Burnt dinner: how wildfires impact the diet of lizards

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Resumo

Nos últimos anos, tem-se verificado uma mudança acentuada nos padrões dos incêndios florestais, caracterizada por um aumento do número de incêndios, da sua intensidade e da extensão de terreno que afetam. Há muito que o fogo é reconhecido como uma força ecológica e evolutiva importante nas comunidades vegetais, mas a sua influência nos animais, particularmente no que diz respeito às interações bióticas, continua pouco estudada. Esta tese teve como objetivo explorar os impactos dos regimes de incêndios florestais nas interações bióticas, analisando a dieta de *Podarcis lusitanicus* Geniez, Sá-Sousa, Guillaume, Cluchier & Crochet, 2014, uma espécie de lagarto que habita áreas propensas ao fogo no norte de Portugal. Usando o DNA metabarcoding, analisámos a dieta de 12 populações diferentes de lagartos em regiões com diferentes histórias de fogo: áreas recentemente queimadas, áreas queimadas há cerca de 8 anos e áreas não afetadas pelo fogo. O nosso estudo realçou a eficácia do metabarcoding no fornecimento de perfis alimentares detalhados, revelando uma vasta gama de taxa de presas, incluindo oito ordens adicionais de artrópodes anteriormente não detetadas pelos métodos morfológicos tradicionais. Esta abordagem molecular também permitiu a identificação de presas com uma resolução taxonómica mais elevada. Como espécie generalista, *P. lusitanicus* apresenta flexibilidade alimentar, e a nossa investigação revela que, embora a riqueza global da sua dieta não seja afetada pelos incêndios florestais, ocorrem mudanças significativas na composição das suas presas consumidas. Com o aumento do tempo desde a perturbação, a diversidade de presas aumenta conforme o habitat vai regenerando, embora a riqueza de espécies nas áreas queimadas tenha permanecido ligeiramente inferior à das áreas não queimadas, mesmo após oito anos. Os resultados sublinham a adaptabilidade de *P. lusitanicus* às perturbações causadas pelo fogo, refletindo provavelmente a capacidade da espécie para prosperar em ambientes de sucessão pós-fogo. Além disso, este estudo sublinha a importância de estudos de dieta a longo prazo para compreender as consequências ecológicas dos incêndios florestais e as trajetórias de recuperação dos ecossistemas afetados. Estes estudos são também fundamentais para avaliar a diversidade de artrópodes, uma vez que as dietas dos predadores fornecem informações valiosas sobre as comunidades de presas.

Palavras-chave: Ecologia do fogo; Alterações Climáticas; Podarcis, Metabarcoding; Portugal

Abstract

Over recent years, there has been a marked shift in wildfire patterns, characterized by a rise in the number of fires, their intensity, and the extent of land they affect. Fire has long been recognized as an important ecological and evolutionary force in plant communities, but its influence on animals, particularly regarding biotic interactions, remains understudied. The aim of this thesis was to explore the impacts of wildfire regimes on biotic interactions by examining the diet of the lizard *Podarcis lusitanicus* Geniez, Sá-Sousa, Guillaume, Cluchier & Crochet, 2014, a species inhabiting fire-prone areas in northern Portugal. Using DNA metabarcoding, we analysed the diet of 12 different lizard populations across regions with varying fire histories: recently burned areas, areas burned around 8 years ago and areas unaffected by fire. Our study highlighted the effectiveness of metabarcoding in providing detailed dietary profiles, revealing a wide range of prey taxa, including eight additional arthropod orders previously undetected in this lizard by traditional morphological methods. This molecular approach also allowed for the identification of prey at a higher taxonomic resolution. As a generalist species, *P. lusitanicus* exhibits dietary flexibility, and our research reveals that while the overall richness of its diet remains unaffected by wildfires, significant shifts occur in the composition of consumed prey. As time has passed since the disturbance, prey diversity increased as the habitat regenerated, although species richness in burned areas remained slightly lower than in unburned sites even after eight years. The results emphasize the adaptability of *P. lusitanicus* to fire disturbances, likely reflecting the species' ability to thrive in post-fire successional environments. Furthermore, this research underscores the importance of long-term dietary studies for understanding the ecological consequences of wildfires and the recovery trajectories of affected ecosystems. Such studies are also beneficial for assessing arthropod diversity, as predator diets provide valuable insights into prey communities.

Keywords: Fire Ecology; Climate Change; Podarcis; Metabarcoding; Portugal.

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List of Abbreviations

AL	Álvora
AM	Ante Meridiem
ANOVA	Analysis Of Variance
BOLD	Barcode Of Life Data Systems
BU16	Burned In 2016
BU22	Burned In 2022
COI	Cytochrome C Oxidase Subunit I
DNA	Deoxyribonucleic Acid
eDNA	Environmental Deoxyribonucleic Acid
EU	European Union
F	females
GCMS	Global Climate Models
GLM	Generalized Linear Model
GS	Gerês-Soajo
ICNF	Instituto da Conservação da Natureza e das Florestas
IQRS	Interquartile Ranges
M	males
MC	Marco De Canaveses
MTC	Mediterranean-Type Climate
N	North
NCBI	National Center of Biotechnology Information
NGS	Next Generation Sequencing
NW	Northwestern
OTU	Operational Taxonomic Unit
PCR	Polymerase Chain Reaction
PE	Paired-End
PEAR	Paired-End Read Merger
PERMANOVA	Permutational Multivariate Analysis of Variance
PM	Post Meridiem
rRNA	Ribosomal Ribonucleic Acid
SVL	Snout Vent Length
UN	Unburned
VC	Viana Do Castelo

W	West

1. General Introduction

1.1 Natural disturbances in shaping the ecosystems

Disturbance is a fundamental component of ecological systems, affecting terrestrial and aquatic ecosystems at a wide range of scales. Disturbances can alter the state of the system and the trajectory of an ecosystem, and are therefore, key drivers of spatial and temporal heterogeneity (Turner, 2010). Their characterisation is complex since the casual factors can be both endogenous and exogenous, and because they operate over a wide range of frequency, size, predictability, timing (season of the year) and magnitude of impact. As for their origins, disturbances may be abiotic (e.g., hurricanes, tornados, or volcanic eruptions), biotic (e.g., the spread of a non-native pest or pathogen), or both such as fires. Fires are mostly caused by lightning or humans, and occasionally by spontaneous combustion and volcanic activity. The spread and intensity of fire depends not only on abiotic conditions suitable for ignition (e.g., humidity, temperature, and wind) but also on endogenous factors, such as the amount of fuel and its combustibility (Attiwill, 1994; Turner, 2010).

There are biomes around the world that periodically suffer disturbances and have become naturally adapted to them, such as Mediterranean vegetation in response to repeated fire regimes(Keeley et al., 2011a; Pausas & Verdú, 2005), or the adaptation of plants to flood stress (Jackson & Colmer, 2005). The ability of the ecosystems to regenerate after a disturbance depends on the sources of resilience that operate at multiple scales (Hughes et al., 2005).

1.2 Fire as a worldwide disturbance

Wildfires have played a decisive role in shaping the evolution and function of many ecosystems around the world (Ferreira et al., 2019). Approximately one third of the Earth's land surface is affected by intensive and frequent burning, causing substantial and recurrent disturbances to forest ecosystems (Catry et al., 2009; Hosseini et al., 2016; Keeley et al., 2011a). These fires have been occurring for millennia (Pausas et al., 2008), and, more than just local ecological disturbances, they represent a global-scale ecosystem process (Bradshaw et al., 2018; Keeley et al., 2011a).

Fire selectively shapes most of the traits of plants growing in fire-prone environments and has shaped the development of some biomes (Bond & Keeley, 2005; Escudero et al., 2000; Ripa et al., 2023). Fire has played a role throughout the history of land plant evolution and is not exclusively a recent phenomenon; Mesozoic fossils

provide evidence of fire-adaptive traits, and in certain lineages, these traits persist to the present day as adaptations to fire (Keeley et al., 2011b). In fire-prone environments, adaptive characteristics such as the ability to resprout, the formation of fire-resistant structures (serotiny), germination stimulated by heat shock or smoke, allow plants to adjust to a particular fire pattern (Ripa et al., 2023). However, no species is intrinsically "fire-adapted", but rather adapted to a specific fire pattern, which includes frequency, intensity and combustion patterns (Keeley et al., 2011a). Species that exhibit adaptations to a specific fire regime can be threatened when that regime changes. For example, many of the shrub species characteristic of Mediterranean-type climates, are tolerant to periodic high-intensity fires at intervals of several decades or more. However, when the frequency of fires increases, these species can be rapidly decimated (Bradstock, 2008; Keeley et al., 2011a).

Human alteration of fire regimes, particularly through increases in fire frequency, can have significant impacts on plant and animal species (Keeley et al., 2011b; O'Connor et al., 2011). This is evident in Mediterranean-climate ecosystems, where higher population densities are associated with increased fire frequency, posing a conservation concern (Syphard et al., 2009). Similarly, shifts in fire regimes can affect bird species distributions, with predicted increases in fire frequency likely to have a negative impact (Reside et al., 2012). The potential for human control over historic fire regimes is observed in the boreal forest, with different cultures having varying impacts on fire regimes (Granström & Niklasson, 2008). However, in some high-elevation tropical forests, frequent surface fires are a natural and integral part of the ecosystem, and a decrease in fire frequency could lead to negative consequences (Yocom & Fulé, 2012). Although climate continues to be an important factor in global fire regimes, a shift from predominantly climate-controlled to human-controlled fire regimes has been observed around the world in recent decades (Bowman et al., 2013; Marlon et al., 2008).

Climate, biology and human influences are the three main factors that interact in a complex form in contemporary patterns of fire activity. Due to this complexity, it is difficult to make global generalisations about the phenomenon (Bowman et al., 2013). Although local factors such as land use and vegetation type exert a great influence on the occurrence of fires, their impacts are noticeable on a global scale when considering changes in vegetation cover, atmospheric emissions and ecosystem functions and services (Oliveira et al., 2014). In particular, human activity plays a significant role in determining the frequency, extent, and intensity of fires in landscapes (Coughlan & Petty, 2012). Therefore, understanding the biotic and abiotic controls on current fire regimes is

made more difficult by human intervention, which alters landscapes to make them more or less susceptible to fires, as well as directly influencing fire ignition and extinction (Chuvieco et al., 2008; Krawchuk et al., 2009).

1.3. Fire-prone ecosystems: the Mediterranean Basin

The Mediterranean basin, located on the border between the arid subtropics and temperate mid-latitudes, is characterised by high interannual variability with low annual rainfall totals, creating a state of semi-permanent water stress (Tuel & Eltahir, 2020). Because of this, ecosystems in the Mediterranean Basin are highly fire-prone, as are the other four mediterranean-type climate (MTC) regions in the world: central Chile, the Cape Region of South Africa, southwestern Australia, and parts of California and northern Baja California in North America (Figure.1). These geographically distinct Mediterranean ecosystems are classic examples of convergence in ecosystem structure and dynamics, with a similar climatic regime (Cody & Mooney, 1978). An essential feature of Mediterranean climate regions (MTC) is that, during the rainy season, the amount of precipitation exceeds the potential evapotranspiration, allowing enough vegetation to grow to form a continuous vegetation fuel. During the dry summer months, this dry vegetation becomes highly flammable, making these regions prone to recurrent high intensity fires, leading to similar ecological responses to fire (Keeley et al., 2011a).

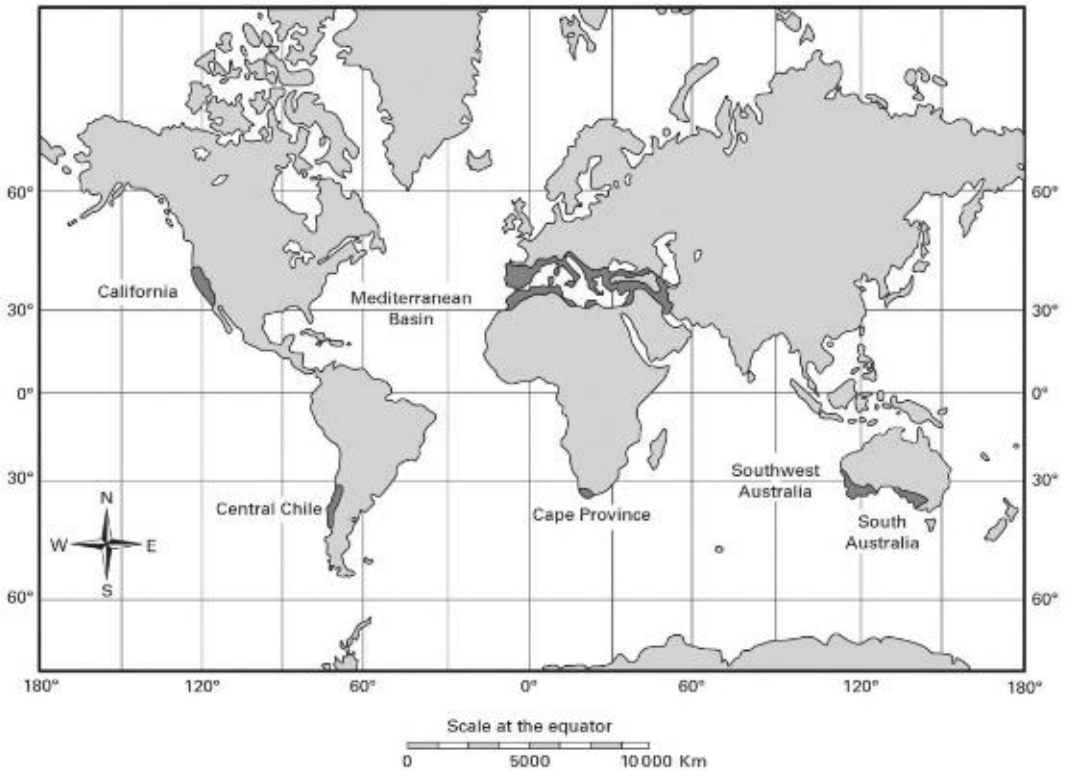

***Figure 1**. The Mediterranean Basin, and the four other mediterranean-type climate (MTC) regions. Adapted from Keeley et al. (2012).*

Fire has played a crucial role in shaping Mediterranean ecosystems over millions of years. Human activity has significantly modified landscapes, influencing the quantity and continuity of available fuel and, consequently, fire regimes, since prehistoric times (Pausas & Fernández-Muñoz, 2012). Transformations in human societies, such as the transition from nomadic to sedentary communities and from pre-industrial to post-industrial eras, together with associated changes in land use, have altered fire regimes throughout history in various landscapes (Delcourt & Delcourt, 1997; Guyette et al., 2002; Keeley, 2002; Pausas, 2004; Pausas & Keeley, 2009). However, in recent decades the changes in fire regime have been rapid. In the Mediterranean basin, the 20th century was marked by a doubling in the occurrence of fires (Le Houérou, 1987), attributed to two factors: anthropogenic fuel changes and climate change (Pausas & Fernández-Muñoz, 2012). Industrialisation resulted in the migration of people from rural areas to industrial urban centres, leading to the abandonment of agricultural practices and a decrease in grazing pressure, without replacement by natural herbivores. This change in land use, combined with the increase in forest plantations, especially of dense conifers, promoted a substantial accumulation of fuel (Pausas & Keeley, 2009). However, today, the area burned is greatly influenced by drought-related climatic conditions (Pausas & Fernández-Muñoz, 2012).

Over the last few decades, the Mediterranean has been identified as particularly sensitive to rising greenhouse gas concentrations in successive generations of global climate models (GCMs), caused by its unique geographical position (Giorgi & Lionello, 2008; Tuel & Eltahir, 2020). These models project a significant reduction in precipitation with an increase in temperature and drought in summer, especially in the northern Mediterranean (Brogli et al., 2019; Giorgi & Lionello, 2008; Planton et al., 2012). These climate changes could result in an increase in the frequency and extent of wildfires, as well as a potential increase in fire danger in large areas of the Mediterranean, with an intensified risk predicted for the end of the century (Arca et al., 2010; Bedía Jiménez et al., 2014). Changes in fire regimes have the potential to cause devastating impacts on the sustainability of many components of Mediterranean ecosystems and can exceed natural adaptations to the previous regime (Pausas & Keeley, 2014).

1.3.1 Wildfires in Portugal: dynamics and causes

Within the EU, Portugal is one of the Mediterranean Member States that periodically undergoes serious damage by wildfires (Mateus & Fernandes, 2014; San-Miguel-Ayanz et al., 2020). The meteorological conditions (episodes of high rainfall followed by dry periods), in conjunction with strong winds, significantly increase the

tendency for natural forest wildfires to occur (Ferreira et al., 2019; Meira Castro et al., 2020; Pausas et al., 2008). However, these natural occurrences of fire in Portuguese forests were only common until the start of the Industrial Revolution in the 19th century. Due to the increasing mechanisation of agriculture and changes in everyday life and social organisation, the traditional use of fire in Portuguese forests began to change from that time onwards (Alves et al., 2006). Later, between the 1950s and 1970s, there was a major change in the fire regime in Portuguese forests, resulting from a significant exodus of the rural population to urban and industrial areas (Meira Castro et al., 2020). As a consequence of these events, there was a progressive abandonment of extensive agricultural areas, as well as a decrease in livestock grazing and in the amount of forest fuels consumed by grazing and firewood collection, which gradually gave way to forest growth and the expansion of large areas of scrubland (Nunes & Lourenço, 2017; Nunes et al., 2016; Nunes, 2012).

These changes in socio-economic patterns have led to a change in the causes of fire ignition in recent decades, which have become predominantly anthropogenic rather than natural in origin, not only in Portugal but also in other Mediterranean countries (Nunes et al., 2016; Parente et al., 2018; Velez, 1993). According to the European Commission (San-Miguel-Ayanz et al., 2018), in the five southern Member States (Portugal, Spain, France, Italy and Greece), the annual number of forest fire occurrences tended to increase between the 1980s and 1990s, partly due to improved recording procedures. This trend continued until the 2000s, when there was a decrease. During this period, Portugal was identified as the country with the highest number of fires (42% of all recorded occurrences).

Human activity and behaviours have been identified as the main causes of wildfire ignitions in Portugal over the last three decades, whether by deliberate actions, negligence, accident or carelessness, accounting for 97% of the fires investigated (Meira Castro et al., 2020). Traditional agricultural practices, such as the use of bonfires to prepare the soil for new crops, dispose of waste and promote the growth of pastures for livestock, also contribute significantly to the problem (Gomes, 2006).

From the dry Mediterranean summer areas in the south to the hyper-humid temperate mountains in the north-west, Portugal has various bioclimatic zones (Mesquita & Sousa, 2009). With very distinct temperature and rainfall patterns, these zones present a clear gradient from north to south (Figure 2) (Fraga et al., 2019). Due to this climatic diversity, the country supports a wide range of vegetation, each adapted to its specific climatic conditions (Lousã, 2004). This diversity, however, is not fully reflected in the composition of its forests. Another relevant factor is that Portuguese forests are predominantly composed by monocultures of pine (*Pinus pinaster*) and eucalyptus (*Eucalyptus* spp.), highly combustible species due to their essential oils, rather than on native species such as oak (*Quercus spp.*), which are better adapted to fire regimes and less flammable (Gomes, 2006). The fire return interval is also crucial for the presence of certain species in forests. Short intervals (< 20 years) favour fast-growing species such as eucalyptus, which is predominant in the country. Higher fire frequencies result in a change in landscapes, which become dominated by scrubland and pastures. Longer intervals (> 40 years) allow for the planting of slow-growing pines or oaks (Mateus & Fernandes, 2014). The mean fire return interval (1975-2005) in Portugal is 36 years, and in most of northern Portugal is less than 25 years (Oliveira et al., 2011). However, areas burned two or more times in the north western mountains of the country are characterized by a fire-free interval of 12-15 years (Fernandes et al., 2012).

