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Demographic and Behavioural Responses of the Roe Deer (*Capreolus capreolus*) to Landscape Modifications and Landscape of Fear

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Chapter 1

Background and Research Objectives

1.1 The ecological concept of landscape

The relationship between landscape complexity and population dynamics is one of the main research topics in ecology and, given the flexible definition of landscape, i.e., an area that is spatially heterogeneous in at least one factor of interest (Turner and Gardner 2015), landscape ecology is applicable across scales and adaptable to different systems (Turner 2005).

A landscape is classically described by its spatial structure, that is the arrangement, composition, and configuration of the different land cover types, features, and habitats composing it. Human development greatly changed the spatial structure of landscapes affecting their suitability for wildlife. A complex branch of research aims at analysing the effects of landscape modifications, i.e., the conversion of native vegetation into agriculture and urban development, on local populations using different approaches (Fischer and Lindenmayer 2007).

Animal species can react differently to landscape modifications; these can lead to altered breeding patterns and social systems and can induce behavioural alterations affecting movements, but also communication. Moreover, they can interfere with species interactions affecting, for example, competition and predation (e.g. Tuomainen and Candolin 2011; Lowry et al. 2013). While for many species these responses are negative, because they imply a reduction in survival, reproduction, and persistence over time, other species can benefit from the new conditions. Indeed, modified landscapes offer abundant human-related resources and a high diversity of anthropogenic habitats. The response of wild species to landscape modifications varies from “avoidance”, for species which are very sensitive to habitat changes, to “exploitation”, for species which are almost completely dependent on anthropogenic resources and habitats, passing through “adaptation”, for species which can utilise anthropogenic resources and habitats but rely predominantly on natural ones (Lowry et al. 2013).

Interestingly, landscape ecological approaches are not limited to land. For example, the landscape of fear is considered a crucial mechanism in structuring communities, having cascading effects from individuals to ecosystems (Laundré et al. 2010). Indeed, the perceived predation risk plays a powerful role in shaping the behaviours of fearful prey, with consequences for individual physiology, population dynamics and demography, and interactions at the community level (Gaynor et al. 2019). The landscape of fear can be defined as the spatial variation in prey perception of predation risk (Gaynor et al. 2019): a sort of “map” with “peaks” and “valleys” describing an animal's perception of the locations as dangerous and safe, respectively (Van Der Merwe and Brown 2008).

Risk perception is manifested in measurable behavioural outcomes, as prey incorporate information about predation risk into decisions about where to go and how to behave. Anti-predator behaviour encompasses any response adopted by a prey species to minimize the likelihood of predation. This behaviour can be either reactive, such as fleeing when faced with a predator, or proactive, like maintaining vigilance to enhance predator detection or improve the chances of evasion upon encountering a threat (Gaynor et al. 2019). Thus, the landscape of fear generates two behavioural strategies to minimise risk:

i) avoidance of high-risk areas on both spatial and temporal scales, and

ii) modulation of anti-predator behaviours to reduce the probability of predation at a given location (Gaynor et al. 2019).

1.2 The target species: the roe deer

The roe deer (*Capreolus capreolus*, Linnaeus 1758), the target species of this research project, is an important species from the ecological point of view representing a prey for carnivores and a game species (Putman et al. 2011). This species can also serve as an environmental bioindicator: its widespread geographic distribution, relatively long-life cycle, and facility to collect biological samples, for example from hunted animals, render it an ideal organism in ecotoxicology (Cappelli et al. 2020).

This small-sized ungulate is classically described as a forest-dwelling species, as woodland contains all the necessary resources for its survival and persistence, like access to cover and feeding sites (Lovari et al. 2017 and references therein). Its small body size is suitable for movements in densely vegetated areas, such as woodlands with thick underwood.

The roe deer show marked plasticity inhabiting contrasting environments (Jepsen and Topping 2004; Morellet et al. 2011). Indeed, the species is renowned for its ability to thrive across a wide range of habitats, from dense woodlands and rugged mountains to open grasslands and agricultural landscapes (Danilkin and Hewison 1996; Lovari et al. 2016).

The ability of a species to cope with landscape modifications depends on its traits, that is on its evolutionary history. The roe deer evolved during the Pleistocene (Sommer et al. 2009) when the ungulate community was rich and counted no less than 21 species, nearly all of them larger than the roe deer. Within this community, the roe deer was probably a subordinate species forced to use margins between forests and open areas, two very different habitats where other species were dominant. These margins corresponded to glades opened by natural events (e.g. fires, floods, landslides), thus short-lasting habitats which tend to close after some years (Lovari et al. 2017).

Such evolutionary pressures could have produced a particularly important trait, which is behavioural plasticity, that is the ability of individuals to behave suitably under new conditions, which is crucial for determining their success or failure (Snell-Rood 2013).

Roe deer densities and distribution strongly increased after the Second World War; the species started to recover after a historical decline with gradual recolonisation of previously occupied areas (Apollonio 2004; Apollonio et al. 2010). The expansion can be mainly explained by the changes in the hunting regulations, the human abandonment of hill and mountain areas with consequent increase of natural vegetation (Meriggi et al. 2009) and, also, the reintroductions of animals for hunting purposes (Randi et al. 2004). Nowadays the roe deer is becoming a stable presence in fragmented and intensive agricultural landscapes (Hewison et al. 2001), where natural vegetation is restricted to small isolated patches or is nearly absent, even though these are considered marginal habitats for this species (Lovari et al. 2016).

Northern Italy is one of the most anthropogenic landscapes in Europe and, in particular, the Po Plain, the intensively cultivated lowland area interposed between the Apennines and the Alps, has been dramatically altered and its natural setting of alluvial plain forests has been lost. Nowadays, small and isolated natural or semi-natural vegetated patches are interspersed within the highly modified anthropogenic matrix and located along the rivers, namely the Po River and its main affluents.

Interestingly, the roe deer is becoming a stable presence in fragmented and intensive agricultural landscapes in several areas within the Po Plain (De Pasquale et al. 2019; Dondina et al. 2019), where natural vegetation is restricted to small isolated patches or is nearly absent. Within this highly modified context, the roe deer shows marked adaptability moving through the landscape relying on small patches of woodlands and sub-wooded habitats (e.g. hedgerows, reforestations, poplar cultivations, and uncultivated fields occupied by shrubs and little trees; Dondina et al. 2019).

In these areas, where top predators are generally absent or occasionally present, the landscape of fear would be primarily defined by human presence and activities. Conversely, in hill and mountainous areas surrounding the Po Plain along the Apennines and the Alps, the landscape of fear would be primarily defined by the presence of the roe deer main predator in Italy, which is the wolf (*Canis lupus*).

1.3 Project aims

In order to comprehend the broader patterns of wildlife behaviour in human-modified landscapes, it is crucial to conduct analyses at larger spatiotemporal scales, considering environmental gradients and various seasons (Neumann et al. 2013). The main aim of this research project was to investigate the relationship between the landscape, meant both as spatial structure of the land cover and perception of risk, and the local responses, in terms of demography and behaviour, of the roe deer in different study areas in north-western Italy, which were described in Chapter 2. In this context, the specific objectives of the study were:

i) To define the importance of the roe deer as a prey species for the wolf. While the roe deer is considered a common prey for wolves (e.g. Zlatanova et al. 2014; Newsome et al. 2016), the patterns of its consumption are highly variable over space and time. Understanding the predation pressure can shed light on roe deer population dynamics; indeed, large carnivores, such as wolves, may have a significant impact on both the demography and behaviour of prey populations (Bonnot et al. 2020).

This first objective, discussed in Chapter 3, has been addressed within the framework of a long-term research project (2007-2022) carried out in the Lombard Apennines focused on predator-prey dynamics involving wolves and wild ungulates.

ii) To analyse the demography of roe deer populations inhabiting different study areas situated along a gradient of landscape composition, ranging from natural to modified, and experiencing differing predator pressures. Both landscape structure and predator presence can significantly influence this species, impacting factors such as population density and demographic parameters (e.g. Focardi et al. 2002; Zannèse et al. 2006; Melis et al. 2009; Randon et al. 2020).

iii) To analyse the anti-predator responses of the roe deer within the same study areas and, consequently, identify the main factors influencing roe deer behaviours. Roe deer exhibit a variety of anti-predator responses that can be finely tuned on local scales based on perceived risk. Among these responses, shifts in activity patterns (Bonnot et al. 2020) and modulation of alarm behaviours (e.g. Benhaiem et al. 2008; Sönnichsen et al. 2013; Eccard et al. 2017) have been examined in this project.

The second and third specific objectives, discussed in Chapters 4 and 5 respectively, have been evaluated within the framework of a specific research project (2020-2022) carried out with a species-oriented approach in seven different study areas located in north-western Italy.

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Chapter 2

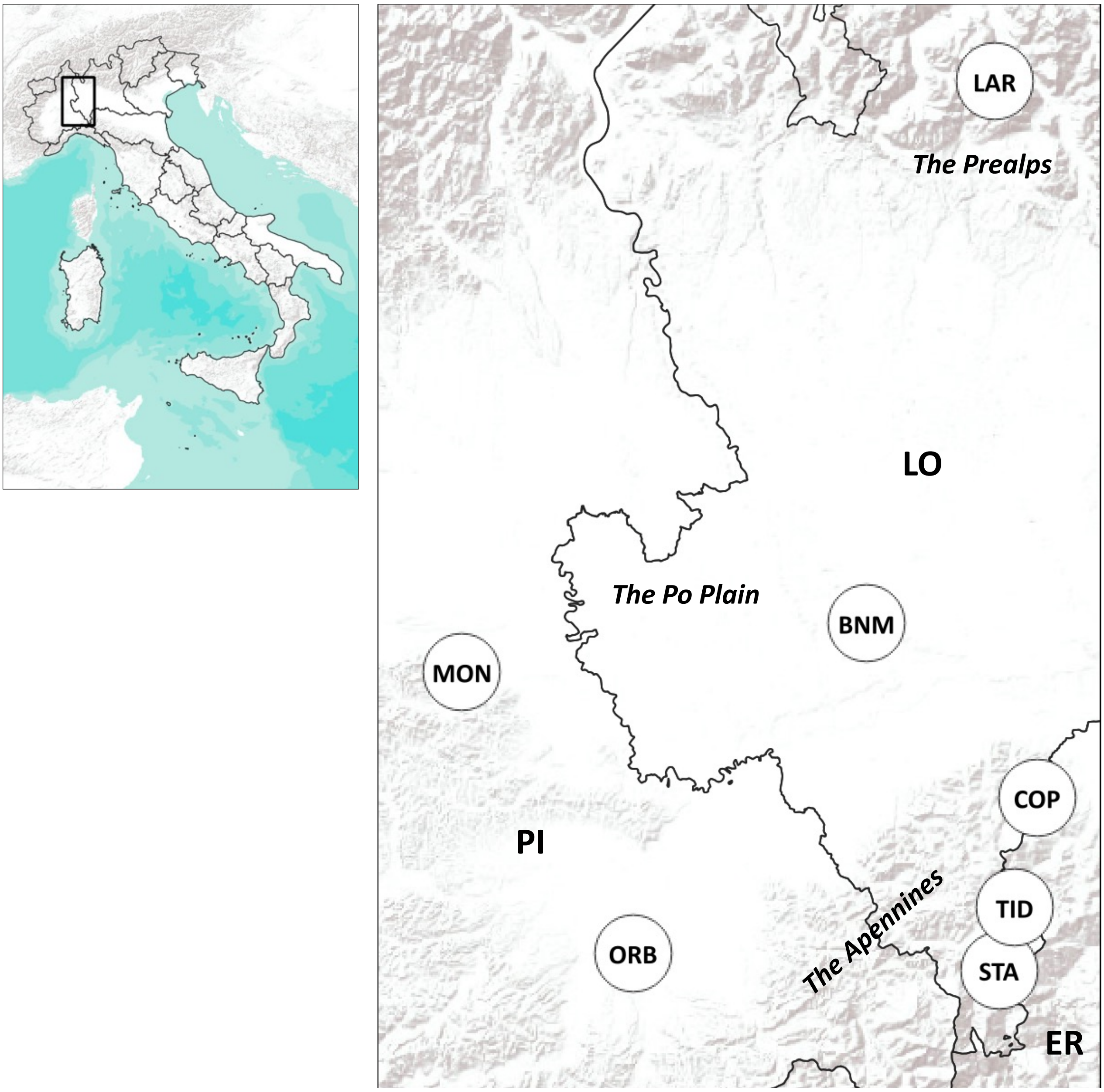
Landscape Composition and Predator Presence in the Study Areas

2.1 Overview of the study areas

This research has been conducted across seven distinct study areas situated in north-western Italy, spanning the regions of Lombardy (LO), Piedmont (PI), and Emilia-Romagna (ER).

The study areas exhibit notable diversity in landscape morphology and composition. In terms of topography, they are distributed, from north to south, encompassing the Lombard Prealps (LAR), the Po Plain (BNM, MON, and ORB), and the Lombard Apennines (COP, TID, and STA) (Figure 2.1.1 and Table 2.1.1).

Figure 2.1.1 - Location of the study areas in north-western Italy



Considering landscape composition, two study areas (LAR and STA) are characterized by a predominant natural landscape with abundant woodlands. A mixed landscape with an alternation of woodlands and cultivated lands characterizes the other two study areas (BNM and TID). Finally, the last three study areas (MON, ORB, and COP) are characterized by a predominant agricultural landscape with restricted patches of woodland (Figure 2.1.2 and Table 2.1.1).

Figure 2.1.2 - Land cover types (derived from Corine Land Cover 2018) characterizing the portion of north-western Italy encompassing the study areas

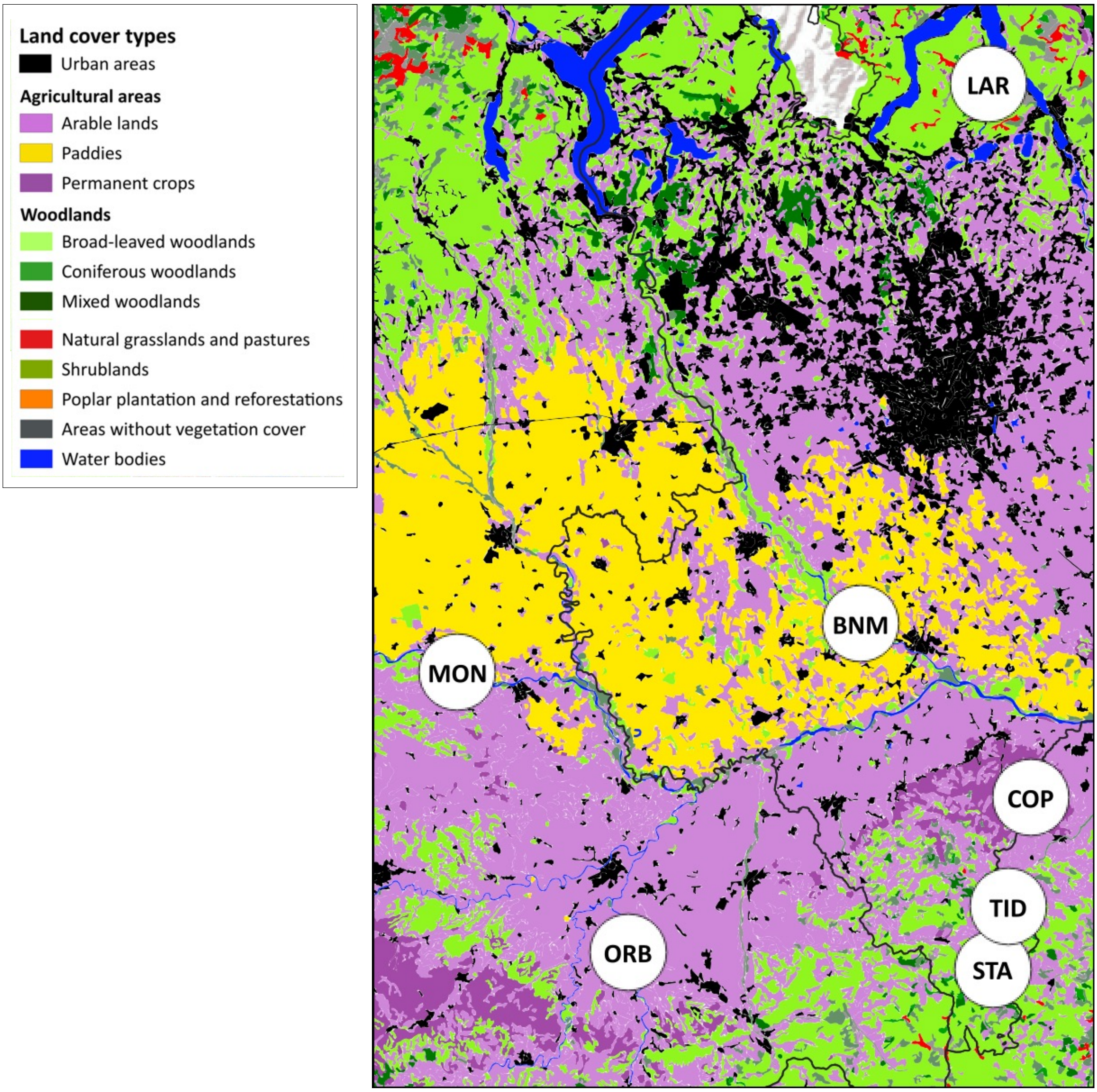


Table 2.1.1 - Main characteristics of the study areas

	LAR	**BNM**	**MON**	**ORB**	**COP**	**TID**	**STA**
	Triangolo Lariano	**Boschi Negri e Moriano**	**Basso Monferrato**	**Torrente Orba**	**Colline Oltrepò Pavese**	**Val Tidone**	**Alta Valle Staffora**
Region	LO	LO	PI	PI	LO - ER	LO - ER	LO - ER
Provinces	CO - LC	PV	AL	AL	PV - PC	PV - PC	PV - PC
Elevation range (m a.s.l.)	200-1100	< 100	100-350	110-145	130-410	385-860	550-1500
Land cover classes:							
Urban areas	8.3%	3.3%	7.1%	5.3%	7.4%	4.6%	2.2%
Agricultural areas:							
arable lands	-	19.3%	13.6%	65.0%	10.7%	30.0%	11.6%
paddies	-	18.7%	10.5%	-	-	-	-
permanent crops	0.1%	0.4%	2.7%	-	69.6%	1.5%	0.5%
Woodlands:							
broad-leaved w.	64.4%	26.6%	26.6%	12.9%	6.5%	37.9%	53.4%
coniferous w.	0.7%	-	-	-	-	2.0%	12.6%
mixed w.	14.3%	-	-	-	-	1.4%	4.1%
Natural grasslands and pastures	9.3%	1.3%	7.0%	7.8%	2.0%	7.9%	6.3%
Shrublands	1.4%	3.8%	4.9%	2.6%	3.1%	13.0%	8.5%
Poplar plantation and reforestations	-	16.6%	15.3%	0.9%	0.7%	0.2%	0.4%
Areas without vegetation cover	0.0%	3.4%	0.5%	1.7%	-	1.0%	0.4%
Water bodies	1.1%	6.8%	11.9%	3.9%	-	0.4%	-
Range of annual temperature (°C)	2-19	1-23	2-23	3-23	2-23	1-21	1-19
Range of annual rainfall (mm)	51-141	41-105	41-94	32-125	37-105	52-139	64-188

2.2 Description of the study areas

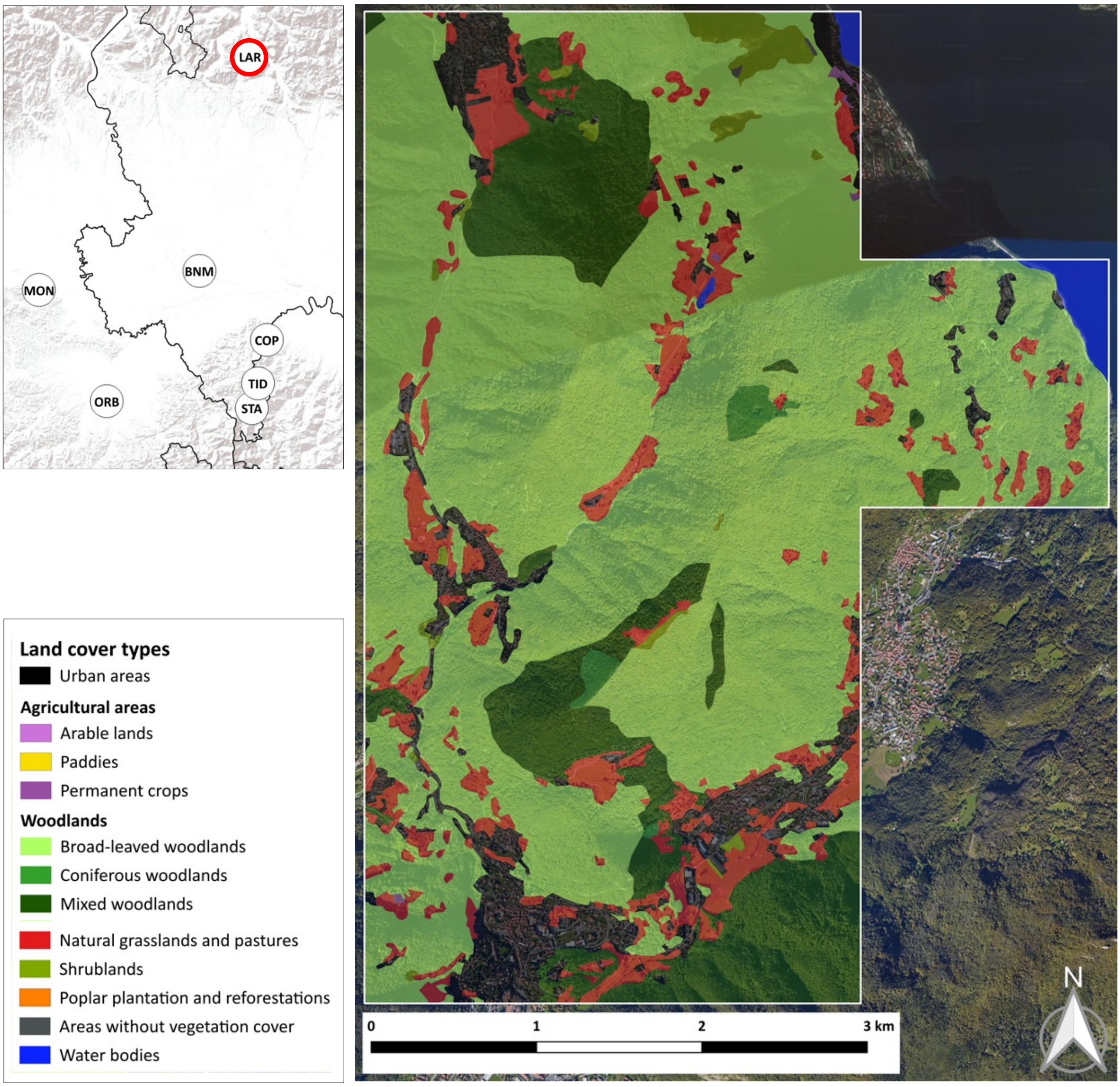
The land cover layers compiled by Regions were used to describe the landscape composition of the seven study areas (Lombardy: https://www.geoportale.regione.lombardia.it; Piedmont: https://www.geoportale.piemonte.it; Emilia-Romagna: https://geoportale.regione.emilia-romagna.it); in particular, the reported detailed categories were summarised into broad classes mainly describing the land use categories.

2.2.1 The Lombard Prealps

The Lombard Prealps form a natural border between the plain of the Po Valley and a fault line that separates them from the southern slopes of the Italian Alps in the Lombardy region. The pre-alpine landscape is highly diverse, featuring partly wooded and partly rugged or rocky reliefs, abundant with flowing waters and both large (including Garda, Maggiore, and Como,) and small lake basins throughout. The Lombard Prealps encompass elevations ranging from approximately 200 m to over 3000 m above sea level. The climate varies with elevation, but generally, lower valleys and foothills have a temperate climate with mild winters and warm summers, similar to the surrounding lowland areas. Higher elevations experience cooler temperatures and substantial precipitation levels, with snowfall common in winter. Average temperatures range from around 0°C in winter to 20°C in summer.

Triangolo Lariano (LAR) - The study area named Triangolo Lariano is located between the two branches of the Como Lake and the foothills of Brianza, an area characterised by the presence of many glacial small lakes. The territory is predominantly mountainous with elevations ranging from 180 to 1100 meters a.s.l. (Monte Oriolo 1108 m). The climate is temperate-continental; summers tend to be warm to hot, while winters are cold, often with significant snowfall. The climate is influenced by the proximity to the lakes and the surrounding mountains. Precipitation is evenly distributed throughout the year, with slightly higher rainfall in the spring and autumn months. The study area has a predominant natural landscape with abundant woodland cover (Figure 2.2.1). Broadleaved woodlands are widespread (64.4% of the surface) and mainly composed of thermophilous species (e.g. *Ostrya carpinifolia*, *Acer* spp., *Fraxinus* spp., *Tilia* spp.), beech (*Fagus sylvatica*) and chestnut (*Castanea sativa*). Mixed woodlands (14.3%) are mainly composed of birch (*Betula* spp.) and spruce (*Picea abies*), whereas monospecific coniferous woodlands (0.7%) are very limited and originated from artificial reforestation. Agricultural areas are mainly constituted of arable fodder crops and grasses, which are restricted to the valley bottoms, and pastures on mountain ridges; meadows and pastures cover 9.6% of the surface. The study area overlooks the Como Lake; water bodies (1.1%) are represented by little mountain streams and one natural marsh. Urban areas (8.3%) are located at the valley bottoms and are represented by medium or small villages. The average human population density is 229.8 inhabitants per km^2. Main human activities include extensive farming, livestock (dairy production), and recreational activities such as hunting and outdoor activities. Hunting is typically restricted to a few months during the year, primarily during the autumn season, while outdoor activities (e.g. hiking and rock climbing) are enjoyed throughout all seasons of the year.

Figure 2.2.1 - Land cover classes mainly characterizing the landscape of the area Triangolo Lariano

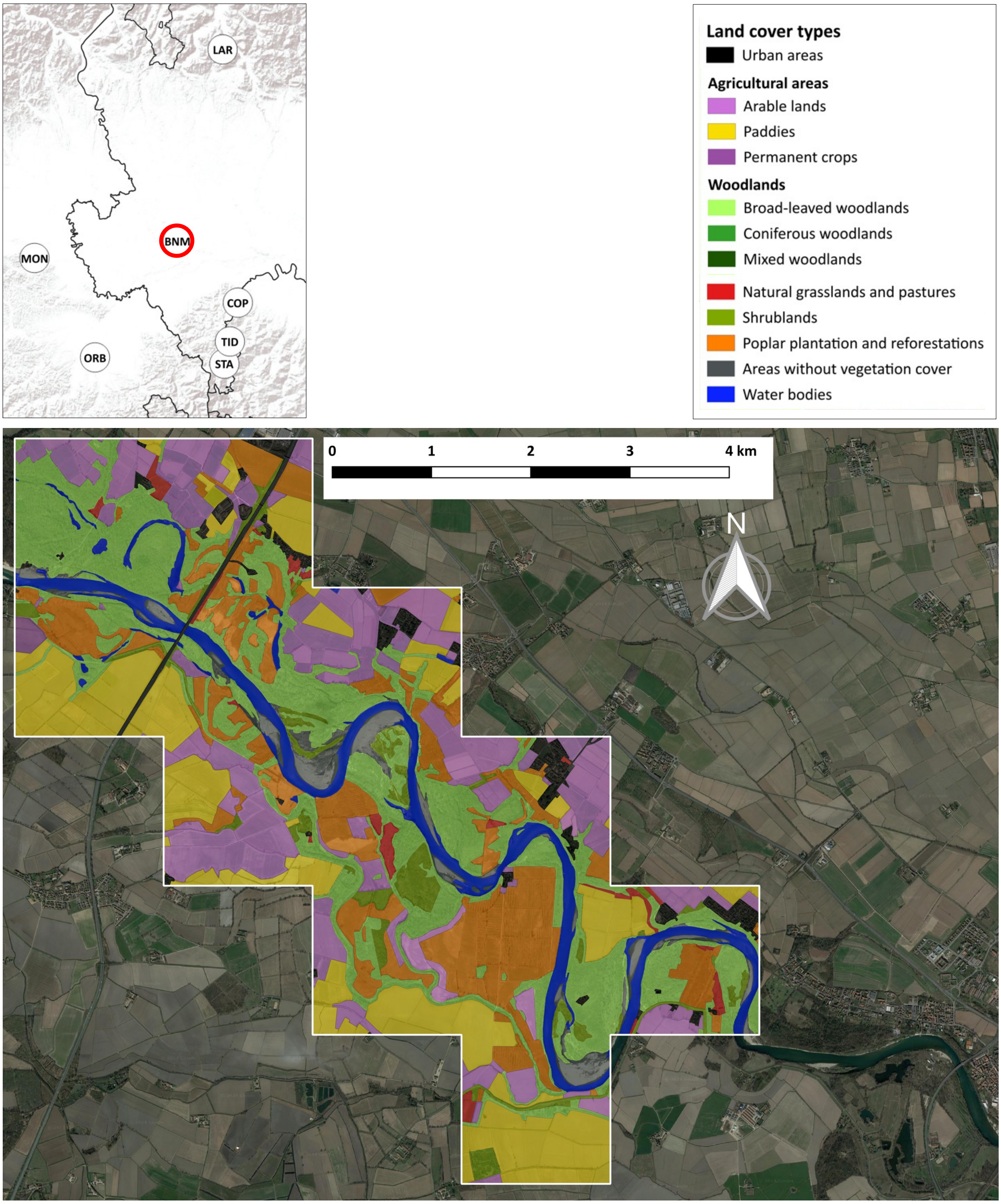


2.2.2 The Po Plain

The Po Plain is a vast lowland region in northern Italy, stretching from the foothills of the Alps in the north to the northern Apennines in the south. The plain is primarily characterised by extensive cultivation and is home to major urban centres. Within this landscape, small, isolated patches of natural or semi-natural vegetation are scattered amidst the heavily modified human-dominated surroundings, often lining the banks of rivers, particularly the Po River and its main tributaries. Enclosed between the Alps and the Apennines, the topography of the Po Plain contributes to consistently high levels of relative humidity year-round. As a result, the climate of the study area can be described as humid continental, featuring cool, foggy winters (with mean temperatures ranging from 0-5 °C during the coldest months) and warm, moist summers (with mean temperatures ranging from 22-25 °C during the hottest months).

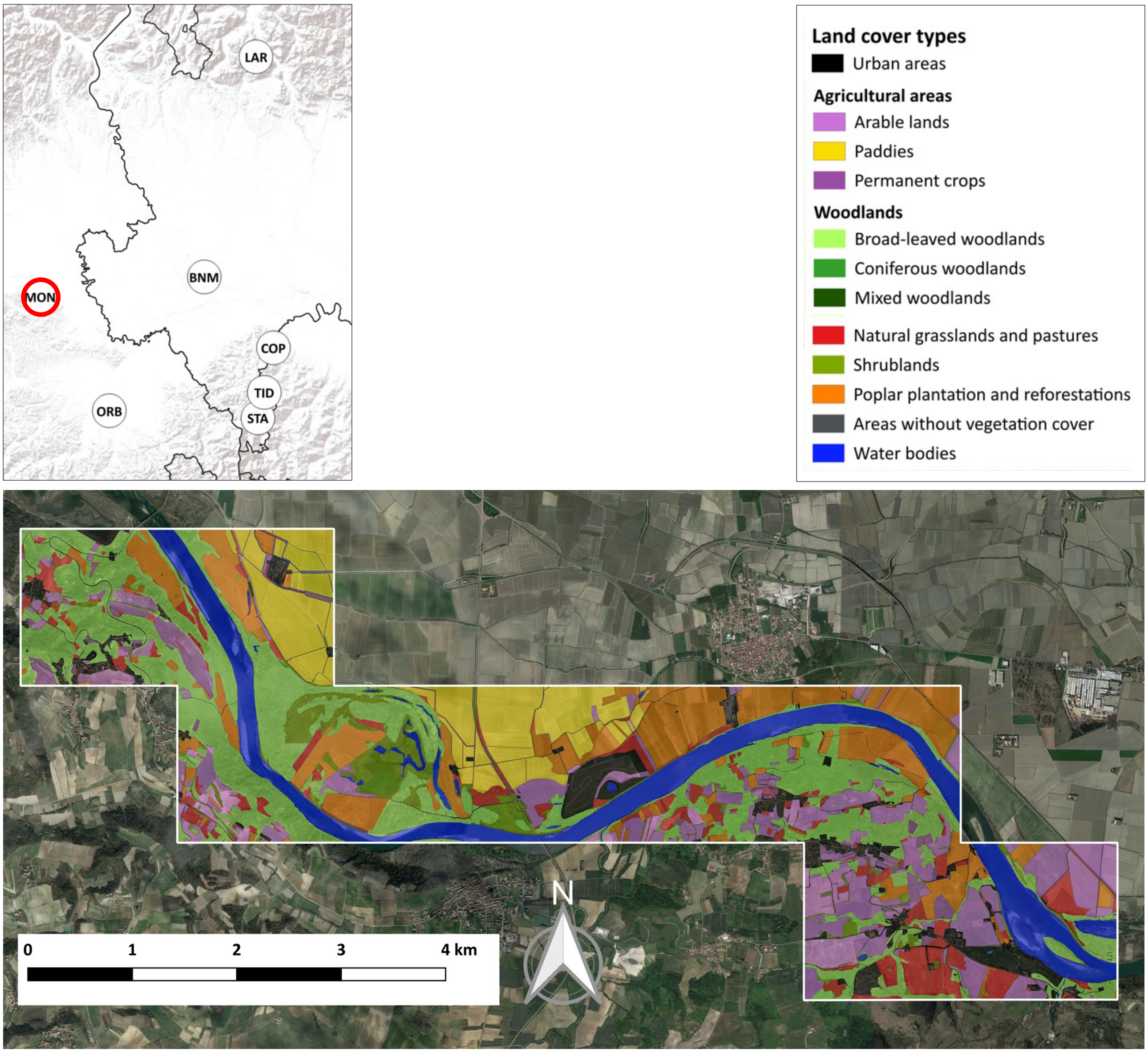
Boschi Negri e Moriano (BNM) - The study area named Boschi Negri e Moriano is located in the Po Plain along the Ticino River in the Lombardy region. The climate is temperate-humid; summers tend to be hot, while winters are cold. The general lack of winds and the abundance of watercourses facilitate a very high level of relative humidity throughout the year; even though rainfalls are higher in the spring and autumn months. Snowfall is occasional. The area is characterized by a mixed landscape with an alternation of woodlands and cultivated lands (Figure 2.2.2). Meso-hygrophile and multi-layered woodlands (*Quercus robur*, *Populus* spp., and *Ulmus minor*), which are the remnants of the native forest of the alluvial plain, and riparian woodlands (*Salix* spp. and *Alnus glutinosa*) represent a continuous canopy along the river (26.6% of the surface). The shrub layer is rich and well-structured being composed of many species (*Crataegus monogyna*, *Corylus avellana*, *Cornus mas*, *Prunus* spp., and *Sambucus nigra*). Arable lands (19.3%; mainly maize and other cereals) and paddies (18.7%) are predominant among the agricultural patches. The area also hosts extensive poplar plantations and, to a lesser extent, mixed broadleaved reforestations (16.6%). The Ticino River and its banks, canals, streams, and marshes cover 6.8% of the surface. Urban areas (3.3%) are represented by medium or small villages. The average human population density is 145.7 inhabitants per km^2. Main human activities are intensive farming (cereals production) and recreational activities, such as hiking and biking. The proximity to the city of Pavia and the climate are conducive to a variety of outdoor activities throughout the year; hiking, often with unleashed dogs, biking, and birdwatching are the most popular activities. BNM is largely included in a Natural Park named “Valle del Ticino Lombardo Regional Park”. Additionally, the study area includes the small Nature Reserve “Bosco Siro Negri”, where no human activities are permitted except for scientific research. The Protected Area encompasses 91.8% of the surface of the BNM study area.

