediterranean climate with cold rainy winters and hot dry summers (Cervellini et al., 2020; Condé et al., 2002). However, there are noteworthy differences between the eastern and western locations. Following the Köppen-Geiger climate classification, Portugal is classified as a type Cs climate (Temperate with dry summer), but can be subdivided in Csa (hot summer) and Csb (warm summer) as seen in the Fig. 2 (Beck et al., 2018; IPMA, 2020). Thus, the study's eastern locations are classified as Csb, having a higher annual mean temperature (about 17.5°C) and lower annual precipitation (400 to 1000 mm), whereas the western ones, in the Csa subregion, have lower annual mean temperature (10 to 15°C) and higher annual precipitation (1200 to 2600 mm). Furthermore, Costa et al. (1998) subdivide Portugal in several biogeographic regions and subregions based on plant communities (Aguiar et al., 2008). Pampilhosa da Serra, Góis, Serra da Lousã and Mortágua belong to “Gaditano-Onubo-Algarviense” region, the most complex of the Portuguese regions due to their bioclimatic, lithologic and plant diversity. It also has a complex orogeny, with low altitude areas and the highest point is at Serra da Lousã, at 1204 meters high. Due to the ocean proximity, it is a mild environment, without extreme winters (Aguiar et al., 2008; Costa et al., 1998). The “Luso-Estremadurensis” region comprises Fundão, Penamacor, Penha Garcia and Serra da Malcata. This region, far from the ocean,

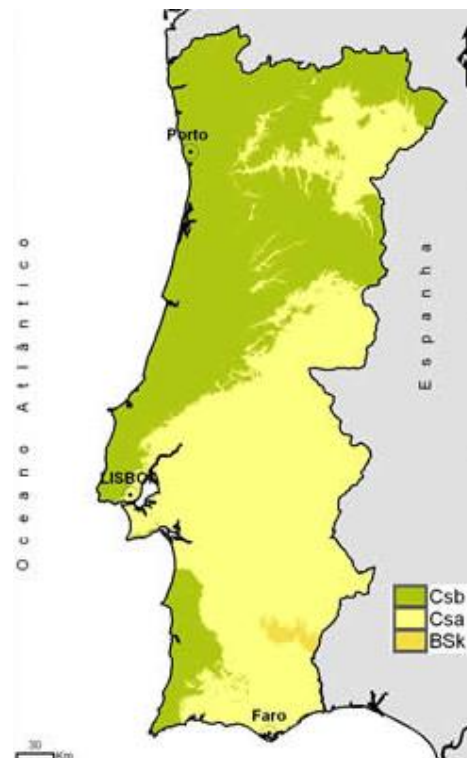


Fig. 2 | Köppen-Geiger climate classification for Portugal. From IPMA, 2020

has a pronounced Mediterranean bioclimatic influence and the woodlands are dominated by oaks and a broad diversity of small shrubs (e.g. *Cistus* sp., *Erica* sp., *Genista* sp.). In addition, the orogeny is simpler with some mountains just over 1000m (Aguiar et al., 2008; Costa et al., 1998).

In addition, eastern and western locations also differ in landscape structure and composition (Fig. 3) - the eastern locations are part of the “Beira Interior” region while the western are included in the “Beira Alta and Maciço Central” region (Abreu et al., 2004). “Beira Alta”, that includes Mortágua and Góis study areas, is characterized as having a wet transitional climate, with low temperature variance and morning fogs in the valleys. Agriculture is also a common practice in this area, resulting in a patchy landscape of farmlands and woodlands. In the transition between the arid ridges and the fertile valleys, the hillsides are covered by eucalyptus and pine trees. Looking more in detail, Góis belongs to “Montes Ocidentais da Beira Alta” landscape unit, without pronounced hills, making the clear transition between the rough relief of “Beira Alta” and the lowlands of “Beira Litoral” (Abreu et al., 2004). On the other hand, Mortágua is at the south end of “Serra do Caramulo” subregion, with steeper and higher slopes, standing out from the surroundings. These subregions are clearly marked by an extremely monotonous landscape due to the extension of pine and eucalyptus plantations. “Maciço Central” region, particularly “Serra da Lousã e Açor” subregion, comprise the Serra da Lousã and Pampilhosa da Serra sampling areas. Due to the rocky and steep nature, the temperature is never too hot, with cold or very cold winters and cool to moderate summers - mean annual temperature between 10°C and 12.5°C and precipitation always above 1200mm. In this unit, eucalyptus and pine tree plantations are also common, as well as extensive shrublands usually as result of fire regimes. In some of the mountains, like Serra da Lousã, it is possible to observe how the rocky nature of this subregion creates microclimates, allowing the mixture of typically Mediterranean ecosystems (*Arbutus* sp. and *Laurus* sp.) with others of Atlantic influence (oaks and chestnut tree; Abreu et al., 2004).

The eastern locations are grouped in the “Beira interior” region, with marked rural environment and a continental influence. Thus, these locations are characterized by a low level of precipitation, but occasional intense rainfall, and marked temperature variation, especially in the valleys, with hot summers but very low temperatures in the winter. In Serra da Malcata the precipitation reaches the highest value (above 1000mm). In this region, the

terrain exhibits smooth slopes, highlighting the residual relief of Penha Garcia, between 500 and 800m, and Serra da Malcata, between 800 and 1260m. “Cova da Beira” subregion, where Fundão sampling sites are located, is a levelled valley protected by the two surrounding mountains with a marked continental climate and high abundance of water. Due to its fertility, it is an area highly patched by agricultural farmlands and some forestry areas. Penamacor and Serra da Malcata belong to the “Penha Garcia e Serra da Malcata” subregion that, in contrast with Cova da Beira, is characterized by irregular terrain and is dominated by woodlands, in particular pine tree, oaks and shrubs especially at the northern end. To the south, the lowlands are also less rocky and shrubland dominates the landscape along with farmlands and eucalypt plantations. Finally, Penha Garcia sites fall within the “Castelo Branco-Penamacor-Idanha” subregion, that is characterized essentially by monotonous eucalyptus plantations and shrublands, with very few farmlands (Abreu et al., 2004).

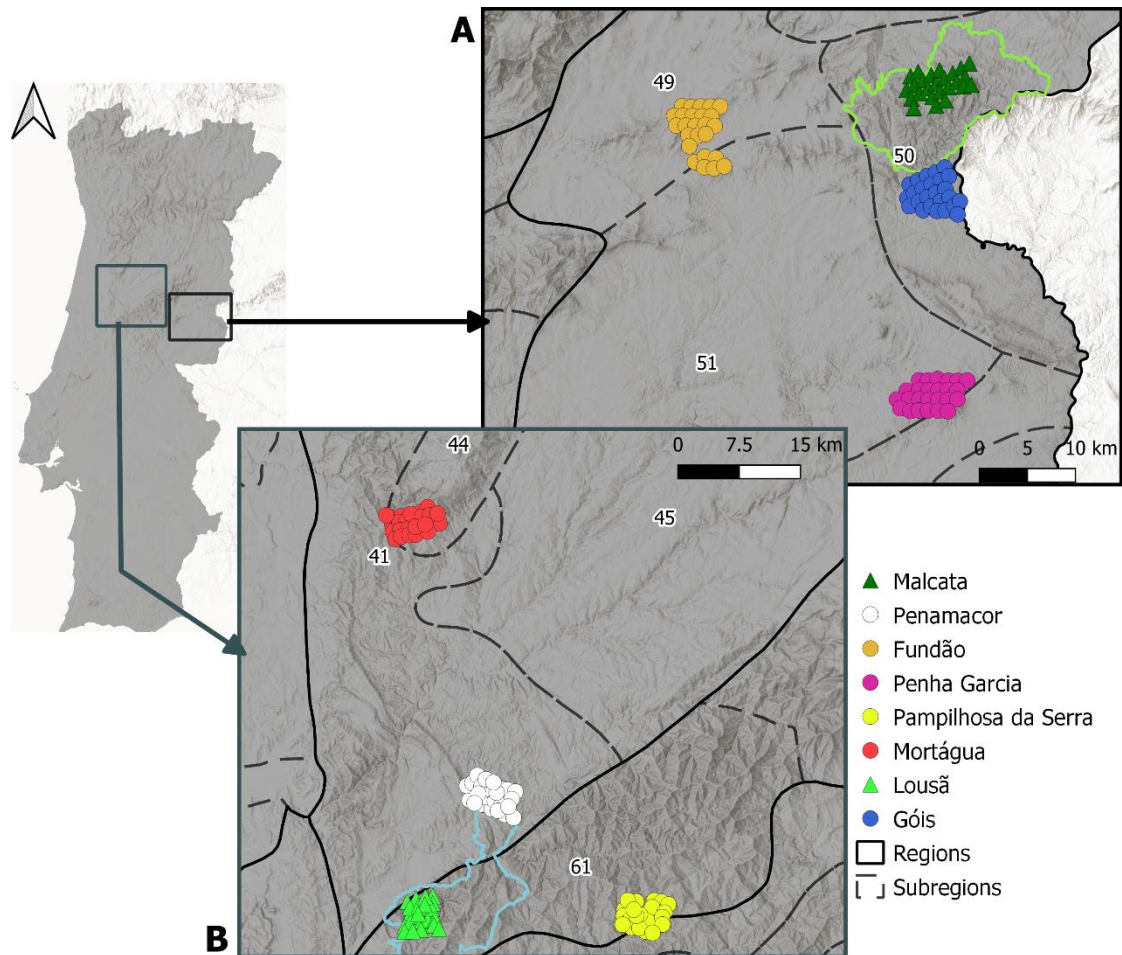


Fig. 3 Study areas locations relative to landscape units described by Abreu, Pinto-Correia and Oliveira (2004). **A**, eastern subareas; **B**, western subareas. Subregions: **41**, “Montes Ocidentais da Beira Alta”; **44**, “Serra do Caramulo”; **45**, “Dão e Médio Mondego”; **49**, “Cova da Beira”; **50**, “Penha Garcia e Serra da Malcata”; **51**, “Castelo Branco-Penamacor-Idanha”; **61**, “Serra da Lousã e do Açor”. Regions: **41**, **44** and **45** - “Beira Alta”; **49**, **50** and **51** - “Beira Interior”; **61** - “Maciço Central”. The relief is also depicted through shading.