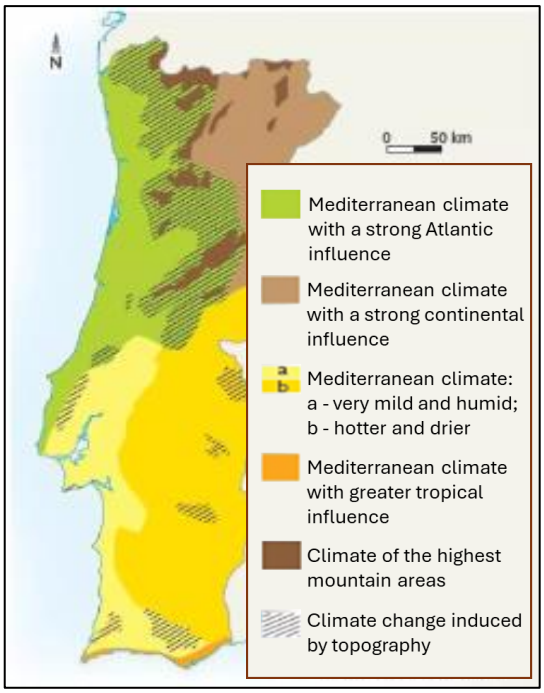

Figure 2. *Portugal's bioclimate zones. Adapted from Medeiros (2005).*

Over the last 18 years, the average percentage of forest area burned in Portugal is approximately 1.02% per year (JRC/EU, 2024), representing the highest incidence of wildfires in Europe (Figure 3), with almost 3 million hectares burned since 1980 (San-

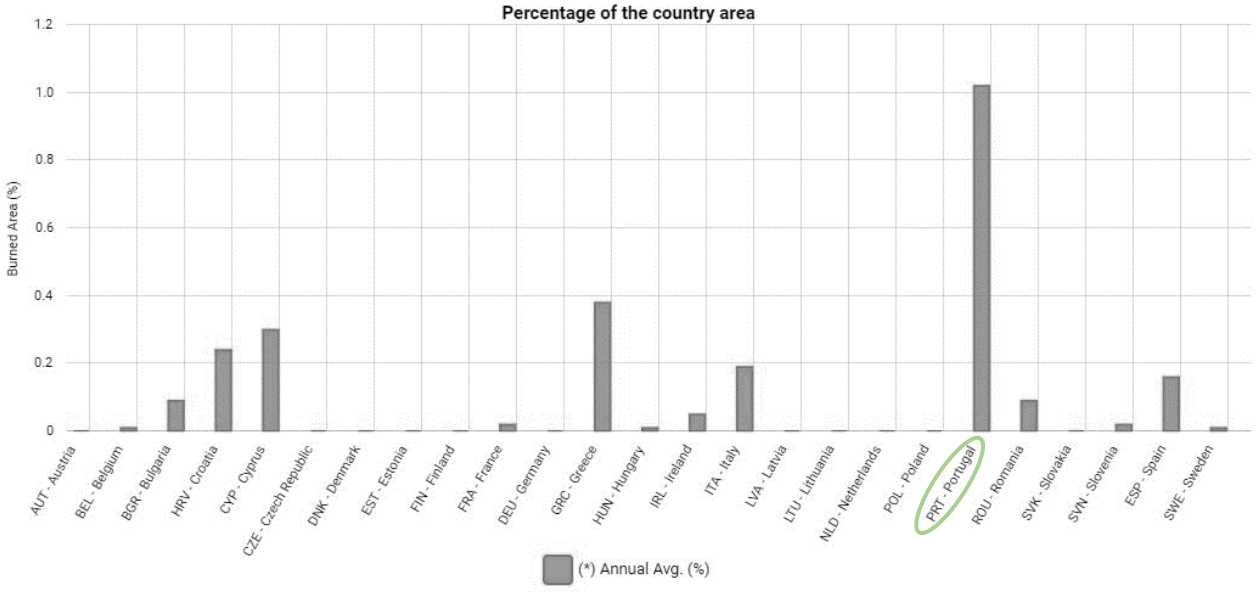

Figure 3. *Average percentage of forest area burned annually from 2006 to 2023 in the European Union. Portugal (green) is the country with the highest percentage of burned area. The highest percentages correspond to countries in the Mediterranean region. Adapted from JRC/UE, 2024. (*) percentage of the country area.*

Miguel-Ayanz et al., 2020). During the period 1980-2010, more than 3.2 million hectares burned in Portugal, representing around 33% of the country's area (Rego and Silva, 2014). Between 2003 and 2005, the area burned reached its highest annual figures since 1980, totalling 750,932 hectares (Martins et al., 2012). Using remote sensing data from 1975 to 2007, it was possible to calculate that the area burned in a single year ranged from 15,000 hectares in 1977 to 440,000 hectares in 2003, with the largest fire covering 58,000 hectares in 2003 (Marques et al., 2011). The year 2017 was the worst year on record, with the Portuguese government recording around 21,000 forest fires, which burned an area of 539,920 hectares. The biggest fire that year covered 296,000 hectares of forest, scrubland and farmland (San-Miguel-Ayanz et al., 2020).

The greatest fire pressures occur in the north of Portugal, where there is the highest frequency of fires and large fires, responsible for most of the country's burned area (Nunes, 2012). This is mainly due to the mountainous topography of the districts above the river Tagus and the predominance of tree species (*Pinus pinaster* and *Eucalyptus* spp.) and shrubs (genera *Erica*, *Calluna*, *Ulex* and *Cytisus*) prone to fire (Meira Castro et al., 2020; Nunes, 2012). Between 2002 and 2023, Viana do Castelo recorded the highest number of fires, followed by Braga and Porto (Figure 4) (GWIS, 2024; Meira Castro et al., 2020).

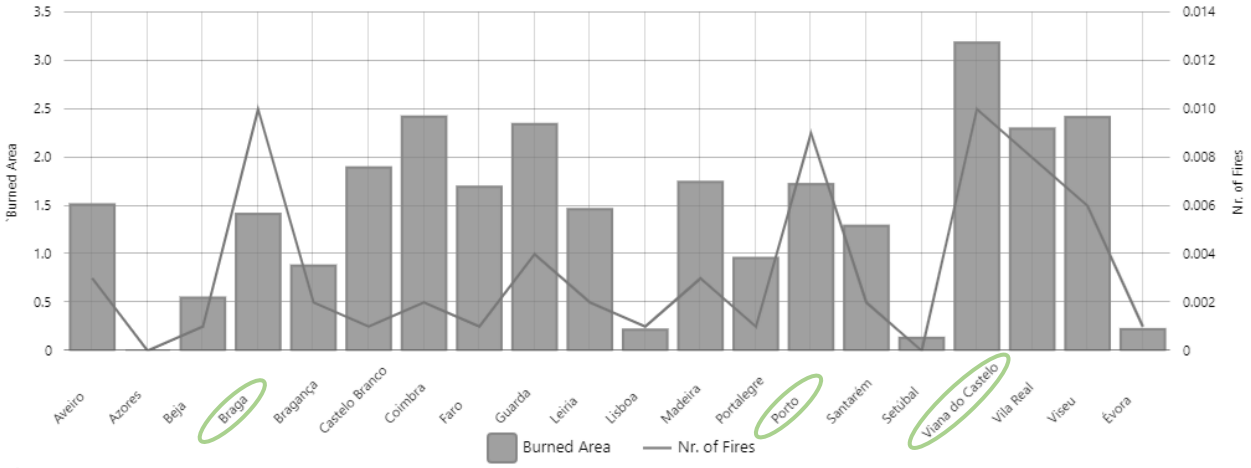

Figure 4. *Average burned area (ha) and average number of fires per region area (Km²) by district in Portugal between 2002 and 2023. The areas more affected by wildfires (green) are in the north part of Portugal. Adapted from GWIS, 2024.*

1.4 Ecological consequences of wildfires: impacts on fauna

Over longer time scales, fire moulds the composition of communities and affects the ecology of populations and the evolution of species, creating resilient and resistant communities, especially due to its great effect on habitat structure (Ferreira et al., 2019). Several researchers have documented the effects of fire on plant communities and revealed the connections between plants and animals following a disturbance caused by fire (Butler et al., 2021; Just et al., 2016; Moyo, 2022). Therefore, fire regimes, vegetation structure and composition, and animals form a direct feedback loop, where fire regimes shape vegetation patterns and vegetation, in turn, affects fire regime attributes (such as frequency and severity). In addition, animals influence vegetation structure and composition, while vegetation modifies animal occupancy, survival and reproduction rates, altering habitat characteristics (Ferreira et al., 2019; Moyo, 2022).

Wildfires are recognised as fundamental disturbance agents, both on a small scale (within the habitat) and on a large scale (ecosystem/landscape) and can have direct and indirect effects on individual organisms. The direct effects result from the fire itself, which causes emigration and/or mortality. Indirect effects result from changes in resource availability (e.g. food and shelter) and habitat structure, leading to the creation of a mosaic of fragmented habitats, variably affected by the intensity of the fire, from unburned to severely burned areas (Izhaki, 2012; Sarà et al., 2006). These changes in the conditions and structure of the environment after the fire determine the temporal and spatial dynamics of the fauna and flora, both in the short and long term (Sarà et al., 2006).

Fire can have positive, negative and neutral effects on soils, organisms and plants (Izhaki, 2012). After a fire, the responses of faunal populations are likely to vary between species with different behaviours or life history characteristics, such as their mode of reproduction (e.g. parthenogenesis), dispersal capacity, morphological adaptations and body size, as well as the characteristics of the fire regimes to which they are exposed, which enable them to persist during the fire (Ferreira et al., 2019; Izhaki, 2012; Moyo, 2022). The specific responses of each species also differ according to the time elapsed since the fire (Izhaki, 2012).

Among the possible deleterious effects of fire are respiratory and neurological disorders due to the inhalation of toxic smoke compounds, physical injuries, emigration or forced immigration into the burned area, and even the death of organisms (Bradshaw et al., 2018). In the long term, fire can have prolonged indirect effects on organisms, altering vegetation succession patterns and the subsequent occurrence of key plants and landscape structure (Bradshaw et al., 2018; Moyo, 2022). This can increase the influence of biotic factors, such as predation, or the invasion of fire-tolerant weed species favoured by fire (Doherty et al., 2022; Letnic et al., 2013). However, studies on vertebrates have shown that fires rarely cause total mortality and that residual populations can remain in burned areas (Banks et al., 2011; Suárez et al., 2012), especially in species specialised in cliffs and rocks, whose habitat is not destroyed by fire (Chuvieco et al., 2008; Ferreira et al., 2019). In addition, some organisms are good dispersers and can often move away from the fire, while others can burrow into the soil (Moyo, 2022). In a recent study, it was shown that fire can increase the abundance and richness of pollinating insects, except for short fire intervals that negatively affect a selected group of Lepidoptera (butterflies and moths) (Carbone et al., 2019). Thanks to their ability to fly, many insects are able to recolonise or forage in burned areas as soon as flowers are present. Ground-dwelling arthropods that survive fire, as well as eusocial insects, that can also benefit from burned environments due to less competition for food resources and a lower risk of predation (Karpestam et al., 2012; Mola et al., 2020). Furthermore, in general, omnivorous fauna tend to recolonise burned sites more quickly than nectar-dependent organisms (e.g. pollinators), since the latter need specific floral resources that may not be readily available immediately after a fire (Kelly et al., 2018).

Many vertebrate species, such as reptiles, birds and mammals, in Mediterranean forests are fire-associated. For these species, recently burned forests offer high quality habitats due to the creation of new environmental conditions. These habitats include open shrublands and post-fire edges, which are favoured by pioneer species. However,

as the vegetation regenerates and succession progresses, many of these pioneer species end up disappearing (Izhaki, 2012). For several reptile species, the benefits of new open habitats after fires are related to their thermal qualities (Santos & Poquet, 2010).

The recolonisation of burned areas occurs mainly in two ways: (1) by the permanence and early arrival and generalist colonisers of the surrounding habitat from earlier post-fire stages; and (2) by the limited contribution of less mobile and specialist species that survive by chance, especially in the larger wildfire fragments present in the burned matrix (Ferreira et al., 2019; Sarà et al., 2006). Although fires reduce the availability of habitat for forest inhabitants, the overall impact of fire is not necessarily negative. Wildfire creates heterogeneous landscapes with forest patches that may be sufficient to sustain forest inhabitants, as well as providing open habitats essential for the maintenance of open habitat species and species that depend on the mosaic of these habitat types (Brotons et al., 2005). Consequently, the most abundant species in burned habitats are typically associated with open areas, while the most abundant species in unburned habitats tend to avoid these areas. However, there are a wide variety of succession paths for vertebrate species, and the responses of specific species depend on the post-fire biotic and abiotic conditions in the burned habitat (Izhaki, 2012).

1.5 Study species: *Podarcis lusitanicus*

Wall lizards lacertids of the genus *Podarcis* Wagler, 1830, are a group of reptiles that evolved and diversified along the Mediterranean Basin (Carretero, 2008; Harris & Sa-Sousa, 2002). They represent an important herpetofauna element of Mediterranean ecosystems, playing an important ecological role in food chains (Carretero, 2004). The genus ranges from Central Europe to the northern fringe of the Sahara in North Africa and from the Iberian Peninsula to Crimea, currently comprising around 27 or 28 species, although not all are universally accepted (Uetz, 2024).

The taxonomy of *Podarcis* has been controversial since it was first described. This group of wall lizards has been studied more from a phylogenetic and phylogeographic point of view than almost any other group of reptiles in Europe (Kaliontzopoulou et al., 2011; Pinho et al., 2006), with the Iberian *Podarcis hispanicus* (Steindachner, 1870) having been considered a species complex (Harris & Sa-Sousa, 2002). *Podarcis guadarramae* (Boscá, 1916), was formally recognized as a species by Geniez et al. (2014), where the subspecies *Podarcis guadarramae guadarramae* was assigned to the eastern populations of the called Type 1B lineage of *Podarcis hispanicus* (Pinho et al., 2006; Pinho et al., 2008), and the western populations of the called Type 1A lineage of *Podarcis*

hispanicus (Pinho et al., 2006; Pinho et al., 2008) were named as a new subspecies, *Podarcis guadarramae lusitanicus* . The later was recently considered as a full species, through a multilocus phylogenetic study (Caeiro-Dias et al., 2021). In that same study, *P. lusitanicus* Geniez, Sá-Sousa, Guillaume, Cluchier & Crochet, 2014 appeared as sister to *P. bocagei* Seoane, 1884, rather than to *P. guadarramae* (Caeiro-Dias et al., 2021). *Podarcis bocagei* and *P. lusitanicus* have been treated as distinct species for a long time, as they maintain easily observable morphological differences in sympatry (Caeiro-Dias et al., 2021). This is not the case with *P. guadarramae* and *P. lusitanicus* as they have no known clearly diagnostic morphological differences (Carretero et al., 2022).

The Lusitanian wall lizard is endemic to the Iberian Peninsula, being widespread in northern Portugal, northwest Spain, and northeast to Picos de Europa (Figure 5) (Speybroeck et al., 2016). In Portugal, *Podarcis lusitanicus* is found in the northwest, reaching the Serra da Estrela region in the south. *Podarcis lusitanicus* and *P. bocagei* are often found in syntopy in the northwest of their range (Loureiro, 2010), although reproductive isolation is almost complete (Arntzen & Sá-Sousa, 2007). The Lusitanian wall lizard tends to use open rocky habitats more frequently than *P. bocagei* (Sillero & Goncalves-Seco, 2014), associated with a greater flattening of the head in *P. lusitanicus* when compared to *P. bocagei*, but not a difference in their locomotor capacity (Kaliontzopoulou et al., 2012a). In Serra da Estrela, the Lusitanian wall lizard can be found in syntopy with *Iberolacerta monticola* (Boulenger, 1905) at sites between 1400

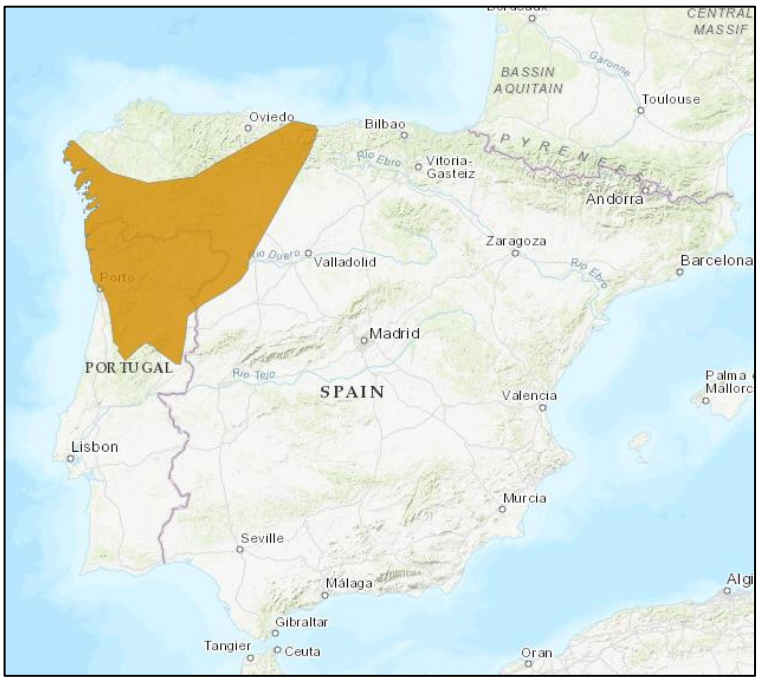

Figure 5. *Geographic range of the wall lizard Podarcis lusitanicus (Bowles, 2024).*

and 1850 meters and with *P. carbonelli* (Pérez Mellado, 1981) at 1450 meters (Carretero et al., 2022).

Podarcis lusitanicus is a saxicolous, insectivorous, diurnal and small lizard, with head and body lengths varying between 41.5 and 62.5 mm in males and between 40 and 60 mm in females (Carretero et al., 2022). The average weight is 2.8 g in adult males and 2.1 g in adult females (Galán, 1986). Males have a predominantly reticulated dorsal pattern, while females show a pattern with longitudinal lines with straight edges (Figure 6). There is also territoriality between males, with the larger ones dominating the smaller ones (Carretero et al., 2022). Behavioural thermoregulation in *P. lusitanicus* is mainly characterized by the adoption of warming postures and alternation between sun and shade (Díaz et al., 1996). *Podarcis lusitanicus* is quite resistant to desiccation, despite being smaller in size and having a higher surface-to-volume ratio compared to *P. bocagei* (Sannolo et al., 2018). These ecophysiological differences seem to contribute to the predominance of *P. lusitanicus* in Mediterranean environments and in burned areas of the north-eastern peninsula (Ferreira et al., 2019).

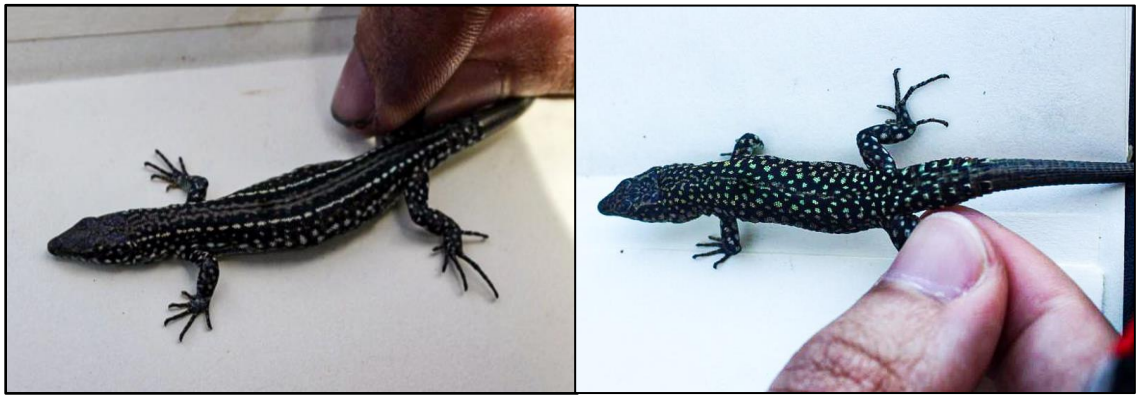

Figure 6. *Differences in patterns between females (on the left) and males (on the right) of Podarcis lusitanicus. Pictures taken by DS Vasconcelos.*

In northern Portugal, this lizard has an irregular distribution, with populations located on open natural rocky outcrops and on artificial stone walls surrounding agricultural fields (Geniez et al., 2014). Due to its low dispersal capacity through vegetated areas, populations tend to become isolated by the forest and shrub matrix (Ferreira et al., 2019). As a result of this requirement for open fields (Ferreira et al., 2017), the Lusitanian wall lizard benefits from recurrently burned landscapes, dominated by granitic rocks, where lizard populations reach high effective sizes (Diego-Rasilla & Perez-Mellado, 2003; Ferreira et al., 2016). The positive response of *P. lusitanicus* to fire in northern Portugal (Ferreira et al., 2018; Ferreira et al., 2016) showed that recurrently burned landscapes offer ecological opportunities for this wall lizard, associated with increased genetic variability in populations exposed to repeated fires. Fire clears vegetation and exposes previously unsuitable rocky outcrops, creating new

habitats for the wall lizard. This dynamic favours not only an increase in the effective size of the local population, but also allows for the dispersal of individuals between metapopulations and the potential recruitment and settlement of migrants from outside the affected area. Mobility and migration can positively increase genetic diversity at a population level. In this way, burned areas can benefit lizard populations, as indicated by the increase in genetic diversity in recurrently burned populations (Ferreira et al., 2019).