Figure 2.2.2 - Land cover classes mainly characterizing the landscape of the area Boschi Negri e Moriano



Basso Monferrato (MON) - The study area named Basso Monferrato is a foothill area, with elevations ranging from 110 to 360 meters a.s.l., located along the Po River in the Piedmont region. The area has a continental climate, characterised by hot summers and cold winters. Precipitation is moderate and distributed throughout the year, with slightly higher rainfall in the spring and autumn months. Snowfall is occasional. The Po River crosses the study area longitudinally; the two banks of the Po River exhibit distinct characteristics: the left bank is predominantly flat and characterised by extensive cultivated fields, while the right bank, situated at the foothills of the Monferrato hills, presents a more diverse landscape (Figure 2.2.3).

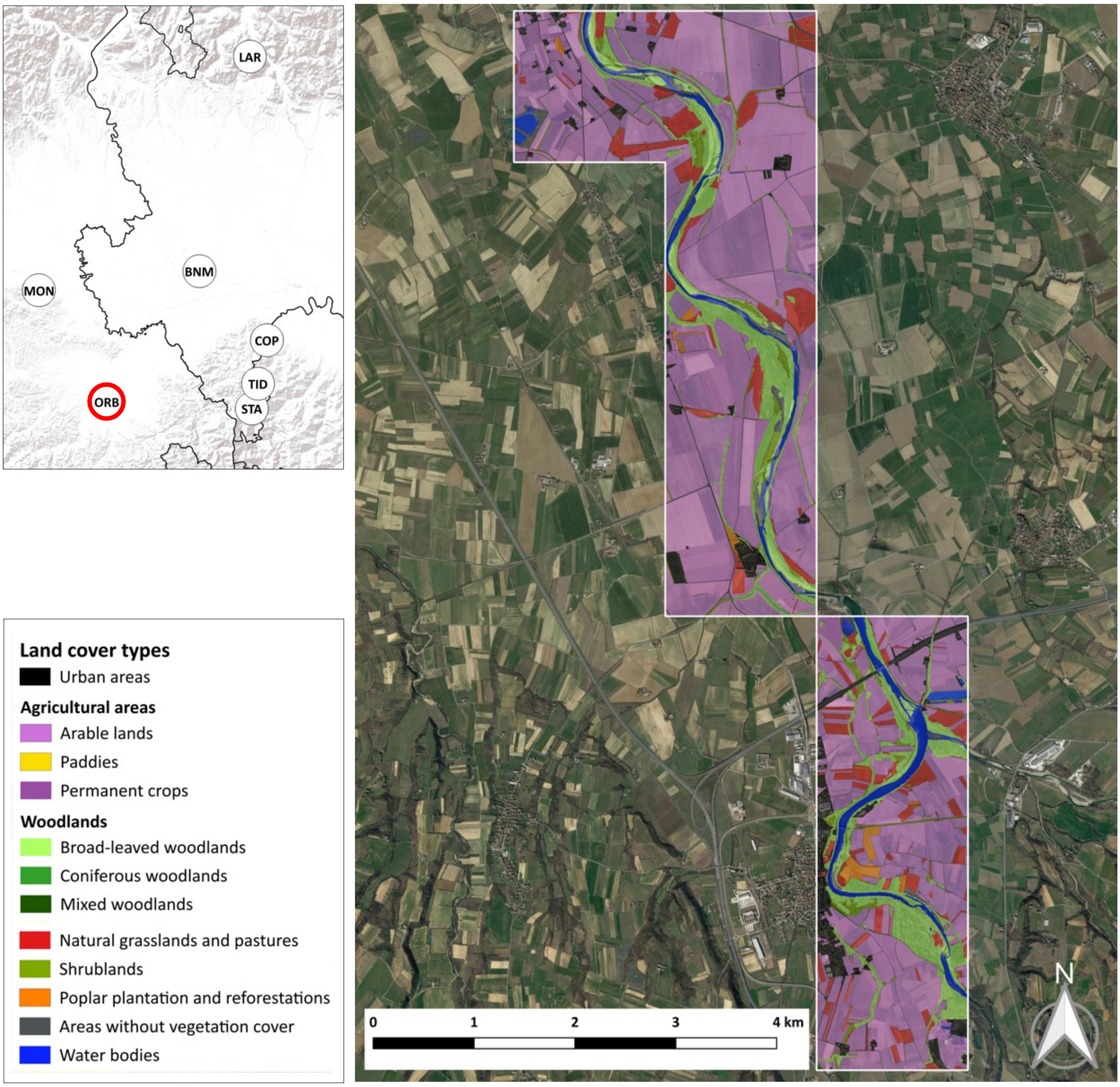
Figure 2.2.3 - Land cover classes mainly characterizing the landscape of the area Basso Monferrato



This area lies in an intensive agricultural landscape, where arable lands (13.6%; mainly maize) and paddies (10.5%) are cultivated over very large fields and poplar plantations (15.2%) are widespread. Within this agricultural landscape, there is a floodplain natural area hosting heterogeneous habitats, such as riparian woodlands, mainly composed of *Salix* spp., and meso-hygrophile woodlands, mainly composed of *Quercus* spp. and *Robinia pseudoacacia*, wetlands, and semi-natural xeric grasslands (i.e. Habitat 6210). Considering the entire study area, broadleaved woodlands and grasslands cover 26.6% and the 7.0% of the surface respectively. The Po River covers 11.9% of the surface. Urban areas (7.1%) are represented by small villages. The average human population density is 39.4 inhabitants per km^2. Main human activities are intensive farming (cereals production) and hunting. Hunting is typically restricted to a few months during the year, primarily during the autumn season. MON is partially included in a Natural Park named “Po Piemontese Regional Park”. Additionally, the study area includes the Special Conservation Area “Ghiaia Grande (Fiume Po)”. The Protected Area encompasses 43.5% of the surface area of the MON study area.

Torrente Orba (ORB) - The study area known as Torrente Orba is situated in the Po Plain alongside the Orba Stream, which is a tributary of the Tanaro River, within the Piedmont region. This area is characterized by a completely flat terrain. The area has a temperate-continental climate, characterized by warm summers and cold winters. Precipitation is distributed throughout the year, although there may be slightly drier periods during the summer months. Snowfall is occasional. ORB is characterized by an agricultural landscape composed of numerous small parcels with diverse crops (Figure 2.2.4). Arable lands (65.0%) are allocated, for example, to maize and other cereals, alfalfa, vegetables, fruits, and sunflowers. These agricultural areas are interspersed with arable fodder crops and grasses (7.8%). Most of the natural areas are located along the course of the Orba Stream, where woodlands are relatively continuous and diversified. In the floodplain, riparian woodlands are nearly intact, dominated by species such as *Salix alba* and *Populus nigra*, while in drier areas woodlands are dominated by *Quercus* spp. and *Robinia pseudoacacia*. Woodland covers 12.9% of the surface. Despite extensive cultivation, the landscape remains heterogeneous due to the mix of cultivated parcels and natural habitats. Urban areas (5.3%) are represented by small villages. The average human population density is 57.2 inhabitants per km^2. Main human activities are farming and hunting, which is typically restricted to a few months during the year, primarily during the autumn season. A small portion of the study area, corresponding to 14.2% of the surface, is included in the Nature Reserve of the Orba Stream.

Figure 2.2.4 - Land cover classes mainly characterizing the landscape of the area Torrente Orba

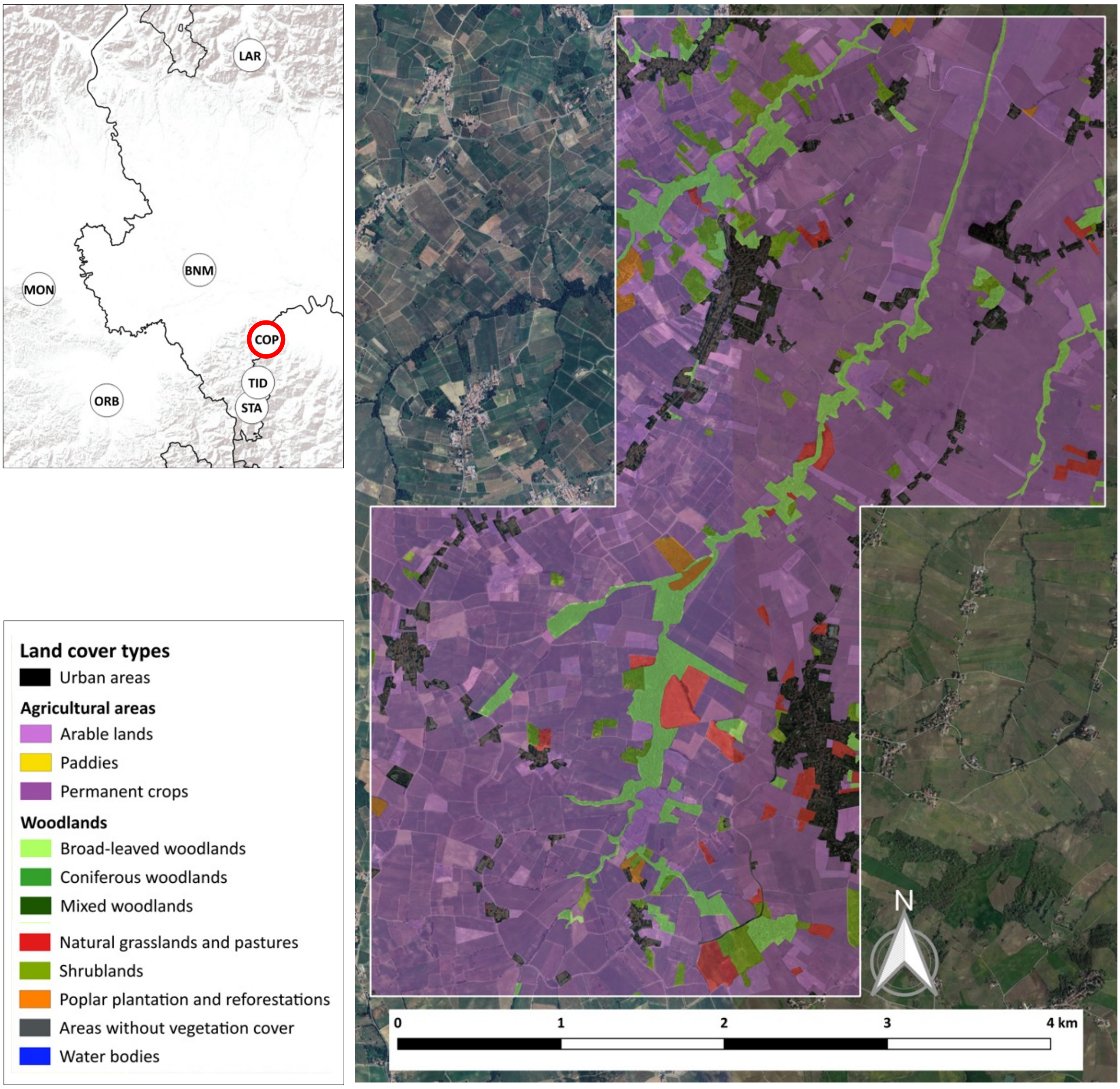


2.2.3 The Lombard Apennines

The Lombard Apennines are a restricted portion of the Northern Apennines, localized in the southwestern part of the Lombardy region, which lies to the south of the Po River. The study area ranges from 200 m to 1724 m a.s.l. (M. Lesima). The climate is sub-Mediterranean with an average yearly temperature of 9 ◦C (January: av. temp. = −1.1 ◦C, July: av. temp. = 18.1 ◦C). Mean annual precipitation ranges from 950 mm at lower elevations to 1300 mm at higher elevations, mostly concentrated in two rainy seasons (April-May and November). The study area can be subdivided into three elevational zones well characterised by different land cover classes. The northern portion of the study area, localized just behind the Po Plain, is the lower-hill zone, which ranges from 200 m to 500 m a.s.l. and is characterised by cultivated lands. Vineyards and annual crops are predominant (37.6% and 26.2% of the surface, respectively) and urbanised area covers 10.3% of the surface. Woodlands are restricted to small patches along streams (14.7%), and uncultivated lands cover 5.5% of the surface. It follows the upper-hill zone, which ranges from 500 to 800 m a.s.l. and where annual crops are predominant (24.8%, mainly fodder crops such as cereals and leguminous plants), but vineyards are still present (11.5%), and uncultivated lands reach 8.8%. Broad-leaved woodlands cover 40.0% of the surface. The most representative species are *Quercus* spp. and *Castanea sativa*. Urban areas represent 4.2% of the surface. The southern portion of the study area is the mountainous zone (> 800 m a.s.l.) mainly covered by natural vegetation (78.7%), where broad-leaved woodlands (54.7%; main species: *Fagus sylvatica*) are predominant on conifer reforestations (1.5%; *Pinus* spp. and *Picea abies*) or mixed woodlands (1.3%). Uncultivated lands reach 10.2%, and pastures–meadows reach 7.4%. Cultivated lands cover 14.3% of the surface (mainly annual crops), and urban areas are restricted to 2.8% of the surface. The average human population density of the whole study area is 27.43 inhabitants per km^2.

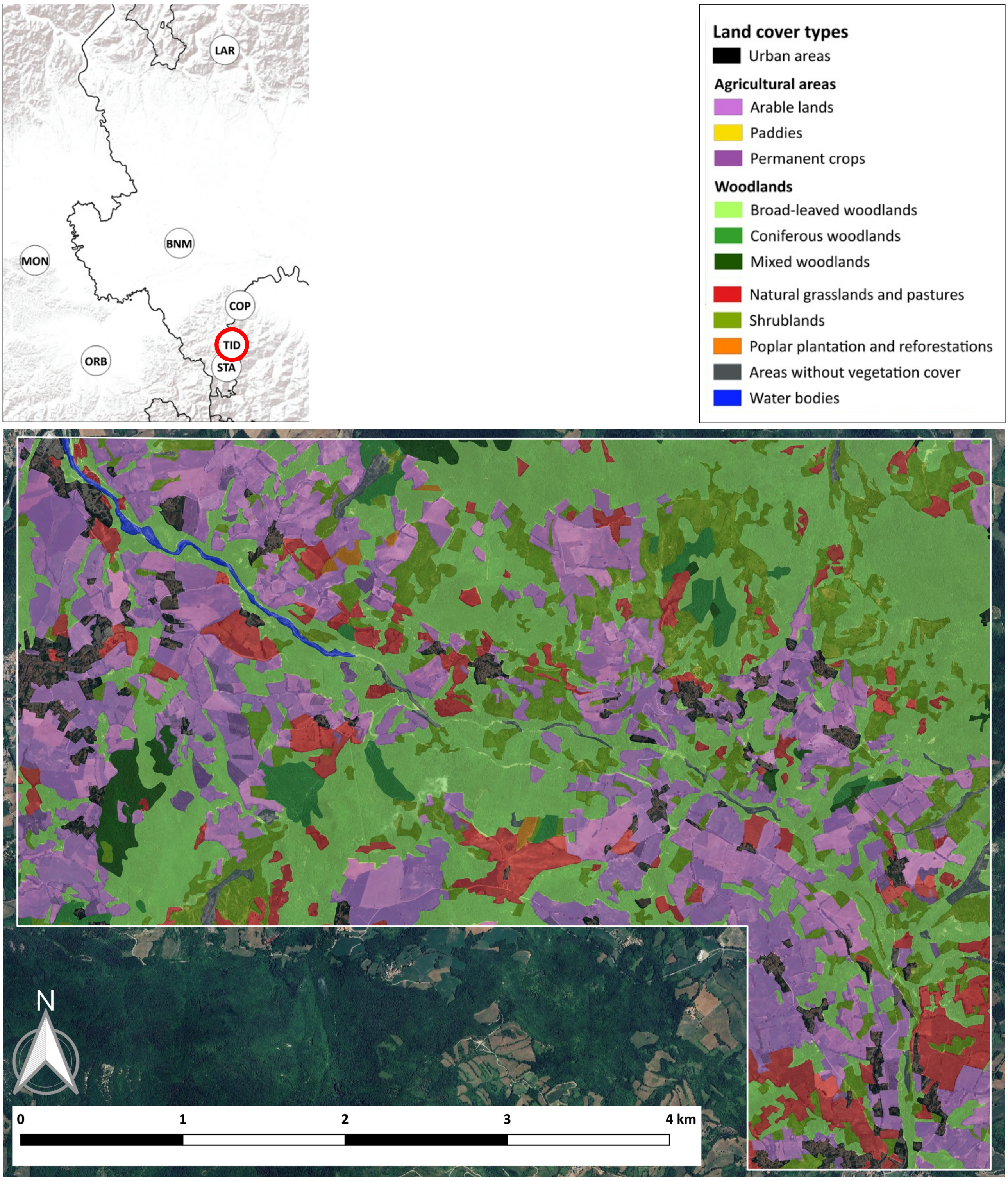
Colline Oltrepò Pavese (COP) - The study area named Colline Oltrepò Pavese is a hill area located in the northern Apennines between Lombardy and Emilia-Romagna regions with elevations ranging from 130 to 410 meters a.s.l. The climate is typically temperate, with warm summers and cold winters. Precipitation is evenly distributed throughout the year, with slightly drier conditions in the summer months. It is characterized by vast and intensive vineyard cultivations (69.6%), but agricultural lands also include non-irrigated arable lands (10.7%). Such cultivated lands are mainly constituted of permanent or seasonal fodder crops and grasses. Areas covered by natural vegetation, on the other hand, are limited, with only 6.5% of the surface occupied by broad-leaved woodlands, 2.8% by shrublands, and 2.3% by meadows. Woodlands, in particular, are represented by small and isolated patches along vineyard borders or along little streams used for agricultural purposes and are dominated by *Robinia pseudoacacia*. Watercourses mainly consist of streams and canals that are used for agricultural purposes; they tend to be dry during the summer months. Urban areas, comprising 7.4% of the total area, consist of small villages situated atop the hills (Figure 2.2.5). The average human population density is 91.0 inhabitants per km^2. Main human activities are intensive farming (wine production) and hunting. Both these activities imply a higher human presence in the environment during the autumn season.

Figure 2.2.5 - Land cover classes mainly characterizing the landscape of the area Colline Oltrepò Pavese



Val Tidone (TID) - The study area named Val Tidone lies between the upper-hills and the mountains zones of the northern Apennines between Lombardy and Emilia-Romagna regions. The territory has elevations ranging from 390 to 860 meters a.s.l. (Monte Lazzarello 864 m). The climate is temperate, with warm summers and cool winters. Precipitation is evenly distributed throughout the year, with slightly higher rainfall in the autumn months. Snowfall is recurring during the winter. The area is characterized by a mixed landscape with an alternation of woodlands and cultivated lands (Figure 2.2.6).

Figure 2.2.6 - Land cover classes mainly characterizing the landscape of the area Val Tidone

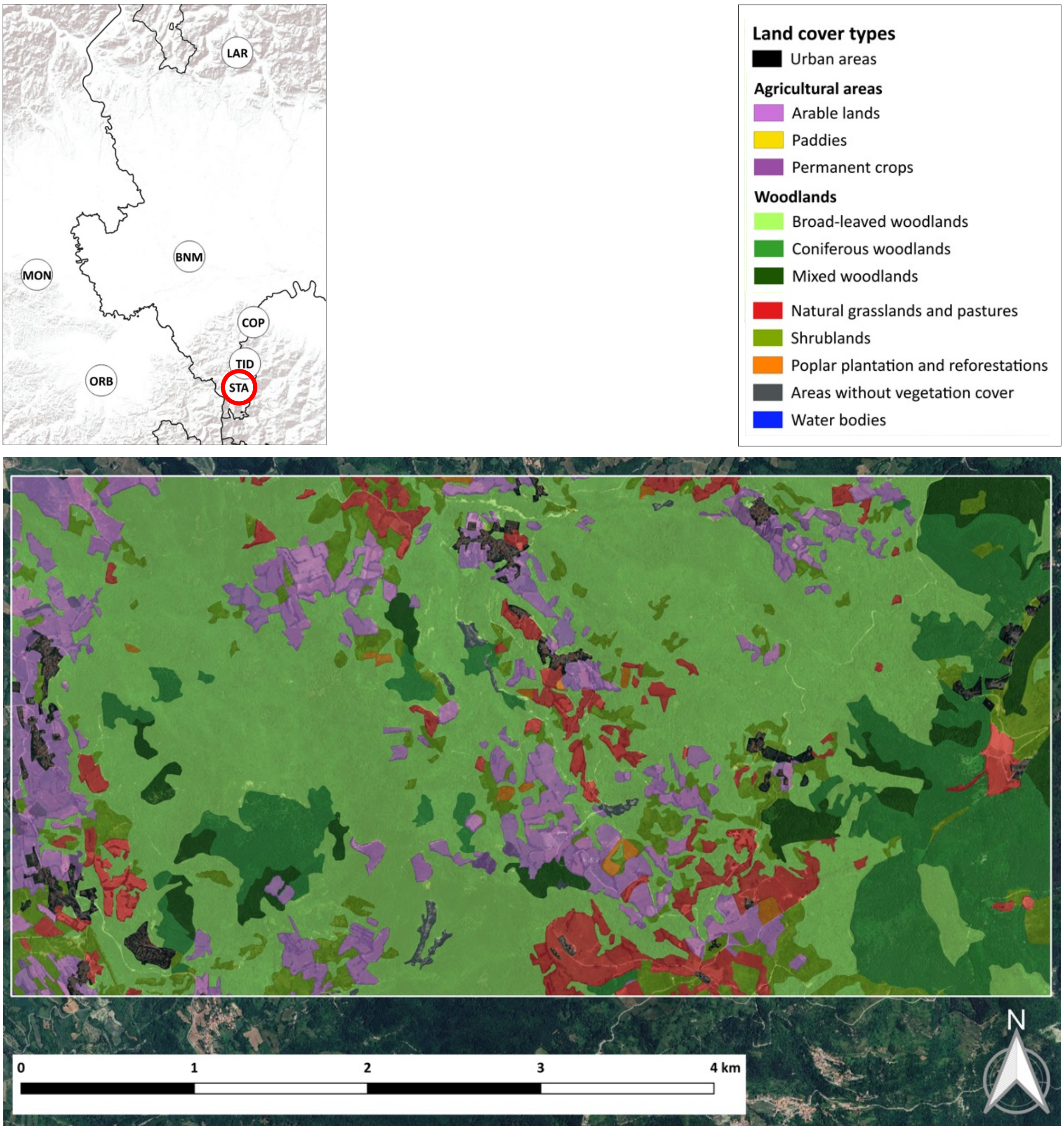


Cultivated lands are mainly constituted of permanent or seasonal fodder crops and grasses (30.0%). Broad-leaved woodlands are predominant (37.9%) and composed of thermophilous species dominated by *Quercus pubescens* in association with *Quercus petraea*, *Quercus cerris*, *Fraxinus ornus* and *Acer campestre*. Coniferous and mixed woodlands are restricted to 2.0% and 1.4% of the surface respectively. Conversely, shrublands natural grasslands and pastures are widespread (13.0%

and 7.9% respectively). Watercourses are represented by mountain streams, which can be seasonally dry. Urban areas (4.6%) are represented by small villages. The average human population density is 25.9 inhabitants per km^2. Main human activities are extensive farming, livestock rearing (mainly cattle) and, among recreational ones, hunting, motocross, and hiking. Hunting is typically restricted to a few months during the year, primarily during the autumn season. Motocross and hiking are carried out, especially during the milder months of spring and summer.

Valle Staffora (STA) - The study area named Valle Staffora is a mountainous area located in the northern Apennines between Lombardy and Emilia-Romagna regions with elevations ranging from 550 to 1550 meters a.s.l. (Monte Penice 1460 m). The climate is temperate, with warm summers and cool winters. Precipitation is evenly distributed throughout the year, with slightly higher rainfall in the autumn months. Snowfall is recurring during the winter. The study area has a predominant natural landscape with abundant woodland cover (Figure 2.2.7). Indeed, woodlands are widespread and form a continuous canopy (70.1%): among broad-leaved ones (53.4%), monospecific beech (*Fagus sylvatica*) and chestnut (*Castanea sativa*) woodlands are dominant; among coniferous ones (12.6%), associations of *Pinus nigra*, *Picea abies*, and *Larix decidua* are widespread. Mixed woodlands are also present (4.6%). Alongside woodlands, well-structured shrublands and natural grasslands and pastures on mountain ridges are present (8.5% and 6.3% respectively). Agricultural areas are mainly constituted of permanent or seasonal fodder crops and grasses (11.6%). Open areas, including both natural clearings and cultivated fields, are scattered amidst the woodlands. Watercourses are represented by mountain streams, which can be seasonally dry. Urban areas (2.2%) are represented by very small villages. The average human population density is 12.5 inhabitants per km^2. Main human activities are extensive livestock (mainly cattle) and, among recreational ones, hunting, and motocross. Hunting is typically restricted to a few months during the year, primarily during the autumn season. Motocross is carried out, especially during the months of spring and summer.

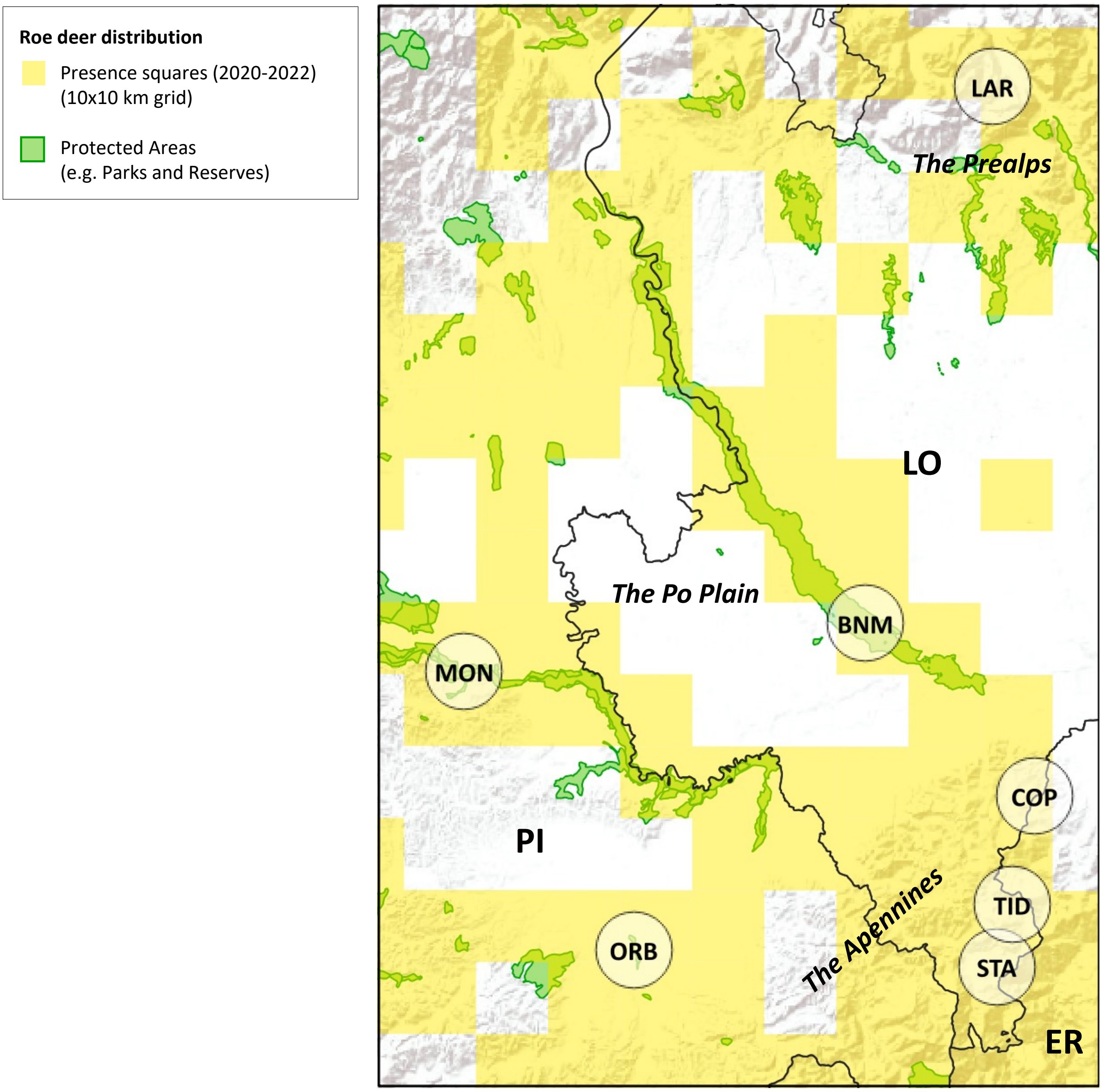
Figure 2.2.7 - Land cover classes mainly characterizing the landscape of the area Valle Staffora



2.3 Faunal assessment

The roe deer has a significant presence in northern Italy, with populations spread across the various regions (Figure 2.3.1).

Figure 2.3.1 - Roe deer distribution* (1) *in the portion of north-western Italy encompassing the study areas



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[1] Occurrence data of the roe deer were obtained from the citizen-science platform iNaturalist (validated data accessed on 18th May 2024) and occasional data recorded during the study period (December 2020 - November 2022).

In the alpine and pre-alpine areas, extensive woodland cover interspersed with meadows provides ideal conditions for the species. The hilly regions, such as the Oltrepò Pavese in Lombardy and the Langhe and Monferrato in Piedmont, also support substantial roe deer populations. Even in the plains and lowland areas, where urbanization and intensive agriculture are predominant, roe deer can still be found. Here the Protected Areas, such as the “Valle del Ticino Lombardo” Regional Park, help maintain viable populations.

Regarding wild ungulates, besides the roe deer, another species present in every study area is the wild boar (*Sus scrofa*). The wild boar can be found throughout Italy, spanning the entire Apennines and Alps mountain chains, as well as coastal and plain areas. This species is highly adaptable and can inhabit various habitats, ranging from undisturbed natural areas to highly urbanized ones. The fallow deer (*Dama dama*) is present in BNM, MON, TID, and STA; this species has an almost continuous distribution along the Northern Apennines, whereas presents a spotted distribution in the plain areas of Lombardy and Piedmont. Suitable habitats for the species consist of broad-leaved woodlands, ranging from plains to hills, with a good alternation of shrublands and clearings. The red deer (*Cervus elaphus*) is present in TID and STA. Similar to the fallow deer, this species has an almost continuous distribution along the Northern Apennines but is absent or sporadic in the Po Plain. The presence of the species is associated with extensive mature woodlands, preferably consisting of broad-leaved species, interspersed with wide clearings. The mouflon (*Ovis aries musimon*) is present only in LAR study area. This species has a patchy distribution in Italy, confined to the areas where it was introduced due to its limited dispersal behaviours. Suitable habitats for the species are hilly areas with an alternation of broad-leaved woodlands and shrublands (Varuzza 2019).