From personal observations and field records, Serra da Lousã and Serra da Malcata sampled sites are characterized as semi-natural forest and, as above mentioned, they are considered control sites. These locations vegetation is composed by deciduous trees, like *Quercus* spp., *Castanea sativa* Mill, *Fraxinus angustifolia* Vahl, *Fagus sylvatica* L., *Salix alba* L., *Pinus* spp. and several shrubs and herbs such as *Pteridium aquilinum* (L.) Kuhn in Kersten, *Pterospartum tridentatum* (L.) Willk, *Cistus* spp., *Lavandula* spp., *Ulex* spp., *Erica* spp., *Rubus* spp., *Cytisus* spp. and *Daphne* spp. These species were not present in all sites and their abundance also varied among sites. Near water bodies, *F. angustifolia* and *S. alba* were the dominant species. However, overall dominant tree species were *Pinus* spp. and *Quercus* spp, although some areas were dominated by pastures and shrublands. In some of the locations, disperse exotic species such as *Acacia* sp. and *Pseudotsuga* sp. were also

present, but never dominant. Additionally, Serra da Lousã is a relevant touristic location, classified as a Site of Community Importance within the Natura 2000 network (PTCON0060), whereas Serra da Malcata is a Natural Park, also included in the Natura 2000 network (PTZPE0007) as a Special Protection Area (European Commission, 2021). The remaining sampling locations were predominantly dominated by exotic forests of *Eucaliptus* spp. (mainly *E. globulus*) in several stages of production (initial, intermediate and pre-harvested) and some shrublands and pastures, but with lower densities.

Red fox and stone marten co-occur within our sampling areas and where the chosen species for this study (Bencatel et al., 2018).

2.2 Sampling design

In each study site we defined a 16km² grid (1x1km cell). In each grid, 25 grid nodes, 1 km apart, were used as sampling points, totalizing 200 over the entire eight study areas. Along each point, a 300m transect was monitored to collect scats, covering a total extension of 60km for the entire study area. The sampling was performed each year from February 2019 until September 2020 in two survey periods, rainy and dry. The rainy period sampling was implemented between February and May and the dry sampling between June and September. Sampling was performed once each season, in each location. The transects were surveyed along dirt roads or trails near the defined sampling point and on foot by one or two observers in search of scats.

All scats collected were visually identified in the field with the help of a field guide (Navarro, 2012), based on their morphology, odour, location on the transect and dimension. Two samples of each scat were collected. A quarter of the scat was collected to a tube for genetic analysis and two quarters to another tube for diet analysis, leaving about a quarter in the field. For each scat, we recorded the day, time, location, site of collection, scat morphological characterization and identification (ID).

To prevent the contamination of samples sent for molecular analysis, sterile tubes and gloves were used in scat manipulation, and before each collection the material (tweezers and scapula) was sterilized with alcohol and fire (Foran et al., 1997). While in the field, these samples were stored with 96% ethanol (about 3 parts of ethanol to one part scat) in a

polystyrene box with ice pads to prevent the degradation of the DNA in the sample (Panasci et al., 2011). Then, in the lab, the samples were stored in the freezer at -20°C, until processing.

Older, weathered and whitened scats were not collected due to the low probability of recovering conserved genetic material worth of posterior genetic identification (Foran et al., 1997; Panasci et al., 2011). To increase the probability of collecting scats from different species, attempts were made to target morphologically different scats during field work (Navarro, 2012).

2.3 *Genetic analysis*

Scat species identification based solely on morphological aspects has been highly criticized so the accuracy of field identification was tested using molecular scatology. DNA was isolated from the collected scats and a fragment of mtDNA was amplified and sequenced to allow species identification of the scat donor. Whenever the samples were not in use, they were stored in the freezer at -20°C. The isolation was performed in a specific room for DNA extraction from non-invasive samples. This room is equipped with a UV light used to sterilize the space before and after each procedure to prevent contamination. This light was turned on each time for about 30 minutes. The space and equipment were also rubbed with a commercial disinfectant and with ethanol before using the UV light. For DNA isolation, we used the QIAamp Fast DNA Stool Mini Kit (QIAGEN®) protocol with some modifications (Appendix I). A negative control (with no genetic material added, only the reagents) was included in every extraction and throughout the isolation procedure to keep track of potential contaminations.

After the isolation procedure, the samples were quantified in a separate room using a NanoDrop UV/Vis spectrophotometer. For each sample, including for the extraction controls, the following parameters were registered: concentration (ng/μl), 260/280 and 260/230 absorbance ratios. This data allows to speculate about the amount of DNA, and relative amount of other biological molecules, such as proteins, on our DNA isolates (Green & Sambrook, 2018).

For the amplification, the reagent mix for the Polymerase Chain Reaction (PCR) was prepared in DNA UV-cleaner box, using sterilized materials and aerosol resistant pipette tips. The amplified region, a non-coding region of the mitochondrial DNA (D-loop region), contains the main regulatory elements for replication and expression of the mitochondrial genome and it is known to vary in length and sequence composition between species (Sbisà et al., 1997). These characteristics make this region a useful marker for species identification.

Two pairs of primers were used: L-Pro (Mucci et al., 1999) and MelCr6 (Marmi et al., 2006), more specific for mustelids; Thr-L 15926 and DL-H 16340, mainly used for canids (Vilà et al., 1999). The first pair was used mainly with samples identified in the field as belonging to stone marten (*Martes foina*) and the second pair with samples identified as belonging to red fox (*Vulpes vulpes*). When the amplification failed with one of the pairs of primers, the other pair was used. A negative control was always included, where ultrapure and sterile water was used instead of the DNA sample. Amplification was attempted at least twice with each primer, before discarding the sample from further analysis, if the amplification failed. The amplification mix was performed for a final volume of 25µL per sample, using 5µL of the DNA extraction. For the first pair of primers the mix also contained: 4mM of MgCl₂, 2mg/mL of BSA and 0.16mM of each primer solution; for the second pair: 4mM of MgCl₂, 1.6mg/mL of BSA and 0.12mM of each primer solution. Furthermore, the solution was saturated with dNTPs to ensure the activity of the Taq polymerase, also added to each solution. The thermocycling conditions for both pairs of primers were: an initial activation step at 95°C for 4min followed by a 45 cycles step of denaturation at 95°C for 1min, annealing at 48°C for 2min and extension at 72°C for 1.5min and a final extension step for 10min at 72°C. An electrophoresis was performed to confirm the success of the amplification. The matrix was a 2% agarose gel with a fluorescent DNA binding tag that allows the observation of the DNA bands under UV light. Only 5µL of amplified product was used and the negative controls of the amplification were included to check for possible contamination. Electrophoresis was run for approximately 35min at 100 volts.

Whenever the amplification was proven successful (visualization of a clear distinct band), the remaining amplified product (approximately 20µL) was purified using ExoSap-IT®. The purified samples were then sent to sequencing with the above-mentioned primers, separately. Software MEGA X (Kumar et al., 2018) was used to align the forward and

reverse sequences in order to search for a consensus sequence, manually accessing major discrepancies. Good-quality sequences were subsequently compared with the ones deposited in GenBank® using BLAST (NCBI, 2017). A match was acceptable if the “Percent Identity” value was above 95% and if the top 10 results were of the same species.

2.4 *Diet analysis*

In total, 57 scats were used for diet analysis, 34 of which were genetically identified. An attempt was made to balance the number of analysed scats by species and habitat. There was a need to add scats that were not genetically identified to increase sample size: 22 from stone marten and 1 from red fox. These extra scats were selected based on the high success of morphological identification, a result discussed in the “Discussion” section.

First, the scats were soaked in water and broken up with the help of tweezers to facilitate the separation of the components. Then, the samples were rinsed in water through a sieve of about 1mm mesh to retain preys’ indigested remains like feathers, seeds, hairs, bones, teeth and invertebrate exoskeletal elements (Kruuk & Parish, 1981; Reynolds & Aebischer, 1991). The retained remains were analysed and separated by food item category/type. The percentage of each food item type, per scat, was visually estimated. After dried at 50°C for at least 2 hours, the undigested remains were separately weighted. The identification of arthropods was performed to order level, and the remaining items were identified to species level, whenever possible.

For seeds, two approaches were taken according to their size and number detected in scats. For large-size species or when in a low amount, they were individually counted and weighted; if very small and in a high number, they were only weighted. After species identification, the minimum number of fruits eaten was estimated using the average number of seeds per fruit and, when needed, the average weight per seed (Balmori et al., 2013). The minimum number of arthropods eaten was estimated based on the number of equivalent number of legs, elytra or thorax colour and form, and other distinctive remains found (e.g., scorpion telson). Identification was performed by comparing the detected body parts structures with those described in several field guides (Barrientos, 2004; Di Palma & Massa, 1981; Lourenço et al., 2009; Thyssen, 2010). To identify mammalian hairs, a small sample

of each type of hair found in the scat was analysed. Hairs were observed under a microscope to register the structure of the medulla and cross section shapes, which allow mammal species identification. Mammalian prey were identified using reference guides for hairs identification (Day, 2009; Teerink, 1991). Other unique prey remains, like scales, bones, teeth and feathers were also identified using several identification keys (Brom, 1986; Carmona et al., 2010; Day, 2009; Delfino, 2004; Di Palma & Massa, 1981; Santero & Alvarez, 1985).

2.5 Data analysis

To estimate amplification success, the number of successfully amplified samples (see “Genetic analysis” section) was divided by the number of extracted DNA samples. Molecular identification success was calculated by dividing the number of successful identifications by the number of samples in which DNA was successfully amplified. Morphological identification accuracy was estimated by dividing the number of correct identification (“matches” between morphological and genetic identification) by the number of samples morphologically attributed to the species. A One-way analysis of variance (ANOVA) was performed to test the statistical significance of the differences found in the success rate of molecular identification between the seasons, habitats, species and scat age.