1.6 Diet studies

Diet is an essential component of an organism's biology, and variation in dietary characteristics between species plays a key role in various ecological and evolutionary processes (Perez-Cembranos et al., 2016; Rato et al., 2022). Dietary studies can uncover the role of species in food webs and the relevance of food resources to their population sustainability (Alonso et al., 2014). This knowledge is particularly significant in the current scenario of environmental changes, such as the increase in wildfires, which influence the distribution, abundance and diversity of consumers and resources (Thuiller et al., 2011).

Unravelling the details of species' feeding habits can be a challenge, especially in the case of species with highly diverse diets (Alemany et al., 2022). In particular, for omnivorous species that consume a wide range of animal and plant foods, understanding the interactions between predators and prey becomes more complex (da Silva et al., 2019; De Barba et al., 2014; Tercel et al., 2021). In lizards, the diet can be complex, with dietary variations between species depending on evolutionary history, microhabitat characteristics and prey availability (Pianka & Vitt, 2003). Most lizards are generalist predators, consuming a diversity of food items, including plants, although they are predominantly opportunistic insectivores (Alemany et al., 2023). Foraging ecology involves a balance between the benefits of acquiring prey and energy (such as caloric value, water content, nutrients and secondary compounds) and the costs associated with doing so (such as the risk of predation, time for other activities and food digestibility) (Rato et al., 2022). In lizards, there are two foraging strategies within a recognized continuum, each offering different advantages depending on the situation: active foraging and the "sit and wait" strategy (Perry, 1999; Pianka & Vitt, 2003). Active foragers move over large areas and actively search for their prey, which leads them to find sedentary, aggregated and unpredictably distributed prey. In contrast, "sit and wait" predators remain in a fixed location, waiting for more active prey to approach, and then make a quick attack to capture them (Huey & Pianka, 1981; Pereira et al., 2019;

Verwaijen & Van Damme, 2007). Consequently, the types of prey consumed can vary depending on the foraging strategy adopted.

The traditional methodologies used for the taxonomic identification of the diversity of prey consumed by animals are not always adequate, as they often do not allow for the complete identification of the food items ingested (De Barba et al., 2014). During the first diet studies, assessment was carried out through direct observation in the field, which posed a significant challenge, especially in relation to generalist predators (Pompanon et al., 2012) or when the prey was difficult to identify visually (Kartzinel & Pringle, 2015). In lizard diet studies, the morphological identification of prey in stomach contents, often obtained by sacrificing animals (Díaz & Carrascal, 1990) or from specimens preserved in herpetological collections (Pianka, 1986), has traditionally been the most common approach. As alternatives to these methods, techniques such as stomach washing (Luiselli et al., 2011) and the analysis of faecal samples (Perera et al., 2006) have also been used. These methods have significant limitations, as they require extensive taxonomic knowledge to identify prey items from undigested partial remains (Pereira et al., 2019). In addition, due to their high digestibility, soft-bodied prey often goes undetected and therefore, are not included in diet analyses (Pompanon et al., 2012). In fact, the lack of a clear trophic niche structure observed in many lizards may be related to the difficulty of identifying prey at more specific taxonomic levels than broad categories such as order or family (Luiselli, 2008), due to an oversimplification of the diversity of prey consumed, especially in taxonomically complex groups (Hawlena & Pérez-Mellado, 2009; Pereira et al., 2019). Furthermore, analysing stomach contents by dissection raises ethical issues, as it requires the sacrifice of the specimens studied, which is especially problematic when dealing with protected or endangered species. Similarly, the stomach washing technique, although less lethal, is still considered invasive and can cause stress or harm to the animals, making it unsuitable for studying protected species (Alemany et al., 2023).

Recent advances in high-throughput DNA sequencing techniques have made it possible to generate a large number of sequences in a single analysis. Next-generation sequencing (NGS) platforms introduce new approaches to assess biodiversity, with the potential to revolutionise scientific knowledge in the areas of genomics and transcriptomics (Pereira et al., 2019). Initially applied mainly in microbiology, this technology is becoming increasingly common in the identification of plants, invertebrates and vertebrates, from DNA extracted from heterogeneous samples or environmental DNA (Deagle et al., 2014). The technique of amplifying DNA barcodes from DNA

mixtures is known as "metabarcoding" (Yu et al., 2012). Currently, DNA metabarcoding offers a fast and effective alternative to traditional microscopy methods, making it possible to detect prey DNA in digested samples and proving efficient at identifying previously undetected prey groups (Jarman et al., 2013; Sousa et al., 2016), which makes it extremely useful for diet studies.

1.7 DNA Metabarcoding

Due to the limited number of professional taxonomists available and with millions of species in the world, species identification has become an increasingly complex challenge. Furthermore, the task of identification becomes even more difficult for taxonomists when specimens are damaged, poorly preserved or in immature stages of development (Pompanon et al., 2012). However, the use of genetic barcoding makes it possible to effectively identify a specimen with a small amount of tissue.

Molecular scatology is a genetic technique that analyses faeces to study animal ecology and is considered a type of environmental DNA sample (Ando et al., 2020). Environmental DNA is a complex mixture of genetic material that can be degraded and comes from environmental samples such as soil, water or air, without the need to directly isolate the DNA of the target organisms (Taberlet et al., 2012). Since the 1990s, faecal samples have been widely used to obtain genetic information in a non-invasive way, helping to estimate phylogeny, geographical distribution and population size (Kohn & Wayne, 1997). Simultaneously, molecular techniques have been developed to detect food from the DNA present in faeces, overcoming the limitations of traditional morphological methods. The first DNA-based diet studies identified specific foods using PCR amplification targeted at each taxon. Later, Sanger cloning and sequencing were applied to isolate food DNA sequences from faecal DNA mixtures, identifying them through DNA barcode databases (Ando et al., 2020). Although this method allowed for more accurate taxonomic identification, it was expensive and inefficient for processing a large number of samples, which is necessary in large-scale biodiversity studies (Ando et al., 2020; Yu et al., 2012). This challenge was overcome with the introduction of next-generation sequencing, which enabled the development of "DNA metabarcoding" (Alemany et al., 2022). This high-throughput sequencing technology makes it possible to process hundreds or thousands of samples in a short period of time, revolutionizing the ability to monitor biodiversity efficiently and on a large scale (da Silva et al., 2019).

This technique makes it possible to identify specimens of unknown origin by comparing a short, standardized DNA sequence, known as a DNA barcode, with a library of barcodes from organisms whose taxonomy is known (Bell et al., 2016; Deiner et al.,

2017; Wilson et al., 2019). This allows for precise identification of the specimen, which can go down to family, genus or even species level (Pereira et al., 2019). In addition, metabarcoding makes it possible to identify practically all the species consumed by a predator or herbivore (Hope et al., 2014; Nielsen et al., 2018), including those that are rare or have a soft body (da Silva et al., 2019). However, the effectiveness of this identification depends on the quality of the DNA available and the existence of DNA reference databases. The process involves the mass collection of samples, which are then homogenized to extract the genomic DNA. This DNA is amplified in mass using PCR (Polymerase Chain Reaction) for the specific barcode gene and subsequently sequenced on machines capable of distinguishing individual DNA molecules (Williams et al., 2014). Bioinformatics tools are then used to process the large volume of sequences generated, reducing it to a manageable, high-quality dataset suitable for further analysis (Yu et al., 2012).

The design of universal PCR primers is one of the most critical factors in using DNA metabarcoding to assess species diversity, as these primers must amplify the target group without amplifying other taxa present in the environmental samples (Elbrecht et al., 2016). The choice of genetic markers should be guided by the question being addressed and knowledge of the species being studied (Deagle et al., 2014; Pompanon et al., 2012). This choice becomes even more challenging in the case of omnivorous diets (da Silva et al., 2019). By using taxon-specific primers, high taxonomic resolution is expected, and markers with smaller fragment sizes, usually less than 300 bp, are used for more accurate prey detection (Kartzinel & Pringle, 2015). The region most commonly used as a DNA barcode in animals is subunit I of the mitochondrial cytochrome c oxidase (COI) gene, which is particularly useful in metabarcoding because it is almost universal among animals, with the exception of a few protozoa, while its substitutions allow the differentiation at the species level (Hebert et al., 2003). This gene also has a higher substitution rate compared to others, such as ribosomal RNA (rRNA), which improves taxonomic resolution. Thus, it has been possible to develop extensive taxonomically verified reference databases for DNA metabarcoding (Yu et al., 2012). Although there is a lack of consensus regarding some groups, the vast amount of sequence data available in public databases such as BOLD and GenBank makes the use of COI markers advantageous (Wilson et al., 2019).

A review conducted by Ando et al. (2020) revealed that the number of annual publications in international journals related to metabarcoding studies on faecal samples has increased significantly in the last 10 years, with a particularly pronounced increase

from 2017 to 2018, when the number of publications almost doubled. The review also indicated that mammals were the most studied group, accounting for more than half of the annual publications, while invertebrates were the least investigated group. This disparity can be attributed to difficulties in extracting DNA from small quantities of faecal samples, as well as the difficulty in finding primers capable of amplifying dietary items without simultaneously amplifying the host's DNA in the case of invertebrates.

Although metabarcoding solves many of the problems associated with traditional species identification, it also presents several challenges. Among the main challenges are processing and analysing the large volumes of data generated, as well as the difficulty of applying this data in practical contexts. Another significant problem is the need to minimize errors, such as false positives, which can occur due to cross-contamination and technical errors, something common in many ecological studies. In addition, metabarcoding only indicates the presence of certain species in the samples, without providing information on their relative abundances. Another challenge is the possibility that the sequences obtained may be biased in favour of certain taxonomic groups, depending on the markers used during amplification. It is therefore essential to use appropriate controls, carry out replications and validate the results to ensure accuracy in metabarcoding approaches (Alberdi et al., 2019; da Silva et al., 2019; Deagle et al., 2014; Piper et al., 2019).

1.8 Diet studies in *Podarcis lusitanicus*

The lizards of the Lacertidae family are typical examples of generalists, feeding mainly on a variety of terrestrial invertebrates (Arnold, 1987; Sagonas et al., 2014). However, some lacertids can be omnivores or even herbivores (Martin et al., 2005; Pérez-Mellado & Corti, 1993; Vervust et al., 2010). Although lizards can include plants in their diet, this usually occurs only when arthropods, their main food source, are not enough to meet their energy needs. For this reason, lizards living on islands are expected to consume more plant material than those living on the mainland (Van Damme, 1999).

Previous studies on the diet of *P. lusitanicus* have used morphological identification through stomach contents (Bas, 1982; Pérez Mellado, 1982) or pellets (Kaliontzopoulou et al., 2012b). They revealed that the main prey groups include Hemiptera, Coleoptera, Araneae, Hymenoptera and Diptera. To a lesser extent, Orthoptera, Lepidoptera (including larvae), Opiliones, Miriapoda, Isopoda, Collembola, Diptera larvae, Trichoptera larvae, Mecoptera, Blattodea, Thysanoptera, Dermaptera, Embioptera, as well as vertebrates, Oligoquetes and Gasteropods were also identified (Bas, 1982; Braña, 1984; Galán, 2003; Kaliontzopoulou et al., 2012a; Pérez Mellado, 1982).

An efficient COI primer set for eDNA or gut content samples is “fwhF2” in combination with “fwhR2n”, which generates a 205 bp amplicon and stands out for its high efficiency in taxonomic recovery (Bylemans et al., 2018). Although the use of multiple primer sets or marker genes has been proposed to improve taxon recovery (Alberdi et al., 2018; Zhang et al., 2018), in the case of terrestrial arthropods, a single well-designed primer set can be sufficiently effective, making the use of multiple sets unnecessary. This primer set showed effective performance in metabarcoding terrestrial arthropod samples (Elbrecht et al., 2019). Although the 16S marker is also used in diet studies (Pereira et al., 2019), a bias was reported in the identification of Araneae, a group of arthropods with a high incidence in the diet of *P. lusitanicus* (Pereira et al., 2019). In addition, the abundance of sequence data available in public databases favours the use of COI markers over others (Wilson & Primack, 2019).

1.9 Research objectives

The main aim of this thesis is to use the well-document fire regime in Northern Portugal as a natural laboratory to assess the impact of fires on the diet of the Lusitanian wall lizards, by applying a metabarcoding approach. With the fire regime shift due to climate change, the urge to understand how these ecosystems are affected arises, especially considering the limited knowledge currently available. Fires in this particular geographic region are an ideal system to study the impact of this disturbance, due to two factors: 1) their frequent occurrence in certain regions and 2) the precise delimitation of the affected areas and the detailed recording of the time of occurrence. Lizards have been model organisms for ecological and evolutionary studies, from the individual to the community level, because they share attributes relevant to the study of many biological processes and are often abundant and easy to manipulate, which has led to an accumulation of knowledge on demography, adaptive ecomorphology and ecophysiology (Camargo et al., 2010). Furthermore, in the particular case of *Podarcis lusitanicus*, even in cases of small-scale fires it is possible to collect samples from the same locality in both burned and unaffected areas, enabling direct comparisons.

These data will allow us to analyse how and if fires impact the diet of these lizard species and, as a proxy, the diversity of prey species. Moreover, by sampling repeatedly burned areas, the obtained results will allow the assessment of whether ecological resilience leads to a reduction in differences over time since the disturbance occurred (i.e., since the last fire).

2. Manuscript I

Burnt dinner: how wildfires impact the diet of lizards

Abstract

In recent years, there have been significant changes in wildfire regimes, including an increase in the number of fires, their severity and the area affected. Fire has long been recognized as an important ecological and evolutionary force in plant communities, but its influence on animals, particularly regarding predator-prey interactions, remains understudied. This study focuses on the impact of wildfires on the diet of *Podarcis lusitanicus* Geniez, Sá-Sousa, Guillaume, Cluchier & Crochet, 2014, a lizard species inhabiting a fire-prone region in the Iberian Peninsula. Faecal samples were collected from lizards in Portugal from recently burned areas, in areas burned around 8 years ago and in areas unaffected by fire, with four replicate areas for each of the three fire regimes within Northern Portugal. The lizards' diet was analysed using a metabarcoding approach, employing high-throughput sequencing. As a generalist species, *P. lusitanicus* exhibits dietary flexibility, and our research reveals that while the overall richness of its diet remains unaffected by wildfires, significant shifts occur in the composition of its consumed prey. The results show the resilience of the species and its ability to adapt to environmental changes induced by fires. The subtle variations in diet composition indicate that ongoing ecological transformations are taking place. This study further demonstrates the advantages of using DNA metabarcoding over traditional morphological methods to analyse diet. DNA metabarcoding not only provides a more detailed and accurate picture of predator-prey dynamics but also opens up new ways to investigating ecological interactions in fire-prone ecosystems with greater precision and gives notable insights on the distribution of prey taxa within the study region.

Key words: Fire Ecology; Climate Change; Podarcis; Metabarcoding; Portugal.

Introduction

Disturbance is a fundamental component of ecological systems that alter the state of the system and the trajectory of an ecosystem and are therefore key drivers of spatial and temporal heterogeneity (Turner, 2010). Caused by both biotic and abiotic factors, wildfires affect approximately one third of the Earth's land surface, causing substantial and recurrent disturbances to forest ecosystems (Catry et al., 2009; Hosseini et al., 2016; Keeley et al., 2011). Occurring for millennia (Pausas et al., 2008), there are biomes around the world that are naturally disturbance-prone adapted, such as the Mediterranean vegetation, which is well adapted to a repeated fire regime (Keeley et al., 2011; Pausas & Verdú, 2005). However, no species is intrinsically "fire-adapted", but rather adapted to a specific fire pattern, which includes frequency, intensity and combustion patterns (Keeley et al., 2011). These adaptations to a specific fire regime can be threatened when that regime changes. Human alteration of fire regimes, particularly through increases in fire frequency, can have significant impacts on plant and animal species (Keeley et al., 2011; O'Connor et al., 2011). This is evident in Mediterranean-climate ecosystems, where higher human population densities are associated with increased fire frequency, posing a conservation concern (Syphard et al., 2009). While the occurrence of fires is strongly influenced by local factors such as vegetation cover and land use, their impacts are evident on a global scale when considering atmospheric emissions, changes in vegetation cover and ecosystem functions and services (Oliveira et al., 2014).

Wildfires can have a direct effect, such as emigration and/or mortality, and indirect effects on individual organisms. The indirect consequences result from changes in resource availability (e.g. food and shelter) and habitat structure, with fires often leading to the creation of a mosaic of fragmented habitats (Izhaki, 2012; Sarà et al., 2006). These changes in the conditions and structure of the environment after the fire determine the temporal and spatial dynamics of the fauna and flora, both in the short and long term (Sarà et al., 2006). After a fire, the responses of faunal populations are likely to vary between species with different behaviours depending on the life history characteristics and time elapsed since the fire (Izhaki, 2012). Wildfires rarely result in total mortality among vertebrates (Banks et al., 2011; Suárez et al., 2012), allowing remnant populations to survive in burned areas, especially in species adapted to cliffs and rocks, whose habitats typically remain intact after the fire (Chuvieco et al., 2008; Ferreira et al., 2019), or thanks to the dispersal or burrowing capacity of some organisms (Moyo, 2022). After the fire, many insects, due to their flight capacity, are able to quickly recolonize affected areas, while surviving arthropods may benefit from the post-disturbance

environment through diminished competition or a reduced risk of predation (Karpestam et al., 2012; Mola et al., 2020). In general, omnivorous fauna tend to recolonize these areas more quickly, as they do not depend on specific resources (Kelly et al., 2018). In that sense, diet studies become an important tool in understanding species interactions. This is especially relevant in the current scenario of increased wildfires, to understand the impact of these alterations on the prey communities and how these affect the lizard populations (Alemany et al., 2022).

The Mediterranean climate is characterized by episodes of high precipitation followed by dry periods (Syphard et al., 2009), resulting in plant growth sufficient to produce contiguous fuel loads that are highly flammable during the summer drought, making these regions highly fire-prone (Keeley et al., 2011). Fire has played a crucial role in shaping Mediterranean ecosystems over millions of years. However, in recent decades the changes in fire regime have been rapid. In the Mediterranean basin, the 20th century was marked by a doubling in the occurrence of fires (Le Houérou, 1987), attributed to two factors: anthropogenic fuel changes and climate change (Pausas & Fernández-Muñoz, 2012). Global climate models project a significant reduction in precipitation with an increase in temperature and summer drought, especially in the northern Mediterranean (Brogli et al., 2019; Giorgi & Lionello, 2008; Planton et al., 2012). These climate changes could result in an increase in the frequency and extent of wildfires, as well as a potential increase in fire danger in large areas of the Mediterranean, with an intensified risk predicted for the end of the century (Arca et al., 2010; Bedía Jiménez et al., 2014).

Within the European Union, Portugal is one of the Mediterranean Member States that periodically faces serious damage due to wildfires (Mateus & Fernandes, 2014; San-Miguel-Ayanz et al., 2020). Climatic conditions, such as episodes of high rainfall followed by dry periods, coupled with strong winds, significantly increase the likelihood of natural wildfires (Ferreira et al., 2019; Meira Castro et al., 2020; Pausas et al., 2008). However, changes in socio-economic patterns in recent decades have resulted in a change in the causes of ignition, which have become predominantly anthropogenic rather than natural, both in Portugal and in other Mediterranean countries (Nunes et al., 2016; Parente et al., 2018; Velez, 1993). Over the last three decades, human activity and behaviour have been identified as the main causes of wildfire ignition in Portugal, whether through deliberate actions, negligence, accidents or carelessness, accounting for 97% of the fires investigated (Meira Castro et al., 2020). Traditional agricultural practices, including the use of bonfires, as well as the predominance of monoculture forests composed of highly

flammable species (such as pine trees and eucalyptus), play a significant role in intensifying the problem (Gomes, 2006). The greatest pressure occurs in northern Portugal, harbouring the highest frequency of fires and large fires, responsible for most of the country's burned area (Nunes, 2012).