Considering the potential predator of the roe deer, the red fox (*Vulpes vulpes*) is abundant in every study area. The species is widespread throughout Italy and inhabits very different habitat types. The most important predator of deer, which is the wolf (*Canis lupus*), can be considered absent or occasionally present in LAR, BNM, and MON study areas (2), whereas in ORB, COP, TID, and STA the species is present with stable packs. Whereas in ORB the presence of wolves is very recent (Marucco et al. 2022), in the Lombard Apennines the first unquestionable evidence of wolf recolonization was an illegally killed individual found in 1987; the results obtained by the first research project (1987-1992) reported the presence of two breeding pairs with pups along the southernmost mountain

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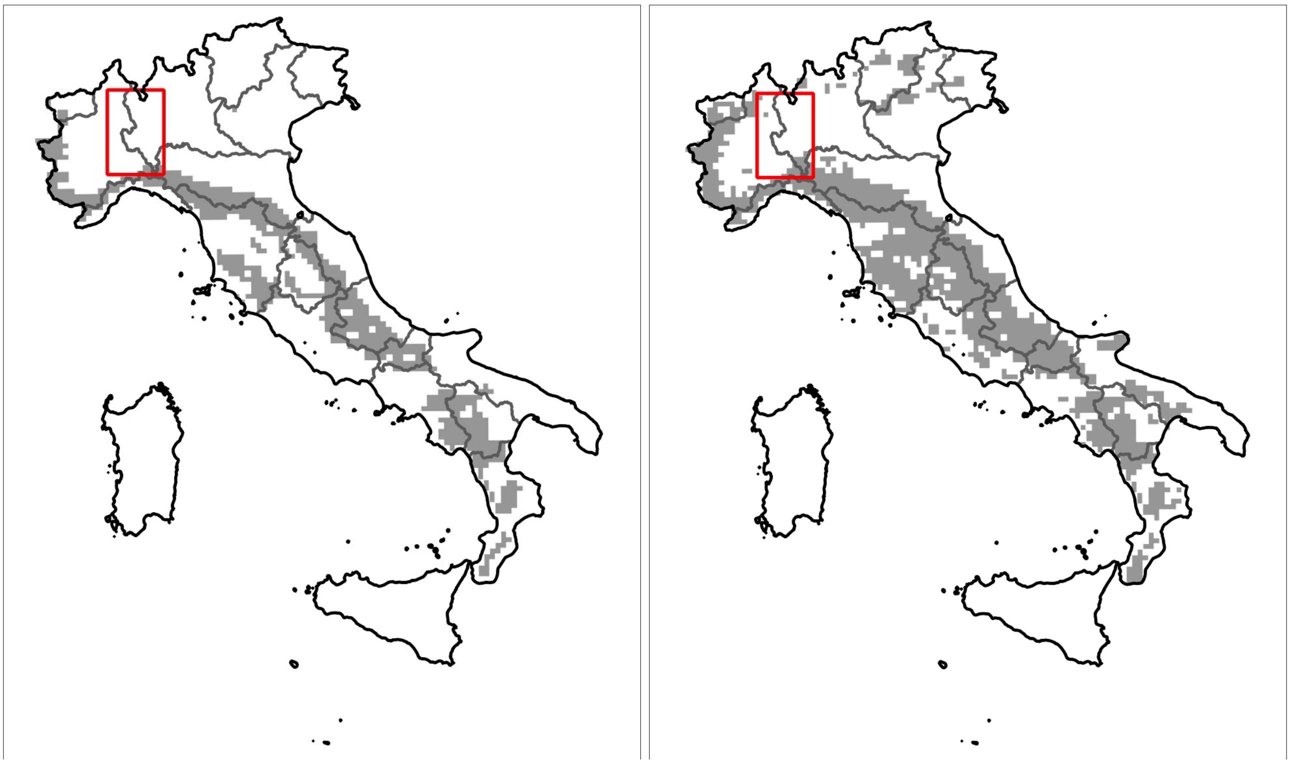
[2] Data referred to the study period (December 2020 - November 2022); by 2023 the presence of the predator also became stable in LAR and MON, where wolf pairs or packs were recorded.

ridge (Meriggi et al. 1991). Since then, the presence of the species has become stable and widespread in the whole area (Meriggi et al. 2020) (Figure 2.3.1).

Figure 2.3.1 - Location of the study areas in north-western Italy (in red) and recent changes in wolf distribution at the national level (in grey) as reported in Habitats Directive Reports (on the left: 3° report 2007-2012; on the right: 4° report 2013-2018)



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Chapter 3

Unveiling the Role of Roe Deer in Wolf Ecology

3.1 Introduction

3.1.1 The theoretical effects of predation on prey population

Predation plays a pivotal role in shaping the behaviour of individuals, the population dynamics of prey species, and the overall structure of ecological communities.

Generally, at the population level, prey populations can withstand predation pressure meaning that predators do not necessarily limit prey population growth by increasing mortality rates. When predation is density-dependent, its impact is compensatory and prey population dynamics may remain relatively stable (Errington 1946; Sinclair and Krebs 2002). Indeed, for most species, bottom-up control, through the food supply, is the primary limiting factor of population growth, which can be modified by secondary factors, such as top-down control represented by predation, intra- and inter-specific interactions and environmental disturbances (Sinclair and Krebs 2002; Melis et al. 2009). Moreover, when considering large carnivores and their herbivore prey, the significant difference in spatial scale between their ranging behaviours and their respective population densities implies that large predators may have limited impacts on the population growth rate of prey, especially if they are generalists capable of switching between different prey species (Skogland 1991). However, the effects of predation on prey populations are highly variable depending on the context. In less productive environments, predation may significantly impact prey population growth rates, becoming additive to other sources of mortality (Melis et al. 2009).

3.1.2 The wolf as predator

The wolf is a generalist and opportunistic predator expected to adjust its feeding habits according to the local abundance and accessibility of food sources (Mech 1970; Peterson and Ciucci 2003).

Although showing remarkable adaptability to various prey and ecological contexts, wolves primarily consume large and medium-sized wild ungulates (Mech 1970; Okarma 1995; Meriggi and Lovari 1996; Peterson and Ciucci 2003; Zlatanova et al. 2014; Newsome et al. 2016; Meriggi et al. 2020; Janeiro-Otero et al. 2020). Smaller wild prey species, such as medium-sized and small mammals, generally contribute less to wolf diets and rarely represent a majority proportion of their diet (but see Gable et al. 2018), whereas livestock tends to increase in areas or periods where wild ungulates are scarce (Meriggi and Lovari 1996; Sidorovich et al. 2003; Torres et al. 2015; Petridou et al. 2019). Thus, when a rich and diverse wild ungulate community is available, the wolf diet is dominated by ungulate (Meriggi and Lovari 1996), although wolves utilize all available food resources and exhibit flexibility in their foraging behaviour.

Zlatanova et al. (2014), revealed two ecological trends in wolf diet in Europe: (i) wolves inhabiting natural habitats with abundant wild ungulates primarily feed on wild prey, and (ii) in highly anthropogenic habitats, with low abundance of wild prey, wolves turn to livestock consumption, supplemented by plant matter, smaller prey (such as rodents and lagomorphs), and scavenging on garbage. Therefore, diet breadth increases as the presence of large prey decreases in the diet.

These trends have been documented also in the Italian Peninsula, where wolf feeding habits have been extensively studied.

After being nearly eradicated from their original range and facing extinction, wolves survived in small, fragmented areas within the remote, sparsely populated, mountainous regions of the Central and Southern Apennines. The Italian wolf population reached a historical minimum, with an estimated 100 individuals remaining (Zimen and Boitani 1975; Boitani 1992). However, after full legal protection was established in 1971 (Boitani 1992), wolves swiftly began to recolonize their range, gradually expanding from their surviving areas (Fabbri et al. 2007). During the first phase of this recolonization process the expanding wolf population has locally adapted to feed on most available resources, such as livestock, small- and medium-sized mammals, fruits, and garbage (Meriggi et al. 1991, 1996). Subsequently, as wild ungulate populations increased in abundance and expanded their distribution range, the diet of wolves shifted towards a higher consumption of wild ungulates (Meriggi et al. 2015, 2020).

However, wolves do not hunt according to prey abundance only; the wolf diet varies depending on several factors, including landscape characteristics, the availability and accessibility of domestic prey, the composition of the wild prey community, prey species local ecology and behaviours, previous hunting experience and cultural transmission (Meriggi et al. 1996; Peterson and Ciucci 2003; Janeiro-Otero et al. 2020; Martins et al. 2020).

Generally, one or two main prey species, selected within a multi-prey system, characterize the wolf diet at a local scale (Peterson and Ciucci 2003). Focusing on a small number of species, wolves simplify their hunting strategies and optimize their energy expenditure. This hunting behaviour allows them to efficiently exploit the most profitable prey within their territory.

3.1.3 The roe deer as prey

The roe deer, a ubiquitous ungulate species found across much of Europe (Lovari et al. 2016), represents an important prey species for different carnivores, such as the red fox (Panzacchi et al. 2008), the Eurasian lynx (Okarma et al. 1997; Andrén and Liberg 2015), and, clearly, the wolf.

Its widespread distribution, allowed by its adaptability to diverse habitats (Danilkin and Hewison 1996; Hewison et al. 2001; Morellet et al. 2011), and its relatively high densities in most of its range (Burbaitė and Csányi 2009; Apollonio et al. 2010), make it a common prey species for predators due to the increased encounter rates. This is particularly true in areas of large-scale forest exploitation (Jędrzejewska et al. 1994), where it can reach very high densities because of its ability to exploit landscapes with smaller, fragmented woodlands and a high amount of woodland-open area edges (Saïd and Servanty 2005). Moreover, its small body size, ranging between 18 to 32 kilograms (Danilkin and Hewison 1996), makes it an ideal prey due to the required relatively short handling time (Meriggi et al. 2020), especially when compared to larger ungulate species like the red deer and the wild boar. Furthermore, despite its typically elusive behaviour and low tendency to aggregate, roe deer vulnerability increases during the winter season, when, especially in open habitats and under severe climate conditions, large groups or herds are observed (Danilkin and Hewison 1996), making it easier for predators to locate and capture.

At the national level, the roe deer represents the second most consumed species among wild ungulates by wolves, after the wild boar (Mori et al. 2017; Meriggi et al. 2020). However, the extent of its consumption varies considerably across different regions of the Italian Peninsula. It reaches the highest proportion in the Northern Apennines and in the Western Alps, where roe deer constitute a primary component of the wolf diet being the first or the second most consumed species after the wild boar or the red deer. However, in the Central and Southern Apennines, the roe deer is considered either a secondary or accessory prey species, depending on the specific study area. In areas of recent recolonization (e.g. the Po Plain or coastal areas or the Eastern Alps) the roe deer represents a secondary prey species (Table 3.1.1).

3.1.4 Expanding ranges: the wolf and the roe deer across Italy

The presented results clearly highlight the importance of the roe deer as prey for the wolf; furthermore, by comparing the results obtained in the study areas examined over long periods of time (e.g. Lombard Apennines and Tuscany), a close interconnection in the population dynamics of the two species emerges.

Since the 1970s, a combination of factors has driven a significant expansion of both roe deer and wolf populations across the Italian peninsula. The abandonment of hill and mountain agriculture, along with the migration of human activities from rural to urban areas, has facilitated the recovery and enlargement of undisturbed natural habitats, thus providing expanded territories suitable for wildlife. Furthermore, advancements in hunting practices, the reintroduction and restocking of deer in specific areas, and the implementation of enhanced conservation laws aimed at protecting carnivores have all played pivotal roles in bolstering the numerical growth of these populations (Apollonio et al. 2010; Chapron et al. 2014; Linnell et al. 2020; Cimatti et al. 2021).

Once the areas characterized by high naturalness and a low human population density, such as mountainous and hill areas, have been recolonized, both roe deer and wolf populations have begun expanding towards nearly urbanized and agricultural areas, such as the Po Plain in Northern Italy (Meriggi et al. 2020).

Table 3.1.1 - Roe deer consumption by wolves in Italy reported in published papers and other technical documents (e.g. theses and reports) on wolf food habits estimated through scat analyses

Geographical region	Study area	Study period	Roe deer consumption	References
Western Alps	Ligurian Alps	2012-2014	42.5%	Torretta et al. 2017
		2017-2018	27.1% - 51.1%	Boni 2019
	Pesio Valley	2020-2021	28.4%	Macario 2022
	Po Valley	2016	22.0%	Giachello 2016
	Susa Valley	2000-2003	14.4% - 51.1%	Gazzola et al. 2007
	Aosta Valley	2007-2009	18.6% - 42.7%	Palmegiani et al. 2013
	Piedmont (VCO)	2022	18.8%	Bigarella 2022
Eastern Alps	Lessinia	2016-2017	≅ 5%	Selva 2018
Northern Apennines	Ligurian Apennines	2008-2013	11.3% - 35.2%	Imbert et al. 2016
	Apennines (PV-PC)	2007-2012	23.1%	Meriggi et al. 2015
	Lombard Apennines	2007-2008	10.4%	Milanesi et al. 2012
		2015	65.9%	Meriggi et al. 2020
		2018	70.2%	Torretta et al. 2018
	Tuscany (AR)	2000-2009	20.1% - 48.2%	Bassi et al. 2017, 2020; Ståhlberg et al. 2017
	Tuscany (PI)	2018-2021	16.4 - 48.6%	Di Rosso et al. 2023
Southern Apennines	PNALM	2006-2009	11.7%	Ciucci et al. 2020
	Pollino National Park	1999-2003	2.9%	Ciucci et al. 2018a
Po Plain	Ticino River Natural Park	2017-2018	7.5%	Dondina et al. 2021
Tyrrhenian coast	Maremma Regional Park	2016-2018	5.8%	Ferretti et al. 2019
		2019-2023	< 10%	Lazzeri et al. (submitted)

3.1.5 Case studies

The following two case studies delve into particular aspects of this predator-prey system.

i) **Recent changes in wolf occupancy and feeding habits in the Northern Apennines** - The ongoing range expansion of the wolf in Italy is currently leading the species closer to highly anthropized landscapes. A long-term research project (2007-2022) carried out in a focal area along the Apennines provides the opportunity to finely monitor the dynamics of this expansion and to predict the new scenarios of wolf-human coexistence. The project revealed that both the wolf range and its feeding habits greatly changed during the study period. Wolves first settled in the mountains of the study area and, once it had been saturated, they began to occupy the hill zones. These recently occupied zones are markedly different from the undisturbed mountains: woodlands are restricted to small patches, and agricultural lands are predominant. Nevertheless, wolves rapidly colonized the hills, driven by roe deer abundance. This widespread species became very abundant during the last few decades and supported the wolf range expansion; indeed, its consumption greatly increased until it became the staple of the wolf diet. Obtained results suggest that wolf expansion will follow that of the roe deer across the Po Plain with the settlement of wolf packs in roe deer high-density areas;

ii) **Feeding habits of the wolf packs inhabiting the Po Plain and the Lombard Apennines** - Wolf packs are the social units of the wolf population; packs typically consist of a breeding pair and their offspring, along with other adult or subadult wolves. Packs generally establish territories in areas with high prey availability and employ cooperative hunting strategies, honed over generations, to capture their preferred prey. This section sheds light on the social structure and feeding habits of four wolf packs settled in north-western Italy. Obtained results revealed the roe deer as the primary dietary component across all monitored packs. This preference can be attributed to factors such as the abundance of roe deer populations, the relatively small body size of individuals which allows for efficient consumption by wolves, and cultural transmission of hunting techniques among related neighbouring packs. These findings suggest a complex interplay between predator behaviour, prey availability, and social dynamics within the wolf population.

3.2 Recent changes in wolf occupancy and feeding habits in the Northern Apennines

3.2.1 Introduction

The Italian Peninsula harbours one of the largest wolf populations persisting in Europe, which is estimated to be ca. 3300 wolves (range: 2945-3608) distributed over ca. 150,000 km^2 (La Morgia et al. 2022). The Apennines, the mountain system extending for ca. 1200 km along the Italian Peninsula, host the largest portion of this population, corresponding to ca. 2400 wolves (range: 2020–2645) continuously distributed across the entire mountain system, from the Aspromonte in the south (Calabria region) to the Ligurian and Piedmont Apennines in the north (La Morgia et al. 2022). The species is widespread along mountainous valleys and ridges, i.e., the most undisturbed yet saturated areas (Bassi et al. 2015), and its expansion is currently ongoing in flat areas, such as the urbanized and agricultural Po Plain (Meriggi et al. 2020; Dondina et al. 2020).

Thanks to a long-term monitoring project carried out in the area of the Northern Apennines located in the Lombardy region, we are able to retrace the dynamics of wolf recolonization. The Lombard Apennines represent a focal area for the study of the wolf in Italy because they have been monitored since the late 1980s (Meriggi et al. 1991, 1996, 2015, 2020; Milanesi et al. 2012; Torretta and Meriggi 2018; Torretta et al. 2018; Meriggi and Torretta 2021) and because of their strategic location: lying along the Apennine mountain chain and facing the Po Plain near its most functional ecological corridor (e.g., the Ticino River; Dondina et al. 2020), they represent a sort of junction for the expanding wolf population. Thus, the comprehension of factors that have influenced the recent changes in wolf range and diet may clarify the ongoing process of expansion. Indeed, although there are notable differences in landscape composition and prey availability between the Apennines and the Po Plain or the Prealps (Dondina et al. 2020), the main drivers of wolf expansion, such as human presence, woodlands, and prey species, just to mention a few, could play a comparable role in the different areas.

Aims - This section aimed to analyse (i) range dynamics and (ii) changes in wolf feeding habits that recently occurred in the Northern Apennines in the Lombardy region, focusing, in particular, on the period 2007-2022. Regarding the first aim the prediction was that (i) the range expansion would follow a specific gradient from less human-inhabited zones to more human-dominated ones and (ii) different factors would drive the different stages of wolf range expansion, with the availability of natural habitats driving the early stages within less human-inhabited zones and the magnitude of human pressure driving the recent stages within more human-dominated zones. As for the second aim, the prediction was that, following the general trend observed in Europe (Meriggi et al. 2020), (iii) the wolf diet would increasingly be dominated by wild ungulate consumption, resulting in a narrowing diet breadth. A reduction in livestock consumption was expected both in less human-inhabited zones due to the availability of wild prey and livestock protection measures and in more human-dominated zones where livestock is typically housed. Additionally, (iv) among the available ungulate species, the roe deer was expected to constitute the staple of the wolf diet because of the species' range expansion and its increased profitability for the predator.

3.2.2 Materials and methods

Data collection - Wolf presence was monitored using a standardized protocol from 2007 to 2022 by carrying out eight monitoring sessions; each session of data collection included at least two seasonal surveys, but generally, it consisted of four seasonal surveys covering an entire year (winter: December-February; spring: March-May; summer: June-August; autumn: September-November). We were not able to carry out the monitoring continuously during the study period; thus, some years (e.g., 2013, 2014, 2016, 2017) are lacking.

During each seasonal survey, we searched for indirect signs of wolf presence along routes. We adopted a Tessellation Stratified Sampling design (Barabesi and Fattorini 2013), subdividing the study area into sample squares of 25 km^2 (5 × 5 km) and randomly selecting at least one route among the existing footpaths and dirt roads within each square. Thus, selected routes were representative of the different habitat types and uniformly distributed within the study area. The sampling effort, quantified by the number of investigated sample squares, increased from the first to the last session to follow the spread of the species throughout the area: from 2007 to 2012, we only investigated sample squares within the mountainous zone ($n = 11$); in 2015, we added the sample squares within the upper-hill zone ($n = 9$) and, in 2018, those within the lower-hill zone (in 2018: $n = 9$ + in 2020: $n = 5$; total: $n = 14$). In total, we had 34 sample squares covering an area of 850 km^2 (Table 3.2.1). The decision to add sample squares was prompted by the first evidence of occasional wolf presence in each respective zone.

During each survey, we walked the selected routes to record indirect signs of wolf presence, mainly corresponding to scats, footprints, ground scratchings, and feeding remains. We identified them by size, shape, and location (Bartol et al. 2019). Signs of wolf presence are almost always easily identifiable, especially in the absence of other wild large predators; nevertheless, scats and other signs of presence of uncertain origin were discarded. The adoption of the described method facilitated the detection of permanently occupied areas because most of the detected indirect signs of presence corresponded to scent markings (i.e., scats and ground scratchings) left by stable wolves within their territories (Zub et al. 2003; Llaneza et al. 2014). Recorded data were georeferred with GPS (WGS 84 UTM zone 32N).

Table 3.2.1 - The sampling effort for wolf monitoring in the Lombard Apennines (Northern Apennines, Italy) from 2007 to 2022

Monitoring session	Year	Seasonal surveys [1]	N. of surveyed sample squares	Routes		
				N°	Mean (± SD) length	Total length
1	2007	w sp **su** **a**	11	12	6.8 ± 2.5 km	81.9 km
	2008	**w** **sp** su a				
2	2008	w sp su **a**	11	12	6.8 ± 2.5 km	81.9 km
	2009	**w** **sp** **su** a				
3	2010	w **sp** **su** **a**	11	13	6.8 ± 2.3 km	87.9 km
	2011	**w** sp su a				
4	2011	w sp su **a**	11	13	6.8 ± 2.3 km	87.9 km
	2012	**w** **sp** **su** a				
5	2015	w **sp** **su** a	20	26	5.4 ± 2.3 km	139.2 km
6	2018	w sp **su** **a**	29	37	5.4 ± 2.6 km	199.1 km
	2019	**w** **sp** su a				
7	2020	**w** sp **su** a	34	39	5.3 ± 2.6 km	205.8 km
8	2021	w **sp** **su** **a**	34	42	5.1 ± 2.6 km	215.0 km
	2022	**w** sp su a				

[1] In bold the sampling seasons within each year (w = winter, from December to February; sp = spring, from March to May; su = summer, from June to August; a = autumn, from September to November).

Occupancy modelling - To model the range dynamics of the wolf, we used dynamic occupancy models (MacKenzie et al. 2003), where sites corresponded to 34 sample squares of 25 km^2. These models allowed us to study the species range dynamics by incorporating specific covariates to describe the parameters governing occupancy: the probability of occurrence during the 1st year of the survey (i.e., initial occupancy, ψ), the probability of colonization (γ), the probability of extinction (ε), and the probability of detection (p).

We decided to include all sampling seasons in the analysis as (i) the ecological state of a site (whether a sample square is occupied or not) ideally remains unchanged through occasions (i.e., sampling seasons: winter, spring, summer, autumn) within the primary period (i.e., the year); (ii) we assumed that the majority of detected indirect signs of presence were most likely attributable to stable wolves rather than to dispersers (Zub et al. 2003; Llaneza et al. 2014); (iii) dispersals have been documented during autumn, winter, and spring (Morales-González et al. 2022); thus, excluding one season without having in-depth knowledge of the phenomenon in the study area would have been misleading. We excluded 2022 from the analysis, as we only had one sampling season.

We considered, as site-specific covariates, those variables that, according to the literature, are relevant to the species ecology and might influence its distribution. We used the land cover layers compiled by three Italian regions falling within the study area (Lombardy: https://www.geoportale.regione.lombardia.it; Piedmont: https://www.geoportale.piemonte.it; Emilia-Romagna: https://geoportale.regione.emilia-romagna.it) and summarized the reported detailed categories into broad classes mainly representing the key factors likely to influence wolf distribution: woodlands, open areas (e.g., grasslands, meadows, and pastures), uncultivated areas, arable lands, permanent crops, and urban areas. We also considered road networks made up of primary and secondary roads (https://www.openstreetmap.org). We considered, as yearly-site-level covariates, the estimated abundance of the two widespread wild ungulate species, roe deer and wild boar, in each monitoring session. Following the approach proposed by Sanz-Pérez et al. (2018), we used wild ungulate occurrence data - which, in our case, were the indirect signs of presence identified in the field recorded during seasonal surveys - to delineate their utilization distributions (UDs) through kernel density estimation (KDE) (Worton 1989). Using a probabilistic approach, this method describes the intensity of the use of geographical space by animals, generating a surface that describes the probability of being at a particular location in any part of the study area. We used a fixed kernel estimator and applied the reference smoothing factor (h_{ref}). Site-specific and yearly site-specific covariates were referred to each sample square composing the study area. Lastly, seasons and years were considered observation-level covariates, both as categorical variables to see if there was any trend in detection over time (Table 3.2.2).

We checked potential multicollinearity among covariates using the variance inflation factor (VIF); we retained VIF = 3 as the threshold value (Zuur et al. 2010; Dormann et al. 2013). We used the "usdm" package (Naimi et al. 2014) in R (R Core Team 2023).

Table 3.2.2 - Description and expected effects of covariates used to describe the range dynamics of the wolf in the Lombard Apennines (Northern Apennines, Italy)

Parameter	Covariate	Description	Effect	References
Site level				
Initial occupancy (Ψ)	Woodlands	Percentage cover of mixed, coniferous, and broad-leaved woodlands	+	Massolo and Meriggi 1998; Theuerkauf et al. 2003a; Jędrzejewski et al. 2004; Capitani et al. 2006
	Open areas	Percentage cover of open areas (grasslands, meadows, and pastures)	+	Jędrzejewski et al. 2008; Nakamura et al. 2021
	Uncultivated areas	Percentage cover of unutilized areas	+	Meriggi et al. 1991
	Arable lands	Percentage cover of cultivated fields	−	Dondina et al. 2020
	Permanent crops	Percentage cover of vineyards and fruit trees	−	Dondina et al. 2020
Extinction (ε)	Urban areas	Percentage cover of built-up areas	+	Theuerkauf et al. 2003a; Llaneza et al. 2012; Louvrier et al. 2018
	Road density	Length of primary and secondary roads (km/km^2)	+	Theuerkauf et al. 2003a; Llaneza et al. 2012; Louvrier et al. 2018
Yearly site-level				
Colonization (γ)	Roe deer abundance	KDE based on occurrence data recorded during seasonal surveys	+	Massolo and Meriggi 1998
	Wild boar abundance	KDE based on occurrence data recorded during seasonal surveys	+	Massolo and Meriggi 1998
Observation level				
Detection (p)	Year	Year of monitoring	+	
	Season	Season of survey	−/+	

We built models following a stepwise procedure: in the first model (the null model), we assumed that all four parameters (ψ, ɣ, ε, p) were constant across sites and surveys; no covariates were included in this model. Subsequently, we proceeded by fitting covariates for the detection parameter (p), and the best detection model structure was carried forward to estimate additional drivers of other parameters (ψ, ɣ, ε), which were modelled using the standardized (z-score) covariates. We followed an information–theoretic approach for comparing models containing different combinations of covariates for each of the four parameters (ψ, ɣ, ε, p). Model comparisons were made on the basis of AICc scores and Akaike weights (wi) (Burnham and Anderson 2002). Therefore, we used the best model(s) in the estimation of detection probability, initial occupancy, colonization, and extinction of wolf presence in relation to predictive covariates. We averaged coefficients for covariates in models with Δ AICc ≤ 2 (Burnham and Anderson 2002). We assessed the fit of our model using the MacKenzie-Bailey goodness-of-fit test based on bootstrapping (100 iterations). We performed dynamic occupancy models using the "unmarked" package (Fiske and Chandler 2011) in R (R Core Team 2023).

Diet - The wolf diet was studied from 2007 to 2022 through scat analysis. Scats were analysed to identify the consumed items from undigested remains: hairs, bones, hoofs, and claws (medium- and large-sized mammals); hairs and mandibles (small mammals); and seeds and leaves (fruits and plants). Remains were identified by comparison to a private reference collection (hairs, mandibles, and seeds collected in the field from carcasses or plants) and an atlas (Brunner and Coman 1974; Teerink 1991; De Marinis and Asprea 2006). We observed the hairs with an optical microscope (Leica DM750) to identify the consumed species from the characteristics of cortical scales, medulla, and roots. From undigested remains, the proportion of consumed items for each scat was calculated and then converted into a percent volumetric class, following the procedure used in previous researches of the same project to allow the comparison of results (Meriggi et al. 1991, 1996, 2015, 2020; Milanesi et al. 2012). We determined the diet composition for each monitoring session. To assess the adequacy of sample size, i.e., the number of analysed scats, in describing the diet of wolves, we used the Brillouin index (Hb) (Brillouin 1956). Variations between monitoring sessions of the consumed categories were tested via nonparametric multivariate analysis of variance (NPMANOVA) using the post hoc Bonferroni correction of the p-values for pairwise comparisons (Anderson 2001). Moreover, variations between monitoring sessions within categories were tested via nonparametric analysis of variance (Kruskal-Wallis test with Dunn test for pairwise comparisons). Lastly, we assessed wolf diet breadth in each monitoring session using a normalized Levins' B index (Feinsinger et al. 1981).

Diet analyses were performed by subdividing the samples based on the three elevational zones, i.e., mountainous, upper-hill, and lower-hill zones.

3.2.3 Results

Range dynamics - From 2007 to 2021, we confirmed the presence of wolves at 33 of 34 sites. The naive occupancies, that is, the proportion of sites where the wolf was detected at least once based on the conducted surveys, were relatively high in each monitoring session (mean: 0.70 ± SE 0.05; range: 0.40-0.91).

The VIF values of the considered covariates indicated that two (woodlands and road density) had serious multicollinearity; thus, we excluded them from the analyses.

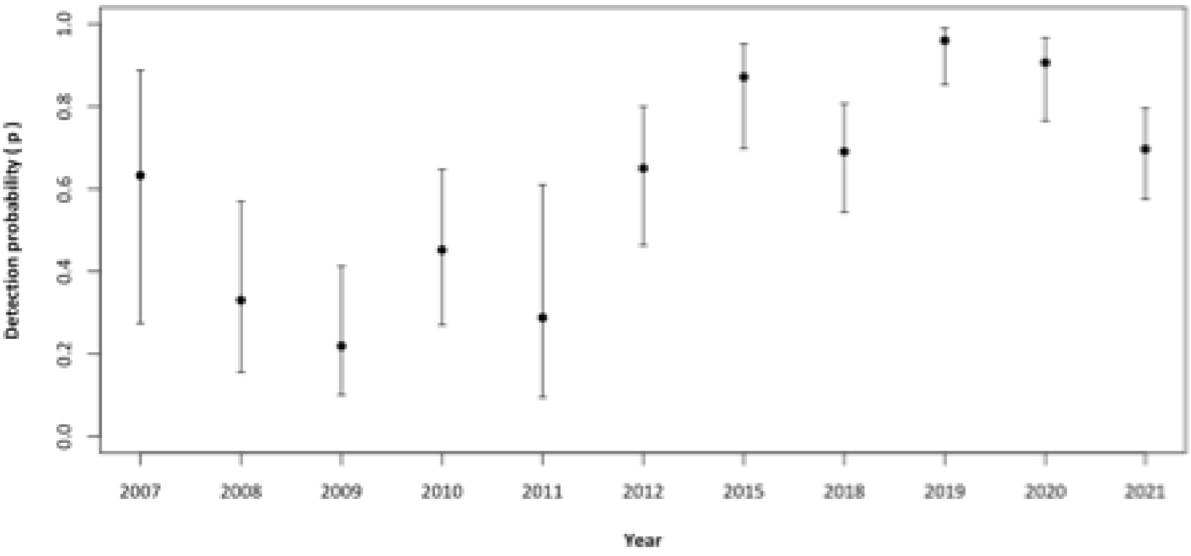
We obtained three models with a ΔAIC < 2; the obtained model set included redundant models; thus, we decided to consider the most parsimonious one (Grueber et al. 2011). This model included roe deer and wild boar abundances as covariates influencing colonization, urban areas cover as a covariate influencing extinction, and the year of monitoring as a covariate influencing detection (Table 3.2.3). The MacKenzie-Bailey goodness-of-fit test indicated that the model adequately fit the data ($\chi^2 = 272.0$, $p = 0.08$).

Table 3.2.3 - Estimated regression coefficient (β) and associated standard error (SE) values for occupancy (ψ), detection (p), colonization (γ), and extinction (ε) from the top model

Parameter	**Term**	**β**	**SE**
Initial occupancy (Ψ)	Intercept	0.04	0.68
Colonization (γ)	Intercept	1.63	1.21
	Roe deer abundance	5.09	2.66
	Wild boar abundance	−10.67	4.76
Extinction (ε)	Intercept	−3.64	0.91
	Urban areas	1.14	0.56
Detection (p)	Intercept	0.55	0.77
	Y 2008	−1.25	0.92
	Y 2009	−1.82	0.90
	Y 2010	−0.74	0.87
	Y 2011	−1.45	1.04
	Y 2012	0.07	0.87
	Y 2015	1.36	0.94
	Y 2018	0.25	0.84
	Y 2019	2.63	1.06
	Y 2020	1.72	0.95
	Y 2021	0.29	0.82

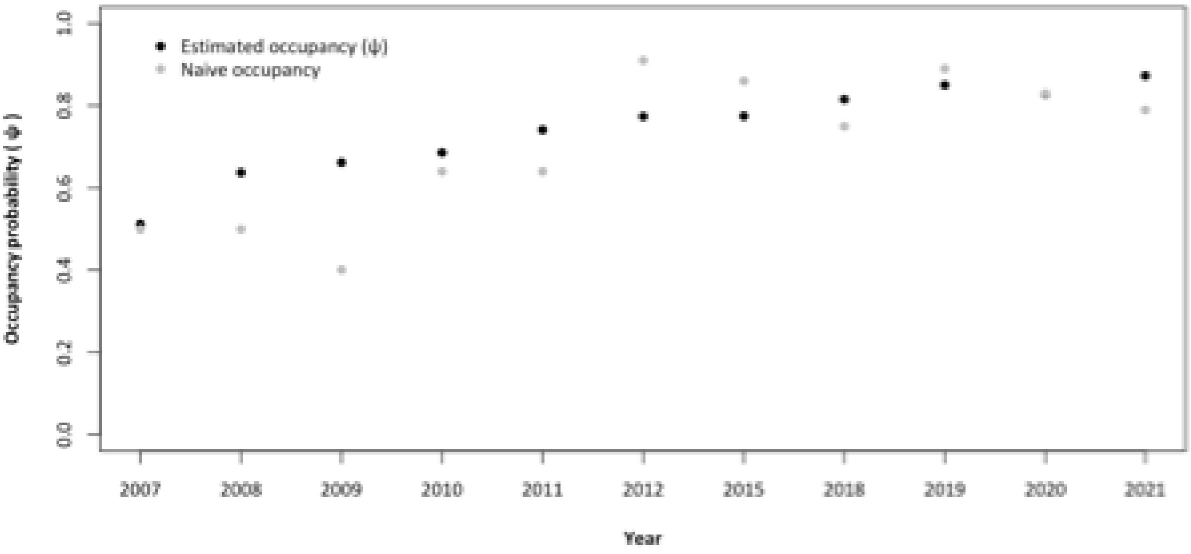
The detection probability as a function of year had the strongest support. The effect of year on detection probability was negative from 2008 to 2011; overall, our model demonstrated high detectability (0.61 ± SE 0.08), although it was slightly lower from 2008 to 2011 (0.22-0.45) and increased thereafter (0.65-0.96; Table 3.2.3 and Figure 3.2.2).

Figure 3.2.2 - Estimated mean probability of detection (±95% CI) of the wolf from 2007 to 2021 in the Lombard Apennines (Northern Apennines, Italy)



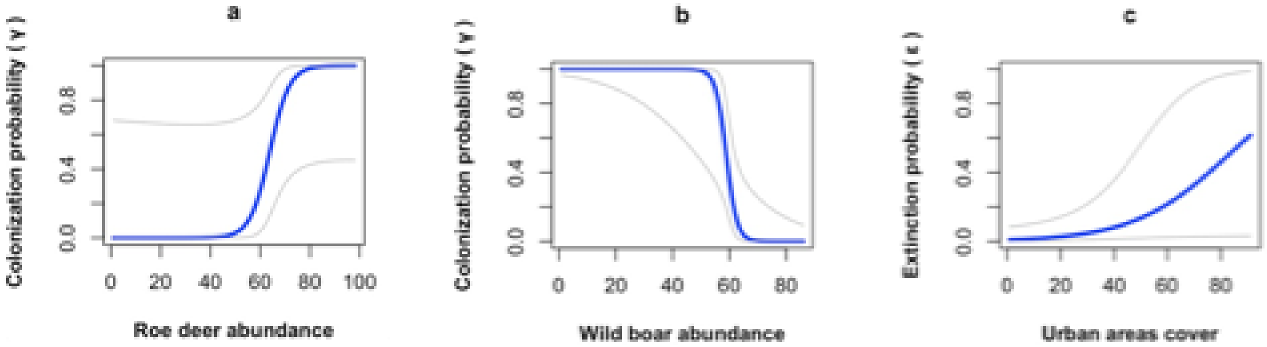
The estimate of occupancy (0.74 ± SE 0.03) did not vary substantially from the naive occupancy because, as previously stated, the detection probability was relatively high; therefore, estimates are reasonably unbiased (MacKenzie et al. 2003). There was a major difference between the model-based estimated occupancy and the naive occupancy in 2009 (Table 3.2.3 and Figure 3.2.3).