Diet was expressed as:

- Frequency of Occurrence (FO) = $\frac{\text{Number of scats with the food item } i}{\text{Total number of scats}} \times 100$
- Percent of Occurrence (PO) = $\frac{\text{Number of occurrences of the food item } i}{\text{Total number of occurrences of all the items}} \times 100$
- Percent of Biomass (PB) = $\frac{\text{Biomass of food item } i}{\text{Total biomass}} \times 100$

The biomass of each food item in both predator’s diet was calculated using correction factors (CF) estimated for red fox, based on the principle that martens eat in a similar manner to foxes (Lockie, 1961). Correction factors allow the estimation of the fresh weight of the consumed prey based on the dry weight of undigested remains of prey in scats (Rosalino et al., 2003). Table 1 resumes the CF values used and their source. FO and PB were calculated only for the main defined categories: arthropod (adults), arthropod (larva), arthropod (eggs),

rodents, insectivores, lagomorphs, birds, reptiles, *Rubus* sp., *Ficus* sp., *Morus* sp, Rosaceae and other non-identified fruits.

Table 1 | Correction factors (CF) used to estimate biomass ingested according to previous studies.

| Food item | CF | Reference |
|---------------------|------|--|
| Arthropods (adults) | 5 | (Lockie, 1961) |
| Arthropods (larva) | 12 | (Lockie, 1961) |
| Small mammal | 28 | (Rühe et al., 2008) |
| Lagomorphs | 31,8 | (Ferrerias & Fernandez-de-Simon, 2019) |
| Birds (small) | 45 | (Lockie, 1959) |
| Fruits | 48,1 | (Ferrerias & Fernandez-de-Simon, 2019) |
| Reptiles | 29,5 | (Jędrzejewska & Jędrzejewski, 1998) |

FO was only used for comparison with diet studies that only provided results expressed using this metric. Comparisons between season, habitat, location (eastern and westerns) and species, and statistical analysis were made with PO and PB. Sub-sample size homogeneity was tested using Chi-square tests (Zar, 2009). Trophic niche breadth was calculated using Levins index (B), ranging from 0 (specialists) to n (generalists, n = number of food item categories) and Shannon-Wiener index (H'), ranging from 0, in the case of specialists, to $\ln(\text{number of categories})$, for generalist species (Krebs, 1999; Levins, 1968). Levins index was subsequently standardized on a scale from 0 (narrow niche amplitude) and 1 (broadest niche amplitude) by the following equation: $BA = (B - 1)/(n - 1)$ (Levins, 1968). Shannon-Wiener index was also standardized through the calculation of Evenness (J') by the formula: $J' = H'/H'_{\max}$ (Krebs, 1999). Niche overlap was calculated using Morisita index (C), a measure least biased by sampling conditions (e.g. evenness of resource distribution and sample size), ranging from 0 (no overlap) to 1 (full overlap) (Morisita, 1959; E. P. Smith & Zaret, 1982). A modified version of t-test was performed to test for statistical significance in differences between H' values (Zar, 2009).

All statistical analyses were performed on R (R Core Team, 2019), except when mentioned otherwise, with the help of “*ecolTest*” and “*spa*” packages (Salinas & Ramirez-Delgado, 2021; Zhang & Ma, 2014). A difference was considered to be statistically significance when $\alpha < 0.05$.

3 Results

Along 200 transects and a two-year sampling period, 215 scats were collected. Most of the scats (n=165) were collected in Eucalyptus plantations, whereas only 50 were collected in the semi-natural native forest areas. Despite this difference, the average number of collected scats is similar between the two sets of areas when considering the number of areas sampled in each habitat (27.5/area in exotic forests and 25/area in native forests). More scats *per* sampling site were collected for red fox (20/area) than for stone marten (5.5/area). In the dry season were also collected more scats (20/area) than in rainy season (7/area). Mortágua was the area where less scats were found, with none being collected during the rainy season.

The majority of the scats (n=204, 95%) were morphologically identified as belonging to either red fox (*Vulpes vulpes*, n=160) or stone marten (*Martes foina*, n=44). The remaining were attributed to other non-target species (n=7) or not assigned due to uncertainty on the identification (n=4).

3.1 Species identification

Genetic identification was attempted in 153 samples (71% of the 215 collected scats) aiming the balance between number of analysed scats per species, sampling area and habitat (native vs plantations). The number of scats selected for molecular analysis by season and location can be seen in Table 2.

Table 2 | Number of samples selected for molecular analysis in the eight study areas, by season and habitat. PS, Pampilhosa da Serra; G – Góis; MR, Mortágua; F, Fundão; P, Penamacor; PG, Penha Garcia; L, Lousã; M, Malcata

| | Exotic forest | | | | | | Native forest | | |
|--------------|---------------|----|----|----|----|----|---------------|----|--------------|
| | PS | G | MR | F | P | PG | L | M | Total |
| Dry | 11 | 14 | 2 | 14 | 28 | 21 | 11 | 12 | 113 |
| Rainy | 4 | 3 | 0 | 17 | 1 | 4 | 4 | 7 | 40 |
| Subtotal | 15 | 17 | 2 | 31 | 29 | 25 | 15 | 19 | |
| Total | 119 | | | | | | 34 | | |

From those, only 51 scats (33%) were molecularly identified, from which 50 were successfully attributed to *V. vulpes* (n=37) and *M. foina* (n=13), with a single scat assigned to domestic dogs, *Canis lupus familiaris* (Table 3). In two of the scats morphologically assigned to red fox, molecular analysis detected the preys (rodents), rather than predators and, thus, were not successful identifications and were not considered for this analysis. The morphological identification accuracy varied between 92% (33 out of 36) in *V. vulpes* and 71% (10 out of 14) in *M. foina*. Morphological identification errors can be categorized in two ways: underrepresentation or false negative, when a scat of a specie is misassigned to another; overrepresentation or false positive, when a scat is morphologically misassigned to a species (Table 3). Agreement between morphological identification in the field and molecular scatology varied among species, but the rates of underrepresentation and overrepresentation were similar within species.

Table 3 | Matches and mismatches between morphological and genetic identification of the 51 genetically identified scats. Correct morphological assignment are marked in bold.

| Genetic identification | Morphological identification | | | Total | % of false negative |
|-------------------------------|------------------------------|---------------|---------------|-------|---------------------|
| | red fox | stone marten | not assigned | | |
| <i>Vulpes vulpes</i> | 33 | 4 | - | 37 | 4/37 (11%) |
| <i>Martes foina</i> | 3 | 10 | - | 13 | 3/10 (30%) |
| <i>Canis lupus familiaris</i> | - | - | 1 | 1 | 1/1 (100%) |
| Total | 36 | 14 | 1 | 51 | |
| % of false positive | 3/36 (8%) | 4/14 (29%) | 1/1 (100%) | | |

Three (8%) of the 40 analysed samples collected in the rainy season were molecularly identified. From the 113 analysed samples collected in the dry season, 48 were molecularly identified (42%). Differences in amplification success were statistically relevant ($p < 0.05$). No more statistically relevant differences were found regarding other variables (e.g. habitat type, age of the scat, species).

3.2 Diet composition

Red fox and stone marten had a globally diverse diet, with 26 different food items identified in the diet of red fox and 22 in the diet of stone marten. (Table 4). However, three

items constitute the bulk of these carnivore diet: arthropods and fruits are the most frequent resources consumed by both species (FO in Table 4), followed by rodents.

Rubus sp. (*R. idaeus* and *R. ulmifolius*) and adult arthropods were the most frequently eaten resource by stone marten (FO_{*Rubus* sp.}=33.3%; FO_{adult arthropods}=30.3%), with fruits contributing to the bulk of their diet (PO=74.6% and PB=81.2%; Table 4; Figure 4). Secondary resources include arthropods (adults and larvae, PO=16.4%) and rodents (PB=10%), indicating they might be an important food resource for this mustelid. Among insects, larvae (mainly Scarabaeoidea) were the most abundant resource (PO=5.2%), followed by adult beetles (order Coleoptera) (PO=4.7%). Reptiles also recorded noteworthy PB values (5.4%) (Table 4; Figure 4).

Prunus sp. was the dominant fruit in the diet of red fox (PO= 21.7%) and contributed considerably for the ingested biomass (PB=78.5%), while *Ficus* sp. was the less consumed fruit (PO=0.9%; PB=0.6%). However, in terms of the number of consumed preys the most consumed item by red fox were arthropods (PO=52.8%), whereas adult beetles (order Coleoptera) and eggs made up the majority of the arthropod ingested (PO=20.8% and 17.9%, respectively). Adult arthropods were also the most frequent resource eaten by red fox (FO=79.2%). Small mammals (orders Rodentia and Eulipotyphla) contributed to 8.1% of the ingested biomass, despite their low contribution in number (PO=4.3%).

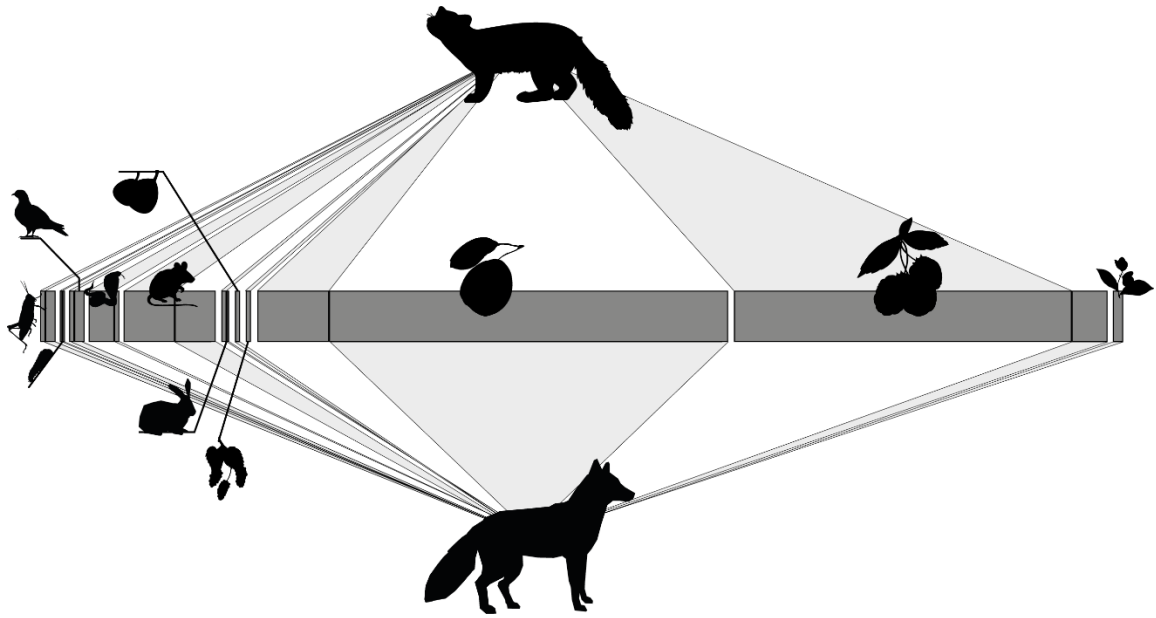
Chi-square tests showed that sample size was not homogenous between all subsamples. Red fox sub-sample between habitats and season and stone marten subsamples between longitudes were not homogeneous ($p < 0.01$). Thus, comparisons were only made between species in both habitats, in the dry season and in the western location, when sample size homogeneity was assured.