Wall lizards, lacertids of the genus *Podarcis* (Wagler 1830), are a group of reptiles that evolved and diversified along the Mediterranean Basin (Carretero, 2008; Harris & Sa-Sousa, 2002). They represent an important herpetofauna element of Mediterranean ecosystems, playing an important ecological role in food chains (Carretero, 2004). The Lusitanian wall lizard *Podarcis lusitanicus* Geniez, Sá-Sousa, Guillaume, Cluchier & Crochet, 2014 is endemic to the Iberian Peninsula, being widespread in northern Portugal, northwest Spain, and northeast to Picos de Europa (Figure 7) (Speybroeck et al., 2016). *Podarcis lusitanicus* is a saxicolous, insectivorous, diurnal and small lizard that tends to use walls, logs and areas with less vegetation for thermoregulation, foraging and shelter (Carretero, 2008). Across its Portuguese range, this lizard has an irregular distribution, with populations located on open natural rocky outcrops and on artificial stone walls surrounding agricultural fields (Geniez et al., 2014). As a result of this requirement for more open areas (Ferreira et al., 2017), *P. lusitanicus* seem to benefit from recurrently burned landscapes, dominated by granitic rocks, where lizard populations reach high effective sizes (Diego-Rasilla & Perez-Mellado, 2003; Ferreira et al., 2016). Fire removes vegetation and reveals rocky outcrops, creating new habitats, which favours population expansion, dispersal between metapopulations and the

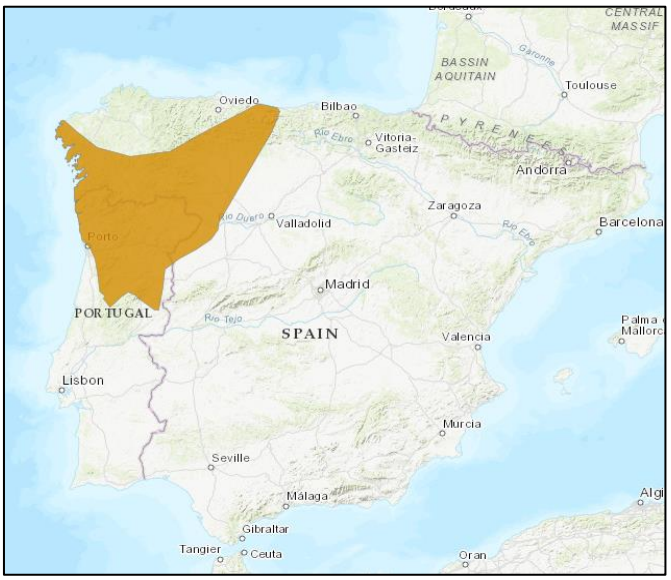

Figure 7. *Geographic range of the wall lizard Podarcis lusitanicus (Bowles, 2024).*

recruitment of migrants. Consequently, burned areas can benefit some lizard populations by increasing genetic diversity (Ferreira et al., 2019).

In this study, in order to assess the impact of fires on the diet of the Lusitanian wall lizard, we used the fire regime as a natural laboratory that is well documented in northern Portugal, applying a metabarcoding approach. Fires in this particular geographical region are an ideal system for studying the impact of this disturbance, due to two factors: their frequent occurrence in certain regions; and the precise delimitation of affected areas and detailed recording of the time of occurrence. Lizards have been widely used as model organisms in ecological and evolutionary studies, from individual analyses to the community level, as they possess important characteristics for understanding various biological processes (Camargo et al., 2010). Furthermore, even in the case of small-scale fires, in the particular case of *Podarcis lusitanicus*, it is possible to collect samples from the same locality in areas affected by the fire and unaffected areas, making it possible to make direct comparisons. This data will provide a detailed analysis of the impact of the fires on the diet of this lizard species, as well as on the diversity of its prey. In addition, by repeatedly sampling areas that have suffered from wildfires, we will be able to assess whether ecological recovery reduces differences over time since the disturbance occurred (i.e. since the last fire).

Material and Methods

Study area

This study was performed in northwestern Portugal confined by the latitudes 41º58' to 41º09' N and longitudes -8º49' to -7º56' W (Figure 8), a region of the country particularly affected by frequent fire regimes (Nunes, 2012).

While locations of fires can be identified through fieldwork or through historic records, these can also be selected using records collected by satellites. Here, we used this approach, known as remote sensing. Remote sensing, using multispectral information collected by satellites, offers the possibility of obtaining data with medium and high spatial resolution. Recent studies using remote sensing show a high correlation for both burned areas and levels of burn severity, when compared with direct evidence from field work (Llorens et al., 2021). Four locations were chosen, corresponding to Viana do Castelo (VC), Marco de Canaveses (MC), Gerês-Soajo (GS) and Álvora (AL) (Figure 8), based on previous knowledge regarding the last fires that took place there, and because they harboured similar characteristics before the fire (such as topography).

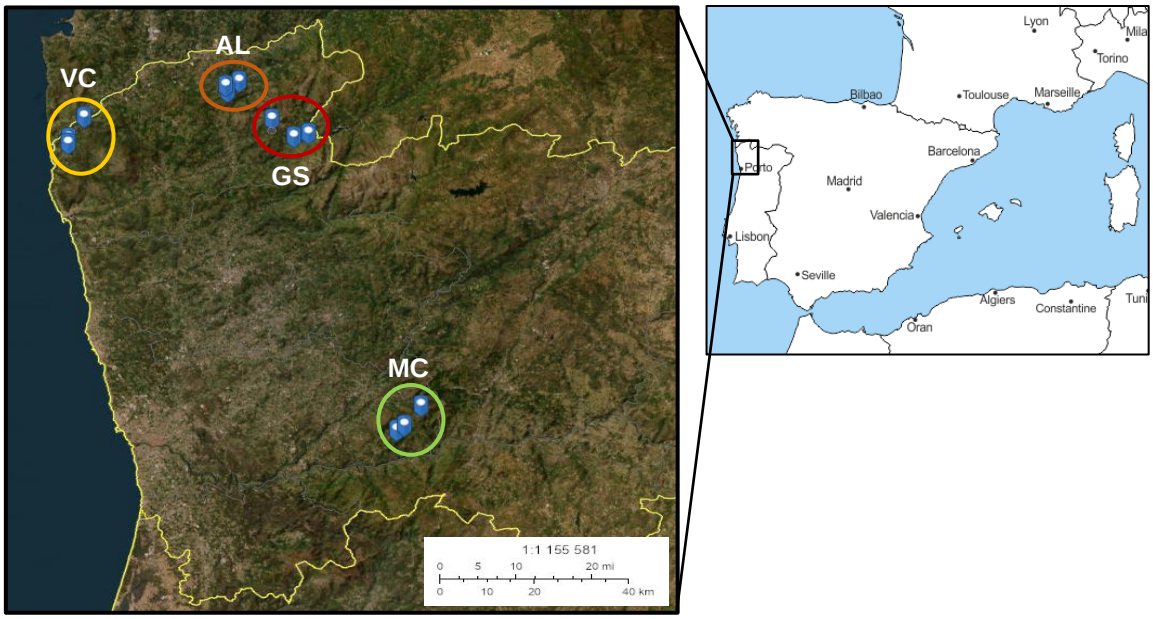

***Figure 8**. The study area is located in the NW extreme of Portugal, the area most affected by fires in Europe (Bowles, 2024; Nunes, 2012). Each location sampled - Viana do Castelo (VC, yellow), Álvora (AL, orange), Gerês-Soajo (GS, red) and Marco de Canaveses (MC, green)- is formed by three populations each with different historic fire conditions.*

At each site we selected a region that burned recently – burned 2022 (BU22) –, another that burned 8 years ago – burned 2016 (BU16) –, and an unburned site (no fires recorded since 2015) – unburned (UN)-, meaning that we have a total of 12 populations (Figure 9). Therefore, this study had four replicates (i.e., locality), each harbouring a distinct fire regime. This sampling scheme is feasible as it is carried out in a small area, with BU16, BU22 and UN in proximity with each other (< 10Km radius), reducing possible variables caused by distance, and each has an abundance of *Podarcis lusitanicus*. After identifying the sites using the remote sensing database of the Portuguese Institute for Nature Conservation and Forests (ICNF; https://geocatalogo.icnf.pt/catalogo_tema5.html), each site was visited prior to the study to confirm that they showed the appropriate characteristics, and that the overall landscapes were similar.

Sampling methods

Sampling of *Podarcis lusitanicus* was carried out during the day (10 AM - 3 PM) in May and June 2023, where they could be found on natural open rocky outcrops and artificial rock walls surrounding agricultural fields. *Podarcis lusitanicus* is often found in syntopy with *P. bocagei* in the northwest of their range (Loureiro, 2010). However, *P. lusitanicus* tends to use open rocky habitats more exclusively than *P. bocagei* (Carretero et al., 2015). Therefore, the sites chosen for sampling were carefully selected and characterised by being open rocky areas. While there is no known syntopy between the

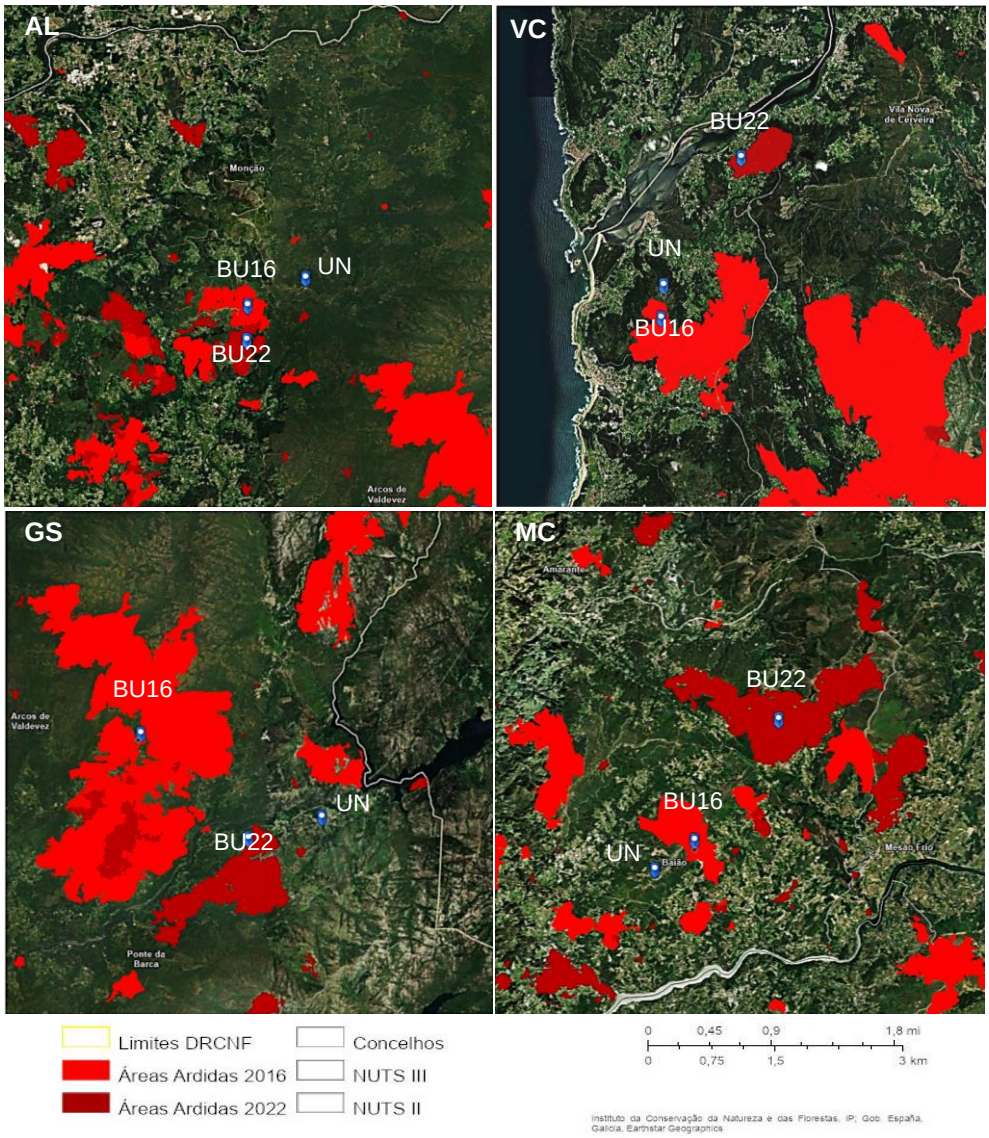

Figure 9. *Sampling sites of the four locations - Álvora (AL), Viana do Castelo (VC), Gerês-Soajo (GS) and Marco de Canaveses (MC) -, with 3 opposite conditions: unburned (UN), burned in 2016 (BU16) - bright red- and burned in 2022 (BU22) - dark red. The pinpoints correspond to the centre of the areas sampled.*

species at the sites sampled, the diagnostic morphological characters, such as size, colour pattern and head shape, were carefully checked to avoid misidentification of the species, with subsequent genetic confirmation using the tail samples taken.

At each of the designated sites, lizards were captured with a noose at the end of a fishing rod, a method widely used by herpetologists because it is both effective and does not harm the animal during capture. After capture, the lizards were removed from the noose as quickly as possible to cause them the least possible stress. Generally, lizards defecate soon after capture, otherwise a simple abdomen massage is enough to obtain a faecal sample. All faecal samples were preserved in 96% ethanol for later diet analysis. A total of 183 *P. lusitanicus* pellets were collected during field work: 38 in Viana do Castelo, 50 in Marco de Canaveses, 53 in Álvora and 42 in Gerês-Soajo (Table 1).

Table 1. *Samples size of each population*

Population	Sample size
Álvora UN	17
Álvora BU16	20
Álvora BU22	16
Gerês- Soajo UN	19
Gerês- Soajo BU16	13
Gerês- Soajo BU22	10
Marco de Canaveses UN	11
Marco de Canaveses BU16	15
Marco de Canaveses BU22	24
Viana do Castelo UN	18
Viana do Castelo BU16	7
Viana do Castelo BU22	13

The tail tip of each individual was collected and stored in 96% ethanol at room temperature. Sex was determined by the presence of developed femoral pores, and the robustness of the head (Barata & Harris, 2015; Perera et al., 2006), as well as standard morphological measurements, including weight and snout ventral length (SVL) using a digital scale and calliper, respectively. Photographic documentation was also conducted. All measurements were taken by the same person (D.S. Vasconcelos) to eliminate inter-observer error. Afterwards, the captured individuals were released back at the capture site.

DNA Extraction and amplification

DNA extraction was performed in a non-invasive extraction room to minimise cross-contamination. This room has a controlled positive pressure environment designed to limit contamination from the outside environment.

The 183 pellet samples were extracted using the E.Z.N.A. Tissue DNA Kit (Omega Bio-Tek, U.S.A.), following the manufactures' instructions with a minor modification in the digestion step of using 800 µL of Gordon buffer, instead of the 200 µL of TL buffer (from the kit) to improve DNA extraction of both hard and soft tissue (Maudet et al., 2004). All samples were vortexed to disrupt the faecal mass and digestion occurred over 3 hours. Extracted DNA was eluted twice with 50 µL of Elution buffer and stored at -20ºC. Eight negative control samples were also obtained and stored at -20ºC. To evaluate the success of the extractions, an electrophoresis in 0.8% agarose gel stained with GelRed (Biotium, U.S.A.) was conducted, and a DNA ladder NZYDNA Ladder V (NZYTech, Portugal) was added. The resultant gels were visualized in an UV transilluminator.

A short fragment (~205 bp) of the mitochondrial cytochrome c oxidase subunit I (COI) was amplified by Polymerase Chain Reaction (PCR) with the Fwh2 primers (Vamos et al., 2017), which have been proposed as the most appropriate COI primer pair for the study of insectivorous diets (Elbrecht et al., 2019; Tournayre et al., 2020). The primers were modified to include Illumina adaptors and a 0–5 bp addition of N bases between the adaptor and the primer to increase sequencing diversity and quality. The different primer variations were then combined before PCR reactions, resulting in mixed forward and reverse primer single solutions. The PCR reaction consisted of 5 µL of QUIAGEN Multiplex PCR Master Mix (Quiagen, Crawley, UK), 0.3 µL combination of 6 Forward primers, 0.3 µL combination of Reverse Primers, 2.4 µL of ultra-pure water, and 2 µL of DNA extract. Cycling conditions used consisted of an initial denaturing at 95°C for 15 min, followed by 45 cycles of 95°C denaturing for 30s, annealing at 52°C for 45s, extension at 72°C for 20s, and a final extension at 60°C for 5min. PCR negative controls were used to detect possible contaminations. All amplifications were checked through electrophoresis by running the PCR products in 2% agarose gels.

Library Preparation

This process consisted of an initial PCR clean-up to remove free primers and primer dimers, using the Agencourt AMPure XP beads (Beckman Coulter, Brea, CA, USA), since the samples showed signs of primer-dimer. This cleaning was performed using a proportion of 0.85 µL of magnetic beads to 1 µL of PCR product. To attach the barcodes in each sample, a second PCR was performed with a unique combination of barcodes per samples. This PCR index was performed using 2.8 µL of ultra-pure water, 7µL of 2x Kapa HiFi, 1.4 µL of each index, and 2.8 µL of cleaned DNA. Indexing the PCR required an initial denaturation of 95°C for 3min, followed by 10 cycles of 95°C for 30s, annealing at 55°C for 30s, extension at 72°C for 30s, and a final extension of 72°C for 5min. In order to confirm the success of the barcodes' incorporation in all samples, they were tested in a 2% agarose gel with the *a priori* knowledge that amplicons should be ~100 bp longer than before this second PCR. A second PCR Clean-Up was then performed, under the same conditions as the first one, except for the ratio of beads used, which was 0.8 µL.

After the second PCR clean-up, all indexed samples were quantified using Epoch™ Microplate Spectrophotometer (BioTek Instruments, Inc.; Winooski, VT, USA), followed by the normalization to obtain the same concentration in all the samples. After the normalization, the samples were pooled together. The pool was tested for quality control in a TapeStation 4200 High Sensitivity D1000 Assay (Agilent, Santa Clara, CA)

and a cleaning was needed using a ratio of beads of 0.78 µL. A second test for quality control in the TapeStation was performed to confirm the success of the cleaning. The final pool was sent to GENEWIZ Next Generation Sequencing laboratory with a final concentration of 27nM to be sequenced in an Illumina MiSeq sequencer with 2x250bp paired-end (PE) configuration. In the GENEWIZ lab, sample quality control was performed using Qubit dsDNA Assay and PhiX (≤30%) was spiked-in to increase sequencing diversity.

Bioinformatics

Once the results were available, the sequences were analysed using various software packages in Ubuntu 24.04, including PEAR (Zhang et al., 2014), OBITools (Boyer et al., 2016) and VSearch (Rognes et al., 2016).

To process the sequencing data in the fastq files, the reads were counted and moved to a raw data file using the command "*grep*". Using PEAR, R1 and R2 were merged into single sequences, discarding single and unassembled reads. Base pairs with less than 26bp of overlapping quality were also rejected. Then, the primers were removed and each assembled read was assigned to its corresponding marker combination, using the command "*ngsfilter*" in OBITools. The reads were once more counted to check for successful rates of the previous step with the "grep" command and then dereplicated into unique sequences using the "*obiuniq*" command. Lastly, sequence cleaning was performed to remove sequences with less than 10 reads and errors such as singletons resulted from PCR errors and chimeras, using the "*obiclean*" command. Sequences with a length between 202bp and 208bp were kept and clustered at a 99% similarity threshold to form Operational Taxonomic Units (OTUs), using VSearch. We removed from each PCR product all OTUs that had a read count < 1% of the total number of reads of that PCR (Mata et al., 2016). This should allow the removal of most PCR and sequencing errors that still passed the "*obiclean*" denoising step. Additionally, all reads identified in the extraction and PCR controls were subtracted from the corresponding sample batch (Evans et al., 2021).