Figure 3.2.3 - Estimated mean probability of occupancy (±95% CI) and naive occupancy of the wolf from 2007 to 2021 in the Lombard Apennines (Northern Apennines, Italy)



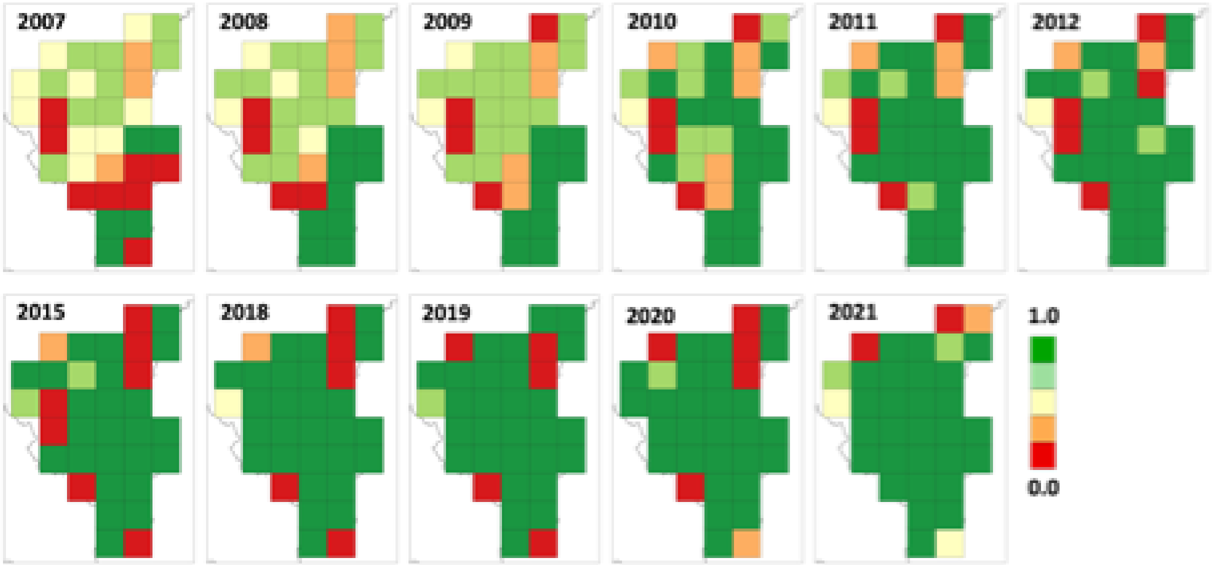
The top model indicated that the range dynamics of the wolf were driven by prey abundance and human presence. The probability of colonization increased with roe deer abundance and decreased with wild boar abundance, whereas the probability of extinction increased with urban area cover (Table 3.2.3 and Figure 3.2.4).

Figure 3.2.4 - Response curves of colonization probability to (a) roe deer abundance and (b) wild boar abundance and response curve of extinction probability to (c) urban area cover estimated based on the top model for the wolf in the Lombard Apennines (Northern Apennines, Italy). Gray lines depict lower and upper 95% CI



The mountainous zone of the study area was the first to be occupied; in this zone, the predicted occupied sites increased from 2007 to 2011, when all sites had occupancy probabilities > 0.80. Starting from 2010, even sites within the upper-hill zone showed an increase in occupancy probabilities and became entirely occupied by 2018. The lower-hill zone was the last to be occupied, and a few sites remained with a low occupancy probability up to 2021 (Figure 3.2.5).

Figure 3.2.5 - Maps of the estimated occupancy probabilities for the wolf from 2007 to 2021 in the Lombard Apennines (Northern Apennines, Italy)



Overall, wolf occupancy in the Lombardy Apennines progressed from the mountainous to lower-hill zones gradually, with very limited extinction probability.

Diet - We analysed 941 wolf scats; in some years, the sample size was not adequate to represent wolf diet as a consequence of the dissimilarities in sampling seasons (Table 3.2.1), so we considered, as subsamples, scats collected during the eight monitoring sessions (2007-2008: 27; 2009-2010: 57; 2011-2012: 70; 2015: 112; 2018-2019: 219; 2020: 203; 2021: 253).

Through scat analysis, we detected nine food categories consumed by the wolf: livestock, wild ungulates, small mammals, medium-sized mammals, birds, invertebrates, fruits, grasses, and garbage (Table 3.2.4).

Table 3.2.4 - Food habits of the wolf in the (a) mountainous (b) upper-hill, and (c) lower-hill zones of the Lombard Apennines (Northern Apennines, Italy) expressed as mean percent volume (VM% ± SE)

Categories and species	**Mountain (n = 696)**		**Upper-hills (n = 156)**		**Lower-hills (n = 89)**	
	VM%	**SE**	**VM%**	**SE**	**VM%**	**SE**
Livestock	**6.55**	**0.88**	**2.39**	**1.09**	**6.08**	**2.44**
Ovis aries	1.04	0.32	0.87	0.67	2.07	1.46
Capra hircus	2.22	0.52	0.73	0.64	1.81	1.30
Bos taurus	3.01	0.61	0.79	0.60	2.20	1.55
Equus caballus	0.29	0.20	0.00	0.00	0.00	0.00
Wild ungulates	**86.70**	**1.11**	**91.68**	**1.72**	**88.44**	**2.90**
Sus scrofa	16.33	1.34	22.88	3.13	16.62	3.71
Capreolus capreolus	62.22	1.74	60.61	3.62	61.91	4.83
Dama dama	4.45	0.77	6.28	1.93	9.91	3.15
Cervus elaphus	3.71	0.70	1.90	1.04	0.00	0.00
Small mammals	**1.70**	**0.46**	**0.63**	**0.63**	**0.00**	**0.00**
Medium-sized mammals	**1.90**	**0.49**	**1.36**	**0.89**	**2.38**	**1.56**
Birds	**0.14**	**0.14**	**0.00**	**0.00**	**0.00**	**0.00**
Invertebrates	**0.01**	**0.01**	**0.00**	**0.00**	**0.00**	**0.00**
Fruits	**0.15**	**0.07**	**0.12**	**0.10**	**1.12**	**0.62**
Grasses	**0.72**	**0.15**	**1.60**	**0.59**	**0.67**	**0.20**
Garbage	**0.004**	**0.004**	**0.003**	**0.003**	**0.00**	**0.00**

The scats analysed for each monitoring session were an adequate enough sample to represent the wolf diet in every considered zone, i.e., mountainous, upper-hill, and lower-hill zones (Supplementary material 3.2: S.1).

In the mountainous zone, the wolf diet was significantly different between monitoring sessions (NPMANOVA: $F = 17.10$; $p < 0.001$); in particular, the diet of the first four monitoring sessions (2007-2008; 2009-2010; 2011-2012; 2015) differed from the diet of the last three monitoring sessions (2018-2019; 2020; 2021) (post hoc pair-wise comparisons with Bonferroni correction). The consumption of livestock increased from 2007-2008 to 2011-2012; then, it rapidly decreased (Kruskal-Wallis test: $H = 116.30$; $df = 6$; $p < 0.001$); conversely, the consumption of wild ungulates, overall the most consumed category, increased from the first to last monitoring sessions ($H = 69.01$; $df = 6$; $p < 0.001$). The consumption of medium-sized mammals was particularly high in 2007-2008; then, it decreased ($H = 29.86$; $df = 6$; $p < 0.001$). Even the consumption of grasses differed between sessions, which was noticeable in 2007-2008 and 2018-2019 ($H = 230.49$; $df = 6$; $p < 0.001$) (Figure 3.2.6 a; Supplementary material 3.2: S.2). In the upper-hill and lower-hill zones, the wolf diet did not differ significantly among monitoring sessions; in both zones, the category of wild ungulates was the most consumed, while other categories were occasional (Figure 3.2.6 a, b; Supplementary material 3.2: S.2).

Among wild ungulates, the wild boar and the roe deer were the most consumed species. In the mountainous zone, wolf consumption of the different species of wild ungulates was significantly different between monitoring sessions (NPMANOVA: $F = 26.44$; $p < 0.001$); in particular, the diet of the first four monitoring sessions (2007-2008; 2009-2010; 2011-2012; 2015) differed from the diet of the last three monitoring sessions (2018-2019; 2020; 2021) (post hoc pair-wise comparisons with Bonferroni correction). The consumption of wild boar generally decreased from 2007-2008 to 2021 ($H = 82.79$; $df = 6$; $p < 0.001$); conversely, the consumption of roe deer generally increased from 2007-2008 to 2021 ($H = 191.20$; $df = 6$; $p < 0.001$). Even the consumption of red deer differed among sessions, being particularly high in 2011-2012 ($H = 61.85$; $df = 6$; $p < 0.001$) (Figure 3.2.7 a; Supplementary material 3.2: S.2). In the upper-hill and lower-hill zones, wolf consumption of the different species of wild ungulates did not differ significantly between monitoring sessions; in both zones, the most consumed species was the roe deer, followed by the wild boar (Figure 3.2.7 b, c; Supplementary material 3.2: S.2).

Figure 3.2.6 - Food habits of the wolf in the (a) mountainous, (b) upper-hill, and (c) lower-hill zones of the Lombard Apennines (Northern Apennines, Italy) expressed as mean percent volume (VM% ± SE) of consumed categories: liv = livestock; ung = wild ungulates; med = medium-sized mammals; sma = small mammals; bir = birds; inv = invertebrates; fru = fruits; gra = grasses; gar = garbage

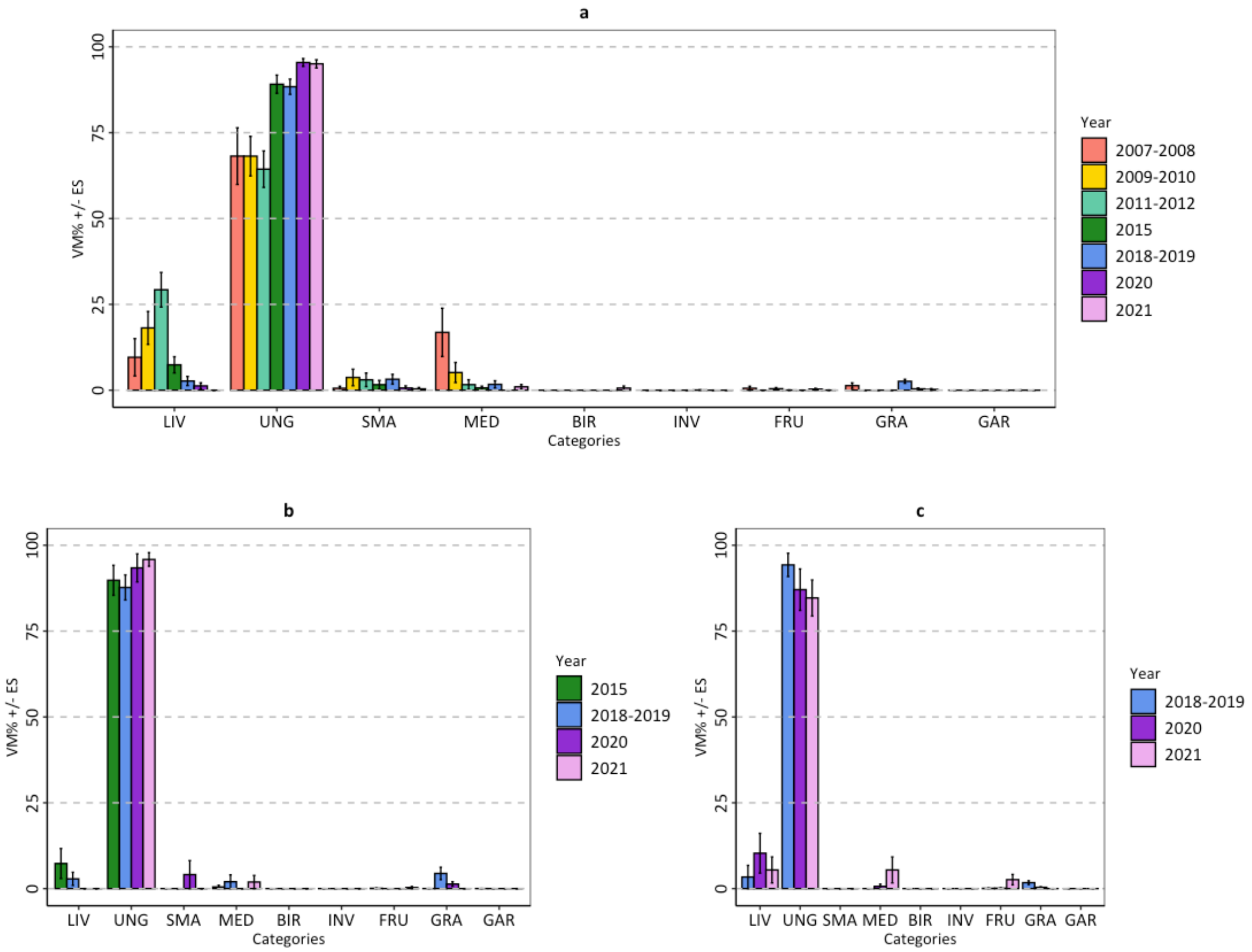
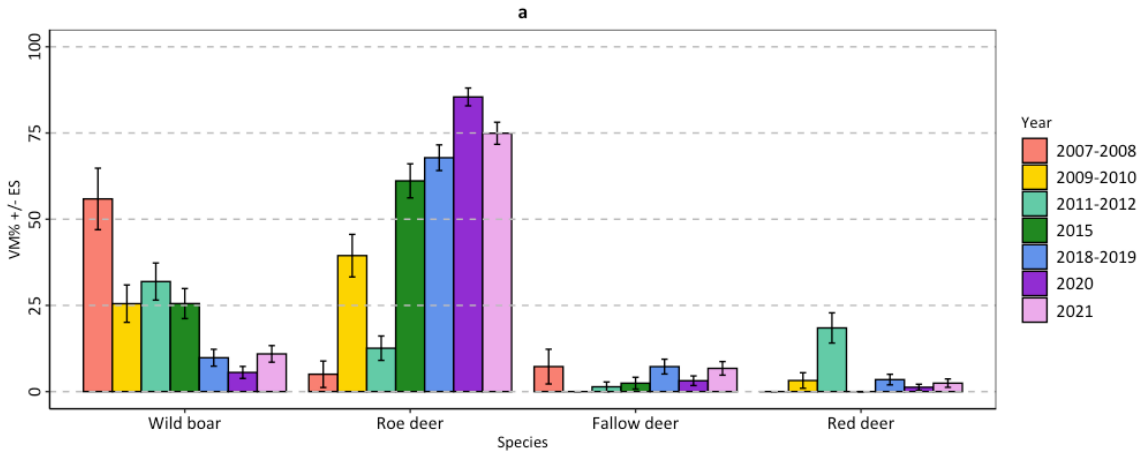
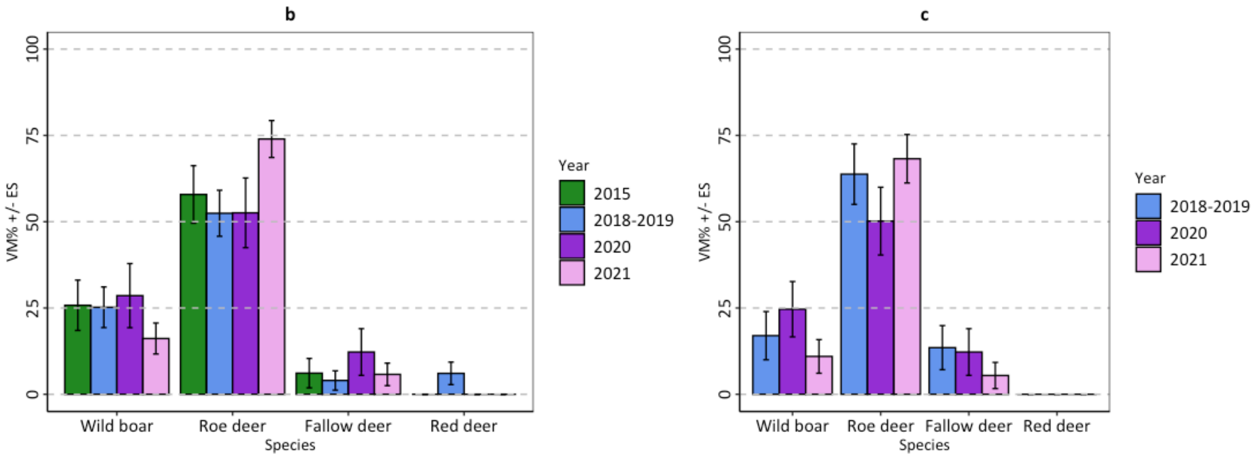


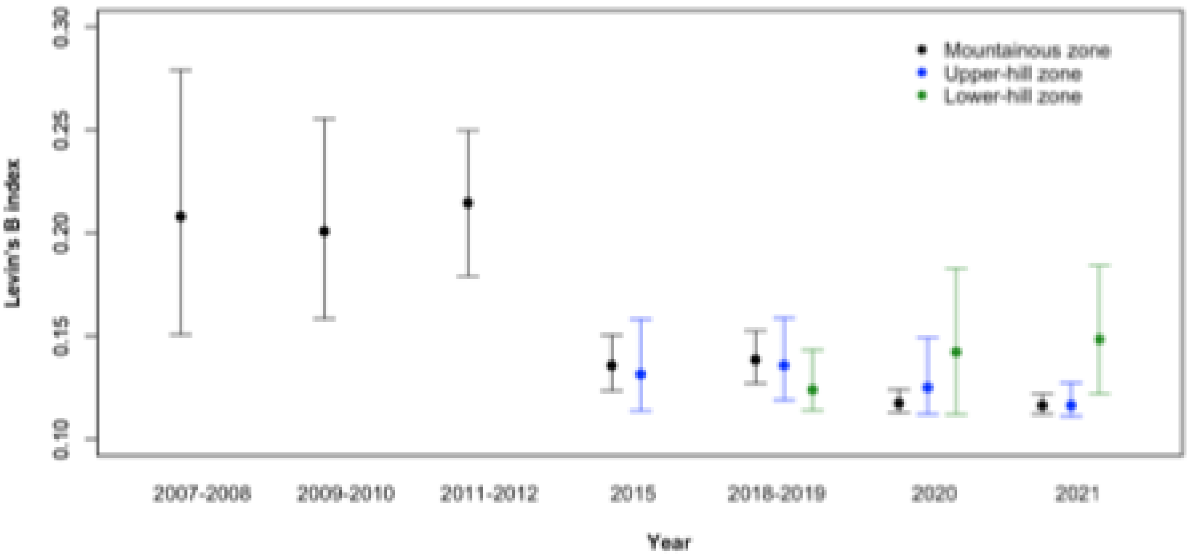
Figure 3.2.7 - Food habits of the wolf in the (a) mountainous, (b) upper-hill, and (c) lower-hill zones of the Lombard Apennines (Northern Apennines, Italy) expressed as mean percent volume (VM% ± SE) of consumed wild ungulate species





Wolf diet was narrow in every considered zone (0.22-0.12); in the mountainous zone it was slightly, but significantly, broader during the first three monitoring sessions and it decreased starting from 2015. No other significant difference emerged (Figure 3.2.8).

Figure 3.2.8 - Breadth of the wolf diet in the three zones of the Lombard Apennines (Northern Apennines, Italy) measured by Levins' B index. Error bars depict lower and upper 95% CI



3.2.4 Discussion

This study describes changes in wolf range and diet that occurred from 2007 to 2021 in an in-depth investigated study area localized in Italy along the Northern Apennines.

As predicted, our results showed a gradual increase in the wolf range during the study period from the mountain to the lower hills. A very similar pattern has been documented in another focal area for the study of the wolf in Italy, that is, Tuscany (Zanni et al. 2023). In the Lombard Apennines, species occupancy steadily increased from 2007 to 2012; starting in 2015, the rate of increase slowed, and occupancy estimates reached a plateau. This might be a consequence of the saturation of our study area; in other words, most of the territory became permanently occupied by resident wolves. As highlighted by Zanni et al. (2023), range expansion suggested that wolves preferred mountains and, once this zone became mostly occupied, started to settle at lower elevations as well. Interestingly, occurrence data in the Po Plain started to be recorded in 2015 and became more and more frequent during subsequent years (Meriggi, unpublished data). The observed pattern, i.e., the plateau in occupancy estimates within the Lombard Apennines and the increasing wolf detections within the Po Plain, suggests that, around 2015, the Lombard Apennines started to act as a source area of dispersers through a density-dependent phenomenon, with individuals being forced to move to lower elevations and more anthropized areas to find available territories. Indeed, dispersers generally move through territories free from packs, where they can eventually settle (Mech and Boitani 2003b). Thus, for dispersers coming from the Lombard Apennines, the best chance to avoid encounters with resident wolves was represented by the few dispersal routes connecting the Northern Apennines to the Central Alps across the highly fragmented and urbanized Po Plain (Dondina et al. 2020).

The expansion of the wolf population toward hills and plains has been documented during the same years (2009-2013) in the entire northern sector of the Apennines (Piedmont, Lombardy, Liguria, Emilia-Romagna, and Toscana regions), where the colonization of new territories in nearly urbanized areas started to be observed, while historical stable territories in remote mountainous areas did not show an increase in the number of individuals (Galaverni et al. 2016). We observed a similar trend in our study area, with increasing colonization of new areas in nearly urbanized landscapes located in the lower-hill zone. This landscape is markedly different from the undisturbed mountainous zone, where wolves settled in the early stages of their recolonization process. In this zone, woodland cover, which provides optimal areas for dens and rendezvous sites (Theuerkauf et al. 2003b; Capitani et al. 2006; Iliopoulos et al. 2014; Ciucci et al. 2018b), is dense and uniform, whereas in the lower hills, it is restricted to small patches along streams. Moreover, woodlands and other natural habitats (e.g., uncultivated lands and shrublands), which are predominant in the mountainous zone, represent suitable habitats for many wild prey. This zone of our study area also includes vast grasslands and pastures along the mountain ridges. Livestock, very abundant on mountainous pastures during the grazing season, represented an easily accessible form of prey for wolves during the first years of the study, as anti-predator measures for livestock protection were not yet implemented at the time (Dondina et al. 2015; Meriggi et al. 2020).

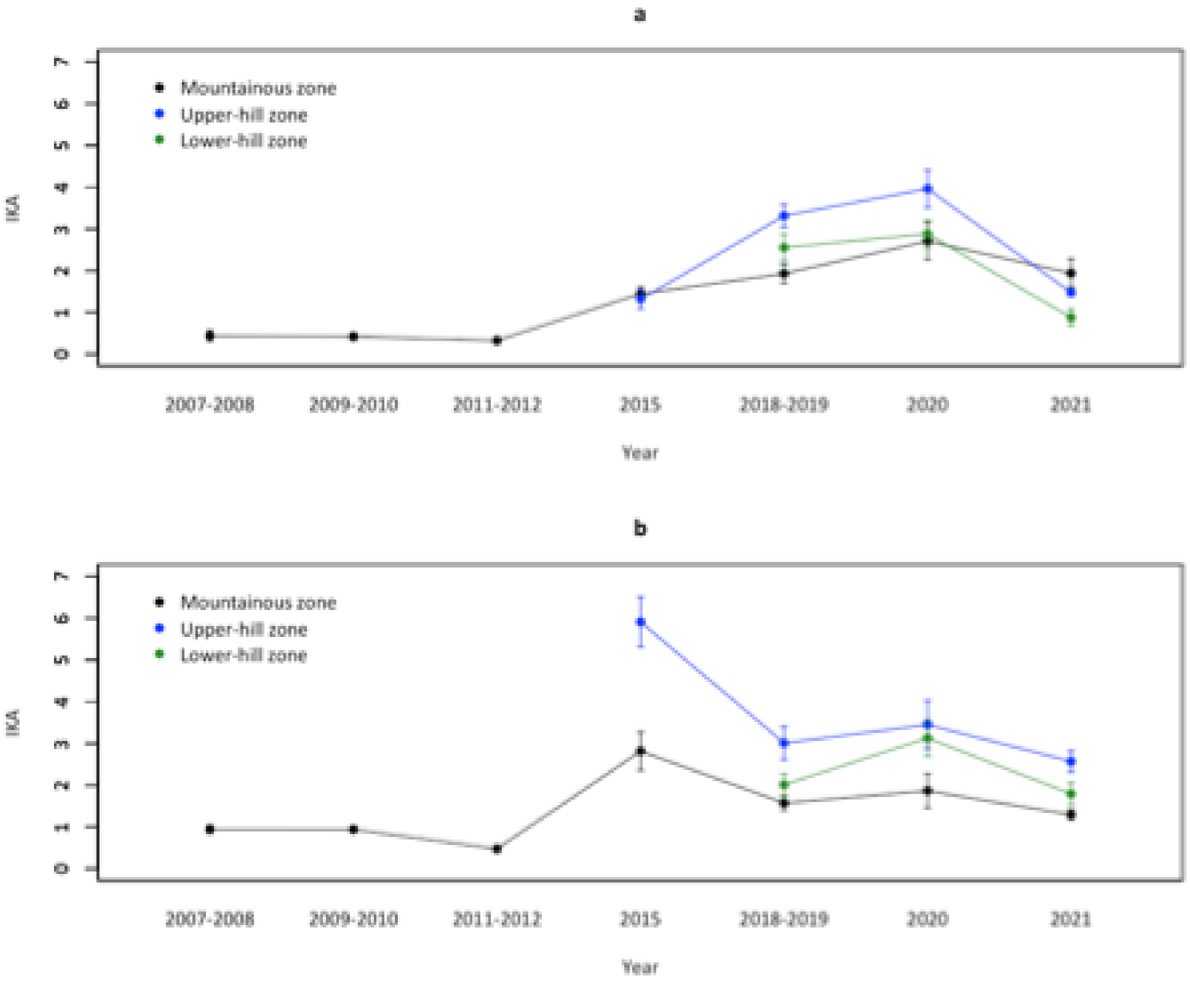
The expansion of the wolf range from the mountains to the hills has most likely been supported by prey availability. Indeed, the most important observed effect of the considered covariates in our models pertains to the colonization parameter, which was significantly influenced by prey abundance. In particular, the probability of colonization was positively influenced by roe deer abundance and negatively influenced by wild boar abundance. We documented very similar trends in the relative abundance values of the two species in the three zones, with lower values during the first monitoring sessions and higher values during the last monitoring sessions, with a slight decrease in the most recent years. Despite this, our data suggested a general increase in both species across the study period (Appendix A). Both the roe deer and the wild boar are classically described as forest-dwelling species, as woodlands contain all the necessary resources for their survival and persistence, like access to cover and feeding sites [roe deer in (Lovari et al. 2017) and references therein; wild boar (Merli and Meriggi 2006; Santilli and Varuzza 2013)]. However, the species are able to exhibit considerable plasticity in terms of habitat selection; over recent decades, they have colonized fragmented landscapes and agroecosystems all over Europe (Danilkin and Hewison 1996; Massei et al. 2015), where they have taken advantage of the presence of scattered natural (e.g., riparian vegetation, residual broad-leaved woodlands) and semi-natural (e.g., hedgerows and arboreal cultivations) vegetated patches [roe deer (Dondina et al. 2019)]. In our study area, we observed that they are less abundant in the mountains compared with the hills, where they successfully exploit a highly heterogenous landscape rich in ecotones with broad-leaved woodlands interposed with vineyards and annual crops; the ecotones are indeed important habitats for both species [roe deer (Lovari et al. 2017); wild boar (Ferretti et al. 2021)]. Despite their similar trends and habitat plasticity, their estimated density distribution had opposite effects on wolf colonization probability. More specifically, the two ungulates showed differences in most frequented areas; in other words, the areas of high-density probabilities of roe deer and those of wild boar are different and not overlapping. Such differences are more marked in the hills of the Lombard Apennines because these zones are characterized by higher habitat heterogeneity if compared with the mountains. This result implies a different use of the space by the two ungulates and suggests specific feeding habits for wolves inhabiting the Lombard Apennines. Indeed, it has been documented that wolves show a complete local adaptation to the ecology of the most consumed ungulate species (Torretta et al. 2017).

Regarding the extinction parameter, we observed the significant positive effect of urban areas. As reported, in the Lombard Apennines, built-up areas mainly correspond to small towns and villages and reach an important percentage of cover only in the most northern sample squares (Figure 3.2.1), where the probability of occupancy was low or irregular across years (Figure 3.2.5). Human presence has been considered for decades to be a factor that negatively influences wolf habitat suitability; recently, it has been argued that this factor has only delayed the wolf recolonization of highly human-dominated landscapes (Zanni et al. 2023), suggesting that wolves are able to exploit even extremely modified landscapes thanks to their behavioural adaptability (Torretta et al. 2022). The diet of wolves in the Lombard Apennines was characterized by the very high consumption of wild ungulates and the secondary consumption of livestock; the other food categories were a negligible fraction of the diet. This result is not surprising, as the study area hosts rich and abundant wild ungulate guilds (Meriggi and Lovari 1996; Zlatanova et al. 2014; Newsome et al. 2016). Moreover, as previous studies of this same research project already showed (Milanesi et al. 2012; Meriggi et al. 2015; Torretta et al. 2018), wild ungulate consumption increased during the study period, following the general trend in the wolf diet observed in Europe (Meriggi et al. 2020). The significant decrease in livestock consumption could also be related to the enhancement of anti-predator measures for livestock protection and/or the general drop in extensive farming with free-grazing animals that has occurred in recent years.

The results regarding wolf consumption of different species of wild ungulates deserve more attention. Conversely to the general pattern observed in recent years in Italy, with the wild boar as the most consumed species (Mori et al. 2017), we found that the roe deer was the staple of the wolf diet in the Lombard Apennines. By focusing on results regarding the mountainous zone, which was monitored for the whole study period, we documented opposite consumption trends for these two widespread wild ungulate species. We observed a gradual shift from the prevalent consumption of wild boar (2007-2008 and 2011-2012) to the prevalent consumption of roe deer, which increased from 2015 onward. These results were unlikely to be related to differences in the abundance of the two species, as we recorded similar trends considering the relative abundance indices; rather, they might be related to a specific adaptation of the predator to the local ecology of the most consumed species (Torretta et al. 2017). The roe deer is generally considered difficult prey for wolves in wooded habitats, as a consequence of its behaviour of living isolated or in small groups (Mattioli et al. 2004). However, in the hills of the Lombard Apennines, this species has reached, in a few years, very high densities, increasing both the encounter and detection chances for wolves. Indeed, in these zones, where wolves have settled in most recent years, predator colonization has followed that of the most consumed species occupying areas with high-density probabilities. Interestingly, very recent studies carried out in Europe have recorded an opposite trend in wolf consumption of the same species, with an increasing predominance of the wild boar over the roe deer [e.g., Spain (Barja et al. 2023); Poland (Mysłajek et al. 2022)].

Appendix A

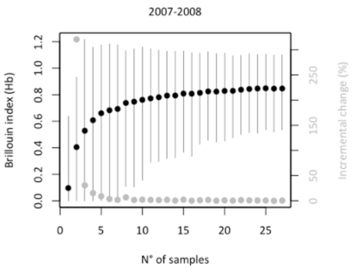
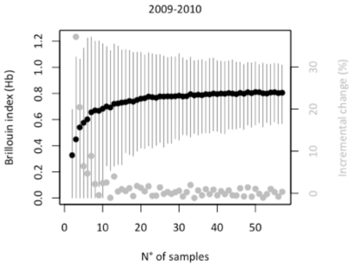
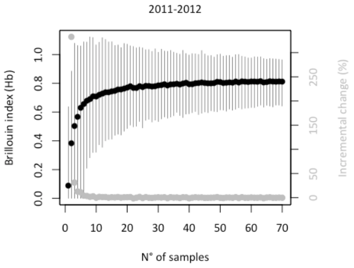
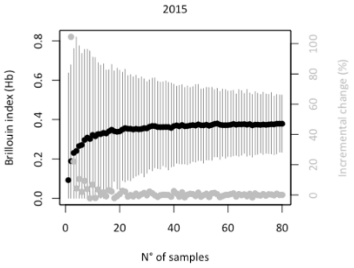
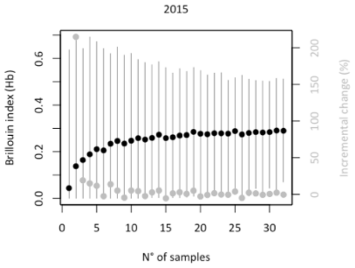
Changes in relative abundance of (a) the roe deer and (b) the wild boar in the three elevational zones characterizing the Lombard Apennines (Northern Apennines, Italy) expressed as Kilometric Abundance Index (IKA)

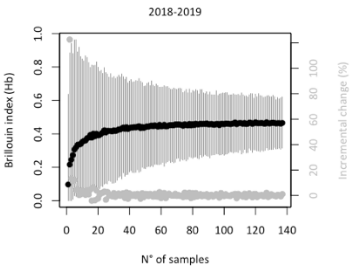


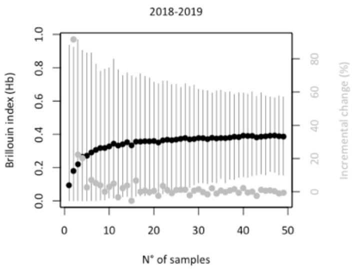
Supplementary material 3.2

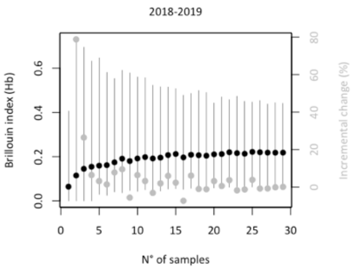
S.1 - Adequacy of sample size used to investigate the wolf diet in the Lombard Apennines (Northern Apennines, Italy)

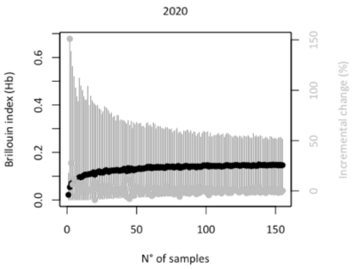
The sample size was adequate to represent the wolf diet. In fact, in every subsample the diversity curve reached an asymptote and the incremental change dropped below 1% with n < 10.