Table 4 | Food items in the diet of stone marten (*Martes foina*) and red fox (*Vulpes vulpes*) expressed as the number of individual items (N), frequency of occurrence (FO), percentage of occurrence (PO) and percentage of consumed biomass (PB).

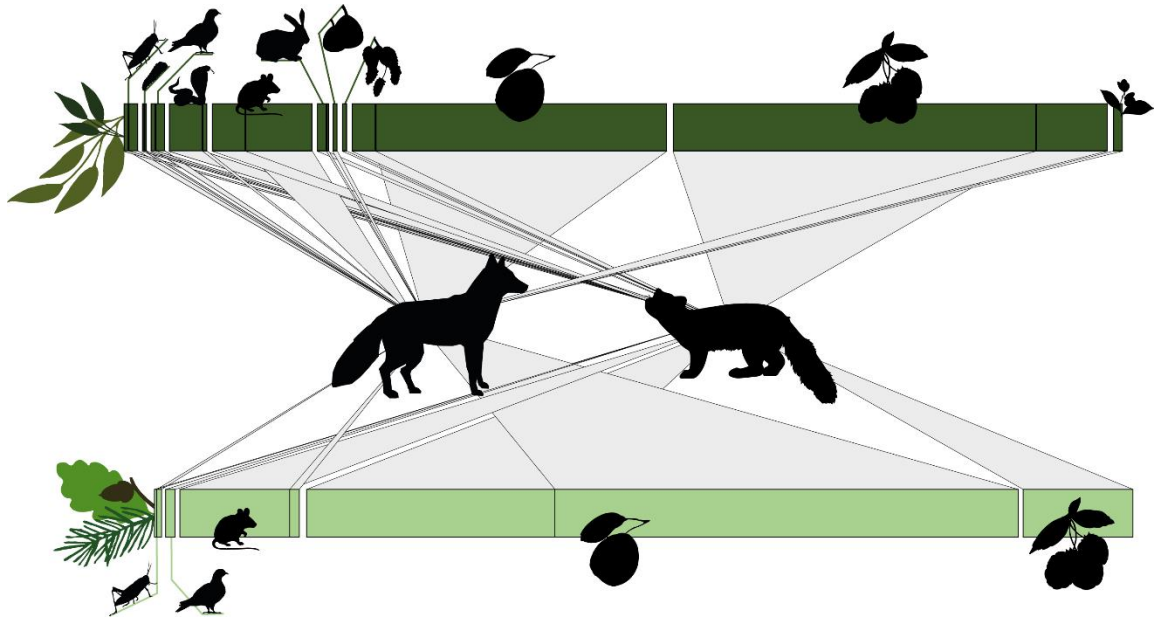
| Food item | <i>Vulpes vulpes</i> | | | | <i>Martes foina</i> | | | |
|--------------------------------|----------------------|-------|-------|-------|---------------------|-------|-------|-------|
| | N | FO | PO | PB | N | FO | PO | PB |
| Arthropod (adults total) | 69 | 79.2% | 32.5% | 1.6% | 26 | 30.3% | 11.2% | 0.6% |
| O. Coleoptera | 44 | | 20.8% | | 11 | | 4.7% | |
| O. Heteroptera | 1 | | 0.5% | | | | | |
| O. Ortoptera | 8 | | 3.8% | | 3 | | 1.3% | |
| O. Diptera | 1 | | 0.5% | | 1 | | 0.4% | |
| O. Mecoptera | 1 | | 0.5% | | | | | |
| O. Isoptera | 1 | | 0.5% | | 1 | | 0.4% | |
| O. Scorpionida | 5 | | 2.4% | | | | | |
| O. Blattodea | 1 | | 0.5% | | | | | |
| O. Plecoptera | | | | | 1 | | 0.4% | |
| O. Hymenoptera | 1 | | 0.5% | | 1 | | 0.4% | |
| N.I. | 6 | | 2.8% | | 8 | | 3.4% | |
| Arthropod (larva) | 5 | 8.3% | 2.4% | 0.0% | 12 | 9.1% | 5.2% | 0.1% |
| Arthropod (eggs) | 38 | 20.8% | 17.9% | a | 3 | 3.0% | 1.3% | a |
| Mammals (total) | 10 | | 9.6% | 8.3% | 8 | | 3.4% | 11.4% |
| O. Rodentia (total) | 8 | 29.2% | 3.8% | 7.0% | 7 | 21.2% | 3.0% | 10.2% |
| <i>Apodemus sylvaticus</i> | 2 | | 0.9% | | 1 | | 0.4% | |
| <i>Mus</i> sp. | 2 | | 0.9% | | 1 | | 0.4% | |
| <i>Microtus</i> sp. | 2 | | 0.9% | | 4 | | 1.7% | |
| <i>Rattus</i> sp. | 2 | | 0.9% | | 1 | | 0.4% | |
| O. Eulipotyphla (total) | 1 | 4.2% | 0.5% | 1.1% | | | | |
| <i>Sorex</i> sp. | 1 | | 0.5% | | | | | |
| O. Lagomorpha (total) | 1 | 4.2% | 0.5% | 0.2% | 1 | 3.0% | 0.4% | 1.2% |
| <i>Lepus granatensis</i> | 1 | | 0.5% | | | | | |
| N.I. | | | | | 1 | | 0.4% | |
| O. Cetartiodactyla* | | | | | 1 | 3.0% | 0.4% | a |
| Birds (total) | 2 | 8.3% | 0.9% | 2.0% | 5 | 15.2% | 2.2% | 1.4% |
| O. Apodiforme | | | | | 2 | | 0.9% | |
| O. Passeriforme | 1 | | 0.5% | | | | | |
| N.I. | 1 | | 0.5% | | 3 | | 1.3% | |
| Reptile (total) | 4 | 16.7% | 1.9% | 0.6% | 4 | 12.1% | 1.7% | 5.4% |
| <i>Psammotromus</i> sp. | 4 | | 1.9% | | 3 | | 1.3% | |
| <i>Malpolon monspessulanus</i> | | | | | 1 | | 0.4% | |
| Fruits (total) | 84 | 66.6% | 39.6% | 87.6% | 173 | 42.4% | 74.6% | 81.2% |
| <i>Prunus</i> sp. | 46 | 20.8% | 21.7% | 78.5% | 12 | 6.1% | 5.2% | 14.1% |
| <i>Rubus</i> sp.** | 19 | 8.3% | 9.0% | 7.4% | 159 | 33.3% | 68.5% | 65.9% |
| <i>Morus</i> sp. | | | | | 2 | 3.0% | 0.9% | 1.2% |
| <i>Ficus</i> sp. | 2 | 8.3% | 0.9% | 0.6% | | | | |
| Rosaceae | 14 | 16.7% | 6.6% | 1.1% | | | | |
| N.I. | b | 12.5% | | b | | | | |
| No. of scats analysed | 24 | | | | 33 | | | |

* *Sus scrofa*; ** *R. idaeus* and *R. ulmifolius* were not distinguished. **a**, PB not calculated due to the absence of conversion factors (CF). **b**, N and PO not calculated due to the uncertainty on the identification.