The obtained OTU table and sequences were further cleaned using the R package LULU (Frøslev et al., 2017) and the taxonomic assignment of OTUs was performed using BOLDigger (Buchner & Leese, 2020), followed by manual inspection and curation. We also used the BLAST function from NCBI implemented in Geneious Prime 2021.1.1 (Kearse et al., 2012), when species-level assignments were not possible with BOLDigger. The taxonomical level assignment was performed according to the following thresholds: to the class level if below 90%; to the family level if between 90 and 95%;

and to the species or genus level (if more than one species from the same genus has a > 95% sequence match) if higher than 95% similarity. Following the completion of taxonomic assignments, the distribution and presence of detected prey species in Portugal were verified using a combination of local databases, that included Naturdata (https://naturdata.com/) and Lusoborboletas (https://www.lusoborboletaspt.com/) and global databases, such as GBIF (http://www.gbif.org/). Additionally, relevant scientific articles were reviewed to confirm whether the presence of specific species within Portuguese ecosystems had already been documented.

Statistical Analyses

For the statistical analysis, R 4.1.0 (R Core Team, 2013) was used to assess differences in dietary descriptors (i.e., diet richness and composition). Dietary analysis was based on two different taxonomic levels: OTU (all taxonomic units identified to the most possibly resolved taxonomic level) and Family. For the OTUs not identified to the species level, we built a neighbour-joining tree in Geneious Prime v. 2024.0.2 (Biomatters), visually inspected the corresponding alignment, and checked for patterns of genetic similarity (~98%) in order to cluster them into distinct taxa (e.g., Carabidae 1, Carabidae 2, and so on).

First, a Shapiro-Wilk test was carried out to assess the normality of the weight and SVL distributions. Although the SVL ($p = 0.452$) had a normal distribution, as this was not the case with the weight ($p = 0.0199$), both SVL and weight were log-transformed to normalize their distribution. The distribution of SVL and weight across fire types and sexes was assessed using boxplots, and their relationship was investigated through a linear regression. Using the function "*aov*", a two-way ANOVA (locality and fire type – nested in locality) was performed to analyse sexual dimorphism in both size (SVL) and body mass (weight).

When tested the richness distribution using the Shapiro-Wilk test, the results indicated that both the OTU and family richness followed a Poisson distribution. Taking into account the hierarchical structure of the sampling scheme, with different localities harbouring distinct fire regimes, a nested Generalized Linear Model (GLM) was implemented to investigate the effects on diet richness. Fire type, sex, SVL and weight were considered as fixed effects, and the fire type nested in the locality. To better understand the effects of the fixed factors, the estimated marginal averages were calculated using the emmeans package 1.10.2 (Russell, 2018).

The iNEXT v. 3.0.1 package (Hsieh et al., 2016) was used to perform rarefaction and extrapolation curves to analyse whether the type of fire (unburned and burned;

burned in 2016 and burned in 2022) and sex had any impact on the differences in OTU and family richness, specifying the datatype as incidence frequencies and setting the confidence level at 84% (MacGregor-Fors & Payton, 2013) with 1000 bootstrap replicates. Further, using the "*ggiNEXT*" function both sample-based and coverage-based rarefaction curves were plotted. However, the estimated richness was compared considering completeness (i.e., sample coverage) instead of sample size (i.e., number of samples), to avoid biases of communities with different levels of richness requiring different sampling efforts in order to be sufficiently characterised (Chao & Jost, 2012).

To compare the OTU and family diet composition among fire type, sex, SVL and weight, a permutational multivariate analysis of variance (PerMANOVA) was used from the R package vegan 2.6-6.1 (Oksanen et al., 2022), considering a stratified design (strata = locality). The presence or absence of each prey item in each sample was used to build a Jaccard dissimilarity matrix using the *vegdist* function from the vegan package. After the PerMANOVA, a pairwise comparison was performed using the "*pairwise.adonis2*" function from the pairwiseAdonis package (Martinez Arbizu, 2020).

A homogeneity of dispersion test (function "*betadisper*") was performed to make sure that the differences observed with PerMANOVA are not due to unequally dispersed values across the different groups, as it evaluates the homogeneity of multivariate dispersions. Afterwards, a similarity percentage analysis of was carried out, also using the vegan package with the function "*SIMPER*", to assess the contribution of each prey item to the differentiation between diets.

Finally, a rank abundance plot was created to visualize the top 10 most abundant prey species in the different fire types, using the "*ggplot*" from the ggplot2 package in R (Wickham, 2016).

Results

Data filtering

From the 183 faecal samples collected, the libraries generated approximately 23 million raw sequence reads, which were reduced to 357,866 reads during bioinformatic processing, resulting in 1,102 OTUs. Non-target amplification (37.39% of total reads) was observed from different sources, both in the samples and in the extraction and PCR negative controls. Most of the non-target OTUs were identified as belonging to Fungi, (mainly Ascomycota and Basidiomycota), accounting for 12.53% of total reads and 33.51% of non-target OTU diversity. An expected presence of *Podarcis lusitanicus* (6.88% of total reads) was observed. After removing negative controls, singletons,

replicates and filtering out taxa, the final lizard diet dataset consisted of 237,402 reads, covering 537 OTUs.

Diet analyses

From a total of 537 prey items considered, five classes of Arthropoda (Arachnida, Collembola, Diplopoda, Insecta and Malacostraca), one class of Mollusca (Gastropoda), and one class of Annelida (Clitellata), were identified. These encompassed at least 26 distinct orders, 49 families and 107 species from 184 OTUs. In general, the order Araneae (21%) was the most consumed prey, with Salticidae being the most frequent family (10%). The other most consumed prey belonged to Coleoptera (12%), Hemiptera (12%), Hymenoptera (11%), Lepidoptera (7%) (Figure 10).

The mean OTU richness in the unburned areas is slightly higher than the burned areas (Figure 11A), with a large overlap in the interquartile range (IQRs), suggesting a considerable similarity in OTU richness distribution between fire types. However, the burned areas present more variability, visible by the spread of OTU richness values. The same tendency is observed regarding the family richness (Figure 11B). The mean and distribution of OTU richness differs between sexes (Figure 12A). Females present a

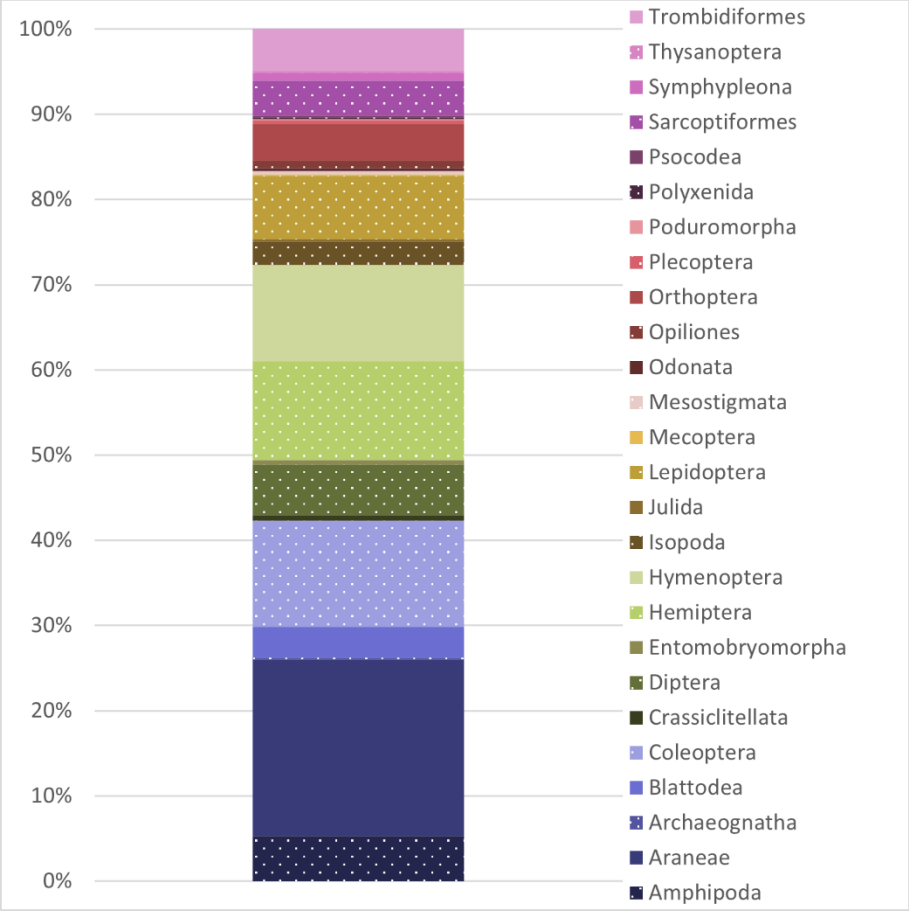

Figure 10. *Frequency of occurrence of the different prey items consumed by P. lusitanicus with a total of 26 identified orders.*

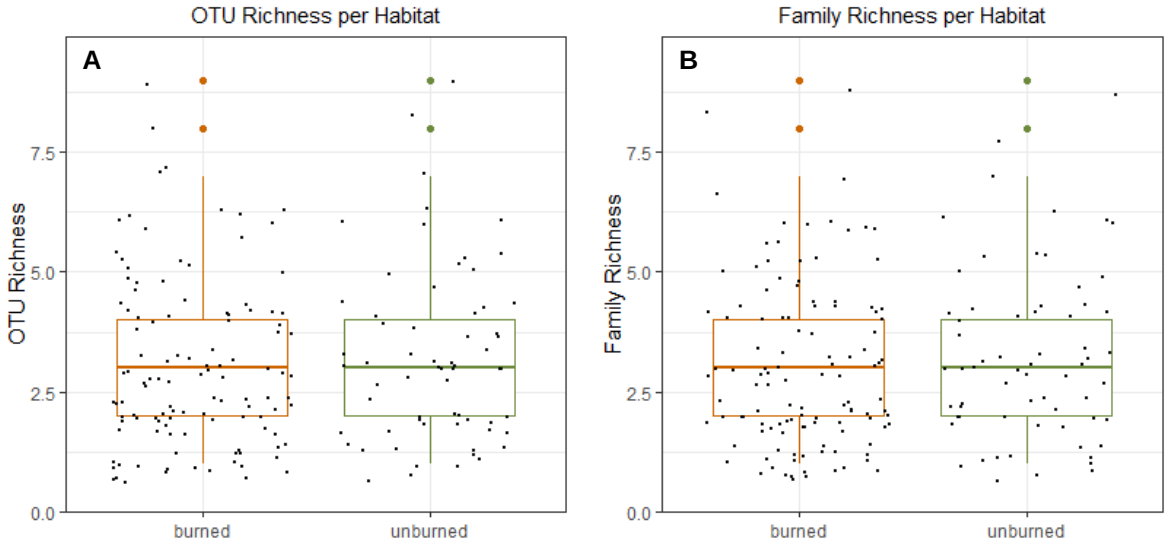

Figure 11. *Boxplot of OTU (A) and family (B) richness per fire type (0-10 Richness Range).*

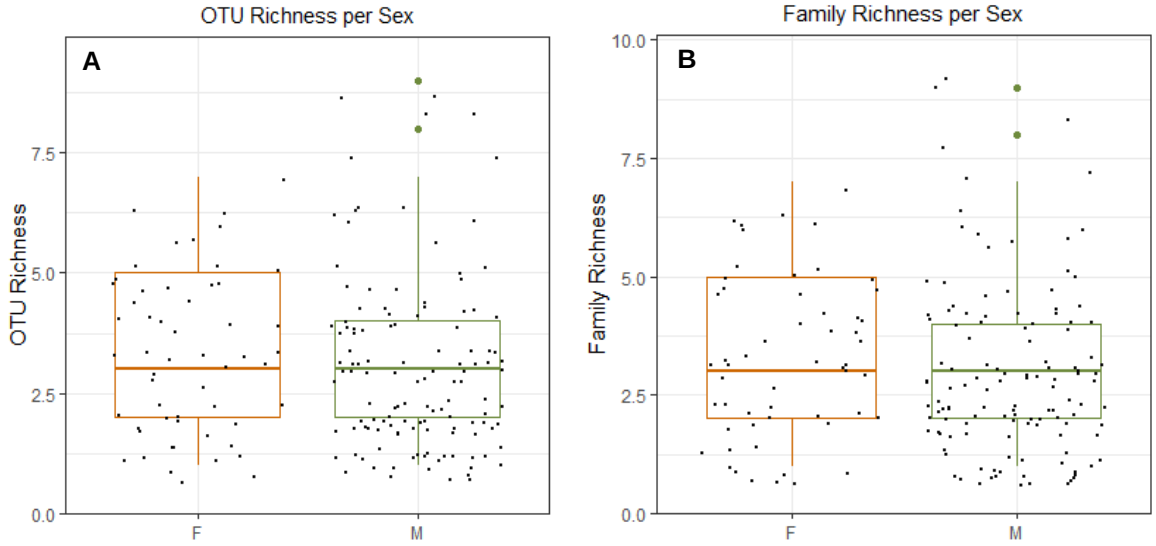

Figure 12. *Boxplot of OTU (A) and family (B) richness in females (F) and males (M) (0-10 Richness Range).*

slightly higher OTU richness than males, with also a higher distribution, exhibited by the greater interquartile range (IQR). The same tendency is observed regarding the family richness (Figure 12B).

Statistical Analysis

The regression for SVL and weight show that these parameters are significantly and positively correlated (Females: $r^2 = 0.427$; Males: $r^2 = 0.491$), i.e. as SVL increases, so does weight and both females and males follow this trend (Figure 13). Neither SVL ($p = 0.348$) nor weight ($p = 0.764$) are affected by the interaction between locality and fire type (Table 2). The only parameter that affects SVL ($p = 0.023$) and weight ($p = 9.32e-06$) is sex, regardless of location or fire type (Table 2; Figure 14).

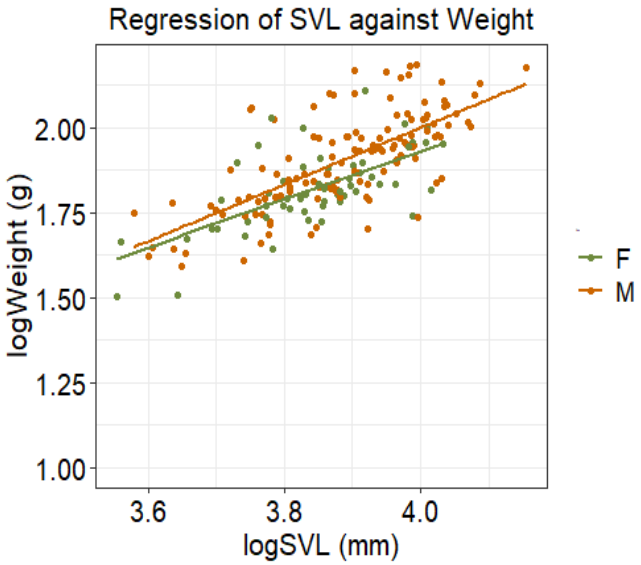

Figure 13. *Linear regression of log-transformed SVL (logSVL) against log-transformed Weight (logWeight) for female (F) and male (M) individuals.*

Table 2. *ANOVA Results for the Effects of Locality and Sex on SVL and Weight. Significant p-values (p < 0.001) are highlighted in bold.*

	Factor	Df	Sum of Squares	Mean of Squares	F-value	p-value
logSVL	Locality:Fire type	1	0.03856	0.03856	1.472	0.264
	Sex	1	0.0749	0.07487	6.15	**0.0141**
logWeight	Locality:Fire type	1	0.0019	0.00189	0.021	0.889
	Sex	1	0.2775	0.27751	22.4	**4.69e-06**

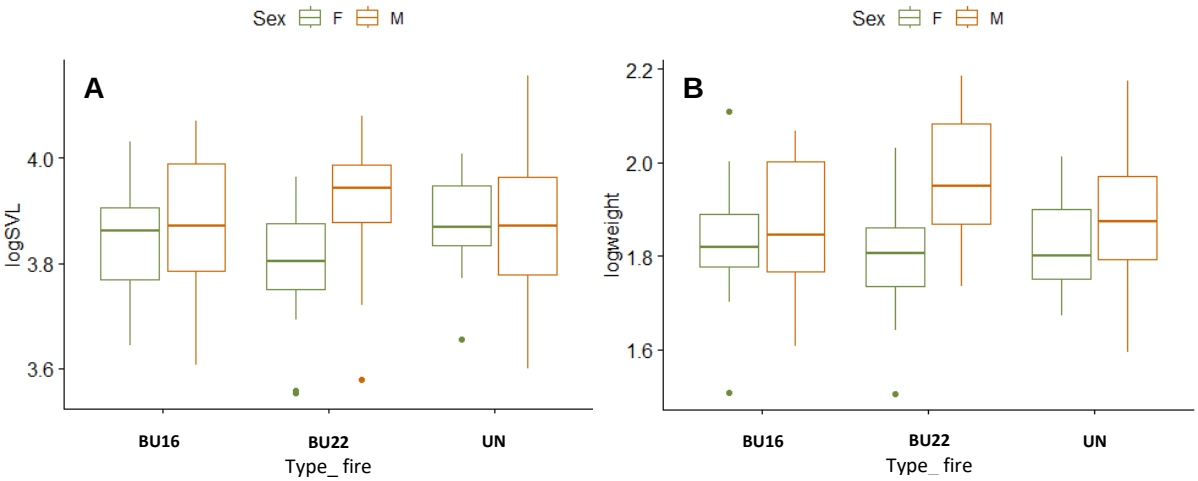

Figure 14. *Boxplot showing the differences between sexes regarding the SVL (A) and weight (B) in the different types of fire.*

The generalized linear models (GLM) showed that none of the variables had a significant effect on either OTU or Family diet richness (Table 3). To further explore the effects of fire type on OTU richness, the estimated marginal means were plotted for each fire type (Figure 15A) and for both sexes (Figure 16A). Although UN presents a slightly higher richness with BU22 being the lower, and the females (F) present slightly higher diversity than males (M), the estimated marginal means for OTU richness show

Table 3. *GLM results for type of fire, sex, log snout-vent length (logSVL) and log weight (logweight) on OTU and family richness in Podarcis lusitanicus.*

	Fixed Values	Estimate	Std. Error	Z-value	Pr(>|z|)
OTU	AL : BU16	0.006393	0.174321	0.037	0.9708
	GS : BU16	-0.244499	0.231150	-1.058	0.2902
	MC : BU16	0.003862	0.198756	0.019	0.9845
	VC : BU16	-0.403868	0.317361	-1.273	0.2032
	AL : BU22	-0.314777	0.218047	-1.444	0.1488
	GS : BU22	-0.135021	0.230954	-0.585	0.5588
	MC : BU22	-0.054532	0.168210	-0.324	0.7458
	VC : BU22	-.0388845	0.252635	-1.539	0.1238
	AL : UN	0.016162	0.190328	0.085	0.9323
	GS : UN	-0.084521	0.183517	-0.461	0.6451
	MC : UN	-0.423329	0.238302	-1.776	0.0757
	VC : UN	NA	NA	NA	NA
	SexM	-0.083272	0.099223	-0.839	0.4013
	logSVL	-0.846077	0.741894	-1.140	0.2541
	logweight	0.483433	0.744248	0.650	0.5160
Family	AL : BU16	0.003352	0.186816	0.018	0.9857
	GS : BU16	-0.140384	0.239678	-0.586	0.5581
	MC : BU16	-0.009437	0.212907	-0.044	0.9646
	VC : BU16	-0.405521	0.338201	-1.199	0.2305
	AL : BU22	-0.261571	0.229485	-1.140	0.2544
	GS : BU22	-0.0389558	0.238301	-0.163	0.8705
	MC : BU22	-0.010504	0.148127	-0.059	0.9530
	VC : BU22	-0.351922	0.266660	-1.320	0.1869
	AL : UN	0.060659	0.201113	0.302	0.7629
	GS : UN	-0.074074	0.195614	-0.379	0.7049
	MC : UN	-0.416521	0.254194	-1.639	0.1013
	VC : UN	NA	NA	NA	NA
	SexM	-0.076292	0.104822	-0.728	0.4667
	logSVL	-0.860708	0.781364	-1.102	0.2707
	logweight	0.492357	0.784596	0.628	0.5303

substantial overlap in their confidence intervals, indicating that any observed differences are not statistically significant. This finding aligns with the GLM results, which also did not identify fire type as a significant predictor of OTU richness. The same conclusions can be drawn regarding to family richness in relation to fire type (Figure 15B) and sex (Figure 16B).