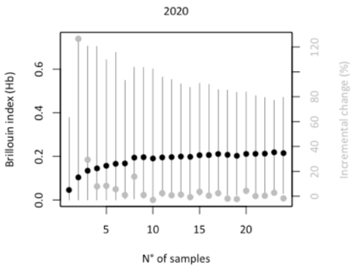
Mountainous zone	**Upper-hill zone**	**Lower-hill zone**
		
		
		
		

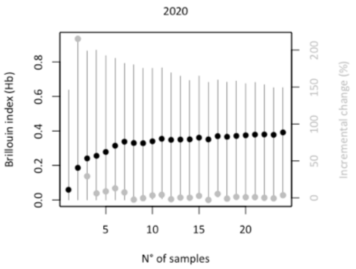

2018-2019
Brillouin index (Hb)
0.0
0.2
0.4
0.6
0.8
1.0
Incremental change (%)
0
20
40
60
80
100
0
20
40
60
80
100
120
140
N° of samples

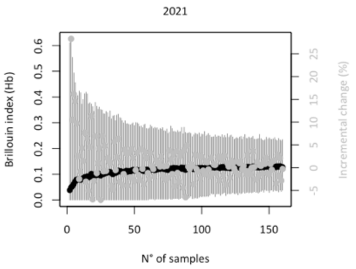

2018-2019
Brillouin index (Hb)
0.0
0.2
0.4
0.6
0.8
1.0
Incremental change (%)
0
20
40
60
80
0
10
20
30
40
50
N° of samples

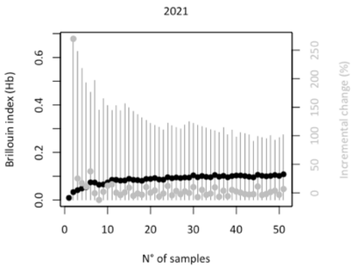

2018-2019
Brillouin index (Hb)
0.0
0.2
0.4
0.6
Incremental change (%)
0
20
40
60
80
0
5
10
15
20
25
30
N° of samples

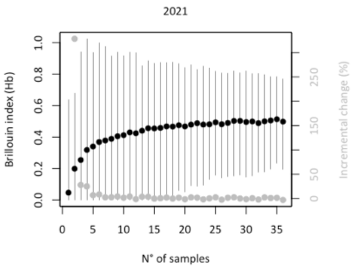

2020
Brillouin index (Hb)
0.0
0.2
0.4
0.6
Incremental change (%)
0
50
100
150
0
50
100
150
N° of samples


2020
Brillouin index (Hb)
0.0
0.2
0.4
0.6
Incremental change (%)
0
20
40
60
80
120
5
10
15
20
N° of samples


2020
Brillouin index (Hb)
0.0
0.2
0.4
0.6
0.8
Incremental change (%)
0
50
100
150
200
5
10
15
20
N° of samples


2021
Brillouin index (Hb)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
Incremental change (%)
-5
0
5
10
15
20
25
0
50
100
150
N° of samples


2021
Brillouin index (Hb)
0.0
0.2
0.4
0.6
Incremental change (%)
0
50
100
150
200
250
0
10
20
30
40
50
N° of samples


2021
Brillouin index (Hb)
0.0
0.2
0.4
0.6
0.8
1.0
Incremental change (%)
0
50
150
250
0
5
10
15
20
25
30
35
N° of samples

S.2 - Details on the food habits of the wolf within the three zones of the Lombard Apennines (Northern Apennines, Italy) expressed as mean percent volume (VM% ± SE)

Categories and species	Mountainous zone													
	2007-2008		2009-2010		2011-2012		2015		2018-2019		2020		2021	
	VM%	SE	VM%	SE	VM%	SE	VM%	SE	VM%	SE	VM%	SE	VM%	SE
Livestock	**9.59**	**5.43**	**18.14**	**4.80**	**29.27**	**5.04**	**7.39**	**2.35**	**2.68**	**1.33**	**1.26**	**0.89**	**0.00**	**0.00**
Ovis aries	3.63	3.63	1.72	1.72	0.54	0.54	4.74	1.69	0.00	0.00	0.63	0.63	0.00	0.00
Capra hircus	2.33	2.33	6.49	3.03	12.51	3.63	1.42	1.24	0.72	0.72	0.00	0.00	0.00	0.00
Bos taurus	3.63	3.63	8.21	3.43	16.21	4.08	1.23	1.22	1.25	0.88	0.63	0.63	0.00	0.00
Equus caballus	0.00	0.00	1.72	1.72	0.00	0.00	0.00	0.00	0.72	0.72	0.00	0.00	0.00	0.00
Wild ungulates	**68.18**	**8.25**	**68.16**	**5.75**	**64.38**	**5.31**	**89.12**	**2.65**	**88.39**	**2.22**	**95.45**	**1.13**	**95.05**	**1.19**
Sus scrofa	55.88	8.92	25.51	5.43	31.9	5.38	25.54	4.36	9.83	2.45	5.57	1.74	10.94	2.40
Capreolus capreolus	5.04	3.84	39.43	6.17	12.6	3.54	61.12	4.94	67.83	3.72	85.46	2.58	74.93	3.20
Dama dama	7.26	5.03	0.00	0.00	1.4	1.40	2.45	1.72	7.25	2.15	3.16	1.40	6.74	1.97
Cervus elaphus	0.00	0.00	3.22	2.26	18.5	4.38	0.00	0.00	3.49	1.54	1.26	0.89	2.45	1.21
Small mammals	**0.57**	**0.57**	**3.71**	**2.42**	**3.02**	**1.97**	**1.61**	**1.25**	**3.20**	**1.42**	**0.63**	**0.63**	**0.39**	**0.39**
Medium-sized mammals	**16.85**	**7.03**	**5.16**	**2.92**	**1.62**	**1.41**	**0.67**	**0.51**	**1.71**	**1.04**	**0.00**	**0.00**	**0.98**	**0.66**
Birds	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.61**	**0.61**
Invertebrates	**0.57**	**0.57**	**0.00**	**0.00**	**0.44**	**0.31**	**0.03**	**0.03**	**0.00**	**0.00**	**0.31**	**0.25**	**0.02**	**0.02**
Fruits	**1.33**	**0.80**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**2.60**	**0.57**	**0.40**	**0.25**	**0.24**	**0.24**
Grasses	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.02**	**0.02**	**0.00**	**0.00**
Garbage	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.05**	**0.03**	**0.00**	**0.00**	**0.00**	**0.00**

Categories and species	Upper-hill zone													
	2007-2008		2009-2010		2011-2012		2015		2018-2019		2020		2021	
	VM%	SE	VM%	SE	VM%	SE	VM%	SE	VM%	SE	VM%	SE	VM%	SE
Livestock	-	-	-	-	-	-	**7.31**	**4.37**	**2.84**	**1.91**	**0.00**	**0.00**	**0.00**	**0.00**
Ovis aries	-	-	-	-	-	-	4.25	3.25	0.00	0.00	0.00	0.00	0.00	0.00
Capra hircus	-	-	-	-	-	-	3.06	3.06	0.32	0.32	0.00	0.00	0.00	0.00
Bos taurus	-	-	-	-	-	-	0.00	0.00	2.52	1.90	0.00	0.00	0.00	0.00
Equus caballus	-	-	-	-	-	-	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Wild ungulates	-	-	-	-	-	-	**89.80**	**4.37**	**87.71**	**3.65**	**93.40**	**4.09**	**95.87**	**1.98**

Sus scrofa	-	-	-	-	-	-	25.78	7.30	25.20	5.89	28.6	9.29	16.16	4.49
Capreolus capreolus	-	-	-	-	-	-	57.89	8.33	52.44	6.67	52.6	10.10	73.95	5.35
Dama dama	-	-	-	-	-	-	6.12	4.26	4.00	2.80	12.2	6.76	5.76	3.26
Cervus elaphus	-	-	-	-	-	-	0.00	0.00	6.06	3.26	0.0	0.00	0.00	0.00
Small mammals	-	-	-	-	-	-	**0.00**	**0.00**	**0.00**	**0.00**	**4.08**	**4.08**	**0.00**	**0.00**
Medium-sized mammals	-	-	-	-	-	-	**0.48**	**0.48**	**2.00**	**2.00**	**0.00**	**0.00**	**1.92**	**1.92**
Birds	-	-	-	-	-	-	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Invertebrates	-	-	-	-	-	-	**0.09**	**0.08**	**0.00**	**0.00**	**0.00**	**0.00**	**0.30**	**0.30**
Fruits	-	-	-	-	-	-	**0.02**	**0.02**	**4.42**	**1.80**	**1.33**	**0.65**	**0.00**	**0.00**
Grasses	-	-	-	-	-	-	**0.02**	**0.02**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Garbage	-	-	-	-	-	-	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**

	Lower-hill zone													
Categories and species	**2007-2008**		**2009-2010**		**2011-2012**		**2015**		**2018-2019**		**2020**		**2021**	
	VM%	**SE**	**VM%**	**SE**	**VM%**	**SE**	**VM%**	**SE**	**VM%**	**SE**	**VM%**	**SE**	**VM%**	**SE**
Livestock	-	-	-	-	-	-	-	-	**3.38**	**3.38**	**10.31**	**5.79**	**5.44**	**3.79**
Ovis aries	-	-	-	-	-	-	-	-	0.00	0.00	7.69	5.33	0.00	0.00
Capra hircus	-	-	-	-	-	-	-	-	3.38	3.38	2.62	2.63	0.00	0.00
Bos taurus	-	-	-	-	-	-	-	-	0.00	0.00	0.00	0.00	5.44	3.79
Equus caballus	-	-	-	-	-	-	-	-	0.00	0.00	0.00	0.00	0.00	0.00
Wild ungulates	-	-	-	-	-	-	-	-	**94.28**	**3.40**	**87.07**	**6.04**	**84.64**	**5.25**
Sus scrofa	-	-	-	-	-	-	-	-	17.0	6.99	24.6	8.02	10.97	4.87
Capreolus capreolus	-	-	-	-	-	-	-	-	63.8	8.75	50.2	9.82	68.23	7.02
Dama dama	-	-	-	-	-	-	-	-	13.5	6.39	12.2	6.76	5.44	3.79
Cervus elaphus	-	-	-	-	-	-	-	-	0.0	0.00	0.0	0.00	0.00	0.00
Small mammals	-	-	-	-	-	-	-	-	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Medium-sized mammals	-	-	-	-	-	-	-	-	**0.00**	**0.00**	**0.65**	**0.65**	**5.44**	**3.79**
Birds	-	-	-	-	-	-	-	-	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Invertebrates	-	-	-	-	-	-	-	-	**0.09**	**0.09**	**0.12**	**0.11**	**2.61**	**1.51**
Fruits	-	-	-	-	-	-	-	-	**1.74**	**0.54**	**0.38**	**0.17**	**0.00**	**0.00**
Grasses	-	-	-	-	-	-	-	-	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**
Garbage	-	-	-	-	-	-	-	-	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**

3.3 Feeding habits of the wolf packs inhabiting the Po Plain and the Lombard Apennines

3.3.1 Introduction

Wolf populations are structured into cooperative social units known as packs. Typically, a wolf pack consists of a breeding pair, comprising the dominant male and female responsible for reproduction, along with their offspring and other adult or subadult wolves, which generally are the offspring of previous years but can also be unrelated adopted individuals (Mech and Boitani 2003a).

Packs are highly territorial and maintain exclusive areas using visual, olfactory, and acoustic communication. Pack territories often remain stable for several successive breeding seasons and breeding pairs. Generally, packs establish their territories in areas with high prey availability, where they can find abundant resources to sustain their members and pups rearing (Fuller et al. 2003).

As expected by a social species, wolves use cooperative strategies while hunting: chasing into bush, heading off fleeing prey, surrounding and ambushing, or taking turns chasing are just a few examples of common cooperative hunting strategies employed by wolves (Peterson and Ciucci 2003). Interestingly, Schmidt and Mech (1997) observed that food acquisition per wolf decreases with pack size, suggesting that larger packs may not necessarily be more effective hunters. Indeed, they noted that pairs of adult wolves are often more efficient at hunting and killing than larger packs. This may be attributed to the presence of young, inexperienced wolves in larger packs, who contribute little to the hunt and primarily follow behind their parents (Peterson and Ciucci 2003).

After weaning, wolf pups join other pack members on hunts, having the crucial opportunity to learn pack hunting techniques (Packard 2003). This period of observation and participation in hunts is essential for young wolves to develop their hunting skills and learn the cooperative strategies employed by the pack and it exemplifies wolf cultural transmission (Jones and Rendell 2022).

Cultural transmission within wolf packs encompasses various aspects of their behaviour and ecology. From the hunting techniques to social behaviours (e.g. communication through vocalizations and body language). Additionally, young wolves learn about their territory and its resources joining the adults in the environment (Mech and Boitani 2003a).

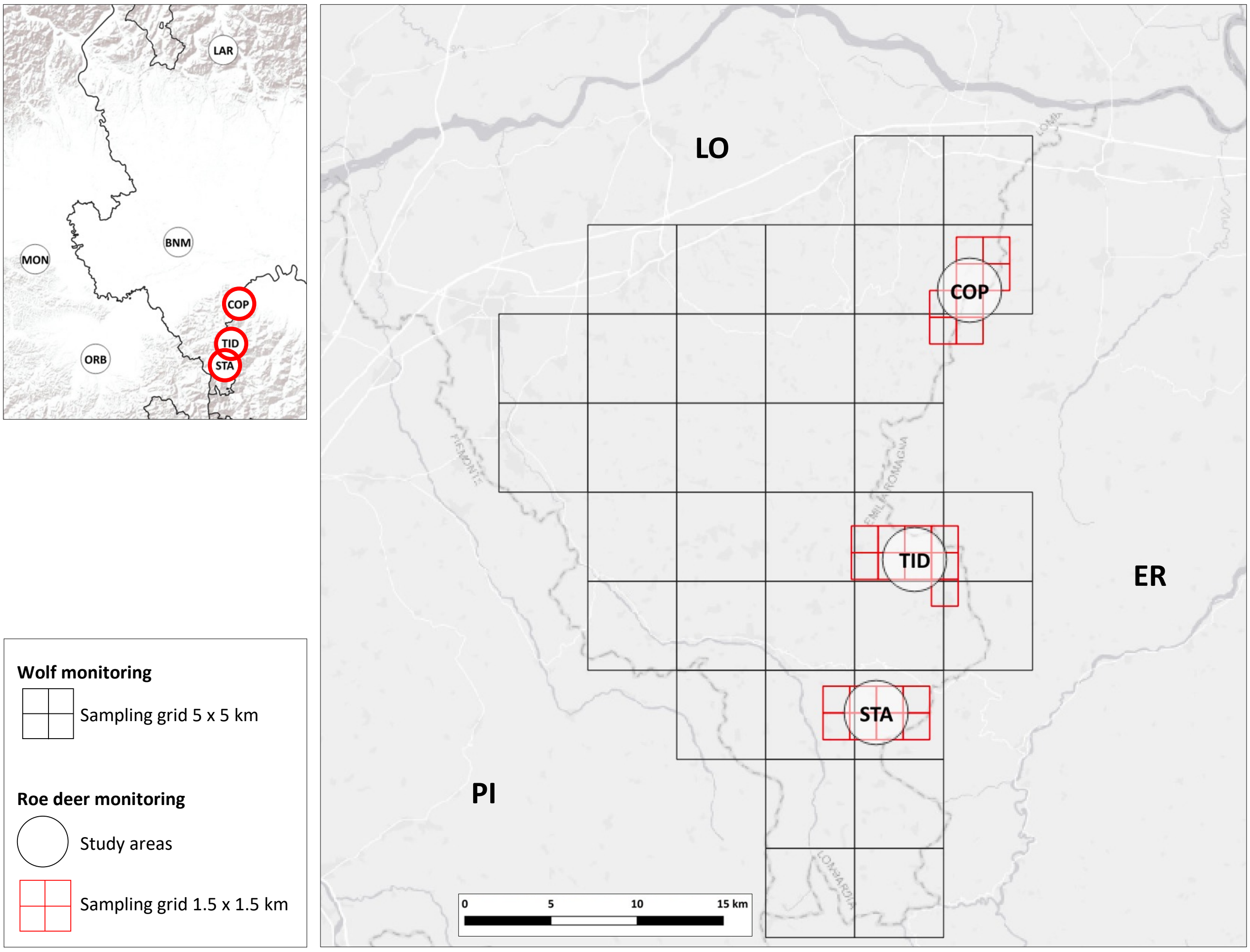
From this emerges how feeding behaviour at the local scale is influenced not only by external factors, such as prey availability and vulnerability, but also by cultural transmission among pack members across generations. This leads the pack itself to become increasingly efficient in hunting and, therefore, to specialize in capturing and consuming the preferred prey.

Aims - The objectives of this section were (i) to describe the composition of packs settled in the study areas of ORB, COP, TID, and STA (i.e. the study areas of the project where the presence of wolves is stable) and (ii) analyse their specific feeding habits. Based on previous results (cfr Section 3.2.3 - Diet), a predominant consumption of the roe deer is expected. Understanding predator food habits is pivotal for elucidating predator-prey dynamics: predation has direct impacts on prey populations, increasing mortality (Hebblewhite et al. 2005), and triggers indirect effects through eliciting antipredator responses (Lima and Dill 1990).

3.3.2 Material and methods

The packs settled in the Northern Apennines were studied within the aforementioned long-term monitoring project (cfr Section 3.2; Figure 3.3.1).

Figure 3.3.1 - Location of COP, TID, and STA study areas in the Lombard Apennines and within the sampling grid used in the wolf monitoring project (2007-2022)



In the area of the Lombard Apennines, the number of packs greatly changed during the period 2007-2022 passing from 4 (Milanesi et al. 2012) to 10 in 2022 (Meriggi, unpublished data).

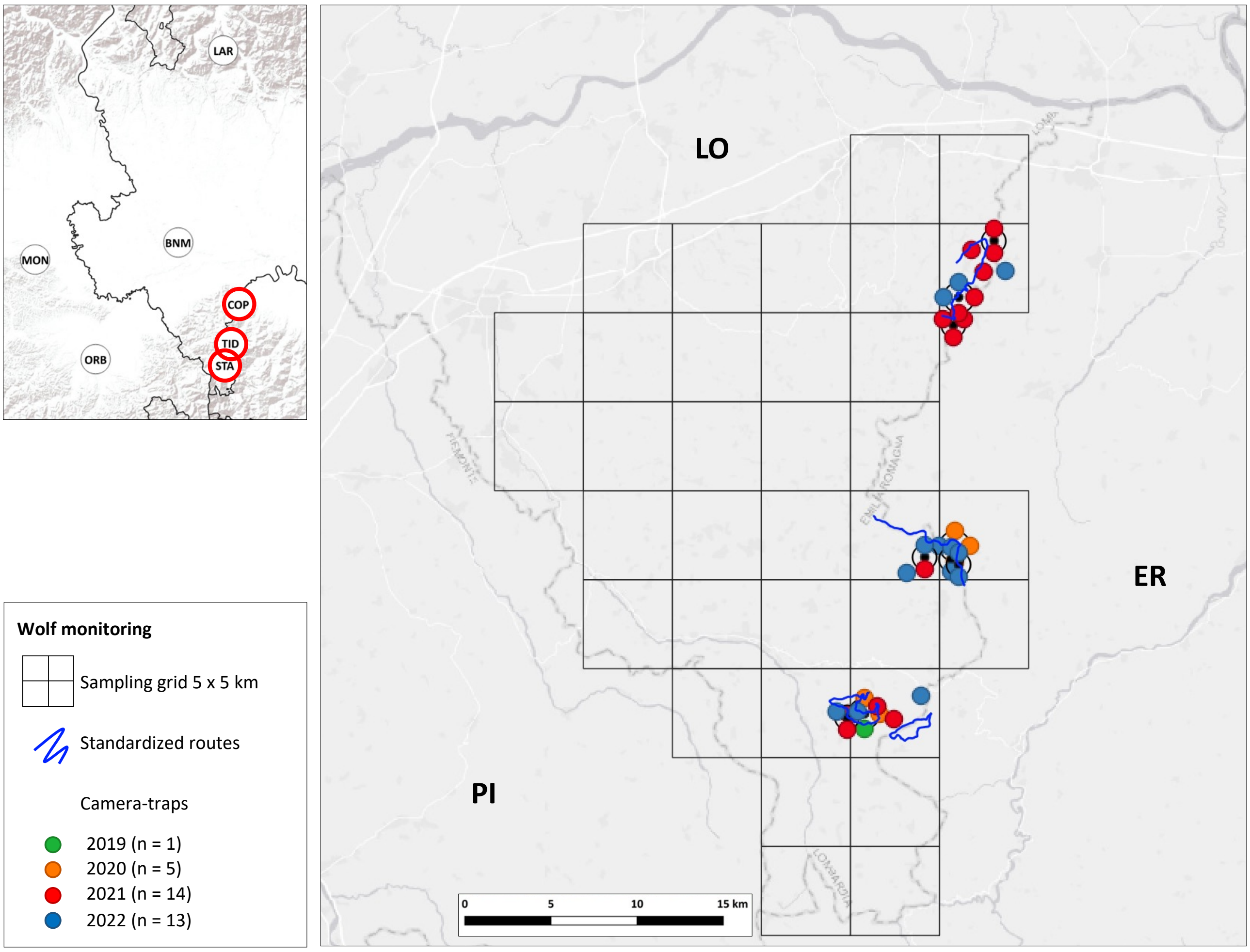
To investigate the presence and composition of packs in COP, TID, and STA study areas, opportunistic camera trapping was conducted starting from 2019. Camera traps were strategically positioned at locations such as marking sites or known travelling routes to maximize the likelihood of capturing the entire pack. Indeed, the wolf is known to be an extremely habitual animal in its movements: pack members show a marked tendency to follow predefined paths within their territory (Mech and Boitani 2003a). Generally, camera traps were kept in the field just for the necessary period to observe the pack composition, then they were relocated to other sites. Starting from 2022, apart from these short camera trap sessions, at least one long-lasting monitoring site was identified in each pack area and camera traps were left in the field for several months. Camera traps were deployed within the same area, and possibly in the same sites, across different seasons over the years to verify the stability of packs presence and composition. From 2019 to 2022, 12 sites were monitored in COP, 11 sites were monitored in TID, and 10 sites were monitored in STA through opportunistic camera trapping (Figure 3.3.2).

Scats, gathered for the diet analyses, were collected and analysed using the methods previously described (cfr Section 3.2.2 - Data collection and Diet). Briefly, wolf scats were systematically searched along standardized sampling routes ($n = 4$; total length = 29.9 km; min. = 7.0 km; max. = 12.1 km), which were surveyed once a season during the sampling sessions. Samples were attributed to a specific pack if collected along the sampling routes within the sample squares where the pack was known to reside based on camera trapping results (Figure 3.3.2).

Scats collected during monitoring sessions lacking strong evidence of pack presence were excluded from the analysis.

The diet composition of the three packs was assessed using the mean percent volume (VM% ± SE) of consumed categories and species, following the previously described procedure (cfr Section 3.2.2 - Diet). Moreover, the consumption of the different wild ungulate species was evaluated using the Manly-Chesson's Index (α; Manly 1972; Chesson 1978). This index compares the proportion of a food item found in the diet, in this case wild ungulate species, to its availability in the environment. The Kilometric Abundance Index (IKA) of the different species, obtained from the presence data recorded seasonally along the sampling routes, was used as a measure of their availability. When selection does not occur $\alpha_i = 1/n$, where n is the number of food items; if $\alpha_i > 1/n$, then the species i is selected, conversely, if $\alpha i < 1/n$, species i is avoided.

Figure 3.3.2 - Camera trap sites and routes used for wolf scat collection investigated for the study of pack composition and their feeding habits in the study areas COP, TID, and STA (Lombard Apennines)



In contrast to other packs, the one established in the Po Plain, specifically within the ORB study area, underwent investigation as part of the Life WolfAlps EU monitoring project. Consequently, insights into the composition and feeding habits of this pack were gleaned from published technical reports and theses.

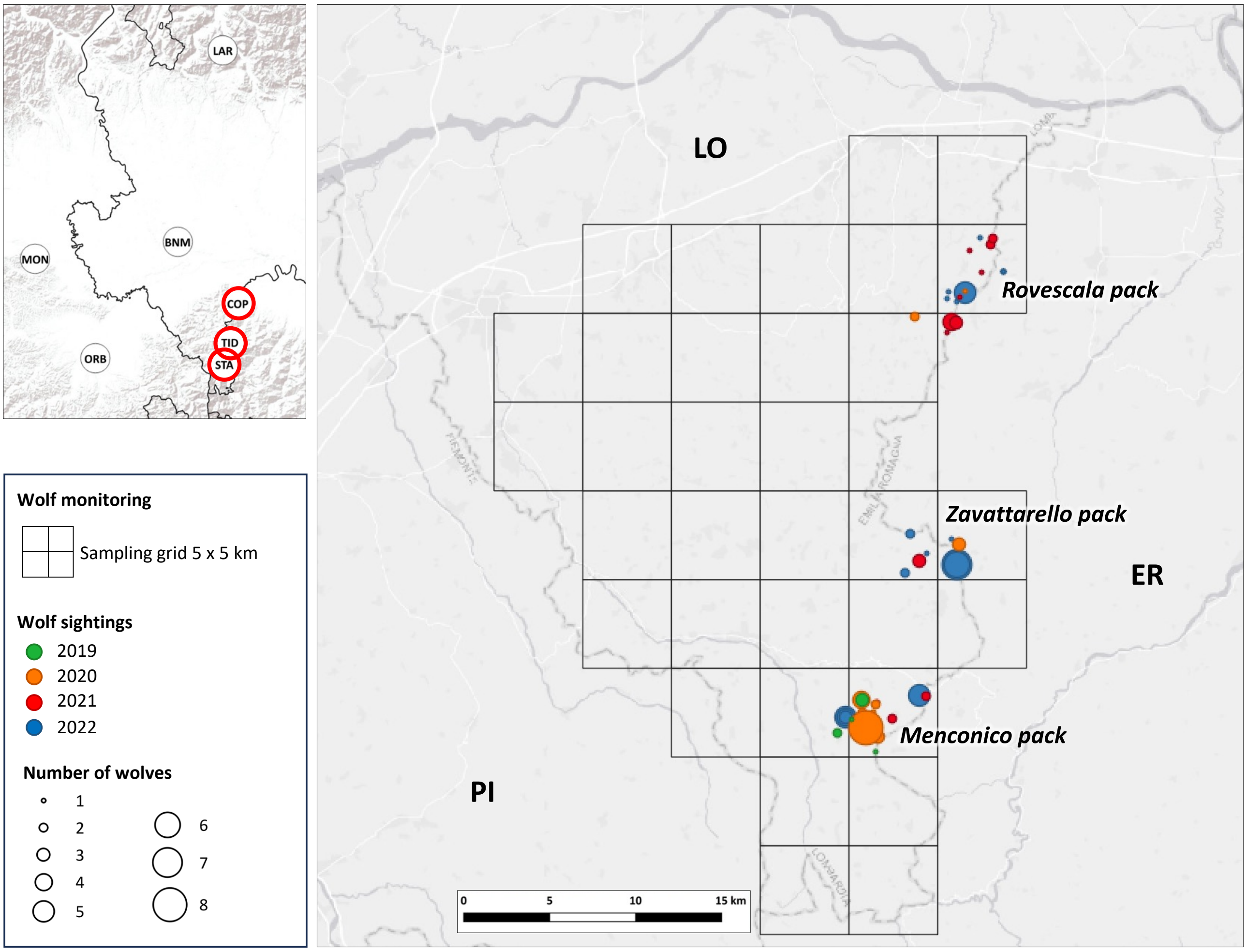
3.3.3 Results

Collected data - A total of 197 wolf events were recorded within the study areas of COP (in 2021 n = 7; in 2022 n = 6), TID (in 2020 n = 7; in 2021 n = 2; in 2022 n = 43), and STA (in 2019 n = 1; in 2020 n = 10; in 2021 n = 17; in 2022 n = 104). Despite the similar number of monitored sites, the recorded events varied significantly among the study areas. This discrepancy was mainly due to the fixed monitoring sites identified in 2022, which contributed the majority of the data. The low number of events in COP study area resulted from the theft of the camera trap shortly after its deployment.

During the sampling sessions (2019 - 2022; cfr Table 3.2.1), a total of 166 scats were collected during the monitoring seasons with evidence of pack presence. Specifically, 37 scats were collected in COP study area, 43 in TID, and 86 in STA.

Packs overview - Wolf events recorded through camera trapping, occasional direct observations, and other indirect data indicating the minimum number of wolves within a pack (e.g. tracks detected on snow), confirmed the presence of three distinct wolf packs. The Rovescala pack established its territory in the area encompassing the COP study area, the Zavattarello pack settled in the area encompassing the TID study area and the Menconico pack settled in the area encompassing the STA study area (Figure 3.3.3).

Figure 3.3.3 - Number of wolves in each sighting within COP, TID, and STA study areas (Lombard Apennines), providing confirmation of pack presence



Food habits overview - The number of analysed scats was an adequate sample representing the wolf diet in every pack area and every season (Supplementary material 3.3: S.1).

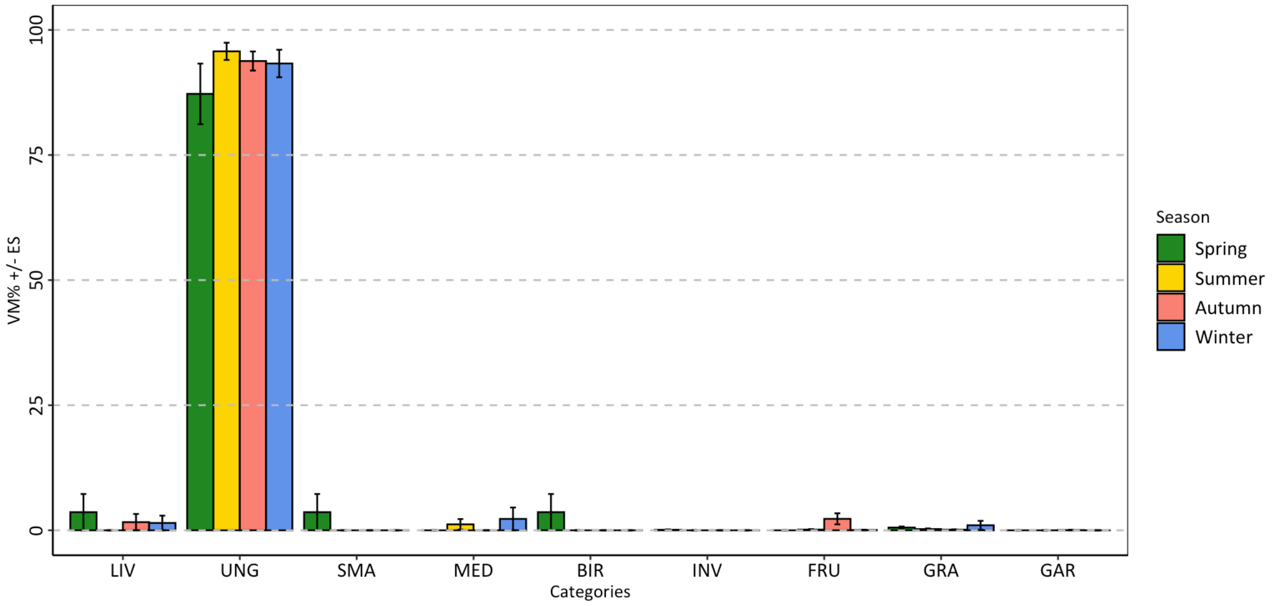
Scat analysis revealed nine food categories consumed by the packs: livestock, wild ungulates, small mammals, medium-sized mammals, birds, invertebrates, fruits, grasses, and garbage.

Considering cumulatively the three wolf packs, the diet did not differ significantly among seasons; throughout the year, that of wild ungulates was the most consumed category, while the consumption of the other categories was occasional (Table 3.3.1 and Figure 3.3.4).