A



B



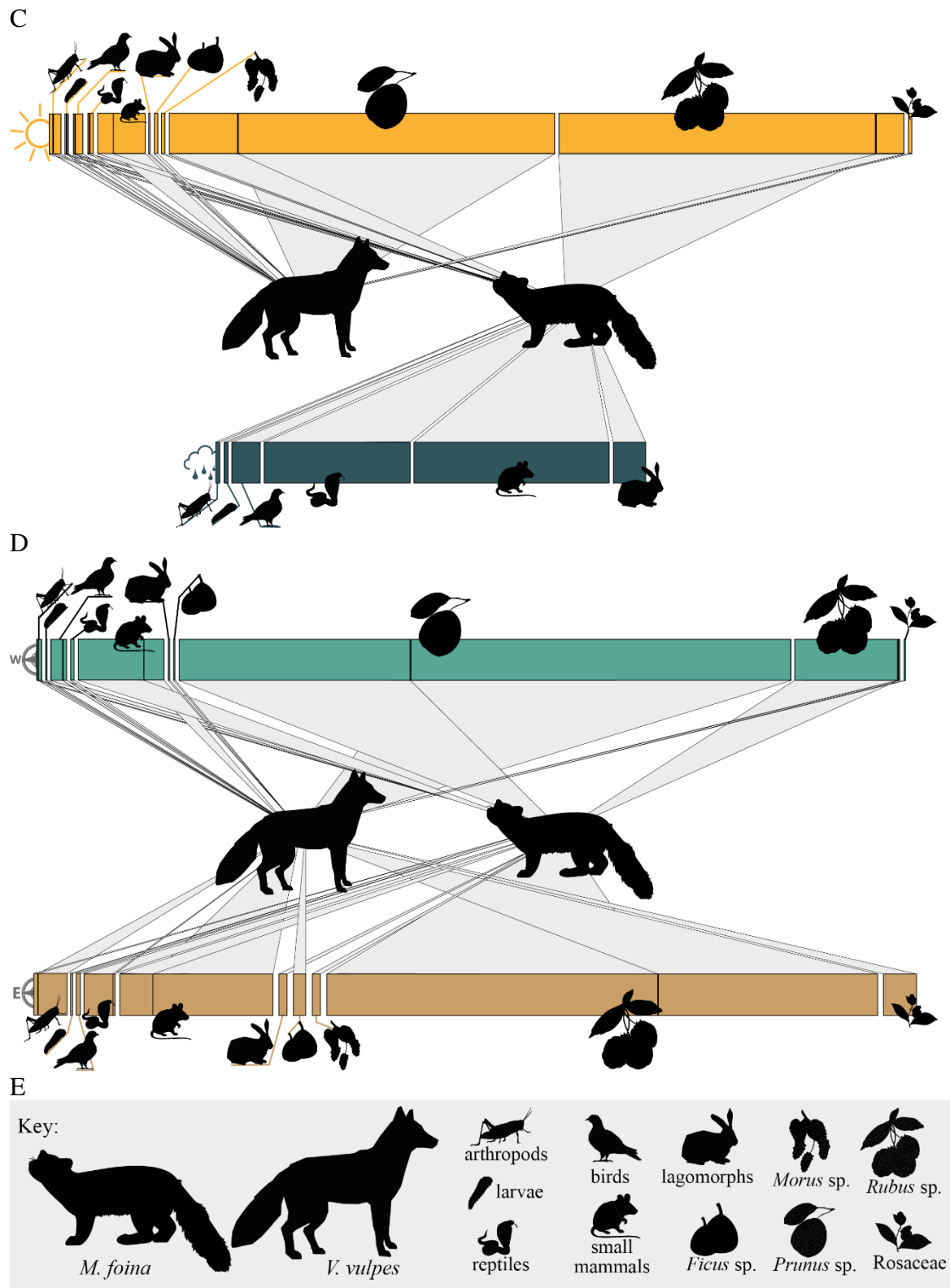


Fig. 4 | PB values by major food items for *M. foina* and *V. vulpes* in various conditions. See Appendix II for values. **A**, Overall diet for both species. **B**, Diet in eucalyptus stands (top bar) and in native forests (bottom bar) for both species. **C**, Diet of *M. foina* in both seasons (dry on the top and rainy on the bottom) and diet of *V. vulpes* in the dry season (top bar). **D**, Variation of the diet of both species according to the longitude of sampled sites (west on the top, east on the bottom). **E**, Key for species and food resources

Although both species focus their consumption on similar type of food resources (e.g. fruits), they seem to target different specific items (Fig. 4). For example, *Prunus* sp. and *Rubus* sp. are eaten by both carnivores, but in different proportions (see also Table 4). Red fox is the main overall consumer of *Prunus* sp., although the ingested biomass of this food item is lower in eucalyptus plantations ($PB_{\text{native}}=97\%$; $PB_{\text{eucalypt}}=61\%$). Similarly, the stone marten consumes higher amount of biomass of this item in native forests ($PB_{\text{native}}=52\%$; $PB_{\text{eucalypt}}=5\%$), but the difference in consumption is higher. The consumption of *Rubus* sp. shows an opposite pattern, since this fruit is mainly ingested by the stone marten ($PB_{\text{red fox}}=7.4\%$; $PB_{\text{stone marten}}=65.9\%$). Inversely to *Rubus* sp., this mustelid ingested a higher biomass of rodents in native forests ($PB=23\%$) than in eucalyptus stands ($PB=7\%$). Red foxes exhibited the opposite pattern, targeting rodents more often in Eucalyptus plantations ($PB_{\text{native}}=2\%$; $PB_{\text{eucalypt}}=12\%$) (Figure 4B).

Some seasonal changes were also detected, with stone martens not consuming fruits during the rainy season. During this season, rodents ($PB=48\%$) and reptiles ($PB=36\%$) constitute the bulk of the biomass ingested by martens, complemented by birds ($PB=7\%$) and Lagomorphs, that appear as a new consumed resource ($PB=8\%$). In the dry season, fruits (*Rubus* sp. and *Prunus* sp.) were the dominant resources in stone marten's diet (Fig.4C).

Finally, we also detected some longitudinal variation in these predators' diets (Fig. 4D). In the western sampling sites, biomass ingested by red fox was mainly composed of *Prunus* sp. ($PB=94\%$), a resource not eaten by either species in the eastern sites. In the later region, foxes' diet is mostly composed by *Rubus* sp. ($PB=55\%$) and rodents ($PB=30\%$), while stone martens main food resource was *Rubus* sp. ($PB=80\%$).

Niche breadth (B_A and J') of both species varied according to habitat type, season and longitude (Table 5). The less diversified diet was recorded for *M. foina* in the dry season ($J'=0.36$ and $B_A=0.07$), while the most diverse diet was recorded also for *M. foina*, but in native habitats ($J'=0.96$ and $B_A=0.85$). Diet diversity (H') was statistically different between habitats and longitudes for *V. vulpes*, but not for *M. foina*. Statistically significant differences are depicted in Table 5.

Trophic niche overlap was higher in native forests when compared to eucalyptus stands, but quite similar between both regions (east and west). Unfortunately, it was not

possible to compare niche overlap between seasons (due to the lack of data for foxes), but niche overlap in the dry season was the lowest of all sub-samples (Table 5).

Table 5 | Niche breadth expressed as Levins standardized index (B_A) and Evenness (J') for stone marten and red fox. Trophic niche overlap between species expressed as Morisita index (C). Nat, native forests. Euc, eucalypt forests.

| Variables | <i>Martes foina</i> | | <i>Vulpes vulpes</i> | | Niche overlap C |
|-----------|---------------------|---------------------|----------------------|---------------------|----------------------|
| | B_A | J' | B_A | J' | |
| Overall | 0.12 | 0.52 ^a | 0.33 | 0.74 ^a | 0.33 |
| Habitat | | | | | |
| Nat | 0.85 | 0.96 ^{b,d} | 0.63 | 0.87 ^b | 0.68 |
| Euc | 0.09 | 0.46 ^{c,d} | 0.35 | 0.76 ^c | 0.34 |
| Season | | | | | |
| Rainy | 0.65 | 0.88 ^e | - | - | - |
| Dry | 0.07 | 0.36 ^{e,f} | 0.33 | 0.74 ^f | 0.28 |
| Longitude | | | | | |
| East | 0.10 | 0.47 | 0.36 | 0.71 ^g | 0.40 |
| West | 0.47 | 0.76 ^h | 0.43 | 0.83 ^{g,h} | 0.44 |

^a to ^h indicate pairs of statistically different H' values ($p < 0.01$) for homogeneous subsamples.

4 Discussion

Understanding the mechanisms that regulate coexistence of species is essential to build and manage more wildlife-friendly environments. Mesocarnivores, as top predators in several areas, including our study areas, may provide insightful information on other species presence and trophic interactions throughout their dietary habits. Therefore, this study on the analysis of trophic niche of red fox and stone marten in two different habitats aims to understand what food resources are they targeting in distinct landscapes contexts and how they manage to coexist. Nevertheless, describing the diet of a species depends greatly on an efficient samples' collection and identification, factors that should be considered prior to interpreting the feeding patterns (see below). Overall, our results show that the trophic niche patterns for both species were similar to those already reported for Mediterranean Regions (Santos et al., 2007), and two different mechanisms seem to allow coexistence in distinct landscapes contexts: resource partitioning in challenging environments (e.g. eucalyptus plantations) and resource share in more favourable ones (e.g. native forests).

4.1 Scat collection

Carnivores leave distinct traces as territorial marks, and their distribution depends on their movement patterns and may vary between species, seasons, and habitat types (Wilson & Delahay, 2001). To maximize data collection in order to test our working hypothesis, this study was focused on two widely distributed species (Bencatel et al., 2018) whose signs are frequently conspicuous, enhancing the survey success detection (Barea-Azcón et al., 2007). In this study, no habitat effect was detected in scat collection, although the number of red fox scats per sampling site was considerably higher than for stone marten. Similarly, the number of collected scats per area in the dry season was also higher for red fox, highlighting the influence of these variables in the number of collected scats (Wilson & Delahay, 2001). In fact, red fox, as the majority of canids, use trails for movements across home ranges and leave scats in these paths, which make this species' scats the easiest to find (Gompper et al., 2006). Furthermore, this is one of the most abundant carnivore species in Portugal (Bencatel et al., 2017). The rainy season is often characterized by a more extreme weather conditions (rain and wind), which enhance scats degradation and decrease their detection during surveys. Thus, the probability to detect scats in the dry season is higher.

4.2 *Success of scat identification*

Scats can be of great use when studying mammalian mesocarnivores, as they allow to collect information on feeding habits and food resource preferences (Hoffmann et al., 2010; Putman, 1984). However, our results indicate that errors in morphological scat identification are common, a pattern already highlighted in previous studies (Alexandre et al., 2020; Monterroso et al., 2013). If a scat is not accurately identified, it can lead to biased results and misleading ecological patterns (Alexandre et al., 2020), such as species distribution range or diet composition. Thus, molecular scatology proved to be a beneficial tool to couple with scat sampling, providing a more accurate identification. Nevertheless, the morphological identification success rate in this study was high (92% for red fox and 71% for stone marten) and within the range of values reported by other studies: 86% for red fox and 78% for stone marten (Monterroso et al., 2013); 96% for red fox and 38% for stone marten (Alexandre et al., 2020). Such high field accuracy in scat identification, supported our decision of using genetically unidentified scats in diet analysis to increase our sample size. Variation in identification success between species was proved once again (Alexandre et al., 2020; Monterroso et al., 2013), and a pattern may be emerging, with scats of stone marten being identified with less accuracy and mainly misidentified as belonging to red fox. Both species are generalists and variations on their diet can interfere with morphological scat identification, by altering the scats shape, size and odour (Panasci et al., 2011). Furthermore, despite similar values for underrepresentation and overrepresentation rates for each species, a counterbalance situation cannot be inferred from this pattern, since additional information retrieved from an erroneously assigned scat can lead to a bias in further results (Alexandre et al., 2020), and erroneous management (e.g. control of a low abundance population) and conservation plans (e.g. promote populations that are already in an acceptable status).