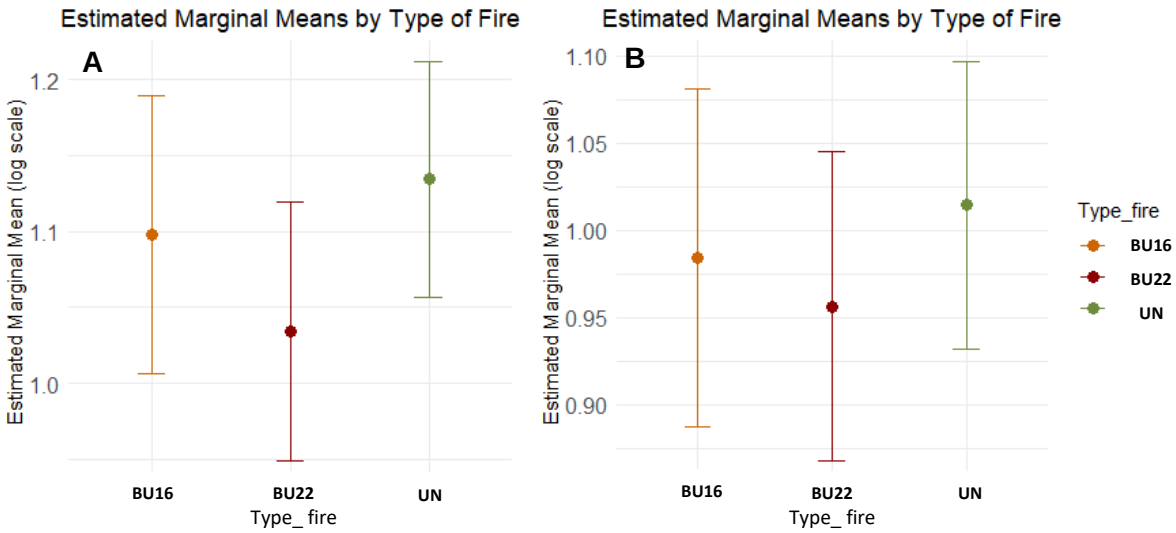

Figure 16. *Estimated Marginal Means of OTU (A) and Family (B) richness by fire type.*

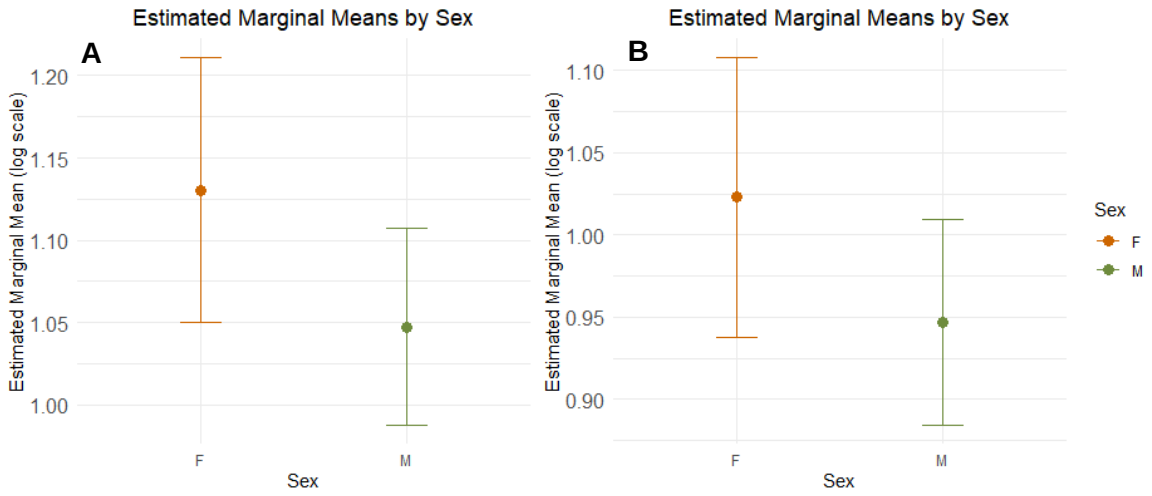

Figure 16. *Estimated Marginal Means of OTU (A) and Family (B) richness by sex.*

From the analysis of the rarefaction and extrapolation curves (Figure 17), we observed no significant differences between burned and unburned areas in terms of niche breadth at both the OTU and family levels (Figure 17A). Similarly, BU16 and BU22 did not present significant differences (Figure 17B). Regarding sex, there were no significant differences in the range of prey items between females and males (Figure 16C) at either the OTU or family levels. For all rarefaction analyses, the minimum observed sample coverage was always above 50%, except for the unburned areas in the OTU diversity analysis (Figure 17A) and the Females (F) in the OTU diversity analysis (Figure 17C).

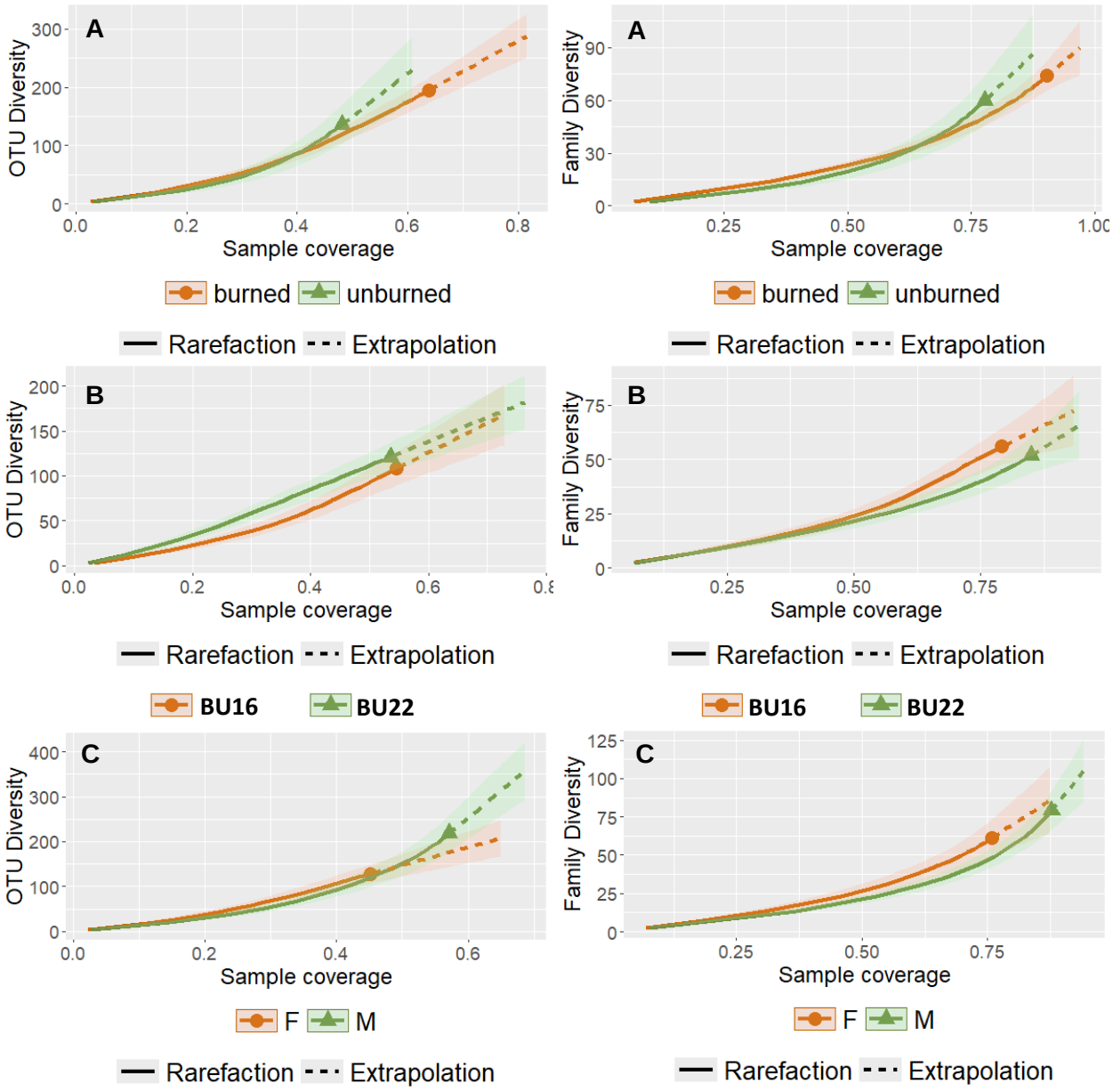

Figure 17. *Diet rarefaction/extrapolation curves for (A) fire type (burned, unburned), (B) burned areas (BU16, BU22) and (C) sex (F, female; M, male), obtained according to the sample coverage.*

The PERMANOVA results indicated that fire type significantly influenced the OTU community composition (F = 1.3405, p = 0.013), while sex, SVL, and weight did not have significant effects (Table 4), and there was no effect of data dispersion on the results (F = 0.755, p = 0.4715). At the family level, there are no significant differences in composition, regarding any of the considered factors (Table 4). The significant differences obtained between the fire types where due to differences between BU22 and UN (p = 0.007; Table 5).

Table 4. *PERMANOVA results for OTU and Family community composition. Significant p-values (p < 0.05) are highlighted in bold.*

	Factor	Df	Sum of Squares	R^2	F	p-value
OTU	Fire type	2	1.295	0.01480	1.3405	**0.013**
	Sex	1	0.416	0.00476	0.8623	0.816
	logSVL	1	0.541	0.00619	1.1209	0.464
	logweight	1	0.743	0.00849	1.5391	0.584
Family	Fire type	2	1.279	0.01581	1.4039	0.057
	Sex	1	0.461	0.00570	1.0131	0.386
	logSVL	1	0.618	0.00764	1.3573	0.223
	logweight	1	1.116	0.01380	2.4508	0.346

Table 5. *Pairwise PERMANOVA Comparisons for OTU by Type of Fire. Significant p-values (p < 0.01) are highlighted in bold.*

Factor	Df	Sum of Squares	R^2	F	p-value
BU 16 vs BU 22	1	0.582	0.01042	1.2001	0.096
BU 16 vs UN	1	0.620	0.01086	1.2846	0.055
BU 22 vs UN	1	0.733	0.01193	1.5096	**0.007**

The OTUs *Salticus scenicus* and "*Tapinoma* sp.1" contributed the most to the differences found in PerMANOVA between the UN and BU22.

The rank abundance plot (Figure 18) shows that the most abundant species present in BU16 and BU22 is *Talitroides topitotum*, while in UN the most abundant OTU is "Arachnida 2", with *Talitroides topitotum* appearing in second. The BU22 line shows a

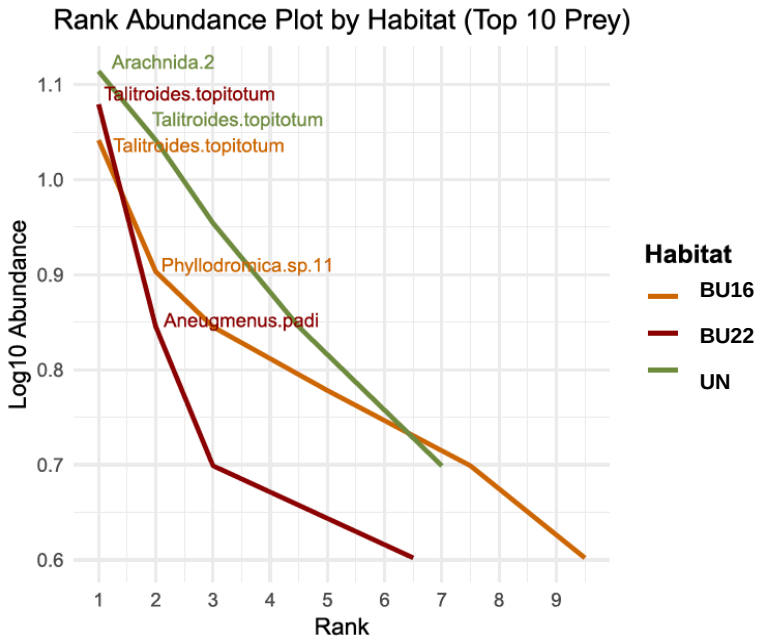

Figure 18. *Rank abundance plot between fire types with the 10 more abundant species in the P. lusitanicus diet.*

steep drop after the first most abundant OTU, indicating high dominance by a few species. When compared to BU16, it is possible to infer that BU16 has a more gradual decline in abundance compared to BU22, indicating change in species composition over time.

Discussion

In this study, the impacts of wildfires in the diet of *Podarcis lusitanicus* were assessed, comparing areas burned in 2022 and 2016, and unburned areas existent across four different localities in Portugal. We assess the richness and composition at the OTU and family levels of the identified prey items, comparing them between fire types, but also considering factors such as sex, SVL and weight of the organisms. Results showed that diet richness does not change significantly across the considered groups, but instead diet composition is distinct between areas with different fire histories.

Based on the 183 samples analysed, the results indicated that the diet of *P. lusitanicus* is quite diverse, with a taxonomic composition in line with previous morphological studies carried out on this species, highlighting the predominant groups, such as Hemiptera, Coleoptera, Araneae, Hymenoptera and Diptera (Carretero et al., 2022). However, a far greater number of prey items were identified in our study compared to previous studies (Carretero et al., 2022; Pérez Mellado, 1982). Diet studies based on morphology tend to underestimate the presence of soft-bodied prey, as they typically only detect items that have not been completely digested (Ingerson-Mahar, 2002). This limitation does not occur in studies using metabarcoding. However, this does not fully explain the additional diversity observed, as we identified several additional orders, including Sarcoptiformes, Psocodea, Archaeognatha and Amphipoda. It is possible that these less common groups are often overlooked in microscopic studies, or that small fragments of remains are wrongly attributed to more common prey families. In addition, some OTUs identified may represent secondary predation, which can overestimate prey diversity estimates when using metabarcoding (da Silva et al., 2019). However, the significant increase in the number of orders identified in this study (26) compared to previous studies using microscopy (15 and 19 in Pérez Mellado (1982) and Carretero et al. (2022), respectively) suggests that the prey spectrum of *P. lusitanicus* is considerably larger than previously reported. In addition, this metabarcoding study provided greater precision in the taxonomic identification of prey, allowing most of them to be identified down to the species level (184 to be precise; see Supplementary Table 6), which is usually not possible in studies based on morphological scatology.

Fire Impact on Richness and Composition

Wildfires in Mediterranean areas cause the fragmentation and destruction of original forest habitats, resulting in a decrease in the size of these areas, increasing the isolation of forest patches and creating a heterogeneous landscape. This forms forest archipelagos dispersed in matrices of open or shrubby vegetation, with different post-wildfire ages (Sarà et al., 2006). In these habitats, both vegetation and fauna recover through ecological succession, with a gradual increase in species richness and diversity, from low xeric shrubs to tall evergreen forests (Katsimanis et al., 2006; Moreira et al., 2003; Suárez-Seoane et al., 2002). As a consequence, differences in species richness and composition between areas affected and unaffected by fire would be expected. Although our results indicated that there are no statistically significant differences in richness between the different types of fire, some trends were observed. The results indicate that the unburned areas (BU) have the highest diversity both at OTU and Family level, which may reflect a more diverse prey base in these undisturbed regions. The lower diversity observed in burned areas in 2022 (BU22) reflects the negative impact of recent fires on prey diversity and availability, resulting in a less varied diet for lizards in these regions. However, over time, there is an increase in the diversity of prey available as the habitat recovers from the fire. This process is evidenced by the diversity values observed in BU16, which are slightly higher than in BU22, although they have not yet reached the levels recorded in the unburned areas (BU). Species richness varies according to time and habitat type, resulting in significant differences between studies concerning the time needed for species richness in burned areas to return to pre-fire levels (Izhaki, 2012). The slow development of some resources over time also affects the presence of fauna and the supply of fuel for new fires (Haslem et al., 2011). These results show that disturbances caused by fire reduce diversity in a habitat, but that there is a partial recovery over time, demonstrating the ecosystem's ability to regenerate. However, even after 8 years, in our study species richness in the affected areas is still slightly below the levels observed in unburned areas.

Despite fire slightly reducing the richness of prey species, our results also demonstrate that lizards in burned areas seem to be able to maintain dietary diversity. This can be explained by the ability of these lizards to adjust their diet, including species that are more resistant to fire or those that quickly colonise habitats after fire, which results in a greater diversity than expected in these areas. Lacertid lizards show remarkable dietary flexibility in response to environmental changes. Studies indicate that they can modify their foraging habits and that, although diet composition is not determined solely by prey availability (Carretero, 2004), they can adapt their diet based

on the type of habitat and resources available after a disturbance (Rato et al., 2022; Sagonas et al., 2014). Besides, the recovery of prey populations after major disturbances, such as wildfires, can be attributed to two non-mutually exclusive biological processes: the survival of remaining individuals and post-fire recruitment (either by local reproduction or immigration) (Robinson et al., 2013; Steinitz et al., 2012). Refuges not only increase immediate survival during a fire but can also facilitate the persistence and recovery of populations in affected areas. In the long term, as vegetation regenerates, these refuges can help re-establish populations, acting as sources of expansion within the burned area or facilitating the arrival of new individuals from outside that area, providing both short-term (food and shelter) and long-term (permanent habitat) resources (Banks et al., 2011; Robinson et al., 2013; Watson et al., 2012). Moreover, our study demonstrates that depending on the fire regime, prey composition is significantly different. Specifically, between areas burned in 2022 and unburned areas. These differences were particularly due to the presence of the "*Tapinoma* sp.1" OTU, a species of ant, and the spider *Salticus scenicus* in unburned areas, and their total absence in the 2022 burned sites. The absence of significant differences in diet composition at the taxonomic family level suggests that although fires can alter the composition of specific OTUs, the broader taxonomic structure remains relatively stable, possibly due to the resilience of diversity at the family level or functional redundancy within these families. Given that studies using microscopy to identify taxa rarely, if ever, identify prey items to higher taxonomic levels, again this demonstrates the value of metabarcoding as a more precise tool to determine the impact of fires for dietary assessments.

The finding that diet richness and composition do not differ significantly between males and females suggests that both sexes interact in a similar way with the environment in terms of feeding. This suggests that both males and females have similar access to food resources and use them in a comparable way, possibly due to a shared ecological niche or similar adaptive behaviours. This is somewhat unexpected, given that prey selection can be influenced by morphological characteristics. Kaliontzopoulou et al (2012b) ,identified dietary differences between males and females, with males selecting larger and harder prey, a tendency that correlates positively with head size and bite force. Considering that the studied populations of *P. lusitanicus* show size and weight sexual dimorphism, differences in prey composition were expected between both sexes. However, in dietary assessments using metabarcoding, faecal samples are crushed during DNA extraction, and so size and hardness of the prey are impossible to be determined, which are crucial factors in prey selection by lizards (e.g. Díaz & Carrascal,

1990). Hence, we are unable to verify whether, despite consuming similar taxonomical groups of arthropods, if males and females of *P. lusitanicus* are selecting specimens of different sizes or even from distinct ontogenic stages.

Fire Responsive Taxa

Some studies have shown that certain fire-sensitive species undergo a significant decline in their populations soon after a fire (e.g. Santos et al., 2016). However, other studies indicate that it is rare for wildfires to cause the complete extinction of these species in the affected areas. In many cases, residual populations manage to survive on the burned sites (e.g. Banks et al., 2011; Sanz-Aguilar et al., 2011; Suaréz et al., 2012). This is especially true for organisms that are good dispersers, able to quickly move away from the fire, or that have the ability to burrow into the soil to protect themselves (Moyo, 2022). The chance of an individual immediately surviving a fire depends on the intensity of the fire, the proximity of safe shelters and the behaviours the organism can adopt to avoid the flames and direct heat (Banks et al., 2011; Friend, 1993; Whelan et al., 2002). The dominance of *Talitroides topitotum* in the burned areas suggests that this species may play an important role as a fire tolerant species. *Talitroides topitotum* is a terrestrial amphipod native to Asia, widely found in subtropical and temperate regions around the world (Umaña-Castro et al., 2018). The species has been introduced to countries such as Costa Rica (Alfaro-Montoya & Umaña Castro, 2013; Umaña-Castro et al., 2018), Brazil (Brisotto et al., 2022) and Indonesia (Daneliya & Wowor, 2016). With a wide altitudinal distribution, this species is able to adapt to diverse temperature and humidity conditions (Umaña-Castro et al., 2018). Due to its high capacity for dispersal and establishment in new environments, *T. topitotum* can become potentially invasive in various habitats, posing a threat to native amphipod species (Daneliya & Wowor, 2016; Umaña-Castro et al., 2018). To the best of our knowledge, while this species has not yet been documented in mainland Portugal, it has been recorded in Madeira and Azores archipelagos (GBIF Secretariat, 2023). These results highlight both the metabarcoding technique and dietary studies of lizards as possible tools in the detection of unrecorded alien species (e.g. Martins et al, 2022). While fires create new habitats, allowing for the colonisation of these areas by alien, potentially invasive, species, we conclude that this is not the case, as *T. topitotum* is also abundant in areas not affected by fires (Supplementary Table 7 and 8) (Martins et al., 2022).