Table 3.3.1 - Seasonal food habits of three packs (cumulative) in the Lombard Apennines expressed as mean percent volume (VM% ± SE)

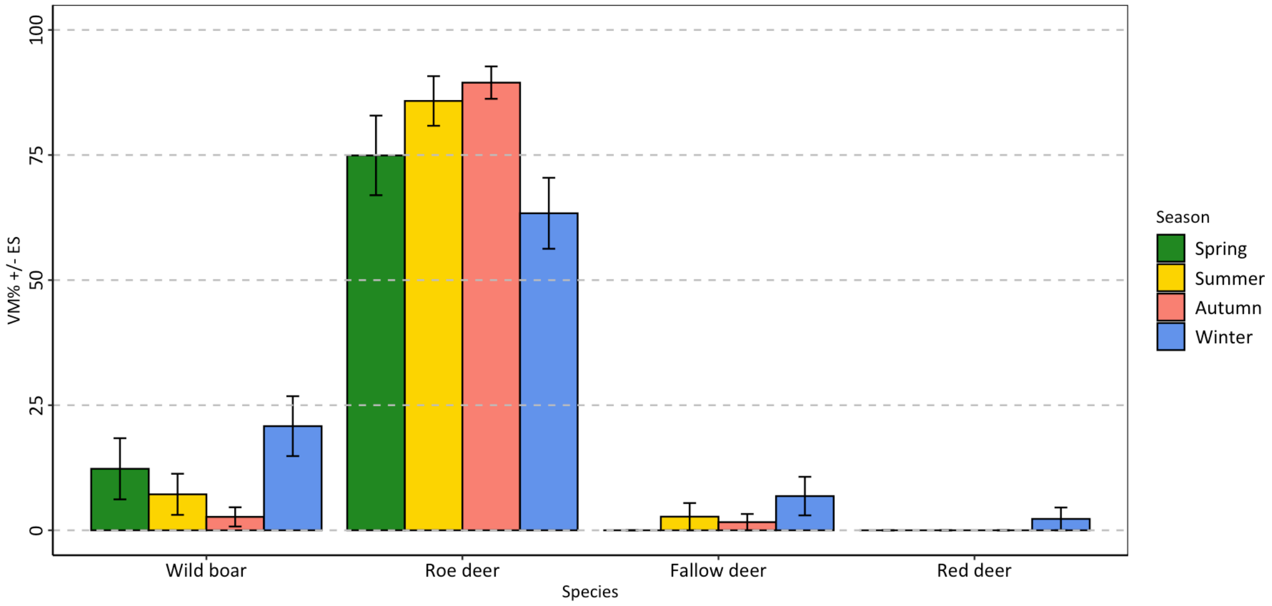
Categories and species	**Spring (n = 27)**		**Summer (n = 36)**		**Autumn (n = 60)**		**Winter (n = 43)**	
	VM%	SE	VM%	SE	VM%	SE	VM%	SE
Livestock	**3.6**	**3.6**	**0.0**	**0.0**	**1.6**	**1.6**	**1.5**	**1.5**
Capra hircus	0.0	0.0	0.0	0.0	1.6	1.6	1.5	1.5
Bos taurus	3.6	3.6	0.0	0.0	0.0	0.0	0.0	0.0
Wild ungulates	**87.2**	**6.0**	**95.7**	**1.7**	**93.8**	**1.9**	**93.3**	**2.7**
Sus scrofa	12.3	6.1	7.2	4.1	2.7	1.9	20.8	6.0
Capreolus capreolus	74.9	8.0	85.8	5.0	89.5	3.2	63.4	7.1
Dama dama	0.0	0.0	2.7	2.7	1.6	1.6	6.8	3.9
Cervus elaphus	0.0	0.0	0.0	0.0	0.0	0.0	2.3	2.3
Small mammals	**3.6**	**3.6**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
ND (vole)	3.6	3.6	0.0	0.0	0.0	0.0	0.0	0.0
Medium-sized mammals	**0.0**	**0.0**	**1.2**	**1.1**	**0.0**	**0.0**	**2.3**	**2.3**
Sylvilagus floridanus	0.0	0.0	0.0	0.0	0.0	0.0	2.3	2.3
Martes sp.	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0
Meles meles	0.0	0.0	1.1	1.1	0.0	0.0	0.0	0.0
Birds	**3.6**	**3.6**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Invertebrates	**0.1**	**0.1**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Fruits	**0.0**	**0.0**	**0.1**	**0.1**	**2.3**	**1.1**	**0.1**	**0.1**
Grasses	**0.6**	**0.2**	**0.3**	**0.1**	**0.1**	**0.1**	**1.0**	**0.9**
Garbage	**0.0**	**0.0**	**0.0**	**0.0**	**0.04**	**0.04**	**0.0**	**0.0**

Figure 3.3.4 - Seasonal food habits of three packs (cumulative) in the Lombard Apennines expressed as mean percent volume (VM% ± SE) of consumed categories: liv = livestock; ung = wild ungulates; med = medium-sized mammals; sma = small mammals; bir = birds; inv = invertebrates; fru = fruits; gra = grasses; gar = garbage



Significant variations in wolf consumption of the different wild ungulate species were noted between seasons (NPMANOVA: F = 4.08; p = 0.003). Specifically, the autumn diet differed from the winter diet (p = 0.006). The consumption of wild boar (H = 10.53; df = 3; p = 0.015) was significantly higher during winter (p = 0.010); conversely, the consumption of roe deer (H = 8.47; df = 3; p = 0.037) was significantly higher during autumn (p = 0.035) (Table 3.3.1 and Figure 3.3.5).

Figure 3.3.5 - Seasonal food habits of three packs (cumulative) in the Lombard Apennines expressed as mean percent volume (VM% ± SE) of consumed wild ungulate species



Each of the three packs primarily consumed wild ungulates, with other categories sporadically and variably consumed by them (Table 3.3.2).

Table 3.3.2 - Food habits of three packs in the Lombard Apennines expressed as mean percent volume (VM% ± SE)

Categories and species	Rovescala pack (n = 37)		Zavattarello pack (n = 43)		Menconico pack(s) (n = 86)	
	VM%	SE	VM%	SE	VM%	SE
Livestock	**7.0**	**4.0**	**0.0**	**0.0**	**0.0**	**0.0**
Capra hircus	4.4	3.1	0.0	0.0	0.0	0.0
Bos taurus	2.6	2.6	0.0	0.0	0.0	0.0
Wild ungulates	**83.0**	**5.2**	**96.7**	**1.4**	**95.5**	**1.3**
Sus scrofa	22.9	6.6	11.1	4.7	3.7	1.9
Capreolus capreolus	57.4	7.6	81.0	5.5	88.3	3.0
Dama dama	2.6	2.6	2.3	2.3	3.4	2.0
Cervus elaphus	0.0	0.0	2.3	2.3	0.0	0.0
Small mammals	**0.0**	**0.0**	**0.0**	**0.0**	**1.1**	**1.1**
ND (vole)	0.0	0.0	0.0	0.0	1.1	1.1
Medium-sized mammals	**2.6**	**2.6**	**0.9**	**0.9**	**0.0**	**0.0**
Sylvilagus floridanus	2.6	2.6	0.0	0.0	0.0	0.0
Martes sp.	0.0	0.0	0.1	0.1	0.0	0.0
Meles meles	0.0	0.0	0.9	0.9	0.0	0.0
Birds	**2.6**	**2.6**	**0.0**	**0.0**	**0.0**	**0.0**
Invertebrates	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**
Fruits	**2.7**	**1.5**	**0.0**	**0.0**	**0.5**	**0.4**
Grasses	**0.2**	**0.1**	**0.1**	**0.1**	**0.7**	**0.4**
Garbage	**0.0**	**0.0**	**0.0**	**0.0**	**0.03**	**0.03**

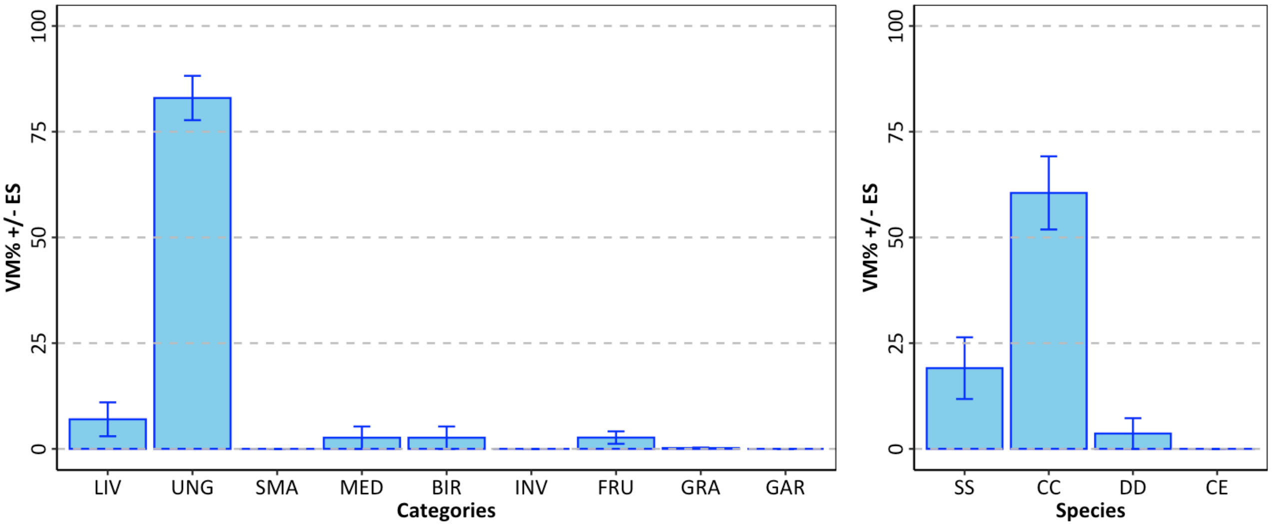
The Rovescala pack - The first data of wolf presence in the study area COP were detected in 2015 (Meriggi et al. 2020). However, the confirmation of the presence of the pack dates back to 2018 with a direct observation of 3 wolves travelling together. During 2022 the pack consisted of at least 5 individuals (1 adult female, 1 adult male, and 3 subadults - of which 1 female) (Table 3.3.3).

Table 3.3.3 - Number of wolf events and number of wolves per event recorded in COP through camera trapping

Year	**N° of wolves**				
	1	**2**	**3**	**4**	**5**
2021	4	2	1		
2022	5				1

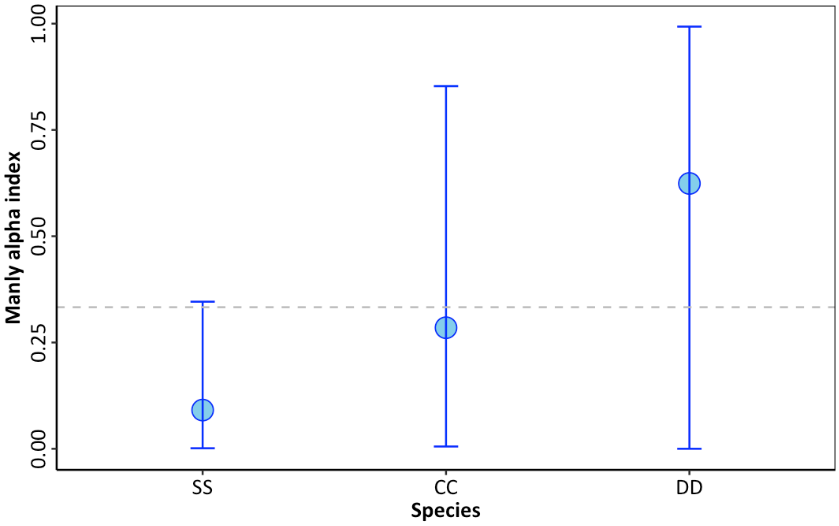
The diet of this pack, derived from the analysis of 37 scats collected from 2018 to 2022, was based on wild ungulates (83.0% ± 5.2), while the consumption of livestock was of secondary importance (7.0% ± 4.0). Among wild ungulates, the roe deer was the most consumed species (57.4% ± 7.6) followed by the wild boar (22.9% ± 6.6) (Figure 3.3.6 and Table 3.3.2).

Figure 3.3.6 - Food habits of the Rovescala pack expressed as mean percent volume (VM% ± SE) of consumed categories (LIV = livestock; UNG = wild ungulates; SMA = small mammals; MED = medium-sized mammals; BIR = birds; INV = invertebrates; FRU = fruits; GRA = grasses; GAR = garbage) and wild ungulate species (SS = wild boar; CC = roe deer; DD = fallow deer; CE = red deer)



Based on the data collected during the same period, the wild boar was the most abundant species, according to the relative abundance index (IKA = 1.425 ± ES = 0.343), followed by the roe deer (1.119 ± 0.435). Although the fallow deer was not recorded on the sampling route itself, its presence was documented in nearby sample squares. The red deer was absent from this study area. Consumption of the three wild ungulate species occurred in proportion to their abundance (Figure 3.3.7).

Figure 3.3.7 - Consumption of wild ungulate species (SS = wild boar; CC = roe deer; DD = fallow deer) by the Rovescala pack; dashed line for α = 0.33 indicating a use proportional to availability. Error bars depict lower and upper 95% CI



The presence of wolves in TID and STA study areas has been detected since the 1990s; however, only in recent years it has been possible to determine the number of individuals within the packs inhabiting these areas, thanks to the deployment of camera traps. Therefore, it is not possible to pinpoint the exact time when a pack settled permanently in these territories.

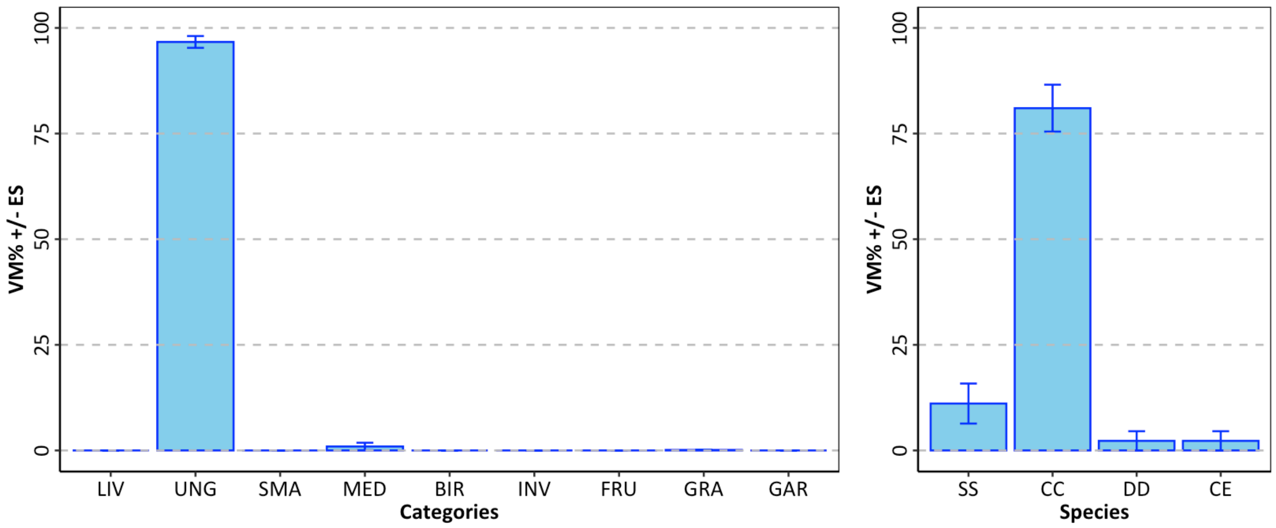
The Zavattarello pack - In 2020 the pack consisted of at least 3 individuals (1 adult female, 1 adult male, and 1 subadult), while in 2022 the pack counted up to 10 individuals, including the breeding pair, their 5 pups, and 3 other wolves (Table 3.3.4).

Table 3.3.4 - Number of wolf events and number of wolves per event recorded in TID through camera trapping

Year	N° of wolves							
	1	2	3	4	5	7	8	10
2020		6	1					
2021	1		1					
2022	22	14	1	2	1	1	1	1

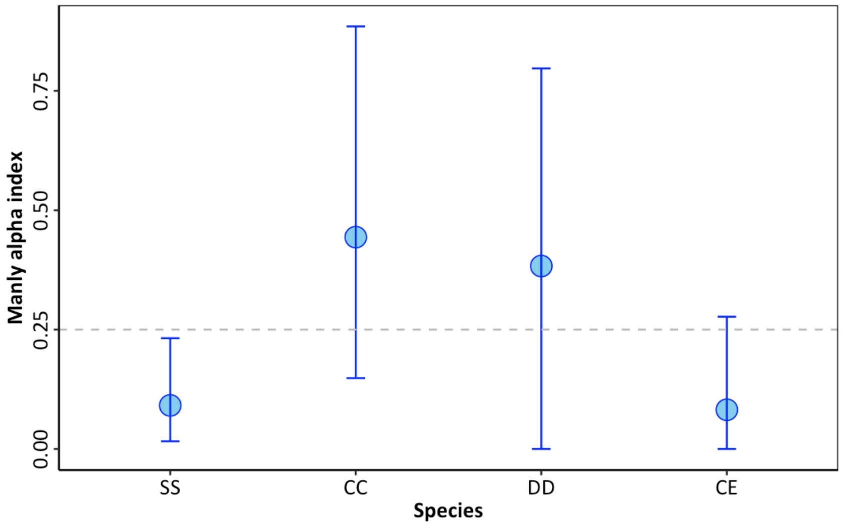
The diet of this pack, derived from the analysis of 43 scats collected from 2020 to 2022, was almost entirely based on wild ungulates (96.7% ± 1.4). The roe deer was the most consumed species (81.0% ± 5.5) followed by the wild boar (11.1% ± 4.7) (Figure 3.3.8 and Table 3.3.2).

Figure 3.3.8 - Food habits of the Zavattarello pack expressed as mean percent volume (VM% ± SE) of consumed categories (LIV = livestock; UNG = wild ungulates; SMA = small mammals; MED = medium-sized mammals; BIR = birds; INV = invertebrates; FRU = fruits; GRA = grasses; GAR = garbage) and wild ungulate species (SS = wild boar; CC = roe deer; DD = fallow deer; CE = red deer)



The roe deer was the most abundant wild ungulate (3.362 ± 0.836), followed by the wild boar (2.163 ± 0.582), and the red deer (0.352 ± 0.188). The fallow deer was the least abundant species (0.051 ± 0.028). While the wild boar was avoided compared with its availability, the consumption of the three deer species was proportional to their abundance (Figure 3.3.9).

Figure 3.3.9 - Consumption of wild ungulate species (SS = wild boar; CC = roe deer; DD = fallow deer) by the Zavattarello pack; dashed line for α = 0.25 indicating a use proportional to availability. Error bars depict lower and upper 95% CI



The Menconico pack(s) - In 2019 the pack consisted of at least 4 individuals: 1 adult female with white forelegs, 1 black adult male, 1 black subadult female, and 1 other wolf of undetermined sex.

In 2020 the pack expanded to at least 8 individuals, including the breeding pair (consisting of the black male and the female with white forelegs) along with their 6 pups; reproduction was confirmed by a direct observation, whereas camera traps recorded only the presence of the pair. In 2021 camera traps captured the presence of 7 different wolves, including the previous breeding pair and 5 others: 1 black adult female, 1 adult female with a particularly short tail, 1 subadult male, and 2 additional adults whose sex could not be determined. Interestingly, in 2022 the breeding pair consisting of the black male and the female with white forelegs ceased to be recorded and, presumably, a new pack consisting of 6 individuals (the breeding pair and their 4 pups) settled in the area (Table 3.3.5). Likely, a pack replacement occurred in this area, but the causes have not been determined.

Table 3.3.5 - Number of wolf events and number of wolves per event recorded in STA through camera trapping

Year	N° of wolves					
	1	**2**	**3**	**4**	**5**	**6**
2019				1		
2020	8	2				
2021	5	8	2			2
2022	48	18	22	11	5	

The diet of the pack(s) inhabiting STA study area, derived from the analysis of 86 scats collected from 2019 to 2022, was almost totally based on wild ungulates (95.5% ± 1.3). Within the category, the roe deer was the most consumed species (88.3% ± 3.0); the consumption of other species was negligible (Figure 3.3.10 and Table 3.3.2).

Based on the data collected along the sampling routes, the roe deer was the most abundant species among wild ungulates (2.151 ± 0.579), followed by the wild boar (1.174 ± 0.200), and the fallow deer (0.158 ± 0.084). The red deer was the least abundant species (0.064 ± 0.036). While the wild boar and the red deer were avoided compared to their availability, the roe deer was selected, and the fallow deer was consumed proportionally to its abundance (Figure 3.3.11).

Figure 3.3.10 - Food habits of the Menconico pack(s) expressed as mean percent volume (VM% ± SE) of consumed categories (LIV = livestock; UNG = wild ungulates; SMA = small mammals; MED = medium-sized mammals; BIR = birds; INV = invertebrates; FRU = fruits; GRA = grasses; GAR = garbage) and wild ungulate species (SS = wild boar; CC = roe deer; DD = fallow deer; CE = red deer)

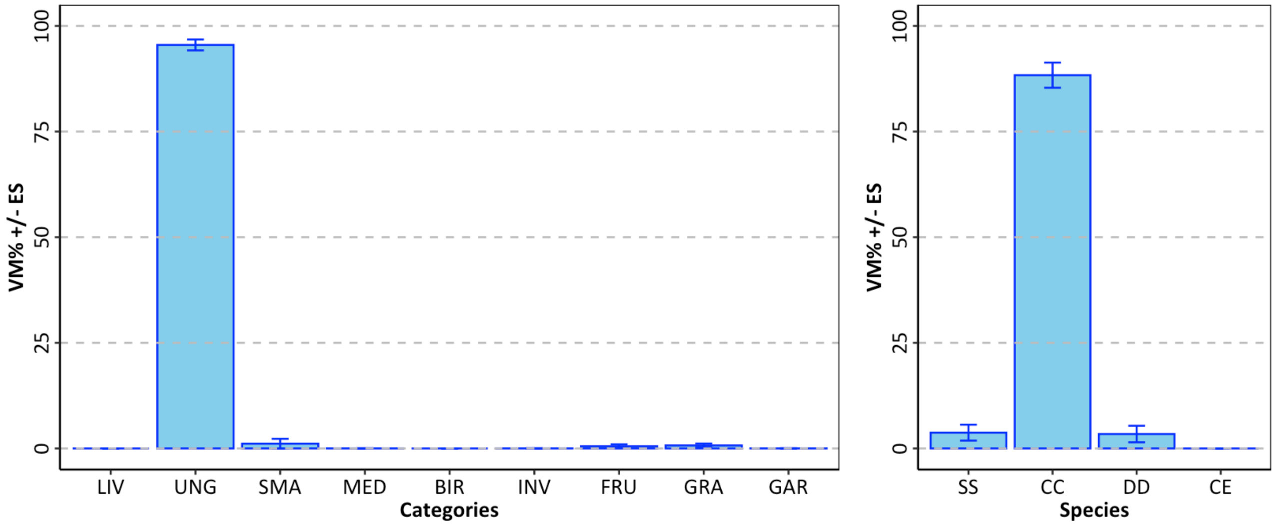
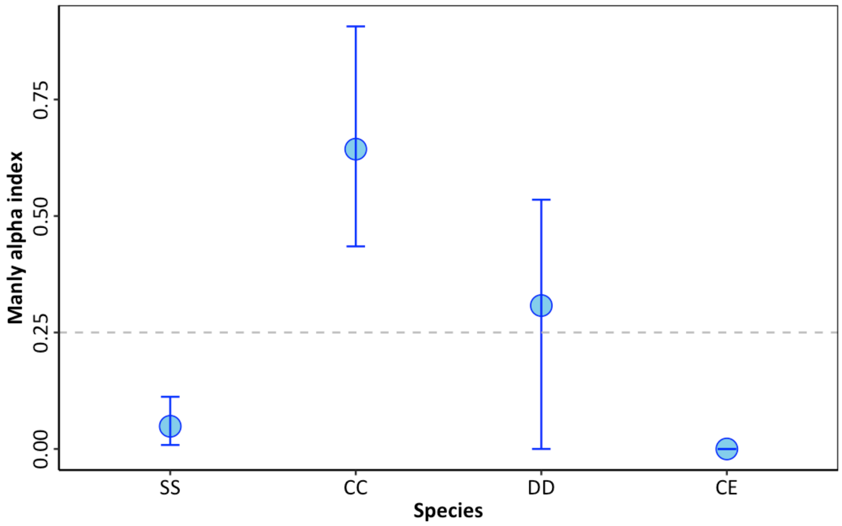


Figure 3.3.11 - Consumption of wild ungulate species (SS = wild boar; CC = roe deer; DD = fallow deer) by the Menconico pack; dashed line for α = 0.25 indicating a use proportional to availability. Error bars depict lower and upper 95% CI



The Orba pack - The first data of wolf presence in ORB were detected in 2018-2019 (Marucco et al. 2020), but the stable presence was confirmed during the summer of 2020 with the first documented reproduction event. During the sampling session of 2020-2021, the pack consisted of at least 7 individuals and, through genetic analysis, the complete genotype of 6 different individuals (the breeding pair and their offspring) was reconstructed (Marucco et al. 2022).

The diet of this pack, expressed as mean percent volume (VM%), was characterized by very high consumption of wild species rather than anthropogenic resources; in particular, the most consumed species was the roe deer (64.9%), followed by the coypus (15.0%), lagomorphs (7.0%), and the wild boar (5.2%). The consumption of livestock, mainly goats, was of secondary importance (≅ 8.0%) (Marras 2022).

3.3.4 Discussion

As predicted based on the recent results on wolf feeding habits in the Lombard Apennines, the roe deer emerged as the primary component of the diet for all four monitored wolf packs. Interestingly, this pattern persisted even in cases where the roe deer was not the most abundant species in the environment, as observed in the COP study area. Moreover, the importance of the roe deer as a primary prey species was emphasized by its selection where it was the most abundant species in the environment, as observed in the STA study area.

This finding could be influenced by a range of factors.

i) Abundance and availability of the roe deer: the roe deer is one of the most widespread and abundant ungulate species in Italy. Although preferring mountainous and hilly landscapes characterized by a mixture of dense cover for shelter and open spaces for grazing (Varuzza 2019), the species is highly adaptable and can inhabit a range of habitats including woodlands, shrublands, and agricultural areas (Danilkin and Hewison 1996; Morellet et al. 2011; Lovari et al. 2016). Indeed, in recent decades, roe deer populations have significantly expanded in terms of range and abundance (Apollonio 2004; Apollonio et al. 2010). This favourable population trend certainly makes the roe deer an ideal prey species for a generalist and opportunistic predator like the wolf.

ii) Size of the roe deer: given the relatively small body size compared to larger prey species, such as red deer or wild boar, wolves can efficiently handle and consume preyed carcasses in a relatively short amount of time; moreover, the size of the species can adequately fulfil the intake requirements of a small pack composed by the reproductive adults and their pups plus, eventually, a few subadults (Meriggi et al. 2020).

iii) Cultural transmission: neighbouring packs tend to be genetically related; the closer one pack lives to another, the greater its chance of being related to the other (Mech and Boitani 2003a). This genetic relatedness may facilitate cultural transmission among neighbouring packs, leading them to adopt similar hunting techniques, sharpened over generations, to hunt and consume the potential prey.

This factor should be regarded as a hypothesis, given the absence of definitive evidence supporting the notion that the wolf packs inhabiting the Lombard Apennines share familial relationships with one another. Further research and genetic analysis are needed to validate this hypothesis.

Nevertheless, this hypothesis would also explain the relatively high pack densities (10 packs in 2022) in the Lombard Apennines (850 km^2), as related packs show greater tolerance towards neighbouring packs due to their familial relationships. This increased tolerance can reduce conflict and competition over resources, allowing for higher pack densities within a given area. In any case, the estimated pack densities fall within the range observed in the Northern Apennines. For instance, Caniglia et al. (2014) reported the presence of 42 packs between Emilia Romagna and Tuscany (19,171 km^2) and Dissegna et al. (2023) estimated the presence of 13 packs in the area of Foreste Casentinesi National Park (362 km^2).

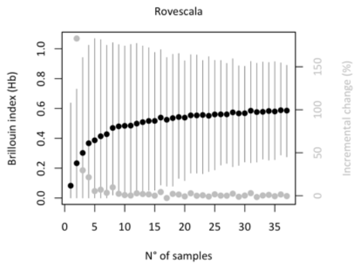
These factors (i.e. abundance and availability of the roe deer and its profitability for the pack, wolf cultural transmission of hunting techniques) are not exclusive rather, they likely interacted synergistically, as expected in the complex dynamics of predator-prey systems.

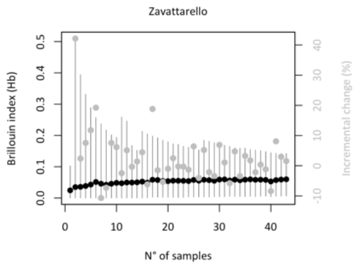
Supplementary material 3.3

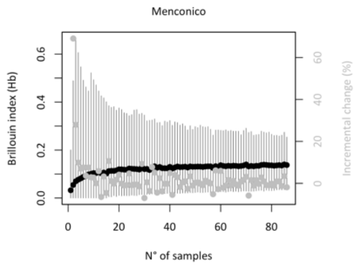
S.1 - Adequacy of sample size used to investigate the diet of three wolf packs in the Lombard Apennines (Northern Apennines, Italy)

The sample size was adequate to represent the wolf diet. In fact, in every subsample the diversity curve reached an asymptote and the incremental change dropped below 1% with n < 10.

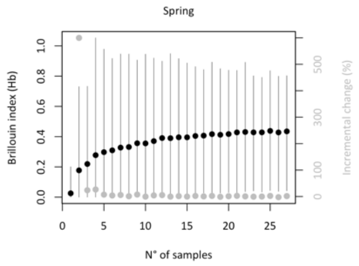
Pack

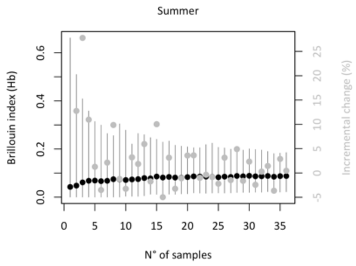


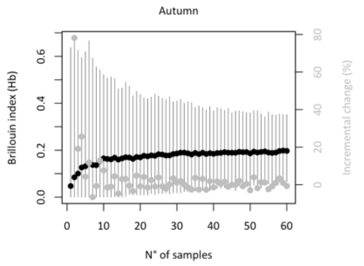


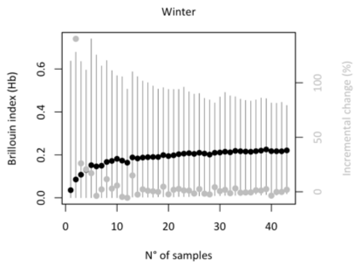


Season









3.4 Conclusions

The significance of the roe deer as a prey species was highlighted by the results of the two case studies. The roe deer played a crucial role in the expansion, feeding habits, and social dynamics of wolf populations inhabiting a wide area in north-western Italy spanning from the Northern Apennines to the Po Plain. In this highly diversified context, roe deer populations have thrived and colonized new areas, giving unique opportunities to the expanding wolf population.

As already pointed out, once prey species are available, wolves are able to settle and persist in any place if human persecution is low (Llaneza et al. 2012). Based on the obtained results, a remarkable expansion of wolves is expected in the Po Plain in the near future, which currently represents the last free-from-packs area accessible for dispersers coming from the Apennines. This area hosts increasing roe deer sub-populations (De Pasquale et al. 2019) despite the high human disturbance, corresponding to dense road networks, continuously built-up areas, and constant human presence. The other side of the coin is that the observed predator pressure on roe deer populations, while not precisely quantified, appears to be considerable. As a consequence, roe deer may experience localized responses aimed at mitigating the impact of predation pressure.

For example, one potential response of roe deer to high predator pressure might be through adjustments in their behaviour and habitat use. For instance, roe deer may exhibit increased vigilance and altered movement patterns to avoid areas with high predator activity. They may also seek refuge in dense vegetation or rugged terrain where predators find it challenging to pursue them effectively. Furthermore, roe deer may undergo changes at the demographic level, such as alterations in reproductive strategies. In areas with intense predation pressure, roe deer may exhibit reduced reproductive rates or altered timing of reproductive events to minimize vulnerability to predation.

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Chapter 4

Understanding Roe Deer Population Demography Across Different Landscapes in North-Western Italy

4.1 Introduction

4.1.1 The fall and rise of the roe deer in Italy

The roe deer is the most abundant deer species in most of the European continent (Burbaitė and Csányi 2009; Apollonio et al. 2010). Despite its widespread current distribution (Lovari et al. 2016), the species underwent a significant decline all over Europe at the beginning of the last century (Apollonio 2004; Apollonio et al. 2010). Excessive and unregulated hunting and the loss of suitable habitats are considered among the main causes of the decline that affected this ungulate (Burbaitė and Csányi 2009).

Since the 1970s, the roe deer population has been undergoing a numerical and distributional recovery (Apollonio et al. 2010). Several factors contributed to a notable expansion of the roe deer population. These included the abandonment of hill and mountain agriculture, which led to a shift of human activities from rural to urban areas. This change favoured the recovery and expansion of natural habitats, providing more territory for wildlife (cfr Section 3.1.4). Additionally, a general improvement in hunting practices has allowed populations of this species to increase; in particular, shorter hunting periods and limited harvest quotas have been established, reintroduction and restocking of deer for hunting purposes have been carried out in specific areas, as well as food provisioning and habitat improvements (Cederlund et al. 1998; Meriggi et al. 2009).

The latest national estimates, albeit from 2010, indicate the presence of at least 460,000 individuals spread across an area of 110,000 km^2 (Carnevali et al. 2009).

Following the spontaneous recolonization of foothill and hilly areas, the species is now expanding its range towards agricultural lowland areas in some regions of northern Italy, including Friuli-Venezia Giulia, Veneto, Lombardy, Piedmont, and Emilia-Romagna (Apollonio et al. 2010).

Moving towards the plain presents new challenges for the roe deer: on one hand, the high level of anthropogenic disturbance, on the other hand, the significant availability of concentrated food resources, represented by agricultural products from crops, which are seasonally more abundant than those provided by woodlands (Abbas et al. 2011; Bonnot et al. 2013).

In agricultural landscapes, which are characterized by high environmental fragmentation, roe deer demonstrate great adaptability; the species can successfully move using the small patches of natural and semi-natural wooded areas (e.g., riparian woodlands, reforestations, poplar cultivations; (Dondina et al. 2019), even though the presence of anthropogenic barriers could hinder or limit dispersion and, consequently, the expansion of the species (Coulon et al. 2004).

4.1.2 Towards an effective management of the roe deer

In Italy, roe deer are hunted in 12 out of the 20 regions, including those located along the Alps (Val d'Aosta, Piedmont, Lombardy, Trentino-Alto Adige, Friuli-Venezia Giulia, and Veneto) and those located along the Northern and Central Apennines (Liguria, Emilia-Romagna, Tuscany, Marche, Umbria). Regarding the Central-Southern Apennines, roe deer hunting occurs only in Lazio, but exclusively within game reserves (Carnevali et al. 2009).

Roe deer hunting in Italy is done through selective harvesting, which means that hunters have to carefully target specific individuals within the population based on factors such as age, sex, and overall health. Selective hunting extends beyond merely targeting sick or injured animals, which is known as sanitary culling. It implies the strategic management of the animal population through culling to achieve and sustain optimal population densities and structures. This approach ensures that the population maintains a dynamic equilibrium with the environment's carrying capacity consistently over time.

The substantial expansion of the species has indeed yielded clear advantages for hunting activities, but it has also resulted in a rise in damage to forestry and agriculture. Consequently, the stakeholders concerned with the management of this species have multiplied (Putman 1986; Putman and Moore 1998; Ward et al. 2004).