DNA degrades over time and the condition on which scats were collected can influence the success of molecular identification. This may be the reason why less scats were molecularly identified in the rainy season, i.e., increased humidity rates can contribute the rapid degradation of scat DNA and the rain can wash of the cells (Farrell et al., 2000; Foran et al., 1997; Panasci et al., 2011). Farrell et al. (2000) reported a 66% rate of successful DNA sequencing in scats collected in dry season and only 28% in the rainy season, a pattern

similar to the one found in this study (44% in the dry season and 8% in the rainy season). However, not all studies support this pattern. A study performed in Mediterranean areas (Portugal and Spain) was not able to find evident seasonal differences in the genetic identification success, despite the marked climate differences between seasons prevailing in this region, a result not expected by the authors (Monterroso et al., 2013). No other variables, like habitat type, species and the age of the scat proved to be significantly influent for the genetic identification success of the scats analysed in our study.

Overall, molecular identification success was very low when compared to other studies: 33% *versus* 83% in Alexandre et al. (2020), 78% in Monterroso et al. (2013) and 53% in Davison et al. (2002). Despite the age of the scat did not statistically prove to impair molecular identification, scats weathering may be the most probable cause, since the great majority of the scats were detected in dirt roads, being thus more exposed to environmental factors that may affect DNA preservation (e.g., direct light).

Even though previous authors advocate that animal tissues is adequately degraded in the digestive tract of carnivores (Foran et al., 1997), in two of the samples of this study the genetic analysis detected the presence of rodents DNA. In fact, not all the genetic material from the prey is degraded during digestion, allowing the identification of some remains through genetic analysis, which is an useful method to improve the accuracy of prey identification (Farrell et al., 2000; Kohn & Wayne, 1997).

4.3 *Overall diet and resource use patterns*

One of the most debated question in ecology is how functionally similar species can coexist (Gordon, 2000). The most widespread principle regarding this question states that complete competitors cannot coexist and must segregate along niche dimensions in order to occupy different ecological niches (Hardin, 1960). Resource partitioning – i.e. species narrow their realized niches to avoid competition - is one of the mechanisms that have been reported as taking part in mesocarnivore coexistence. Thus, to test the validity of this mechanism in allowing co-occurrence in resource poor and new environments (such as plantations; Campinhos, 1999), is of major importance the study of species diet in

ecologically different environments, i.e. native and exotic (Balestrieri et al., 2013; Barrientos & Virgós, 2006).

Red fox and stone marten are species usually characterized as generalists, showing a greater diversity and high frequency of fruits in their diets (Rosalino & Santos-Reis, 2009; Serafini & Lovari, 1993). In this study, blackberries (*Rubus* sp.) and plums (*Prunus* sp.) were frequently eaten by these species, which corroborates the mentioned pattern and highlights the importance of fruits in the diet of generalist mesocarnivores (Rosalino & Santos-Reis, 2009). However, there was a distinct consumption pattern among species: plums were more ingested by foxes, while blackberries were eaten more frequently by martens, supporting the resource partitioning mechanism in fostering co-existence. In addition, stone marten generally invested more in the consumption of blackberries than any other fruit, while red fox has a wider, and more balanced consumption of fruits. In fact, fruits consumption by red fox seems to be widely variable; while some studies report low frequencies of occurrence in diet (Cavallini et al., 1996; Santos et al., 2007), others report high consumptions of Rosaceae fruits, sometimes even higher than for stone marten (Calisti et al., 1990; Serafini & Lovari, 1993). Nonetheless, it seems that stone marten, an inferior competitor (Serafini & Lovari, 1993), eats less of the red fox's most consumed fruits, particularly plums, a resource previously recorded as equally frequent in both species' diets (Monterroso, 2013). In short, fruits are less frequent but consumed in higher quantities by stone marten (i.e. smaller fruits imply eating higher amount), while red fox eats fruits more frequently but in lower quantities (i.e. bigger fruits imply lower numbers), a pattern that can be a result of coexistence mechanisms that drive mesocarnivores to focus on different types of fruits when in sympatry (i.e. resource partitioning).

Arthropods were the second most consumed item by both species, but the pattern of consumption appears to be congruent with other studies, where red fox holds the highest consumption rates (Petrov et al., 2016; Serafini & Lovari, 1993). Arthropod eggs (an unusual item in diet) and larvae also occur with a remarkable frequency. During sampling, there was no evidence of scat colonization by arthropods, although we cannot rule out the possibility of these resources coming from indirect sources, i.e., not eaten by choice. Larvae were in fact bitten as well as some eggs, so, if not eaten by choice, probably eaten by chance when the individual was feeding on carrion.

Small mammals were less consumed by red fox, when compared to some previous studies (Papakosta et al., 2010; Petrov et al., 2016; Serafini & Lovari, 1993), possibly because these preys live in low abundance as a consequence of a poor understory cover in the sampling sites, especially in eucalyptus plantations (Carrilho et al., 2017; Keten et al., 2016; Teixeira et al., 2017). Wild boar (*Sus scrofa*) consumption by the stone marten was also detected, an unexpected food source for this mesopredator due to its size. It was probably consumed as carrion, since predation is an unlikely option due to the species size difference and wild boar behaviour (social ungulates that can evidence aggressive behaviours against possible predators; Berger, 1979) and carrion consumption was already reported for stone marten (Bakaloudis et al., 2012; Carvalho & Gomes, 2004).

The high diversity in both species' diet was reflected in their niche breadth (J') as both species can be considered generalists, with the widest trophic niche belonging to red fox. In addition, B_A values for stone marten were very low when compared to previous studies in other regions (Papakosta et al., 2010 in Greece; Petrov et al., 2016 in Bulgaria; Serafini & Lovari, 1993 in Italy), but in accordance with previous studies performed in southwestern Portugal ($B_A=0.12$; Santos et al., 2007) and in central Greece ($B_A=0.13$; Bakaloudis et al., 2012). In fact, this species has showed the ability to adjust their food requirements depending on environmental conditions (Papakosta et al., 2014). Thus, a narrower trophic niche may be an adaptation to the biotic and abiotic conditions experienced in Portugal or a response to sympatric living with red fox in low resource abundance areas. However, it is important to highlight once again that this index has poor consideration for resources rarely occurring in scats, but nonetheless important in the diet (like rodents).

Only one item (plum) was identified as a key resource for red fox, i.e., a resource that accounts for more than 10% of ingested biomass (Barrientos & Virgós, 2006), in opposition to three identified for stone marten (blackberries, plums and rodents). At first sight, it may seem surprising that with a wider niche, red fox has a lower number of key resources, but with a broader niche, red fox relies less on each food item, thus each contributes less for the biomass ingested. Despite the high frequency of occurrence of arthropods in red fox diet, this resource was not considered a key resource, as occurred in other studies (Calisti et al., 1990; Cavallini et al., 1996). On the other hand, key resources identified for stone martens are congruent with previous studies performed in Central Spain, except for lagomorph consumption that, in this study, was recorded in very low amounts (Barrientos & Virgós,

2006). However, when compared to previous studies on the diet of stone marten, it becomes evident that this species feeds on plum less frequently, and more on blackberries, probably to prevent competition for food with red fox, that markedly prefers larger and more carbohydrate-rich fruits (Bakaloudis et al., 2012; Balestrieri et al., 2013; Debussche et al., 1987).

Overall trophic niche overlap was low, as a predicted condition to allow co-existence, and both species evidence exclusivity in some resources. Thus, food interference and competition should be low between them.

Noteworthy was the lack of consumption of pears (*Pyrus* sp.) and junipers (*Juniperus* sp.), two fruits broadly reported in the diet of both species in previous probably related with their absence in the sampling areas (Bakaloudis et al., 2012; Balestrieri et al., 2013; Calisti et al., 1990; Costa et al., 1998; Santos et al., 2007; Serafini & Lovari, 1993). Also surprising was the low consumption of figs (*Ficus* sp.) and mulberry (*Morus* sp.) by stone marten when compared with other studies in different locations (Bakaloudis et al., 2012; Balestrieri et al., 2013; Barrientos & Virgós, 2006). In fact, these prey items may be lacking or available in very low amounts in the sampling areas, as only blackberry shrubs were broadly spotted in the field. Overall, stone marten adapted their diet in accordance with resource availability, highlighting its generalist behaviour.

4.4 Trophic niche seasonality

The lowest trophic niche breadths and overlaps occurred in the dry season. Although, red fox had a surprisingly high trophic niche breadth in this season. The decrease in niche overlap in the dry season is not consensual with all previous studies with the same species. Carvalho and Gomes (2004) reported a higher overlap in this season when compared to the rainy one. Despite a wide abundance of fruits in this season, small mammals and coleopterans (the main arthropod consumed by both species) decreased their abundance, which may lead to the observed narrowing in the trophic niche of stone marten (Debussche et al., 1987; Moreno & Kufner, 1988; Rosalino et al., 2005). In the dry season, stone marten consumed mainly fruits that were not available in the rainy season – plums ripen between July and October and blackberries ripening occurs around August (Debussche et al., 1987).

Papakosta et al. (2010) also reported the same increase in fruit consumption during the dry season. These conditions lead stone marten to complement their diet in the rainy season with other resources like rodents and arthropods, in line with their availability increase and with what was described in previous studies (Carvalho & Gomes, 2004; Moreno & Kufner, 1988; Papakosta et al., 2010; Rosalino et al., 2005). Stone marten also increased the consumption of lagomorphs, birds and reptiles, a pattern not recorded for this species in other areas where the species is sympatric with the red fox (Carvalho & Gomes, 2004; Papakosta et al., 2010). Nevertheless, as both studies were performed in other regions food resource availability may differ and may be the underlying cause of this pattern. Unfortunately, no samples of red fox were analysed during the rainy season.