Conclusion

The role of fire in the ecology and evolution of flora has been widely assessed, but despite some studies concerning the impact on animals, little is known regarding certain aspects such as biotic interactions (West et al., 2019). This study allowed us to shed new light on the impacts of wildfire in the diet of *Podarcis lusitanicus* population in an area that is constantly affected by them, the Mediterranean Basin. With a generalist diet, this study indicates that the diet of the *P. lusitanicus* is not affected by the wildfires in terms of richness, but very much in terms of composition, showing the capacity of this species to adapt to this specific disturbance. The results of this study also demonstrate that the use of DNA metabarcoding offers a promising window into the interaction between prey and predator with more precision than morphological methods.

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3. General Discussion

The aim of this thesis was to be able to understand more about the impacts of wildfire regimes regarding biotic interactions through the diet of the lizard *Podarcis lusitanicus*, using a metabarcoding approach. We were able to assess the diet of 12 different populations of *P. lusitanicus* inhabiting the northern part of Portugal, and how it changed between different types of disturbance, in a region highly affected by wildfires.

3.1 DNA metabarcoding: marker choice primers and limitations

The traditional approach of microscopic analysis is not always able to identify all the types of food consumed, and in the case of generalist species, obtaining detailed dietary profiles can be difficult, especially for insectivores that eat soft, rapidly digestible prey. However, with the appropriate selection of markers and primers, metabarcoding has been shown to be effective in identifying a wide range of organisms, including those with high digestibility rates (Jarman et al., 2013). Our results confirm previous findings based on morphological identification, especially regarding the most predominant groups. They also provide an extra layer of taxonomic resolution, by identifying prey up to the species level. In addition, metabarcoding revealed 8 additional orders not previously identified: Trombidoformes, Sarcoptiformes and Mesostigmata (Arachnida); Psocodea, Plecoptera, Odonata and Archaeognatha (Insecta); and Amphipoda (Malacostraca), with the latter corresponding to all OTUs identified as *Talitroides topitotum*. These orders include small species that could easily go unnoticed in morphological analyses. The orders Trombidiformes, Sarcoptiformes, Mesostigmata and Psocodea include parasitic species, particularly among mites and lice. Therefore, the presence of these orders may be associated with the fact that the type of sample was faeces and due to the parasitic lifestyle of the orders identified, not necessarily reflecting the diet of the lizards. Of these orders, only Odonata contains larger species that, in principle, would not go unnoticed in morphological analyses. However, as it was found in only two samples in this study, it can be considered a rare occurrence.

Selecting the right primer is crucial in metabarcoding studies because it ensures that the results obtained accurately reflect the true composition of the samples analysed. In a study conducted by Pereira et al. (2019), using a combination of COI and 16S primers, the proportion of non-dietary items identified was relatively high with COI, making it difficult to do an accurate assessment of the diet. When working with pellets, samples are susceptible to contamination by fungi and bacteria. In addition, there may be bias in the detection of certain groups, as described by da Silva et al. (2019), where

Formicidae were completely omitted by one particular primer (“ZBJ”), despite being identified morphologically. This is probably due to ZBJ's known bias in favour of Diptera and Lepidoptera, to the detriment of other arthropod orders. Some animal prey, due to their extremely small size and parasitic nature, can be easily identified visually as non-food objects, but not by some molecular markers (da Silva et al., 2019). In these studies, it proved to be more valuable to use a combination of primers. However, even though we obtained here a considerable amount of non-targeted amplifications (~40%), by using the COI fwhF2 + fwhR2n primer set, it was possible to identify a very diverse diet. As highlighted out by Elbrecht et al. (2019), a single well-designed primer set can be sufficiently effective, making the use of multiple sets unnecessary.

There are still other limitations when using metabarcoding. As previously mentioned, the impossibility of comparing the size and hardness of the prey eaten when metabarcoding is used can be limiting, since this information could be relevant to understanding the results obtained from the diet (Díaz & Carrascal, 1990). Using molecular scatology, it is also impossible to identify cases of cannibalism, known to occur in *P. lusitanicus* (Santos et al., 2023). Another limitation is identifying secondary consumption. By making it possible to identify all the genetic material present in the sample, metabarcoding can also detect residues of prey that were previously consumed by the prey of the animal under study, leading to the occurrence of secondary consumption. The detection of secondary consumption can introduce a significant bias in the interpretation of the diet of generalist vertebrates, especially if other sources of information, such as morphological analysis and behavioural studies, are not integrated to distinguish between primary and secondary consumption. When there are no other sources of information about the diet, it can be impossible to differentiate between these types of consumption. DNA fragments of less than 200 bp can be recovered using molecular markers. Filtering out all taxa with less than 1% of the total number of corresponding PCR reads could theoretically eliminate secondary consumption, although this is not always the case (da Silva et al., 2019). In the present study, the eight additional orders identified could theoretically result from secondary consumption. However, this seems unlikely for a number of reasons. Technically, the length of the fragments (203-207 bp) and the significant number of reads (10-7649 reads) suggest that many of these are not due to secondary consumption. Furthermore, this is more likely for very small prey items. For example, S’Khifa et al. (2023) identified thrips (Thrysanoptera) in the diet of the similar-sized lizard *Atlantolacerta andreanskyi* (Werner, 1929) and accepted that this could be due to secondary predation since ladybirds (Coccinellidae), which were also identified, feed on them. However, in our study, species

of Odonata are generally large, and seem highly unlikely to be present through secondary predation, and furthermore have been reported in other diet studies of lacertid lizards using microscopy (e.g. Busack 1987), albeit in low numbers. In general, therefore, we suggest the differences are due to the ability to process larger numbers of pellets and greater precision, rather than inflation due to the effect of secondary predation for metabarcoding studies.

3.2 The importance of dietary studies

Diet studies based on the identification of prey from faecal samples provide only a snapshot of the last meals consumed, unless they are carried out repeatedly over several years (Alemany et al., 2023). For this reason, the results presented in this thesis are fundamental base-line data. However, it is equally crucial to continue the study in order to better understand the diversity present and monitor possible changes in diet with increasing time since the last disturbance. Although no significant differences in lizard diet richness were observed in different fire scenarios, this may be related to the adaptability of *P. lusitanicus* and its resilience to forest fires. Reptiles show a strong successional response to fire (Smith et al., 2013), a response that is influenced by changes in habitat structure and the life characteristics of the species (Santos et al., 2016). In general, species that prefer open habitats tend to be favoured soon after a fire, while as the vegetation recovers, forest-dwelling species become more abundant and open habitat species begin to decline (Santos et al., 2016; Valentine et al., 2012). The impacts of fire can vary according to the intensity and severity of the fires, and reptile populations can be affected in different ways due to their specific dynamics. However, through the study of replicated lizard populations, it was observed that fire has little impact on the overall richness of the diet of these populations. The differences in diet composition indicate that the ecosystem is affected by fire, but the absence of differences in composition after 8 years suggests that the ecosystem has the capacity of regain the diversity found in the sites not affected by the fire. Thus, continued monitoring of these areas over time will allow for a better understanding of how the ecosystem modulates over time. For future research, it would be beneficial to increase the sample size, especially in comparisons between burnt and unburnt areas. In this study, combining the samples from burned areas in 2016 and 2022 for comparison with the unburned samples resulted in an insufficient number of samples from unburned areas, which could have compromised the robustness of the analyses (based on the sampling coverage obtained with the rarefaction analysis). Furthermore, it would be valuable to carry out additional studies at different times of the year to assess whether the reproductive season

influences the lizards' diet and whether there are subtle differences between the diets of males and females throughout the year. It would also be interesting to collect as many pellets as possible per population to increase the detection of greater taxonomic diversity and rare prey items.

Diet studies are crucial for understanding biotic interactions and understanding their role in shaping species distribution and diversity patterns. Ongoing global environmental changes are modifying species' diets and food interactions in ecosystems, which affects biodiversity and ecosystem functioning. Integrating dietary information into ecological studies is essential for anticipating how food webs will react to environmental changes and identifying early signs of diversity loss. However, more long-term studies on dietary ecology are needed to better understand species' vulnerabilities to changes in environmental conditions (Hallam & Harris, 2023).

3.3 Additional considerations on combining dietary studies with DNA metabarcoding

Dietary studies also serve as a tool for assessing arthropod diversity. Traditionally, arthropod diversity studies have used methods such as Malaise traps, flight-intercept traps and pan traps, as well as behavioural extractors such as Berlese funnels (Marshall et al., 1994). However, there are also studies that assess the diversity of arthropods by analysing the diet of their predators, such as birds (Muñoz et al., 2017), and bats (Zeale et al., 2011). DNA metabarcoding stands out as a powerful tool for identifying prey taxa and has been applied in studies of bat faeces (Zeale et al., 2011), offering greater sensitivity and taxonomic resolution compared to morphological analyses. Although this was not the primary aim of our study, by studying the diet of lizards we obtained data that could be valuable for understanding the diversity of arthropods in northern Portugal, identifying species not previously recorded and updating the available databases. Validating new localities across a wide selection of invertebrates is complex, since public databases often contain taxonomic errors and geographical uncertainties, which can lead to overestimation or underestimation of species richness in certain areas (Maldonado et al., 2015). Local databases are invaluable in this context, as they can provide more accurate and comprehensive information (Meier & Dikow, 2004). For example, while many species were not identified using the Global Biodiversity Information Facility (GBIF, http://www.gbif.org/), specialist sites for Portugal such as Naturdata (https://naturdata.com/) and Lusoborboletas (https://www.lusoborboletaspt.com/), allowed us to greatly refine the number of potentially new records for Portugal. Still several prey species such as *Acanthogethes*

fuscus (Olivier. 1790), *Diplazon tetragonus* (Thurnberg, 1822), *Formica selysi* Bondroit, 1918, *Lasius psammophilus* Seifert, 1992, *Monophadnoides ruficruris* (Brullé, 1832) and *Psilopa marginella* Fallén, 1823, appear to be potentially new records for Portugal, and this information can be used to update the relevant faunistic databases.

Finally, we identified some OTUs classified as non-target taxa, such as nematodes. Although there are a considerable number of studies focussing on the impacts of wildfires on bacteria, fungi, microorganisms and arthropods, a significantly smaller proportion of studies have focused on nematodes. In a meta-analysis that brought together a large number of studies on different organisms regarding the impacts of wildfire, Moyo (2022) concluded that nematodes were all negatively affected by fires. Overall, the effect of fire on underground diversity is largely negative, but more research is needed to confirm this negative impact on different taxonomic groups and ecosystems. However, with such a short fragment of CO1 sequence, drawing taxonomic conclusions regarding nematodes in these populations of lizards is very difficult. Given the information we have already collected, it would be important to follow up this study to better understand the impact of fire on nematodes and to obtain more information about these organisms, for example by identifying which pellets apparently included nematodes and to re-assessing these with other molecular markers, such as the widely used 18S and 28S rRNA genes (e.g. Jorge et al., 2014), which might enable us to adequately identify these important organisms.

4. Conclusion

The results of this study highlight the impacts of disturbances caused by wildfires in the Mediterranean basin on the Lusitanian wall lizards, *Podarcis lusitanicus*, and their prey, by analysing the diet of this species. It was observed that the overall dietary richness of *P. lusitanicus* remained unaffected by wildfires, demonstrating the species' remarkable resilience and adaptability in maintaining a diverse diet. Nevertheless, prey composition of unburned areas was significantly different in comparison with recently burned areas, but not regarding areas burned longer ago. These results may reflect both the lizards' ability to adjust to environmental changes but also the ecosystem's capacity to fluctuation. This adaptability in reptiles is essential for survival in a landscape increasingly marked by frequent fires. The results emphasise the importance of considering both the immediate and long-term ecological impacts of wildfires on species interactions and biodiversity. Although the ecosystem shows signs of recovery over time, the subtle changes in diet composition indicate ongoing ecological changes that require further investigation.

The use of DNA metabarcoding, offering more precise taxonomic identification of prey, has again proved invaluable compared to traditional morphological methods. This method revealed a broader spectrum of food items, especially those that are often not identified in morphological studies due to the rapid digestion of certain prey and their soft constitution. The study also highlights the usefulness of DNA metabarcoding not only for analysing diet, but also for improving our understanding of arthropod diversity in regions affected by fires. This method is also useful for identifying poorly studied and potentially unknown species in Portugal, such as *Psilopa marginella* or *Lasius brunneus*, contributing to a more comprehensive assessment of biodiversity.

As environmental changes continue to impact ecosystems around the world, studies like this are essential for predicting and managing biodiversity loss and ecosystem resilience. Future studies should explore seasonal variations and monitor the evolution of ecosystems, especially in the areas burnt in 2022, which show differences in composition compared to other areas.

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Attachments

Supplementary Table 1. *List of species identified according to their presence in faecal samples, corresponding to 184 OTUs. All the species identified belong to the phylum Arthropoda.*

Class	Order	Family	Genus	Species
Arachnida	Araneae	Agelenidae	*Textrix*	*Textrix pinicola*
		Gnaphosidae	*Callilepis*	*Callilepis concolor*
			Drassodes	*Drassodes pubescens*
			Haplodrassus	*Haplodrassus signifer*
			Nomisia	*Nomisia celerrima*
				Nomisia exornata
			Poecilochroa	*Poecilochroa albomaculata*
			Zelotes	*Zelotes thorelli*
		Linyphiidae	*Agyneta*	*Agyneta rurestris*
		Lycosidae	*Hogna*	*Hogna radiata*
			Alopecosa	*Alopecosa albofasciata*
			Pardosa	*Pardosa hortensis*
				Pardosa accentuata
		Philodromidae	*Pulchellodromus*	*Pulchellodromus pulchellus*
		Pholcidae	*Pholcus*	*Pholcus opilionoides*
		Salticidae	*Ballus*	*Ballus chalybeius*
			Phlegra	*Phlegra bresnieri*
			Salticus	*Salticus scenicus*
			Chalcoscirtus	*Chalcoscirtus infimus*
			Heliophanus	*Heliophanus cupreus*
				Heliophanus flavipes
				Heliophanus kochii
				Heliophanus tribulosus
		Theridiidae	*Theridion*	*Theridion harmsi*
		Thomisidae	*Bassaniodes*	*Bassaniodes bufo*
			Synema	*Synema globosum*
		Zodariidae	*Zodarion*	*Zodarion costapratae*
				Zodarion duriense
	Mesostigmata	Phytoseiidae	*Typhlodromus*	*Typhlodromus transvaalensis*
Diplopoda	Polyxenida	Polyxenidae	*Polyxenus*	*Polyxenus lagurus*
Insecta	Coleoptera	Attelabidae	*Auletobius*	*Auletobius pubescens*
		Carabidae	*Amara*	*Amara aenea*
		Chrysomelidae	*Gonioctena*	*Gonioctena olivacea*
			Calomicrus	*Calomicrus circumfusus*
			Gonioctena	*Gonioctena olivacea*
			Lachnaia	*Lachnaia hirta*
		Curculionidae	*Brachyderes*	*Brachyderes incanus*
			Hypera	*Hypera arator*

		Brachytemnus	*Brachytemnus porcatus*
	Nitidulidae	*Acanthogethes*	*Acanthogethes fuscus*
	Throscidae	*Trixagus*	*Trixagus leseigneuri*
Diptera	Anthomyiidae	*Adia*	*Adia cinerella*
	Ephydridae	*Psilopa*	*Psilopa marginella*
	Muscidae	*Neomyia*	*Neomyia cornicina*
	Syrphidae	*Eumerus*	*Eumerus pulchellus*
	Tachinidae	*Siphona*	*Siphona geniculata*
		Mintho	*Mintho rufiventris*
Hemiptera	Aphididae	*Calaphis*	*Calaphis flava*
	Aphididae	*Capitophorus*	*Capitophorus elaeagni*
		Euceraphis	*Euceraphis punctipennis*
		Geoica	*Geoica utricularia*
		Sitobion	*Sitobion avenae*
	Berytidae	*Apoplymus*	*Apoplymus pectoralis*
	Cercopidae	*Haematoloma*	*Haematoloma dorsata*
	Cicadellidae	*Aphrodes*	*Aphrodes makarovi*
	Coreidae	*Syromastus*	*Syromastus rhombeus*
	Lygaeidae	*Horvathiolus*	*Horvathiolus superbus*
		Spilostethus	*Spilostethus saxatilis*
	Miridae	*Heterocordylus*	*Heterocordylus tibialis*
		Horistus	*Horistus orientalis*
		Pachyxyphus	*Pachyxyphus lineellus*
	Reduviidae	*Rhynocoris*	*Rhynocoris erythropus*
	Rhyparochromidae	*Plinthisus*	*Plinthisus brevipennis*
	Scutelleridae	*Odontoscelis*	*Odontoscelis lineola*
	Formicidae	*Aphaenogaster*	*Aphaenogaster gibbosa*
		Camponotus	*Camponotus cruentatus*
		Formica	*Formica sanguinea*
			Formica selysi
		Lasius	*Lasius brunneus*
			Lasius grandis
			Lasius psammophilus
	Ichneumonidae	*Diplazon*	*Diplazon tetragonus*
	Tenthredinidae	*Aneugmenus*	*Aneugmenus padi*
		Monophadnoides	*Monophadnoides ruficruris*
		Tenthredopsis	*Tenthredopsis nassata*
Lepidoptera	Autostichidae	*Dysspastus*	*Dysspastus fallax*
	Coleophoridae	*Coleophora*	*Coleophora albicosta*
	Depressariidae	*Agonopterix*	*Agonopterix nervosa*
	Gelechiidae	*Aristotelia*	*Aristotelia ericinella*
		Aspitates	*Aspitates gilvaria*
		Isturgia	*Isturgia miniosaria*

			Neotelphusa	*Neotelphusa cisti*
			Rhoptria	*Rhoptria asperaria*
		Noctuidae	*Autographa*	*Autographa gamma*
			Heliothis	*Heliothis peltigera*
			Hoplodrina	*Hoplodrina ambigua*
			Luperina	*Luperina testacea*
			Peridroma	*Peridroma saucia*
			Polymixis	*Polymixis xanthomista*
		Pieridae	*Pieris*	*Pieris rapae*
		Pyralidae	*Acrobasis*	*Acrobasis bithynella*
		Tortricidae	*Acleris*	*Acleris hyemana*
			Cnephasia	*Cnephasia longana*
			Cnephasia	*Cnephasia alfacarana*
	Mecoptera	Panorpidae	*Panorpa*	*Panorpa meridionalis*
	Orthoptera	Acrididae	*Anacridium*	*Anacridium aegyptium*
			Antaxius	*Antaxius spinibrachius*
			Arcyptera	*Arcyptera tornosi*
			Calliptamus	*Calliptamus barbarus*
		Tettigoniidae	*Myrmeleotettix*	*Myrmeleotettix maculatus*
			Lluciapomaresius	*Lluciapomaresius asturiensis*
		Tetrigidae	*Tetrix*	*Tetrix undulata*
	Plecoptera	Perlodidae	*Isoperla*	*Isoperla grammatica*
	Psocodea	Liposcelididae	*Liposcelis*	*Liposcelis bostrychophila*
	Thysanoptera	Thripidae	*Anaphothrips*	*Anaphothrips obscurus*
Malacostraca	Amphipoda	Brevitalitridae	*Talitroides*	*Talitroides topitotum*
	Isopoda	Porcellionidae	*Porcellio*	*Porcellio scaber*

Supplementary Table 2. *Frequence of occurrence of OTU regarding areas burned in 2016 (BU16), areas burned in 2022 (BU22) and unburned areas (UN) present in the diet of Podarcis lusitanicus. When it was not possible to reach the species level, numbers were assigned to aggregate the sequences representing the same species (e.g. sp1).*