In natural and undisturbed habitats like woodlands, the species feeds by selecting the portions of plants that are richer in nutrients, including buds and young leaves (Danilkin and Hewison 1996). This feeding behaviour may affect plant growth and alter the composition of the understory and the tree layer (Moser et al. 2006). In semi-natural and agricultural habitats, the roe deer can cause damage through its foraging activity, as it feeds on the most appetizing portions of crops potentially altering their growth (Danilkin and Hewison 1996).

Last but not least, the roe deer also plays a fundamental role as prey for large carnivores, namely the wolf and the lynx (cfr Section 3.1.3). Several studies have shown that the consumption of wild ungulates by wolves can effectively reduce the depredation of livestock, thereby helping to mitigate conflicts with human activities (Meriggi and Lovari 1996).

4.1.3 The importance of studying population demography

Considering all these factors, effective management of roe deer should aim to strike a delicate balance between hunting and conservation of both habitats and the species itself, while minimizing any adverse effects on human activities. Studying population parameters is crucial for the proper management of the species. This helps prevent bias towards either hunters and breeders, who may advocate for higher population densities, or farmers and foresters, who may prefer lower or even zero densities (Meriggi et al. 2009; Herrero et al. 2013). Accurate estimation of population density and demographic parameters are therefore crucial for gaining insights into the demographic status of the species at a local level and formulating effective management strategies (Flajšman et al. 2013; Herrero et al. 2013; ENETWILD consortium et al. 2020).

The range expansion of the roe deer, which has led the species to occupy the most undisturbed mountainous habitats as well as the highly disturbed flat peri-urban habitats, also influences the choice of the methods used for collecting the data on the field. In recent years, considerable efforts have been dedicated to enhancing the methods employed in monitoring wild populations. The choice of one method over another takes into account various factors, such as management objectives, availability of operators, monitoring duration, associated costs, and, obviously, habitat characteristics (Mayle et al. 1999; Meriggi et al. 2009; Zaccaroni et al. 2018; Hinojo et al. 2022).

Aim - This Chapter aims to analyse the demography of roe deer populations inhabiting different study areas situated along a gradient of landscape composition, ranging from natural to modified, and experiencing differing predator pressures. Both landscape structure and predator presence can significantly influence this species, impacting factors such as population density and demographic parameters (e.g. Focardi et al. 2002; Zannèse et al. 2006; Melis et al. 2009; Randon et al. 2020).

4.2 Material and methods

4.2.1 Population density

Data collection - Roe deer were surveyed using direct observations along road surveys as sampling method. This method implies that trained observers travel along predetermined routes, typically by vehicle, and scan both sides of the route for deer sightings (Meriggi et al. 2009; ENETWILD consortium et al. 2020).

Surveys were conducted during two consecutive winter seasons (2020-2021 and 2021-2022), roughly between November and March of each year. During winter vegetation cover is sparse, making direct observation of animals easier, and roe deer tend to form even very large groups (Danilkin and Hewison 1996), which allows for greater detectability compared to other seasons of the year. Surveys were carried out at dusk, dawn or night hours, when deer are more active and, therefore, visible. These periods coincide with their feeding and movement patterns (Danilkin and Hewison 1996), making it easier to spot them. When surveys were carried out during nighttime hours (e.g., in study areas where human disturbance was high even during crepuscular periods), to locate and count individuals, manually directed 100 W halogen headlights were used.

We considered all existing roads within each study area (except major highways and other high-use paved roads) as routes; thus, we selected a total of 28 routes (Table 4.2.1). During a survey in a given study area, every route was supposed to be covered by car travelling at speeds ≤ 10 km/h. The routes were designated to traverse the various habitats found within each study area.

Table 4.2.1 - Details on the sampling routes used for roe deer direct observations along road surveys in north-western Italy from 2020 to 2022

Study area	N° of routes	Total lengths (km)	Mean (± SD) length (km)	Total area (km^2)
LAR	4	8.9	2.7 ± 1.4	0.7
BNM	3	15.1	7.4 ± 1.5	4.0
MON	5	23.3	5.6 ± 2.5	6.0
ORB	5	14.9	2.5 ± 2.1	12.4
COP	2	15.5	7.8 ± 2.9	5.0
TID	3	17.6	4.9 ± 2.3	3.6
STA	6	23.8	4.0 ± 0.6	2.1

Data collected during these surveys included the number of roe deer groups (solitary individuals included), the number of deer in each group, and their location at the first sighting. Recorded data were georeferred with GPS (WGS 84 UTM zone 32N). Furthermore, for each route, the actual surveyed areas and the perpendicular distance between the observer and the group of deer were mapped using QGIS software.

Density estimation - The most straightforward method to estimate population densities is to relate the number of detected individuals to the total surveyed area along the routes.

However, in cases where there are sufficient observations (min. = 40; Buckland et al. 2015), the method recognized as the most accurate and precise is distance sampling (Buckland et al. 2001). Through a detectability function based on the perpendicular distances between observed individuals and the observer, the method allows for the estimation of population density and abundance. It operates under the assumption that the probability of detecting target individuals decreases as the distance from the observer increases (Buckland et al. 2001).

After a visual inspection of distance distribution, if needed we applied a 5% right truncation of the data to remove outliers as suggested by Buckland et al. (2001). We tested the following combinations of key functions and series adjustments: (1) uniform key with cosine adjustments, (2) half-normal key with cosine adjustments, (3) half-normal key with Hermite polynomial adjustments, and (4) hazard-rate key with simple polynomial adjustments. Cramer von Mises goodness-of-fit test was used to evaluate the adequacy of the models (Buckland et al. 2015). Then we selected the best model according to the coefficient of variation (CV) and the probability of detection. In addition to the density estimation, the Effective Strip Width (ESW) was obtained. ESW is defined as the distance within which the number of unobserved individuals equals the number of observed individuals beyond it.

We performed distance sampling models using the “Distance” package (Miller et al. 2019) in R (R Core Team 2023).

4.2.2 Population structure

Data collection - Roe deer were surveyed using camera trapping as sampling method. In each study area, seasonal camera trapping sessions were conducted over a two-year sampling period from December 2020 to November 2022. These sessions occurred during the four seasons: winter (December to February), spring (March to May), summer (June to August), and autumn (September to November). We maintained camera traps in the field for a putative period of 120 days in each study area.

We subdivided each study area into sample squares using a 1.5 x 1.5 km grid (Table 4.2.2). Within each sample square, one camera trap was deployed randomly during each sampling season. We adopted the sampling design named Tessellation Stratified Sampling (TSS), which allows a better distribution of random samples and increases their representativeness (Barabesi and Fattorini 2013). To reduce spatial correlation, we spaced the camera traps sufficiently apart to minimize the likelihood of capturing the same individual at multiple traps. As a touchstone, Marcon et al. (2019) set the minimum distance between camera traps at 500 m (target species: roe deer; study area: Tuscany, Italy).

Table 4.2.2 - Details on the sampling grid used for roe deer camera trapping in north-western Italy from 2020 to 2022

Study area	N° of sample squares	Total area (km^2)
LAR	9	20.25
BNM	12	27.00
MON	9	20.25
ORB	8	18.00
COP	8	18.00
TID	9	20.25
STA	8	18.00

Camera traps were deployed and activated simultaneously during each seasonal session to ensure continuous monitoring 24 hours a day throughout the sampling period.

We used two camera-trap models (Scout Guard SG520 and Apeman H55) with trigger speed ranging between 0.3 and 0.7 s. Camera traps were set to record 30-second videos with a minimum time delay between consecutive ones. They were mounted on trees and bushes mainly at a height of 50-100 cm (47.2% of deployed camera traps); alternative heights were chosen depending on vegetation and ground conditions (e.g. slope).

All recorded videos were carefully inspected to identify the captured species; consecutive videos regarding the same species were considered as different events only if they were spaced at least 30 minutes apart to ensure capture independence (Kelly and Holub 2008; Monterroso et al. 2014; Torretta et al. 2016). Whenever possible, recorded roe deer were classified by sex and age.

Regarding age classification, three age classes were considered: juveniles (< 1 year old), sub-adults (< 2 years old), and adults (> 2 years old). The date considered for the transition from one age class to the next has been identified as June 1st of each year. To classify the animals, body proportions, including overall size and shape, muscular development, relative head length, chest size, and neck thickness, were considered. Additionally, to estimate the age of females, the shape of the abdomen was observed.

Population structure - The population structure of roe deer in each study area was characterized by selecting a random subsample equal to 50% of the recorded events. This approach aimed to mitigate the issue of double counts, as the same individuals might visit the same trapping site multiple times within a short period.

Population structure was assessed using various metrics:

i) the population pyramid, which visually represents age and sex relative to the number of individuals; significant differences in the number of males and females distributed across different age classes were assessed using the Likelihood Ratio (LR) for contingency tables;

ii) the sex ratio (SR = females/males), which was tested for deviation from the expected sex ratio of 1 using a chi-square goodness-of-fit test;

iii) the age ratio (AR = non-reproductive individuals/reproductive individuals);

iv) the juvenile/doe ratio (J/D ratio = juveniles/adult and sub-adult females), being a synthesis of births and early mortality, this index provides valuable insights into population productivity, independent of early and winter mortality (De Pasquale et al. 2019);

v) the percentage of females of reproductive age (i.e. adult and sub-adult females);

vi) mean litter size, derived from events regarding a female (adult or sub-adult) accompanied by newborns.

Differences between study areas - The existence of any statistically significant differences between the population structures of the roe deer within the study areas was investigated through pairwise comparisons; in particular, the number of males and females in relation to different age classes as well as the sex ratios, the age ratios, and the juvenile/doe ratios were evaluated using the Likelihood Ratio (LR) for contingency tables; the percentages of females in reproductive age were compared by the chi-squared test, and the litter sizes were compared through the Kruskal-Wallis test.

We performed all the analyses using the R software (R Core Team 2023).

4.3 Results

4.3.1 Population density

Sampling effort - A total of 83 sampling sessions were carried out covering a cumulative distance of 621.92 km across the study areas over the two-year period (Table 4.3.1).

Table 4.3.1 - Number of samplings carried out using direct observations along road surveys in north-western Italy from 2020 to 2022

Study area	Total length (km)	2020-2021			2021-2022			Total
		Dawn	Dusk	Night	Dawn	Dusk	Night	
LAR	84.7	2	1	0	7	3	0	13
BNM	69.4	3	0	2	5	2	2	14
MON	72.2	2	1	0	7	3	0	13
ORB	57.3	1	2	0	5	3	0	11
COP	144.5	2	1	0	4	4	0	11
TID	70.4	0	3	0	3	4	0	10
STA	123.5	1	2	0	6	3	0	12

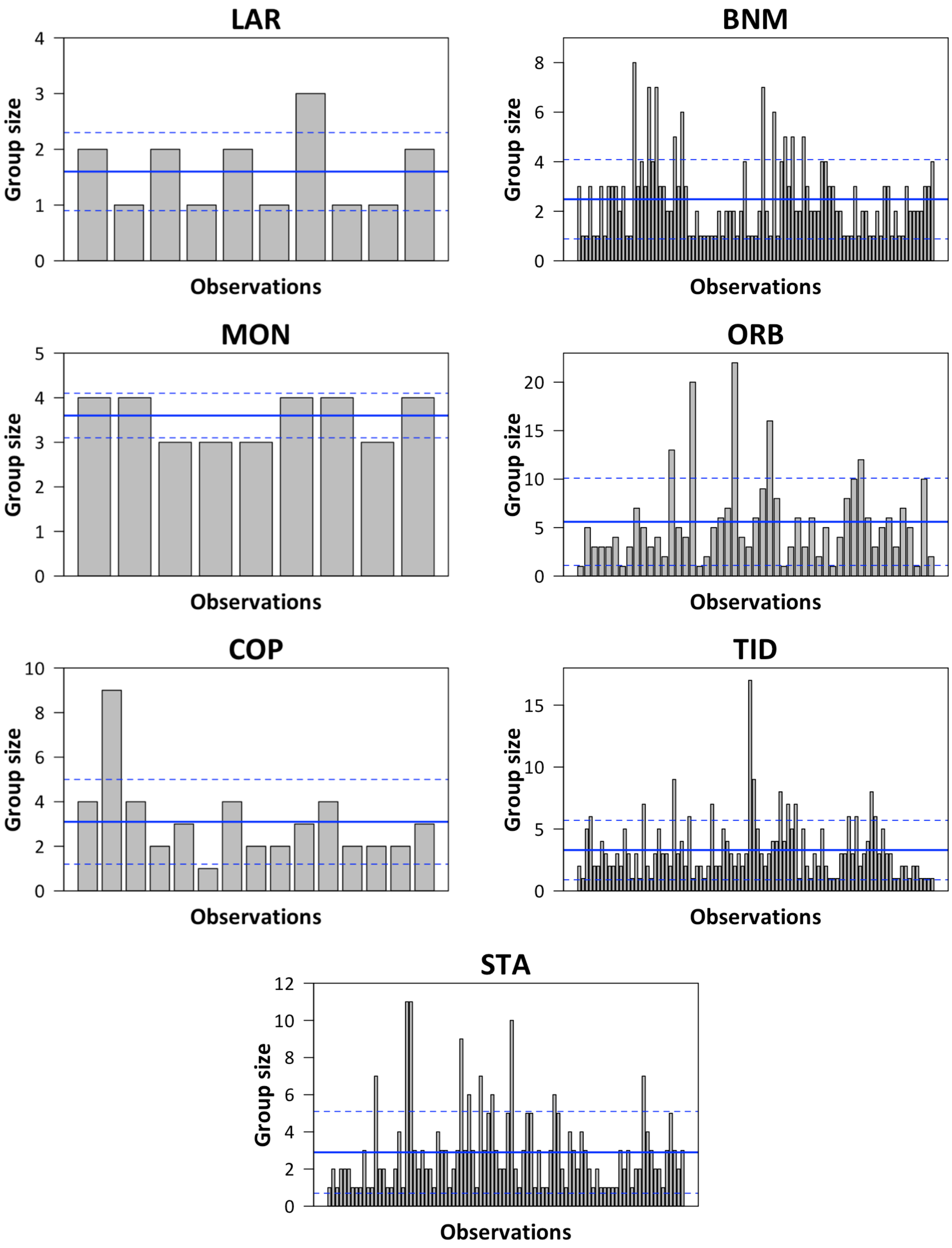
Collected data - Across all study areas and combining data from both sampling seasons, a total of 1200 roe deer, distributed among 368 groups, were observed during road surveys. The number of observed groups and individuals exhibited significant variation across study areas (Table 4.3.2).

Table 4.3.2 - Number of observed groups and individuals and mean (±SD) group size in each study area during the two sampling sessions in north-western Italy

Study area	2020-2021			2021-2022		
	N° of groups	N° of individuals	Mean (± SD) group size	N° of groups	N° of individuals	Mean (± SD) group size
LAR	4	7	1.8 ± 1.0	6	9	1.5 ± 0.5
BNM	34	72	2.1 ± 0.9	63	169	2.7 ± 1.8
MON	0	0	0	9	32	3.6 ± 0.5
ORB	19	99	5.2 ± 3.1	32	185	5.8 ± 5.2
COP	0	0	0	15	47	3.1 ± 1.9
TID	11	15	1.4 ± 0.5	83	298	3.6 ± 2.5
STA	17	48	2.8 ± 1.5	75	219	2.9 ± 2.4

Similarly, the mean (± SD) group size varied between 1.6 ± 0.7 individuals in LAR to 5.6 ± 4.5 individuals in ORB (Figure 4.3.1).

Figure 4.3.1 - Group size of each observation in each study area during the two sampling sessions in north-western Italy; blue lines depict the mean ± SD



Density estimation - The roe deer densities, obtained by relating the number of individuals to the surveyed area, varied significantly across the study areas. Considering the two sampling seasons, it ranged from 2.4 ± 0.2 ind. per km^2 in COP to 26.4 ± 4.6 ind. per km^2 in STA (Table 4.3.3).

Table 4.3.3 - Rough estimate of population density in each study area during the two sampling sessions in north-western Italy

Study area	**Density (mean ± SE)**		
	2020-2021	**2021-2022**	**Total**
LAR	29.7 ± 13.1	6.3 ± 1.2	15.1 ± 6.1
BNM	12.3 ± 7.1	20.5 ± 6.7	17.8 ± 5.0
MON	-	16.4 ± 10.8	16.4 ± 10.8
ORB	19.0 ± 9.7	11.5 ± 2.9	13.4 ± 3.2
COP	-	2.4 ± 0.2	2.4 ± 0.2
TID	9.0 ± 4.2	27.0 ± 4.7	24.2 ± 4.4
STA	22.3 ± 5.4	27.7 ± 5.8	26.4 ± 4.6

For the density estimation using the distance sampling method, analyses were restricted to the study areas with more than 40 observations. Consequently, the study areas LAR, MON, and COP were excluded, as they had only 10, 9, and 15 observations, respectively, over the two sampling seasons (Table 4.3.2).

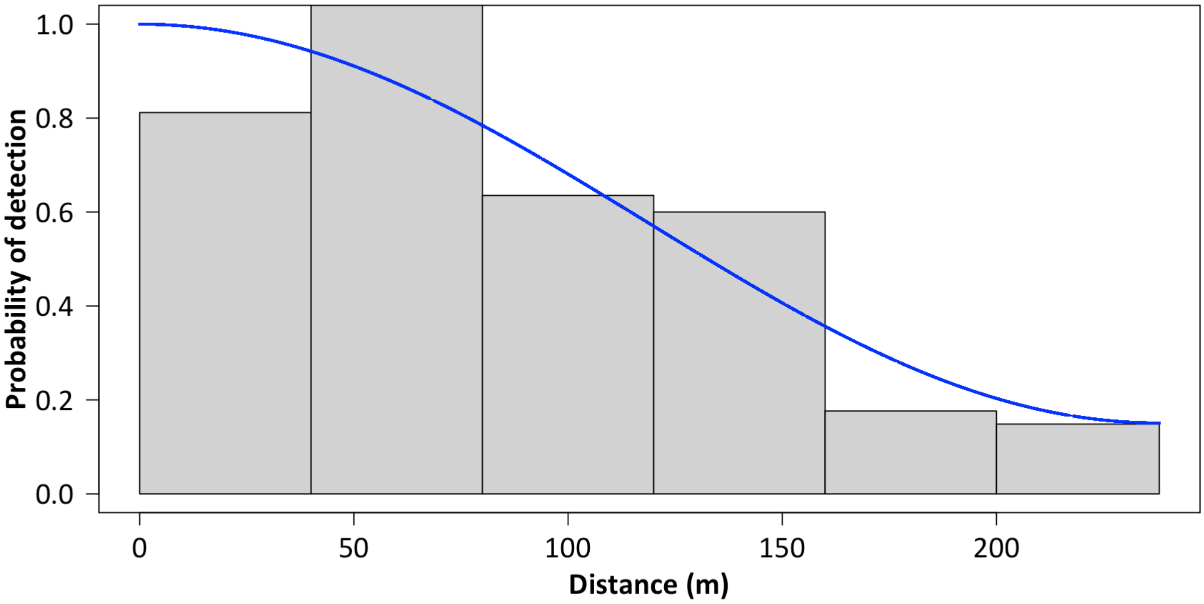
In BNM study area, a total of 97 roe deer groups were counted. Perpendicular distances between the observer and the observed individuals ranged from 0 to 238 m. Among the obtained models (Table 4.3.4), the best one for calculating the detectability function was identified as the uniform model with cosine adjustments (Figure 4.3.2).

Table 4.3.4 - Models obtained through distance sampling for roe deer density estimation in BNM study area. For each model, the coefficient of variation (CV), the probability of detection (Pa ± SE), and the results of the Cramer von-Mises goodness-of-fit test are reported

Key function and adjustments	**CV (%)**	**Pa ± ES**	**χ^2**	**P**
Half-normal key with cosine adjustments	8.5	0.58 ± 0.05	0.07	0.748
Hazard-rate key with simple polynomial adjustments	9.5	0.64 ± 0.06	0.04	0.922
Uniform key with cosine adjustments	**6.1**	**0.58 ± 0.04**	**0.08**	**0.702**
Half-normal key with Hermite polynomial adjustments	8.5	0.58 ± 0.05	0.07	0.748

The estimated density was 11.5 ± 1.2 (SE) ind. per km^2 (95% CI = 9.2 - 14.3 ind. per km^2). The detection probability was estimated at 0.58 ± 0.04, and the Effective Strip Width (ESW) at 136.9 m. The model fit was good (χ^2 = 0.08, P = 0.702).

Figure 4.3.2 - Detectability function of the best model utilized for estimating roe deer density in BNM study area



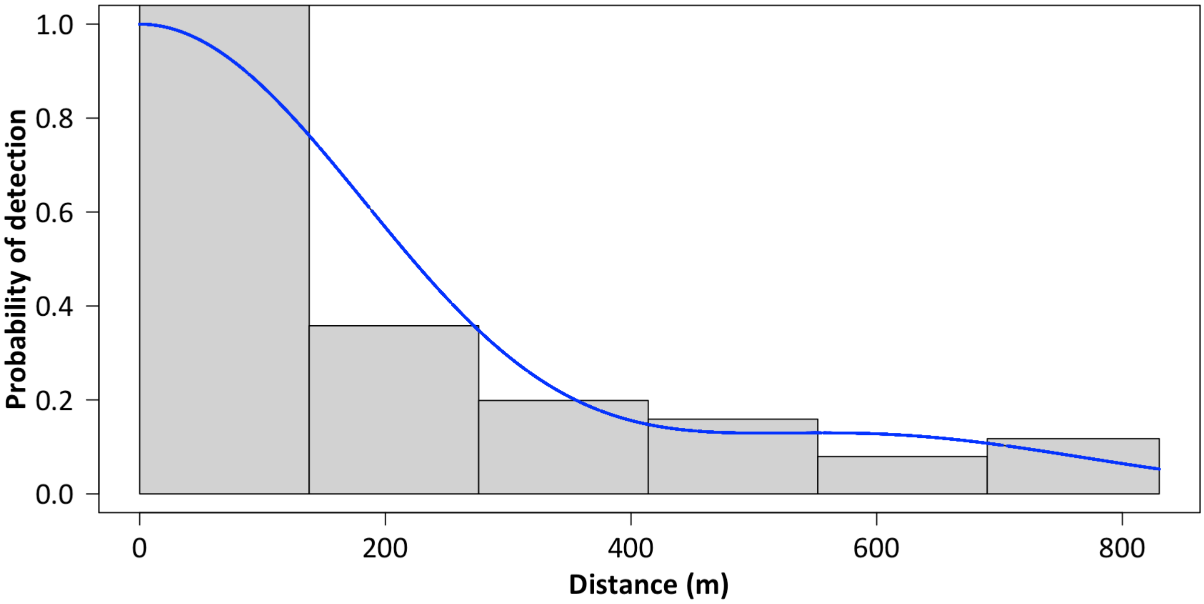
In ORB study area, a total of 51 roe deer groups were counted. Perpendicular distances between the observer and the observed individuals ranged from 0 to 830 m. Among the obtained models (Table 4.3.5), the best one for calculating the detectability function was identified as the half-normal model with cosine adjustments (Figure 4.3.3).

Table 4.3.5 - Models obtained through distance sampling for roe deer density estimation in ORB study area. For each model, the coefficient of variation (CV), the probability of detection (Pa ± SE), and the results of the Cramer von-Mises goodness-of-fit test are reported

Key function and adjustments	CV (%)	Pa ± ES	χ^2	P
Half-normal key with cosine adjustments	**12.4**	**0.34 ± 0.04**	**0.12**	**0.513**
Hazard-rate key with simple polynomial adjustments	23.0	0.31 ± 0.07	0.08	0.679
Uniform key with cosine adjustments	13.2	0.36 ± 0.05	0.15	0.386
Half-normal key with Hermite polynomial adjustments	7.6	0.47 ± 0.03	0.58	0.025

The estimated density was 7.3 ± 1.1 (SE) ind. per km^2 (95% CI = 5.4 - 9.9 ind. per km^2). The detection probability was estimated at 0.34 ± 0.04, and the Effective Strip Width (ESW) at 279.9 m. The model fit was good (χ^2 = 0.12, P = 0.513).

Figure 4.3.3 - Detectability function of the best model utilized for estimating roe deer density in ORB study area



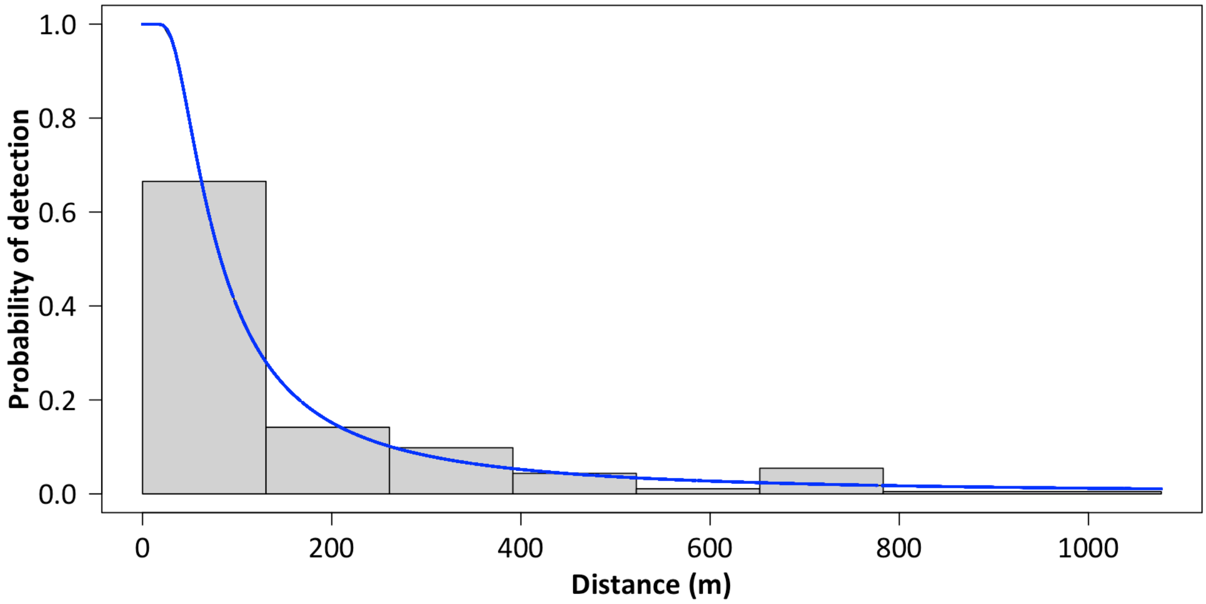
In TID study area, a total of 93 roe deer groups were counted. Perpendicular distances between the observer and the observed individuals ranged from 0 to 783 m. Among the obtained models (Table 4.3.6), the best one for calculating the detectability function was identified as the hazard-rate model with simple polynomial adjustments (Figure 4.3.4).

Table 4.3.6 - Models obtained through distance sampling for roe deer density estimation in TID study area. For each model, the coefficient of variation (CV), the probability of detection (Pa ± SE), and the results of the Cramer von-Mises goodness-of-fit test are reported

Key function and adjustments	CV (%)	Pa ± ES	χ^2	P
Half-normal key with cosine adjustments	7.8	0.29 ± 0.02	1.10	0.001
Hazard-rate key with simple polynomial adjustments	**19.6**	**0.17 ± 0.03**	**0.07**	**0.777**
Uniform key with cosine adjustments	10.5	0.25 ± 0.03	0.61	0.022
Half-normal key with Hermite polynomial adjustments	4.7	0.40 ± 0.02	2.86	< 0.0001

The estimated density was 12.5 ± 3.0 (SE) ind. per km^2 (95% CI = 7.8 - 20.1 ind. per km^2). The detection probability was estimated at 0.17 ± 0.03, and the Effective Strip Width (ESW) at 131.8 m. The model fit was good (χ^2 = 0.07, P = 0.777).

Figure 4.3.4 - Detectability function of the best model utilized for estimating roe deer density in TID study area



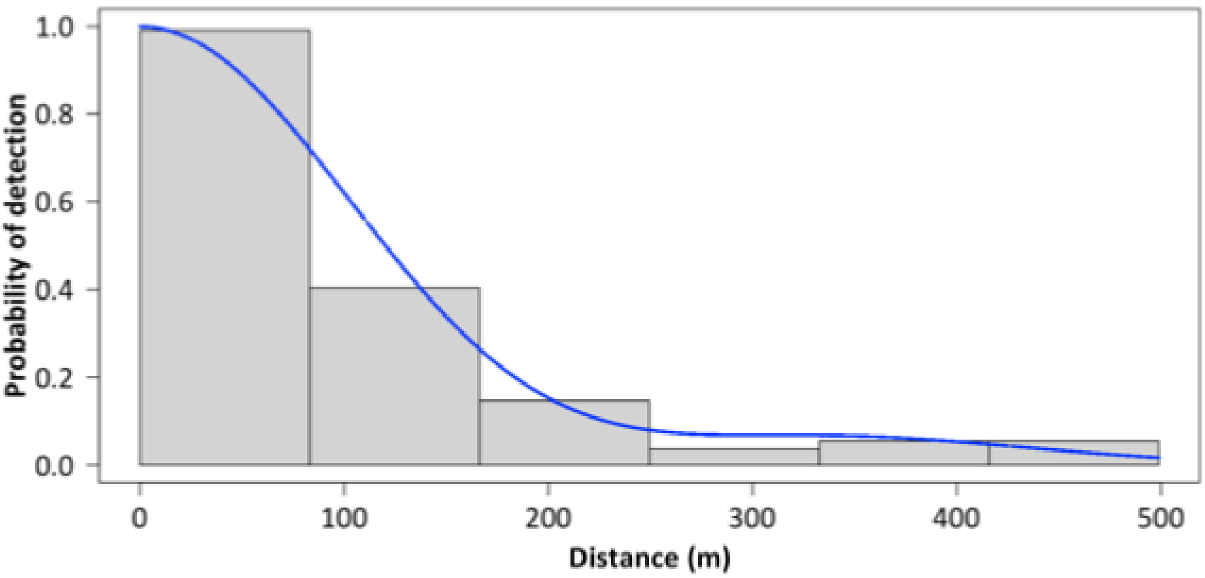
Lastly, in STA study area, a total of 92 roe deer groups were counted. Perpendicular distances between the observer and the observed individuals ranged from 0 to 499 m. Among the obtained models (Table 4.3.7), the best one for calculating the detectability function was identified as the half-normal model with cosine adjustments (Figure 4.3.5).

Table 4.3.7 - Models obtained through distance sampling for roe deer density estimation in STA study area. For each model, the coefficient of variation (CV), the probability of detection (Pa ± SE), and the results of the Cramer von-Mises goodness-of-fit test are reported

Key function and adjustments	CV (%)	Pa ± ES	χ^2	P
Half-normal key with cosine adjustments	**7.8**	**0.28 ± 0.02**	**0.09**	**0.649**
Hazard-rate key with simple polynomial adjustments	13.6	0.27 ± 0.04	0.04	0.918
Uniform key with cosine adjustments	10.1	0.28 ± 0.03	0.09	0.650
Half-normal key with Hermite polynomial adjustments	4.9	0.37 ± 0.02	0.92	0.004

The estimated density was 2.4 ± 0.04 (SE) ind. per km^2 (95% CI = 1.6 - 3.6 ind. per km^2). The detection probability was estimated at 0.28 ± 0.02, and the Effective Strip Width (ESW) at 140.5 m. The model fit was good (χ^2 = 0.09, P = 0.649).

Figure 4.3.5 - Detectability function of the best model utilized for estimating roe deer density in STA study area



4.3.2 Population structure

Sampling effort - In each study area, eight seasonal camera trapping sessions were carried out during the study period.

Due to thefts and/or malfunctions of the camera traps, some camera trapping sessions had durations different from the intended 120 days (Table 4.3.8).

The mean distance (± SD) between camera traps during a sampling session was 1098.2 ± 194.7 m (Table 4.3.9). Thus, it is reasonable to assume that roe deer recorded at different camera trap sites corresponded to distinct individuals. Notably, roe deer exhibiting unique identifying features (e.g. males with a single antler or with abnormal antlers, females with antlers, and individuals with injuries or disabilities) were consistently observed at singular camera trap sites, reinforcing this assumption.

Table 4.3.8 - The sampling effort of camera trapping expressed as mean (± SD) duration (on the first line) and total trapping days (on the second line) during the seasonal sessions in north-western Italy from 2020 to 2022

Study area	Sampling seasons							
	2020-2021				2021-2022			
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
LAR	16.4 ± 1.6 114.5	11.8 ± 6.1 107	14.8 ± 0.4 133	11.3 ± 2.8 113	19.6 ± 0.4 176	11.8 ± 4.6 94	15.0 ± 0.0 105	17.4 ± 0.5 157
BNM	13.0 ± 0.0 156	8.1 ± 2.3 97	14.0 ± 0.0 140	11.6 ± 1.7 105	16.3 ± 1.1 147	11.7 ± 3.9 105	13.9 ± 0.2 126	16.0 ± 0.0 144
MON	18.4 ± 3.3 129	13.2 ± 4.3 132	13.0 ± 0.0 117	12.9 ± 2.5 116	14.1 ± 0.3 127	9.4 ± 5.5 76	15.1 ± 1.7 136	21.0 ± 0.0 189
ORB	16.0 ± 5.8 128	9.2 ± 4.3 74	14.1 ± 3.8 113	13.9 ± 2.5 111	12.3 ± 3.0 99	12.5 ± 4.3 100	10.5 ± 4.4 84	16.0 ± 0.0 128
COP	14.3 ± 1.4 114	11.8 ± 4.2 95	12.6 ± 3.7 113	15.3 ± 3.8 138	8.3 ± 5.6 66	15.2 ± 1.9 107	15.0 ± 0.0 120	15.0 ± 0.0 120
TID	8.8 ± 3.7 79	13.0 ± 0.2 130	14.9 ± 0.2 135	14.8 ± 0.2 134	13.6 ± 4.1 122	13.3 ± 1.6 120	16.0 ± 0.0 144	15.9 ± 0.2 144
STA	13.4 ± 2.1 107	15.3 ± 0.3 122	9.9 ± 5.4 79	19.1 ± 0.3 153	13.4 ± 1.7 107	16.0 ± 7.7 128	15.3 ± 1.0 122	17.4 ± 0.7 139

Table 4.3.9 - Mean distance (± SD) between camera-trap sites during each seasonal session carried out in north-western Italy from 2020 to 2022

Study area	Sampling seasons							
	2020-2021				2021-2022			
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
LAR	1099.0 ± 438.9	760.5 ± 503.3	1009.6 ± 221.0	684.9 ± 433.0	1164.0 ± 417.5	1390.9 ± 233.9	1244.8 ± 276.7	969.2 ± 435.3
BNM	962.5 ± 244.9	1085.5 ± 286.9	1122.5 ± 226.2	867.0 ± 245.3	1138.4 ± 274.3	1419.0 ± 366.0	811.5 ± 267.0	817.1 ± 263.9
MON	1468.9 ± 446.4	1184.9 ± 331.7	936.2 ± 347.3	1379.8 ± 584.4	1103.1 ± 531.2	1122.9 ± 191.6	1025.3 ± 430.8	973.7 ± 600.7
ORB	882.8 ± 426.3	1295.6 ± 317.6	1189.1 ± 457.5	1030.4 ± 436.8	1370.0 ± 251.7	1351.9 ± 224.1	1160.7 ± 636.0	1123.0 ± 466.6
COP	719.1 ± 418.8	861.0 ± 337.9	1164.4 ± 315.1	922.5 ± 273.3	838.7 ± 358.6	1157.6 ± 62.7	1354.7 ± 432.7	918.5 ± 360.8
TID	1025.2 ± 172.1	1105.8 ± 254.8	1138.1 ± 385.6	1174.8 ± 420.8	906.5 ± 510.2	1083.3 ± 198.9	1244.9 ± 344.5	1214.9 ± 509.7
STA	1092.5 ± 750.8	961.8 ± 229.1	1568.8 ± 208.0	1205.2 ± 254.3	1008.3 ± 94.0	1247.5 ± 75.2	1140.5 ± 459.5	1299.1 ± 379.3

Collected data - Across all study areas and combining data from all eight sampling seasons, a total of 2749 roe deer events were recorded (Table 4.3.10).