4.5 *Trophic niche variation between habitats*

Eucalyptus plantations are considered to have a low resource availability or, at least, a narrow pool of resource diversity, thus, it was expected that trophic niche overlap would be higher as a result of these conditions (Calviño-Cancela et al., 2012; Carrilho et al., 2017; Zahn et al., 2010). However, in this habitat the species recorded low trophic niche breadth as well as the second lowest niche overlap. Apparently, these species found a way of coexisting without widely overlapping their diets in these poor resource environments. Stone marten's main food resource was blackberries whereas red fox's diet consists mainly of arthropods and, to a less extent, of blackberries and plums. The number of key resources on the diet of stone marten falls to one in this habitat whereas red fox increases to three when compared with the overall diet. Nevertheless, the same pattern of fruit consumption remains equal to the overall diet, i.e., blackberries are more frequently eaten by stone marten and plums are mainly eaten by red fox. Regarding the consumption of plums by both species, and assuming that this resource is not abundant in eucalyptus plantations (individual of this genera was not recorded in the sampling sites), red fox may be investing in a more active search for this more energetically profitable fruit (has it has a higher pulp-seed ratio) outside the eucalyptus plantations, leaving the more abundant, but less energetically profitable, resources to stone marten (e.g. blackberries; Debussche et al., 1987). Similarly, arthropods are less abundant in eucalyptus stands when compared to native forest (Zahn et al., 2010), but red fox does not change the consumption rate of this resource. Here we assume that red

fox is the dominant competitor that compels stone marten to change their food habits and become more specialized (Laundre et al., 2010; Pereira et al., 2012).

The widest niche for both species was recorded in native forest as well as the highest niche overlap. In native environments, plums and arthropods also become of major importance to stone marten's diet, leading to a higher trophic niche overlap with red fox. In addition, it is interesting that the only case where stone marten niche breadth was higher than the one of red fox (with statistic differences) was in native environments. One explanation could be that in native environments, species have the chance to diversify their diet and share resources, a mechanism facilitated by an increase in resource availability and diversity, when compared to plantations (da Silva et al., 2019), leading to a less competitive environment (Wiens, 1993). Another may focus on a more complex vertical spatial structure in native habitats that allows stone marten to climb on trees and eat the fruits that did not fall yet. This way avoids agonistic encounters with red fox (a terrestrial species) while consuming the same resources.

Differences between the eastern and the western locations were also analysed and there are some noteworthy differences. Differences in diet between the two regions can only be analysed properly for red fox due to sample size homogeneity. This species had a wider niche in the western locations (when compared to the eastern locations and with trophic niche breadth of the stone marten), but that did not seem to influence the trophic niche overlap between both species. Plums are only consumed in the western locations, possibly due to their unavailability in the east, related to the climatic or orographic differentiation between regions (Abreu et al., 2004; Aguiar et al., 2008; IPMA, 2020). Rodent and blackberry consumption by red fox was higher in eastern locations, probably to cope with the absence of the main resource in western locations, the plums. Despite the sample size constraint, the studied species apparently eat more blackberries in the eastern locations. Biogeographic descriptions of this regions highlight blackberries' abundance in the eastern region, which is probably why its consumption increased (Costa et al., 1998).

6 Final remarks

With this work we provide relevant insight into red fox and stone marten trophic niche, when living in sympatry, in natural landscapes as well as in anthropic environments, such as eucalyptus plantations. Stone marten and red fox showed a generalist behaviour, but a wider niche was only registered in native environments and in the rainy season. Furthermore, fruits proved to be an important food resource in the diet of both mesocarnivores, although, these results may be biased for red fox, since no scat from the rainy season was analysed (when the fruit availability is lower). This resource also took part of a niche segregation mechanism: plums were mainly eaten by red fox, whereas stone marten ate mainly blackberries. These patterns were more pronounced in eucalyptus plantations as an increasing need for segregations may be taking part so species can coexist without an agonistic interference, in a poor resource environment (da Silva et al., 2019), following a resource partitioning mechanism (Carvalho & Gomes, 2004). On the other hand, native environments allow for a wider trophic niche and niche overlap through resource sharing, possibly because the resource availability allow species to consume the same type of resources without depleting it in a way that may affect the other species. In conclusion, season and habitat influence the trophic relationships among generalist mesocarnivores, probably through variation in resource availability, a result that contributed to the body of knowledge that show that this mesocarnivore are mainly diet generalists.

Despite the statistical significance of the results, they should be interpreted carefully due to relatively low number of analysed samples. In fact, we had some limitations regarding the sample size that prevented us from deepen the discussion and make more generalized statements. Namely, the differences in trophic niche breadth of red fox between different habitats could not be statistically tested, thus, a broader niche in native habitats for both species is not supported by statistics. Furthermore, the introduction of scats without morphological identification in the diet analysis could lead to potential errors. However, the high accurate morphological identification rate of those scats that were also genetically identified, gives us some confidence that the possible bias that may affect our results due to the inclusion of non-genetically identified samples in the diet analysis is negligible.

From what was recorded in our sampling sites, not all sampled plantations were completely deprived of shrub cover, which translates in the high consumption of blackberries

(a shrub) and rodents (species dependent on understory cover, Carrilho et al., 2017) in this habitat. Additionally, some areas of native forest, especially the ones with Pine trees, had a very low understory cover. These observations, together with the results, may indicate some other mechanism may be driving the results, and local habitat tree structure (i.e. native vs exotic) may not be the main driver influencing the patterns of trophic niche breadth, overlap and consequently, coexistence. Habitat heterogeneity at larger spatial scales may be one of the variables not analysed, but relevant for the species interaction, as species may profit from resources provided by other patches. In addition, habitat heterogeneity inside eucalyptus plantations may be achieved by different aged stands, offering new and different resources that may promote coexistence. Unfortunately, due to our small sample size, we could not test this hypothesis.

Species coexistence does not rely only on food resource partitioning (Gordon, 2000; Schoener, 1974). Individuals of both species may be segregating along other niche axis (e.g. spatial, temporal; Torretta et al., 2016) or at a finer scale (Pereira et al., 2012), both situations not analysed in this work. Furthermore, interactions between individuals are dynamic and multidimensional and become even more complex with an increasing number of species considered (Gordon, 2000; Schoener, 1974). In this study, we only considered two of the species inhabiting these habitats, so the observed patterns can be more complex than discussed, since in the study areas we also detected the presence of other potential competitors, such as the Eurasian badger, the common genet or the Egyptian mongoose.

7 Future research

In order to increase data robustness and allow a better definition of the feeding behaviour patterns and of the mechanisms allowing co-existence, we believe that a higher sample size would be more adequate. Genetic identification of more scats will allow to increase the accuracy of identification and, consequently, of each species' trophic niche. However, scat collection depends greatly on species defecation pattern (which may be affected by diet itself), population abundance and scat detection efficiency, and only the latter factor can be controlled by us. Regarding diet results, analysing more scats will also allow us to increase the robustness of trophic niche differentiation among species (if existing), as well as between species populations inhabiting different habitats, seasons, and longitudes (Trites & Joy, 2005). Consequently, such wider data set will allow for more solid and broad interpretation of the feeding strategies detected patterns.

Furthermore, a more detailed analysis regarding how habitat structure and composition influence species diet could also better inform managers and conservationists when defining actions targeting these carnivores. For example, when assessing how species adapt to forestry plantations, it should be investigated how the age of eucalyptus stands, understory coverage and complexity, which were not taken in consideration in this work, may influence diet choices, as such habitat variations may play an important part in shaping species ecological niches and coexistence (e.g. Smith et al., 2018).

Additionally, to fully understand the ecological mechanisms of co-existence, future research should also consider analysing, simultaneously with the assessment of the feeding behaviour, species spatial and temporal patterns to understand if there is a correlation between an increase in niche overlap and any other segregation mechanism (e.g. Easter et al., 2020). Also, the study of resource abundance in the region where scats are collected would be a relevant information for data analysis, as it would allow inferring about species diet preferences (e.g. Revilla & Palomares, 2002). Resource abundance can be closely linked to the patterns of consumption and trophic overlap between the species (e.g. Creel et al., 2018). Finally, to assess the global impact of exotic plantation on mesocarnivore, diet studies should be extended to other co-occurring species of the predator's guild not covered in this work, like the badger (*Meles meles*) and the Egyptian mongoose (*Herpestes ichneumon*),

whose presence can introduce different nuances on how red foxes and stone martens interact when using food resources.

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Appendices

Appendix I

QIAamp® Fast DNA Stool Mini Kit

Notes before starting:

- Ensure that Buffers AW1 and AW2 have been prepared according to the instructions on the labels.
- Perform all centrifugation steps at room temperature (15–25°C) at 20,000 xg (~14,000 rpm).
- Redissolve any precipitates in Buffer AL and InhibitEX® Buffer by heating and mixing.
- Mix all buffers before use.

1. Weigh 180–220 mg stool in a 2 ml microcentrifuge tube (not provided) and place tube on ice.

2. Add 1 ml InhibitEX Buffer to each stool sample. Vortex continuously for 1 min or until the stool sample is thoroughly homogenized.

3. Incubate at 56°C in a thermal mixer at least 4 hours. Overnight incubation is recommended for old samples. Vortex for 15 s.

4. Centrifuge sample for 1 min to pellet stool particles.

5. 25 µl Proteinase K into a new 2 ml microcentrifuge tube (not provided).

6. 600 µl supernatant from step 4 into the 2 ml microcentrifuge tube containing Proteinase K.

7. Add 600 µl Buffer AL and vortex for 15 s. Note: Do not add Proteinase K directly to Buffer AL. It is essential that the sample and Buffer AL are thoroughly mixed to form a homogeneous solution.

8. Incubate at 70°C for 10 min.

9. Add 600 µl of ethanol (96–100%) to the lysate, and mix by vortexing.

10. Carefully apply 600 µl lysate from step 9 to the QIAamp spin column. Close the cap and centrifuge for 1 min. Place the QIAamp spin column in a new 2 ml collection tube, and discard the tube containing the filtrate. Repeat step 10 until all lysate is loaded.

11. Carefully open the QIAamp spin column and add 500 µl Buffer AW1. Centrifuge for 1 min. Place the QIAamp spin column in a new 2 ml collection tube, and discard the collection tube containing the filtrate.

12. Carefully open the QIAamp spin column and add 500 µl Buffer AW2. Centrifuge for 3 min. Discard the collection tube containing the filtrate.