OTU	BU16	BU22	UN	Grand Total
Acanthogethes fuscus	1	1	1	3
Acleris hyemana	1	1	1	3
Acrobasis bithynella	1	1	1	3
Adia cinerella	2	1	0	3
Aelurillus sp.	2	1	0	3
Agonopterix nervosa	2	1	0	3
Agyneta rurestris	2	3	1	6
Alopecosa accentuata	1	2	2	5
Alopecosa albofasciata	1	1	3	5
Alopecosa sp.	1	1	0	2
Amara aenea	2	2	0	4
Anaceratagallia sp.1	1	3	0	4

Anaceratagallia sp.2	1	1	0	2
Anaceratagallia sp.3	1	1	0	2
Anaceratagallia sp.4	1	1	0	2
Anacridium aegyptium	1	1	1	3
Anaphothrips obscurus	1	1	1	3
Aneugmenus padi	4	7	1	12
Antaxius spinibrachius	1	1	0	2
Aphaenogaster gibbosa	2	1	0	3
Aphididae 1	1	1	0	2
Aphididae 2	1	1	0	2
Aphrodes makarovi	1	2	0	3
Apoplymus pectoralis	1	1	1	3
Arachnida 1	3	1	0	4
Arachnida 2	6	3	12	21
Araneae 1	1	1	1	3
Araneae 2	1	1	1	3
Araneae 3	1	2	0	3
Archaeognatha	1	1	1	3
Arcyptera sp.1	1	1	0	2
Arcyptera sp.2	1	1	0	2
Arcyptera tornosi	2	1	1	4
Aristotelia ericinella	1	1	0	2
Armadillidiidae	1	2	1	4
Arthropoda 1	1	1	1	3
Arthropoda 10	1	1	1	3
Arthropoda 2	1	2	0	3
Arthropoda 3	1	1	1	3
Arthropoda 4	2	1	0	3
Arthropoda 5	1	1	0	2
Arthropoda 6	2	1	0	3
Arthropoda 7	1	2	0	3
Arthropoda 8	1	2	1	4
Arthropoda 9	1	1	0	2
Aspitates gilvaria	2	1	0	3
Atylotus sp.1	2	1	0	3
Atylotus sp.2	3	1	0	4
Atylotus sp.3	3	1	0	4
Auletobius pubescens	2	1	0	3
Autographa gamma	1	2	0	3
Axinotarsus sp.	1	1	1	3
Ballus chalybeius	1	1	1	3
Bassaniodes bufo	3	1	1	5
Bellardia sp.	1	2	0	3
Bibionidae	1	1	0	2
Bourletiellidae 1	3	1	0	4
Bourletiellidae 2	1	2	0	3

Bourletiellidae 3	1	2	0	3
Brachychthoniidae	1	2	0	3
Brachyderes incanus	1	1	0	2
Brachyderes sp.1	1	1	1	3
Brachyderes sp.2	1	1	0	2
Brachyderes sp.3	1	2	0	3
Brachytemnus porcatus	7	1	1	9
Brentidae 1	1	1	0	2
Brentidae 2	2	1	0	3
Brentidae 3	3	1	0	4
Bryotropha sp.	1	1	0	2
Calaphis flava	1	1	0	2
Calaphis sp.	1	1	1	3
Callilepis concolor	1	2	0	3
Calliptamus barbarus	2	3	1	6
Calomicrus circumfusus	1	2	0	3
Camponotus cruentatus	1	2	1	4
Capitophorus elaeagni	1	2	0	3
Capsodes sp.1	6	4	6	16
Capsodes sp.2	1	1	0	2
Capsodes sp.3	1	1	0	2
Cecidomyiidae	2	2	0	4
Ceratozetidae 1	1	1	1	3
Ceratozetidae 2	1	1	0	2
Ceratozetidae 3	1	1	2	4
Ceratozetidae 4	1	1	0	2
Chalcoscirtus infimus	2	2	0	4
Chalcoscirtus sp.	1	1	1	3
Chamobatidae 1	1	1	1	3
Cheiracanthium sp.	1	1	0	2
Chironomidae	1	2	0	3
Chrysocrambus sp.1	1	1	1	3
Chrysocrambus sp.2	1	1	0	2
Chrysocrambus sp.3	1	1	0	2
Chrysocrambus sp.4	1	1	0	2
Chrysocrambus sp.5	1	1	1	3
Chrysomelidae	1	1	0	2
Cicadellidae	1	1	0	2
Cnephasia alfacarana	1	1	0	2
Cnephasia longana	2	1	0	3
Coleophora albicosta	2	1	0	3
Coleoptera 1	1	2	0	3
Coleoptera 2	1	1	1	3
Coleoptera 3	2	3	1	6
Coleoptera 4	1	2	0	3
Coleoptera 5	1	2	0	3

Coleoptera 6	1	2	0	3
Curculionidae 1	1	1	1	3
Curculionidae 2	1	3	0	4
Curculionidae 3	1	2	1	4
Danacea sp.	1	1	0	2
Delphacidae	2	3	1	6
Dicyphus sp.	1	2	0	3
Dilophus sp.1	1	4	0	5
Diplazon tetragonus	1	1	1	3
Dolerus sp.1	2	1	0	3
Dolerus sp.2	2	1	0	3
Dolerus sp.3	1	2	0	3
Dolichopodidae	1	1	1	3
Drassodes pubescens	1	1	3	5
Dysspastus fallax	2	1	0	3
Elateridae 1	1	2	0	3
Elateridae 2	1	1	0	2
Elateridae 3	1	2	0	3
Elateridae 4	1	2	1	4
Eluma sp.1	1	1	0	2
Eluma sp.2	1	1	1	3
Entomobryidae 1	2	2	0	4
Entomobryomorpha	1	2	0	3
Episyrphus sp.	1	1	1	3
Eriophyidae 1	1	2	0	3
Eriophyidae 2	1	1	2	4
Eriophyidae 3	1	3	0	4
Eriophyidae 4	1	2	1	4
Eriophyidae 5	2	1	0	3
Eriophyidae 6	2	1	1	4
Eriophyidae 7	1	2	0	3
Euceraphis punctipennis	1	1	1	3
Euceraphis sp.1	1	1	0	2
Euceraphis sp.2	1	1	0	2
Eucyclodes sp.1	1	1	0	2
Eumerus pulchellus	3	1	0	4
Eupodidae 1	2	2	1	5
Eustigmaeus sp.	1	1	1	3
Eutrombidiidae 1	2	1	0	3
Eutrombidiidae 2	2	1	0	3
Exapion sp.1	1	1	1	3
Formica sanguinea	1	1	1	3
Formica selysi	1	2	0	3
Formica sp.1	2	2	2	6
Formicidae	1	3	1	5
Gastropoda 1	2	1	0	3

Gastropoda 2	1	1	2	4
Geoica utricularia	1	1	1	3
Geometridae 1	1	1	1	3
Geometridae 2	2	1	0	3
Gnaphosidae	1	3	0	4
Gonioctena olivacea	1	3	1	5
Haematoloma dorsata	1	2	0	3
Halticus sp.	2	1	0	3
Haplodrassus signifer	2	1	0	3
Heliophanus cupreus	2	3	4	9
Heliophanus flavipes	2	1	0	3
Heliophanus kochii	2	1	4	7
Heliophanus sp.1	1	1	0	2
Heliophanus sp.10	1	1	0	2
Heliophanus sp.2	1	1	1	3
Heliophanus sp.3	1	1	0	2
Heliophanus sp.4	1	1	0	2
Heliophanus sp.5	1	1	0	2
Heliophanus sp.7	1	1	0	2
Heliophanus sp.8	2	1	1	4
Heliophanus sp.9	1	3	1	5
Heliophanus tribulosus	1	1	2	4
Heliothis peltigera	3	2	1	6
Hemiptera	1	1	0	2
Heterocordylus tibialis	2	1	0	3
Hogna radiata	2	1	0	3
Hoplodrina ambigua	1	2	0	3
Horistus orientalis	3	1	1	5
Horvathiolus superbus	1	3	1	5
Hypera arator	1	2	0	3
Iberattus sp.	2	2	1	5
Ichneumonidae 1	1	1	0	2
Ichneumonidae 2	1	1	0	2
Ichneumonidae 3	1	1	0	2
Ichneumonidae 4	1	2	0	3
Idaea sp.	1	1	1	3
Insecta 1	2	1	1	4
Insecta 2	1	1	0	2
Insecta 3	1	1	1	3
Insecta 4	2	1	0	3
Insecta 5	5	3	1	9
Insecta 6	1	1	0	2
Insecta 7	2	1	1	4
Insecta 8	1	1	0	2
Insecta 9	1	2	0	3
Isoperla grammatica	1	1	2	4

Issidae 1	3	2	2	7
Isturgia miniosaria	1	2	0	3
Julidae	1	1	2	4
Lachnaia hirta	1	1	0	2
Lagria sp.	1	1	1	3
Lasius brunneus	2	1	0	3
Lasius grandis	1	1	0	2
Lasius psammophilus	1	1	0	2
Lasius sp.1	5	3	8	16
Lasius sp.2	1	1	0	2
Lasius sp.3	1	1	0	2
Lepidoptera 1	1	1	0	2
Lepidoptera 2	1	2	0	3
Lepidoptera 3	2	1	0	3
Lepidoptera 4	1	1	0	2
Linyphiidae	1	1	1	3
Liposcelis bostrychophila	1	1	1	3
Lluciapomaresius asturiensis	1	2	0	3
Lluciapomaresius sp.	1	1	0	2
Lophopilio sp.	1	1	0	2
Lumbricidae 1	3	1	0	4
Lumbricidae 2	1	1	1	3
Luperina testacea	1	1	0	2
Lycosidae	1	1	0	2
Minettia sp.	1	2	1	4
Mintho rufiventris	3	1	0	4
Miridae 1	1	2	0	3
Miridae 2	1	1	0	2
Mollusca 1	3	1	0	4
Mollusca 2	1	1	1	3
Monophadnoides ruficruris	1	2	0	3
Mycetophilidae	2	1	0	3
Myrmeleotettix maculatus	1	2	0	3
Nanorchestidae 1	1	2	0	3
Nanorchestidae 2	1	2	0	3
Neanuridae	1	2	0	3
Neomyia cornicina	2	1	0	3
Neotelphusa cisti	2	1	1	4
Nomisia celerrima	1	1	0	2
Nomisia exornata	2	3	3	8
Odonata 1	1	3	0	4
Odontoscelis lineola	1	1	1	3
Oedipoda sp.	1	2	0	3
Olibrus sp.1	2	1	0	3
Olibrus sp.2	1	2	0	3
Oniscidae	1	1	1	3

Oniscus sp.1	3	4	2	9
Oniscus sp.2	1	1	0	2
Oniscus sp.3	1	1	0	2
Oniscus sp.4	1	1	1	3
Oniscus sp.5	1	1	1	3
Oniscus sp.6	2	1	0	3
Oribatulidae 1	1	2	0	3
Oribatulidae 2	1	1	0	2
Pachyxyphus lineellus	1	1	1	3
Panorpa meridionalis	1	1	0	2
Pardosa hortensis	1	1	1	3
Pardosa sp.	2	1	0	3
Pellenes sp.1	1	1	1	3
Periclista sp.	1	2	0	3
Peridroma saucia	1	3	0	4
Phalangiidae 1	1	2	0	3
Phalangium sp.	3	1	2	6
Phlegra sp.	2	1	0	3
Pholcus opilionoides	2	2	1	5
Phyllodromica sp.1	1	1	0	2
Phyllodromica sp.10	1	1	0	2
Phyllodromica sp.11	8	5	3	16
Phyllodromica sp.13	1	1	0	2
Phyllodromica sp.2	1	3	0	4
Phyllodromica sp.3	1	1	0	2
Phyllodromica sp.4	1	1	1	3
Phyllodromica sp.5	1	2	0	3
Phyllodromica sp.6	1	1	0	2
Phyllodromica sp.7	1	1	0	2
Phyllodromica sp.8	1	1	0	2
Phyllodromica sp.9	1	1	0	2
Pieris rapae	1	1	0	2
Platycleis sp.1	1	3	0	4
Platycleis sp.2	2	1	0	3
Platycleis sp.3	2	3	0	5
Platycleis sp.4	1	1	0	2
Platycleis sp.5	1	1	0	2
Platycleis sp.6	1	1	0	2
Platycleis sp.7	1	1	0	2
Plinthisus brevipennis	1	1	1	3
Poecilochroa albomaculata	1	1	1	3
Polymixis xanthomista	1	2	0	3
Polyxenus lagurus	1	1	1	3
Porcellio scaber	2	1	0	3
Pseudeuophrys sp.	1	1	1	3
Psilopa marginella	1	3	0	4

Psyllidae 1	3	3	0	6
Psylliodes sp.1	1	1	1	3
Psylliodes sp.2	1	2	1	4
Psylliodes sp.3	1	1	1	3
Psylliodes sp.4	1	1	0	2
Psylliodes sp.5	1	1	0	2
Pulchellodromus pulchellus	1	1	1	3
Rhogogaster sp.	1	2	0	3
Rhoptria asperaria	1	1	1	3
Rhynocoris erythropus	1	1	1	3
Salticidae 1	1	1	1	3
Salticidae 2	2	1	1	4
Salticidae 3	1	4	1	6
Salticidae 4	1	1	0	2
Salticidae 5	2	1	0	3
Salticidae 6	1	1	1	3
Salticidae 7	2	2	0	4
Salticus scenicus	1	1	4	6
Salticus sp.	1	4	0	5
Sarcoptiformes 1	1	1	1	3
Sarcoptiformes 2	2	2	0	4
Sarcoptiformes 3	1	1	0	2
Sarcoptiformes 4	1	2	0	3
Sarcoptiformes 5	2	1	0	3
Sarcoptiformes 6	2	2	1	5
Sarcoptiformes 7	1	1	0	2
Scheloribatidae	2	1	0	3
Scutacaridae 1	3	2	0	5
Scutacaridae 2	2	1	0	3
Scythrididae	1	1	1	3
Scythris sp.	2	1	0	3
Siphona geniculata	1	1	1	3
Siphona sp.	1	1	0	2
Sitobion avenae	1	2	0	3
Sitona sp.	1	1	1	3
Spilostethus saxatilis	2	1	0	3
Symphypleona	1	1	1	3
Synema globosum	1	1	2	4
Synema sp.6	1	1	0	2
Syromastus rhombeus	2	1	0	3
Talitroides sp.	1	1	0	2
Talitroides topitotum	11	12	10	33
Tapinoma sp.1	1	1	6	8
Tapinoma sp.2	1	1	0	2
Tenebrionidae 1	4	4	0	8
Tenthredo sp.	3	1	0	4

Tenthredopsis nassata	1	1	1	3
Termitidae	2	1	0	3
Tetramorium sp.1	1	1	2	4
Tetramorium sp.2	1	1	0	2
Tetramorium sp.3	1	1	1	3
Tetramorium sp.4	1	1	0	2
Tetramorium sp.5	2	1	0	3
Tetrix undulata	1	1	1	3
Tettigoniidae	1	2	0	3
Textrix pinicola	1	1	1	3
Thereva sp.	1	2	0	3
Theridiidae 1	2	2	0	4
Theridion harmsi	2	3	0	5
Thomisidae 1	1	2	0	3
Thomisidae 2	1	2	2	5
Thomisidae 3	2	1	0	3
Tipula sp.1	3	1	1	5
Tipulidae 1	3	1	1	5
Trixagus leseigneuri	1	1	1	3
Trombiculidae 1	2	1	0	3
Trombiculidae 2	6	3	2	11
Typhlodromus transvaalensis	1	2	0	3
Urodinychidae	2	1	0	3
Utecha sp.1	2	1	0	3
Utecha sp.2	1	1	0	2
Utecha sp.3	1	1	0	2
Utecha sp.4	2	1	1	4
Utecha sp.5	1	1	0	2
Walckenaeria sp.	1	1	1	3
Xysticus sp.1	1	3	3	7
Xysticus sp.2	1	3	0	4
Xysticus sp.3	1	1	0	2
Xysticus sp.4	1	1	0	2
Xysticus sp.5	1	1	0	2
Zelotes sp.1	1	2	0	3
Zelotes sp.2	1	2	0	3
Zelotes thorelli	1	2	0	3
Zodarion costapratae	1	2	0	3
Zodarion duriense	1	2	0	3
Total	**55**	**63**	**65**	**183**

Supplementary Table 3. *Frequence of occurrence of Family regarding areas burned in 2016 (BU16), areas burned in 2022 (BU22) and unburned areas (UN) present in the diet of Podarcis lusitanicus.*

Family	BU16	BU22	UN	Grand Total

Salticidae	10	12	18	40
Formicidae	9	7	18	34
Brevitalitridae	11	12	10	33
Miridae	10	6	7	23
Tenthredinidae	6	11	2	19
Curculionidae	7	6	5	18
Thomisidae	3	5	8	16
Ectobiidae	8	5	3	16
Gnaphosidae	3	7	6	16
Eriophyidae	2	6	4	12
Oniscidae	4	4	4	12
Acrididae	3	5	3	11
Noctuidae	3	7	1	11
Trombiculidae	6	3	2	11
Lycosidae	3	2	6	11
Tenebrionidae	4	4	1	9
Geometridae	3	2	3	8
Tipulidae	5	1	2	8
Chrysomelidae	1	5	2	8
Linyphiidae	2	3	3	8
Tettigoniidae	2	5	0	7
Cicadellidae	2	4	1	7
Theridiidae	3	4	0	7
Issidae	3	2	2	7
Phalangiidae	3	2	2	7
Bourletiellidae	3	3	0	6
Psyllidae	3	3	0	6
Delphacidae	2	3	1	6
Scutacaridae	4	2	0	6
Aphididae	1	3	2	6
Lygaeidae	2	3	1	6
Tachinidae	3	1	1	5
Brentidae	3	1	1	5
Elateridae	1	3	1	5
Lumbricidae	3	1	1	5
Syrphidae	3	1	1	5
Bibionidae	1	4	0	5
Ceratozetidae	1	1	3	5
Armadillidiidae	1	2	2	5
Eupodidae	2	2	1	5
Pholcidae	2	2	1	5
Tabanidae	3	1	0	4
Phalacridae	2	2	0	4
Perlodidae	1	1	2	4
Lauxaniidae	1	2	1	4
Tortricidae	2	1	1	4

Entomobryidae	2	2	0	4
Ephydridae	1	3	0	4
Zodariidae	1	3	0	4
Ichneumonidae	1	2	1	4
Gelechiidae	2	1	1	4
Julidae	1	1	2	4
Scythrididae	2	1	1	4
Carabidae	2	2	0	4
Cecidomyiidae	2	2	0	4
Nanorchestidae	1	3	0	4
Calliphoridae	1	2	0	3
Melyridae	1	1	1	3
Crambidae	1	1	1	3
Brachychthoniidae	1	2	0	3
Tetrigidae	1	1	1	3
Oribatulidae	1	2	0	3
Attelabidae	2	1	0	3
Autostichidae	2	1	0	3
Berytidae	1	1	1	3
Dolichopodidae	1	1	1	3
Agelenidae	1	1	1	3
Cercopidae	1	2	0	3
Therevidae	1	2	0	3
Philodromidae	1	1	1	3
Throscidae	1	1	1	3
Chamobatidae	1	1	1	3
Depressariidae	2	1	0	3
Phytoseiidae	1	2	0	3
Stigmaeidae	1	1	1	3
Polyxenidae	1	1	1	3
Anthomyiidae	2	1	0	3
Porcellionidae	2	1	0	3
Eutrombidiidae	2	1	0	3
Coleophoridae	2	1	0	3
Termitidae	2	1	0	3
Pyralidae	1	1	1	3
Muscidae	2	1	0	3
Reduviidae	1	1	1	3
Mycetophilidae	2	1	0	3
Rhyparochromidae	1	1	1	3
Thripidae	1	1	1	3
Coreidae	2	1	0	3
Urodinychidae	2	1	0	3
Scheloribatidae	2	1	0	3
Chironomidae	1	2	0	3
Liposcelididae	1	1	1	3

Scutelleridae	1	1	1	3
Neanuridae	1	2	0	3
Nitidulidae	1	1	1	3
Cheiracanthiidae	1	1	0	2
Pieridae	1	1	0	2
Panorpidae	1	1	0	2
Total	**53**	**62**	**63**	**178**