13. Place the QIAamp spin column in a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge for 3 min.

14. Transfer the QIAamp spin column into a new, labeled 1.5 ml microcentrifuge tube (not provided) and pipet 45 μ l Buffer ATE (or dd H₂O) directly onto the QIAamp membrane. Incubate for 10 min at 56°C, then centrifuge for 1 min to elute DNA.

15. Add 45 μ l Buffer ATE (or dd H₂O) directly onto the QIAamp membrane and incubate for 10 min at room temperature, then centrifuge for 1 min to elute DNA.

If yield will be quantified by UV absorbance, blank the measuring device using Buffer ATE to avoid false results.

Appendix II

Table 6 | Food items in the diet of stone marten (*Martes foina*) in native forests (Native) and Eucalyptus stands (Eucalyptus) expressed as the number of individual items (N), frequency of occurrence (FO), percentage of occurrence (PO) and percentage of consumed biomass (PB).

| Food item | Eucalyptus | | | | Native | | | |
|-------------------------------|------------|-----|-----|-----|-----------|-----|-----|-----|
| | N | FO | PO | PB | N | FO | PO | PB |
| Arthropod (adults) | 21 | 32% | 11% | 1% | 5 | 27% | 14% | 0% |
| Arthropod (larva) | 11 | 9% | 6% | 0% | 1 | 9% | 3% | 0% |
| Arthropod (eggs) | 3 | 5% | 2% | | 0 | 0% | 0% | |
| Mammals | 6 | | 4% | | 2 | | 6% | |
| O. Rodentia | 5 | 23% | 3% | 7% | 2 | 18% | 6% | 23% |
| O. Lagomorpha | 1 | 5% | 1% | 2% | 0 | 0% | 0% | 0% |
| Birds | 3 | 14% | 2% | 1% | 2 | 18% | 6% | 2% |
| Reptile | 4 | 18% | 2% | 7% | 0 | 0% | 0% | 0% |
| Fruits | 148 | | 75% | | 25 | | 71% | |
| <i>Prunus</i> sp. | 3 | 5% | 2% | 5% | 9 | 9% | 26% | 52% |
| <i>Rubus</i> sp. | 143 | 41% | 73% | 76% | 16 | 18% | 46% | 23% |
| <i>Morus</i> sp. | 2 | 5% | 1% | 1% | 0 | 0% | 0% | 0% |
| Carrion (<i>Sus scrofa</i>) | 1 | 5% | 1% | | 0 | 0% | 0% | 0% |
| Total number of scats | 22 | | | | 11 | | | |

Table 7 | Food items in the diet of red fox (*Vulpes vulpes*) in native forests (Native) and Eucalyptus stands (Eucalyptus) expressed as the number of individual items (N), frequency of occurrence (FO), percentage of occurrence (PO) and percentage of consumed biomass (PB).

| Food item | Eucalyptus | | | | Native | | | |
|------------------------------|------------|-----|-----|-----|----------|-----|-----|-----|
| | N | FO | PO | PB | N | FO | PO | PB |
| Arthropod (adults) | 54 | 79% | 33% | 2% | 15 | 80% | 33% | 1% |
| Arthropod (larva) | 5 | 11% | 3% | 0% | 0 | 0% | 0% | 0% |
| Arthropod (eggs) | 38 | 26% | 23% | | 0 | 0% | 0% | |
| Mammals | 9 | | 6% | | 1 | | 2% | |
| O. Rodentia | 7 | 32% | 4% | 12% | 1 | 20% | 2% | 2% |
| O. Insetivora | 1 | 5% | 1% | 2% | 0 | 0% | 0% | 0% |
| O. Lagomorpha | 1 | 5% | 1% | 0% | 0 | 0% | 0% | 0% |
| Birds | 2 | 11% | 1% | 2% | 0 | 0% | 0% | 0% |
| Reptile | 3 | 16% | 2% | 1% | 1 | 20% | 2% | 0% |
| Fruits | 55 | | 33% | | 29 | | 63% | |
| <i>Ficus</i> sp | 2 | 11% | 1% | 1% | 0 | 0% | 0% | 0% |
| <i>Prunus</i> sp. | 17 | 16% | 10% | 61% | 29 | 40% | 63% | 97% |
| <i>Rubus</i> sp. | 19 | 11% | 11% | 15% | 0 | 0% | 0% | 0% |
| Rosaceae | 14 | 21% | 8% | 2% | 0 | 0% | 0% | 0% |
| N.I. | 3 | 16% | 2% | | 0 | 0% | 0% | |
| Total number of scats | 19 | | | | 5 | | | |

Table 8 Food items in the diet of stone marten (*Martes foina*) in the rainy season (Rainy) and in the dry season (Dry) expressed as the number of individual items (N), frequency of occurrence (FO), percentage of occurrence (PO) and percentage of consumed biomass (PB).

| Food item | Rainy | | | | Dry | | | |
|-------------------------------|-----------|-----|-----|-----|-----------|-----|-----|-----|
| | N | FO | PO | PB | N | FO | PO | PB |
| Arthropod (adults) | 8 | 27% | 24% | 1% | 18 | 33% | 9% | 1% |
| Arthropod (larva) | 11 | 13% | 33% | 1% | 1 | 6% | 1% | 0% |
| Arthropod (eggs) | 0 | 0% | 0% | | 3 | 6% | 2% | |
| Mammals | 7 | | 21% | | 1 | | 1% | |
| O. Rodentia | 6 | 40% | 18% | 48% | 1 | 6% | 1% | 4% |
| O. Lagomorpha | 1 | 7% | 3% | 8% | 0 | 0% | 0% | 0% |
| Birds | 4 | 27% | 12% | 7% | 1 | 6% | 1% | 0% |
| Reptile | 2 | 13% | 6% | 36% | 2 | 11% | 1% | 0% |
| Fruits | 0 | | 0% | | 173 | | 87% | |
| <i>Prunus</i> sp. | 0 | 0% | 0% | 0% | 12 | 11% | 6% | 17% |
| <i>Rubus</i> sp. | 0 | 0% | 0% | 0% | 159 | 61% | 80% | 78% |
| <i>Morus</i> sp. | 0 | 0% | 0% | 0% | 2 | 6% | 1% | 1% |
| Carrion (<i>Sus scrofa</i>) | 1 | 7% | 3% | | 0 | 0% | 0% | |
| Total number of scats | 15 | | | | 18 | | | |

Table 9 Food items in the diet of stone marten (*Martes foina*) in the eastern (East) and in the western (West) sampling areas expressed as the number of individual items (N), frequency of occurrence (FO), percentage of occurrence (PO) and percentage of consumed biomass (PB).

| Food item | East | | | | West | | | |
|-------------------------------|-----------|-----|-----|-----|----------|-----|-----|-----|
| | N | FO | PO | PB | N | FO | PO | PB |
| Arthropod (adults) | 20 | 31% | 10% | 1% | 6 | 29% | 15% | 0% |
| Arthropod (larva) | 12 | 12% | 6% | 0% | 0 | 0% | 0% | 0% |
| Arthropod (eggs) | 3 | 4% | 2% | | 0 | 0% | 0% | |
| Mammals | 7 | | 4% | | 1 | | 2% | |
| O. Rodentia | 6 | 23% | 3% | 8% | 1 | 14% | 2% | 16% |
| O. Lagomorpha | 1 | 4% | 1% | 2% | 0 | 0% | 0% | 0% |
| Birds | 3 | 12% | 2% | 1% | 2 | 29% | 5% | 3% |
| Reptile | 4 | 15% | 2% | 7% | 0 | 0% | 0% | 0% |
| Fruits | 141 | | 74% | | 32 | | 78% | |
| <i>Prunus</i> sp. | 0 | 0% | 0% | 0% | 12 | 29% | 29% | 56% |
| <i>Rubus</i> sp. | 139 | 38% | 73% | 80% | 20 | 14% | 49% | 25% |
| <i>Morus</i> sp. | 2 | 4% | 1% | 2% | 0 | 0% | 0% | 0% |
| Carrion (<i>Sus scrofa</i>) | 1 | 4% | 1% | | 0 | 0% | 0% | |
| Total number of scats | 26 | | | | 7 | | | |

Table 10| Food items in the diet of stone marten (*Vulpes vulpes*) in the eastern (East) and in the western (West) sampling areas expressed as the number of individual items (N), frequency of occurrence (FO), percentage of occurrence (PO) and percentage of consumed biomass (PB).

| Food item | East | | | | West | | | |
|------------------------------|----------|-----|-----|-----|-----------|-----|-----|-----|
| | N | FO | PO | PB | N | FO | PO | PB |
| Arthropod (adults) | 30 | 89% | 29% | 7% | 39 | 73% | 36% | 1% |
| Arthropod (larva) | 1 | 11% | 1% | 0% | 4 | 7% | 4% | 0% |
| Arthropod (eggs) | 38 | 56% | 37% | | 0 | 0% | 0% | |
| Mammals | 2 | | 2% | | 8 | | 8% | |
| O. Rodentia | 2 | 22% | 2% | 29% | 6 | 33% | 6% | 4% |
| O. Insetivora | 0 | 0% | 0% | 0% | 1 | 7% | 1% | 1% |
| O. Lagomorpha | 0 | 0% | 0% | 0% | 1 | 7% | 1% | 0% |
| Birds | 0 | 0% | 0% | 0% | 2 | 13% | 2% | 1% |
| Reptile | 1 | 11% | 1% | 0% | 3 | 20% | 3% | 1% |
| Fruits | 32 | | 31% | | 52 | | 48% | |
| <i>Ficus</i> sp | 1 | 11% | 1% | 3% | 1 | 7% | 1% | 0% |
| <i>Prunus</i> sp. | 0 | 0% | 0% | 0% | 46 | 33% | 43% | 92% |
| <i>Rubus</i> sp. | 18 | 11% | 17% | 53% | 1 | 7% | 1% | 0% |
| Rosacea | 11 | 33% | 11% | 8% | 3 | 7% | 3% | 0% |
| N.I. | 2 | 22% | 2% | | 1 | 7% | 1% | |
| Total number of scats | 9 | | | | 15 | | | |