Table 4.3.10 - Number of roe deer events recorded in the study areas located in north-western Italy during the seasonal sampling sessions carried out from 2020 to 2022

Study area	Sampling seasons								Total
	2020-2021				2021-2022				
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	
LAR	71	33	46	18	39	57	46	59	369
BNM	81	87	152	43	109	90	93	78	734
MON	22	32	22	10	34	31	45	35	231
ORB	46	46	34	78	35	29	35	46	349
COP	24	41	48	51	26	59	48	16	313
TID	23	49	33	39	31	82	145	19	421
STA	11	47	32	25	27	45	106	40	327

Population structure - The population structure of roe deer in each study area was assessed by randomly selecting a subsample comprising 50% of the recorded events. This equated to 185 events in LAR, 367 in BNM, 116 in MON, 175 in ORB, 166 in COP, 211 in TID, and 167 in STA.

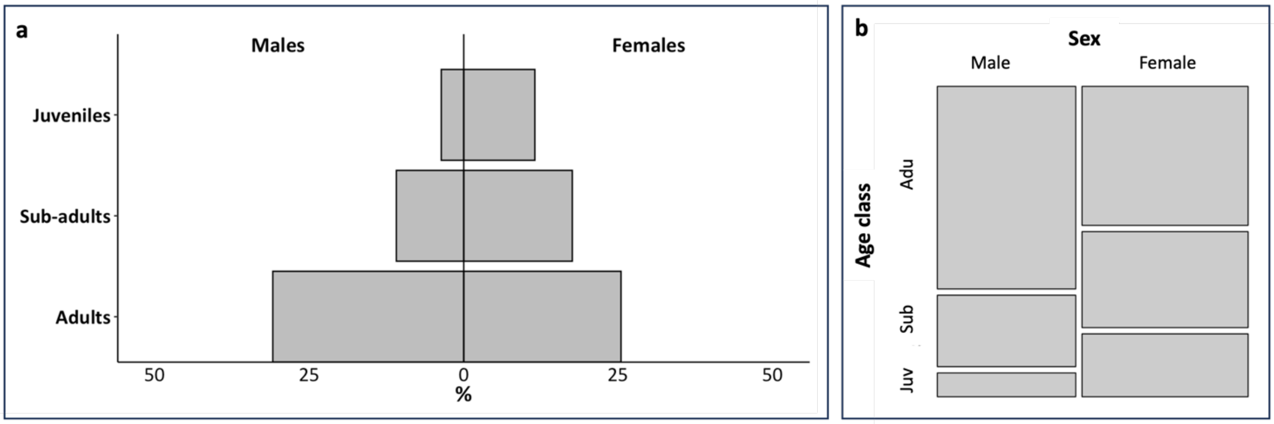
In LAR study area, the population structure of roe deer was characterized based on the observation of 196 individuals identified by sex, age, or both (Table 4.3.11)

Table 4.3.11 - Number of roe deer identified by age and/or sex in LAR study area located in north-western Italy

	Adults	Sub-adults	Juveniles	ND	Total
Males	51	18	6	12	87
Females	42	29	19	9	99
ND	0	0	10	29	39
Total	93	47	35	50	225

Considering the individuals for which it was possible to recognize both the age class and the sex (n = 165), the most represented age class was that of adults (m = 30.9%; f = 25.5%), followed by sub-adults (m = 10.9%; f = 17.6%) and juveniles (m = 3.6%; f = 11.5%) for both sexes (Figure 4.3.6 a). The distribution of the two sexes within the three age classes was found to be significantly different (LR = 9.2; df = 2; P = 0.010) (Figure 4.3.6 b).

Figure 4.3.6 - Roe deer (a) population pyramid and (b) proportional distribution of the two sexes within the three age classes in LAR study area in north-western Italy from 2020 to 2022



No significant deviation from the expected sex ratio of 1 was found, with a sex ratio (SR) of 1.14. The age ratio (AR) was calculated to be 0.52. Moreover, the percentage of females of reproductive age stood at 79.0%, and the juvenile/doe ratio (J/D ratio) was 0.49. The average litter size was 1.19 ± 0.37.

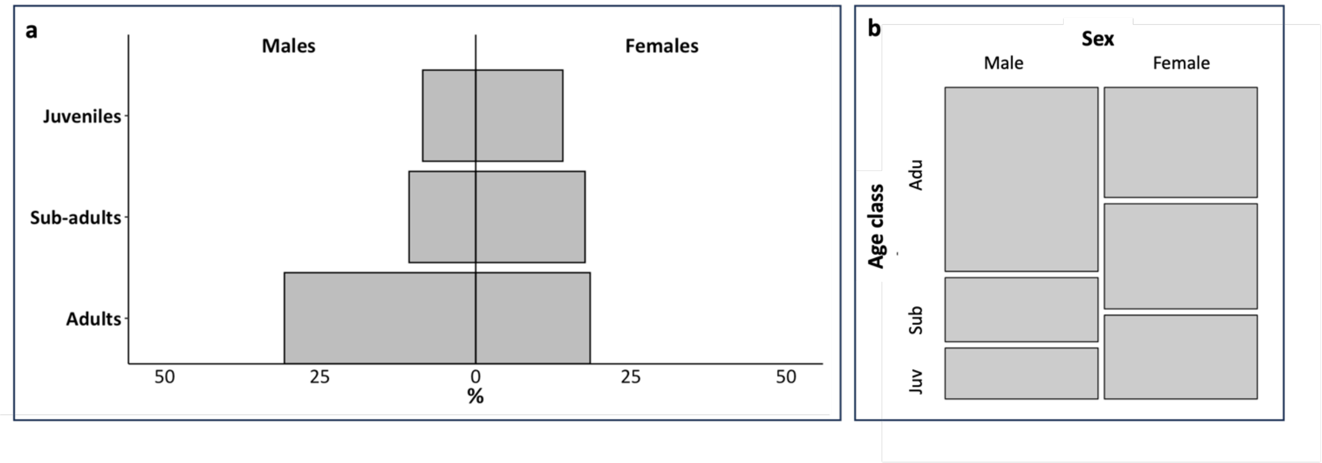
In BNM study area, the population structure of roe deer was characterized based on the observation of 448 individuals identified by sex, age, or both (Table 4.3.12)

Table 4.3.12 - Number of roe deer identified by age and/or sex in BNM study area located in north-western Italy

	Adults	**Sub-adults**	**Juveniles**	**ND**	**Total**
Males	112	39	31	12	194
Females	67	64	51	32	214
ND	1	1	38	59	99
Total	180	104	120	103	507

Among individuals where both age class and sex have been identified (n = 364), adults were markedly the most represented class within males, followed by sub-adults and juveniles (adu = 30.8%; sub = 10.7%; juv = 8.5%), while females exhibited a more balanced distribution (adu = 18.4%, sub = 17.6%; juv = 14.0%) (Figure 4.3.7 a). Indeed, the distribution of the two sexes within the three age classes was found to be significantly different (LR = 22.5; df = 2; P < 0.0001) (Figure 4.3.7 b).

Figure 4.3.7 - Roe deer (a) population pyramid and (b) proportional distribution of the two sexes within the three age classes in BNM study area in north-western Italy from 2020 to 2022



No significant deviation from the expected sex ratio of 1 was found, with a sex ratio (SR) of 1.10. The age ratio (AR) was calculated to be 0.65. Moreover, the percentage of females of reproductive age stood at 72.0%, and the juvenile/doe ratio (J/D ratio) was 0.92. The average litter size was 1.27 ± 0.20.

In MON study area, the population structure of roe deer was delineated through the observation of 156 individuals identified by sex, age, or both (Table 4.3.13)

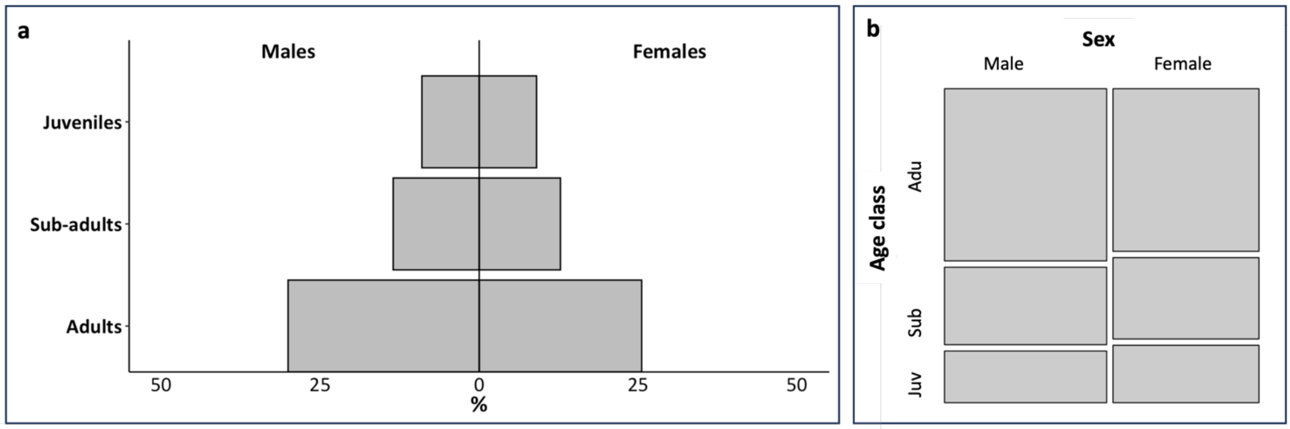
Table 4.3.13 - Number of roe deer identified by age and/or sex in MON study area located in north-western Italy

	Adults	Sub-adults	Juveniles	ND	Total
Males	40	18	12	4	74
Females	34	17	12	7	70
ND	0	1	11	15	27
Total	74	36	35	26	171

Taking into account individuals for which both age class and sex were identifiable (n = 133), adults constituted the majority (male: 30.1%, female: 25.6%), followed by subadults (male: 13.5%, female: 12.8%) and juveniles (male: 9.0%, female: 9.0%) for both genders (Figure 4.3.8 a).

No significant difference was found in the distribution of the two sexes across the three age classes (Figure 4.3.8 b).

Figure 4.3.8 - Roe deer (a) population pyramid and (b) proportional distribution of the two sexes within the three age classes in MON study area in north-western Italy from 2020 to 2022



No significant deviation from the expected sex ratio of 1 was found, with a sex ratio (SR) of 0.95. The age ratio (AR) was calculated to be 0.58. Moreover, the percentage of females of reproductive age stood at 80.9%, and the juvenile/doe ratio (J/D ratio) was 0.69. The average litter size was 1.53 ± 0.37.

In ORB study area, the population structure of roe deer was characterized based on the observation of 247 individuals identified by sex, age, or both (Table 4.3.14)

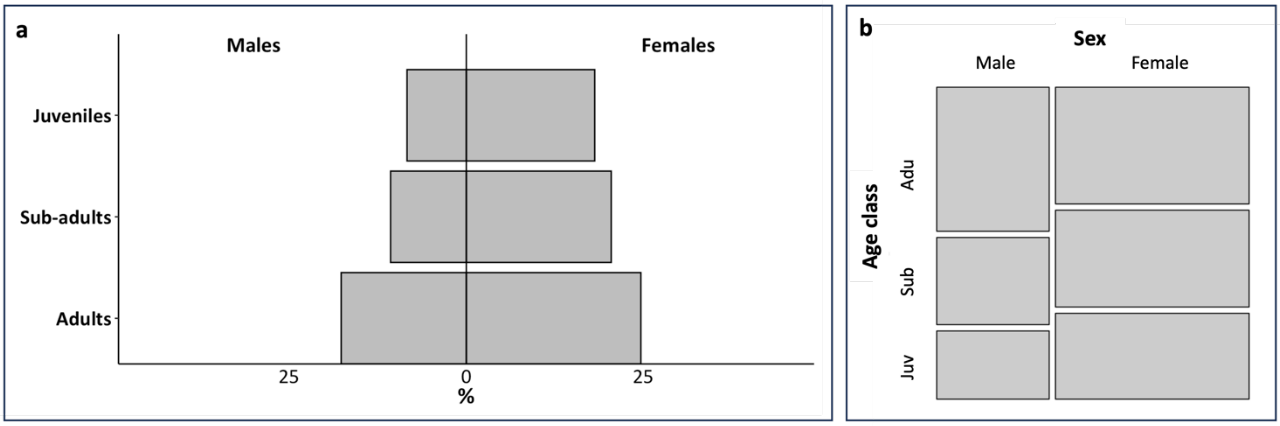
Table 4.3.14 - Number of roe deer identified by age and/or sex in ORB study area located in north-western Italy

	Adults	**Sub-adults**	**Juveniles**	**ND**	**Total**
Males	38	23	18	4	83
Females	53	44	39	17	153
ND	0	0	11	28	39
Total	91	67	68	49	275

Among individuals where both age class and sex were identifiable (n = 215), adults constituted the majority (male: 17.7%, female: 24.7%), followed by subadults (male: 10.7%, female: 20.5%) and juveniles (male: 8.4%, female: 18.1%) for both genders (Figure 4.3.9 a).

No significant difference was found in the distribution of the two sexes across the three age classes (Figure 4.3.9 b).

Figure 4.3.9 - Roe deer (a) population pyramid and (b) proportional distribution of the two sexes within the three age classes in ORB study area in north-western Italy from 2020 to 2022



Even though females were found to be more abundant within each age class, the sex ratio (SR) was 1.84, suggesting no significant deviation from the expected sex ratio of 1. The age ratio (AR) was 0.67. Furthermore, the percentage of females of reproductive age was recorded at 71.3%, and the juvenile/doe ratio (J/D ratio) stood at 0.70. The average litter size was 1.22 ± 0.17.

In COP study area, the population structure of roe deer was determined through the identification of 196 individuals by sex, age, or both among the recorded ones (Table 4.3.15)

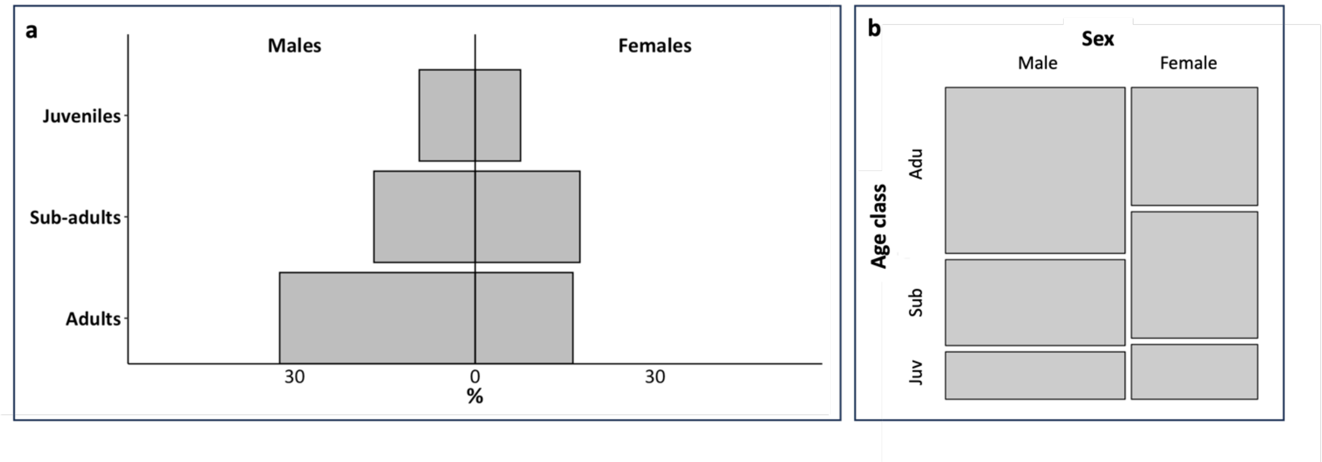
Table 4.3.15 - Number of roe deer identified by age and/or sex in COP study area located in north-western Italy

	Adults	**Sub-adults**	**Juveniles**	**ND**	**Total**
Males	56	29	16	5	106
Females	28	30	13	15	86
ND	0	0	4	20	24
Total	84	59	33	40	216

The individuals for which it was possible to recognize both age class and sex were 172. Considering females, the most represented age class was that of subadults (17.4%), followed by adults (16.3%) and juveniles (7.6%). Conversely, among males, adults were the most represented age class (32.6%), followed by subadults (16.9%), and finally juveniles (9.3%) (Figure 4.3.10 a).

No significant difference was found in the distribution of the two sexes across the three age classes (Figure 4.3.10 b).

Figure 4.3.10 - Roe deer (a) population pyramid and (b) proportional distribution of the two sexes within the three age classes in COP study area in north-western Italy from 2020 to 2022



No significant deviation from the expected sex ratio of 1 was found, with a sex ratio (SR) of 0.81. The age ratio (AR) was calculated to be 0.54. Moreover, the percentage of females of reproductive age stood at 81.7%, and the juvenile/doe ratio (J/D ratio) was 0.57. The average litter size was 1.00 ± 0.00.

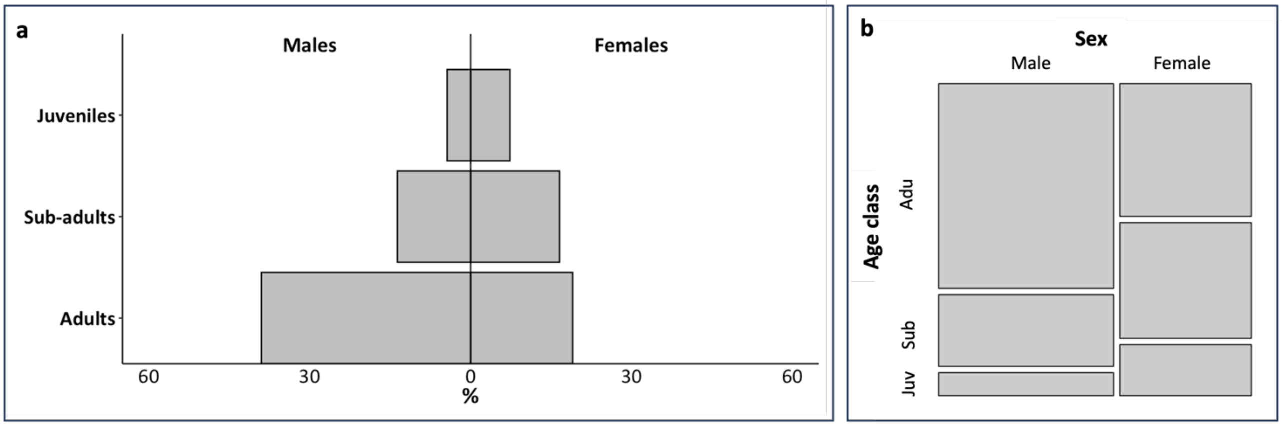
In TID study area, the population structure of roe deer was determined through the identification of 244 individuals by sex, age, or both among the recorded ones (Table 4.3.16)

Table 4.3.16 - Number of roe deer identified by age and/or sex in TID study area located in north-western Italy

	Adults	**Sub-adults**	**Juveniles**	**ND**	**Total**
Males	80	28	9	13	130
Females	39	34	15	8	96
ND	1	0	17	32	50
Total	120	62	41	53	276

205 individuals were identified by both age class and sex. Adults represented the largest portion in both sexes (male: 39.0%, female: 19.0%), followed by subadults (male: 13.7%, female: 16.5%) and juveniles (male: 4.4%, female: 7.3%) (Figure 4.3.11 a). The distribution of the two sexes within the three age classes was found to be significantly different (LR = 12.4; df = 2; P = 0.002) (Figure 4.3.11 b).

Figure 4.3.11 - Roe deer (a) population pyramid and (b) proportional distribution of the two sexes within the three age classes in TID study area in north-western Italy from 2020 to 2022



No significant deviation from the expected sex ratio of 1 was found, with a sex ratio (SR) of 0.74. The age ratio (AR) was calculated to be 0.45. Moreover, the percentage of females of reproductive age stood at 83.0%, and the juvenile/doe ratio (J/D ratio) was 0.56. The average litter size was 1.25 ± 0.21.

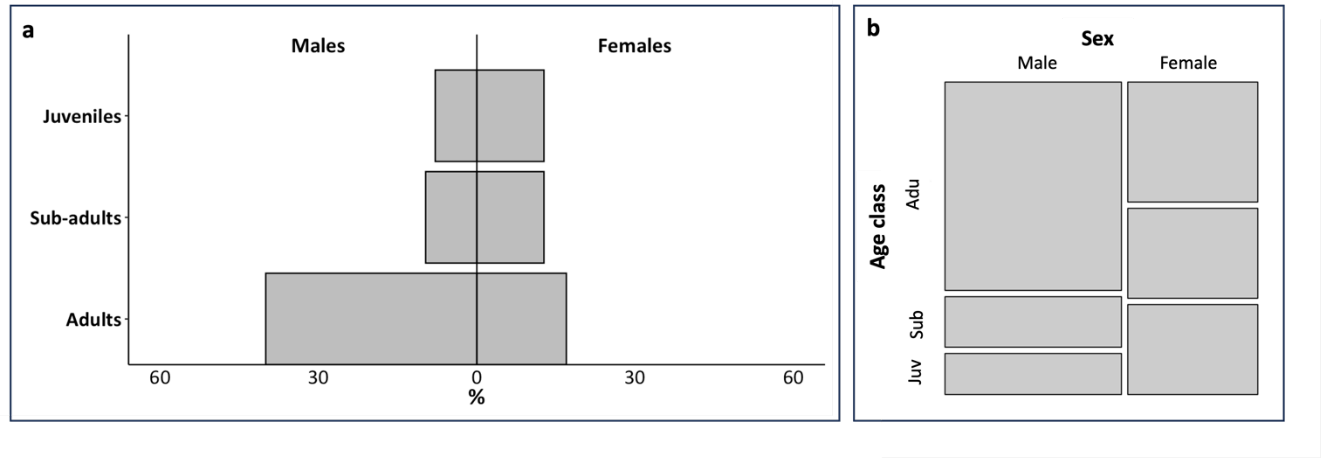
In STA study area, the population structure of roe deer was determined through the identification of 181 individuals by sex, age, or both among the recorded ones (Table 4.3.17)

Table 4.3.17 - Number of roe deer identified by age and/or sex in STA study area located in north-western Italy

	Adults	Sub-adults	Juveniles	ND	Total
Males	66	16	13	5	100
Females	28	21	21	9	79
ND	0	0	2	38	40
Total	94	37	36	52	219

Considering the individuals for which it was possible to recognize both the age class and the sex (n = 165), the most represented age class was that of adults (m = 40.0%; f = 17.0%), followed by sub-adults (m = 9.7%; f = 12.7%) and juveniles (m = 7.8%; f = 12.7%) for both sexes (Figure 4.3.12 a). The distribution of the two sexes within the three age classes was found to be significantly different (LR = 14.5; df = 2; P < 0.001) (Figure 4.3.12 b).

Figure 4.3.12 - Roe deer (a) population pyramid and (b) proportional distribution of the two sexes within the three age classes in STA study area in north-western Italy from 2020 to 2022



No significant deviation from the expected sex ratio of 1 was observed, with a sex ratio (SR) of 0.79. The age ratio (AR) was determined to be 0.45. Additionally, the percentage of females of reproductive age was 70.0%, and the juvenile/doe ratio (J/D ratio) stood at 0.73. The average litter size was 1.07 ± 0.09.

Differences between study areas - Population structures varied significantly across the study areas, with all pairwise comparisons yielding statistically significant differences, except for LAR vs. MON and MON vs. COP. Considering the sex ratio (SR), the study area ORB resulted different from all the others and the study area TID resulted different from the study areas LAR and BNM. Similarly, the age ratio (AR) differed between ORB vs. LAR, ORB vs. TID, BNM vs. TID, and BNM vs. LAR. Lastly, the juvenile/doe ratio (J/D ratio) exhibited a significant difference only for the LAR vs. BNM pair (Table 4.3.18).

The percentages of females of reproductive age varied significantly among the study areas (χ^2 = 68.38; df = 6; P < 0.0001) (Figure 4.3.13).

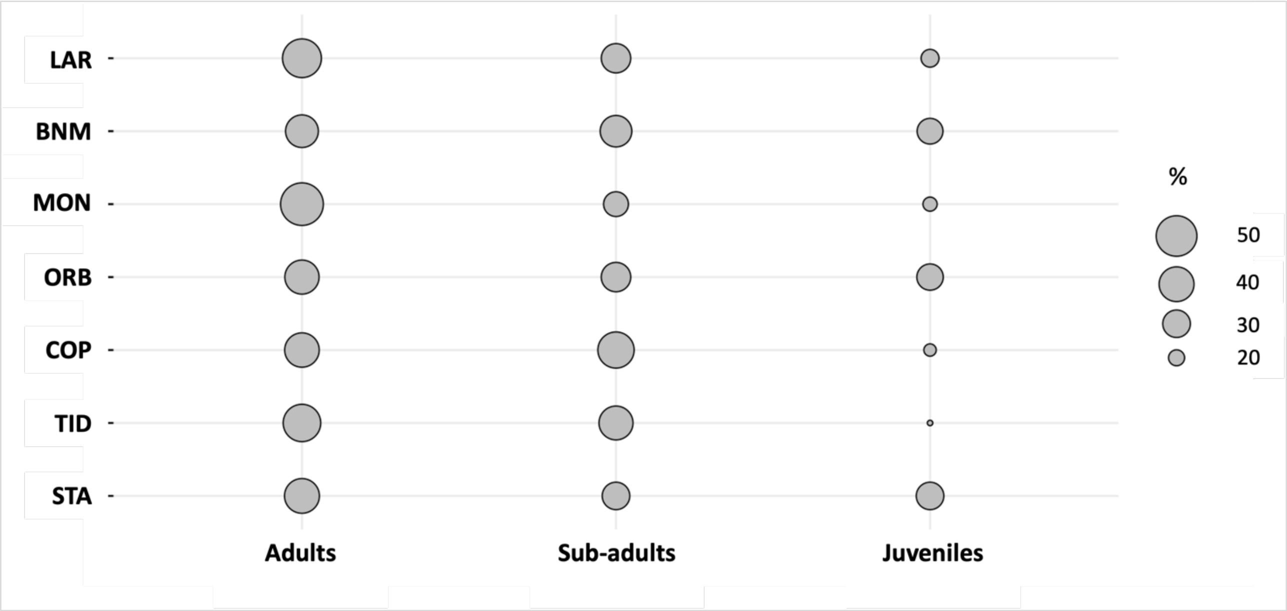
The litter sizes, conversely, were similar among the study areas.

Table 4.3.18 - Pairwise comparisons between the study areas located in north-western Italy regarding the metrics describing roe deer population structure

Study areas	**Population structure**			**Sex Ratio**			**Age Ratio**			**Juvenile/Doe Ratio**		
	LR	**df**	**P**	**LR**	**df**	**P**	**LR**	**df**	**P**	**LR**	**df**	**P**
LAR - BNM	36.99	7	< 0.0001	0.03	1	0.860	4.49	1	0.034	6.75	1	0.009
LAR - MON	11.38	7	0.123	0.69	1	0.405	1.51	1	0.219	1.21	1	0.272
LAR - ORB	23.73	7	0.001	5.82	1	0.016	4.30	1	0.038	1.85	1	0.173

LAR - COP	21.72	7	0.003	2.69	1	0.101	0.97	1	0.323	0.23	1	0.633
LAR - TID	27.52	7	< 0.001	4.73	1	0.030	0.02	1	0.887	0.93	1	0.334
LAR - STA	31.24	7	< 0.0001	3.02	1	0.082	0.03	1	0.864	1.76	1	0.185
BNM - MON	24.78	7	< 0.001	0.63	1	0.428	0.31	1	0.575	1.31	1	0.252
BNM - ORB	36.61	7	< 0.0001	9.44	1	0.002	0.04	1	0.841	1.75	1	0.185
BNM - COP	33.90	7	< 0.0001	3.07	1	0.080	0.93	1	0.334	3.64	1	0.056
BNM - TID	48.51	7	< 0.0001	5.81	1	0.016	4.55	1	0.033	2.10	1	0.147
BNM - STA	42.73	7	< 0.0001	3.45	1	0.063	3.56	1	0.059	0.76	1	0.383
MON - ORB	16.71	7	0.019	9.67	1	0.002	0.01	1	0.967	0.01	1	0.937
MON - COP	8.16	7	0.319	0.48	1	0.487	0.09	1	0.770	0.37	1	0.544
MON - TID	15.94	7	0.026	1.33	1	0.248	1.35	1	0.245	0.03	1	0.858
MON - STA	16.19	7	0.023	0.64	1	0.422	1.11	1	0.292	0.05	1	0.826
ORB - COP	30.33	7	< 0.0001	17.31	1	< 0.0001	1.07	1	0.301	0.60	1	0.437
ORB - TID	49.29	7	< 0.0001	23.40	1	< 0.0001	4.26	1	0.039	0.08	1	0.771
ORB - STA	40.90	7	< 0.0001	17.74	1	< 0.0001	3.48	1	0.062	0.03	1	0.862
COP - TID	20.81	7	0.004	0.22	1	0.634	0.82	1	0.366	0.21	1	0.650
COP - STA	25.11	7	< 0.001	0.02	1	0.899	0.65	1	0.421	0.69	1	0.408
TID - STA	33.72	7	< 0.0001	0.11	1	0.738	0.01	1	0.967	0.17	1	0.684

Figure 4.3.13 - The percentages of females within the three age classes in each study area in north-western Italy from 2020 to 2022



4.4 Discussion

Monitoring large herbivores is often challenging and their densities are often underestimated if monitoring methods are not selected and executed with precision and accuracy (Hinojo et al. 2022). Our results regarding the estimation of population densities of the roe deer across the different study areas in north-western Italy unveiled some constraints that could be addressed in future research. The low number of observations gathered in three out of the seven study areas, despite consistent sampling efforts, underscored the potential limitations inherent in the employed data collection method.

Direct observations along road surveys are among the most reliable and commonly used sampling methods for studying populations of wild ungulates (Meriggi et al. 2009; Herrero et al. 2013; ENETWILD consortium et al. 2020), while, regarding the data analysis, distance sampling is indicated as the most suitable and reliable method for providing precise estimates of density (Smart et al. 2004; Focardi et al. 2005). However, to obtain reliable and precise estimates, some assumptions regarding the data collection must be respected (Buckland et al. 2015). Among the factors that can influence the chances of detecting individuals, and thus obtaining precise density estimates, there are observer-related limitations (e.g. equipment and ability), weather conditions (e.g. rain, fog, and wind), and environmental characteristics (e.g. vegetation cover and slope or roughness of terrain) (ENETWILD consortium et al. 2020; Hinojo et al. 2022).

The low number of observations recorded in LAR study area could indeed be linked to landscape characteristics, such as the steep slopes alongside the roads, which were used as routes for data collection. Moreover, the extensive and dense wooded areas along these slopes likely hindered species detection during the conducted surveys. Additionally, the limited availability of paved and unpaved roads suitable for monitoring and accessible by vehicle further restricted the surveyed area during the sampling.

The problem was different for the other two study areas, which exhibit marked landscape differences compared to this one.

COP study area is located within a hilly environmental context, predominantly characterized by vineyard cultivations. The configuration of the area, characterized by vineyard rows along the hillsides, may have limited the detection of roe deer. The landscape configuration posed no issues in MON study area, where extensive agricultural fields stretch across a flat terrain. Similar to the previous area, wooded patches, and consequently, open areas adjacent to the woods, are scarce and seldom intersected by roads, rendering them inaccessible. As a result, the open areas most likely frequented by roe deer were located in areas that were difficult to reach during the sampling conducted through direct observations along road surveys.

Lastly, the issue in these three study areas could also be the low population densities of roe deer themselves; indeed, the rough density estimate obtained, particularly for COP study area, could suggest this as the most probable explanation.

Regarding the four study areas where it was possible to estimate population density using the distance sampling method, TID study area showed the highest density estimate; the presence of extensive open areas, such as meadows and cultivated fields, and vegetation cover not too dense allowed for optimal application of the sampling method. The roe deer, defined as an ecotonal species, finds its optimal habitat in broad-leaved woodlands interspersed with clearings, as well as semi-natural agricultural areas where crops and meadows alternate with small woodlands (Danilkin and Hewison 1996; Hewison et al. 2001). This study area has exactly these characteristics and can therefore be considered ideal for the presence and persistence of the species. In addition, it is located along the Lombard Apennines in an environmental context surrounded by extensive wooded areas in all directions; therefore, it is not isolated. As reported by Hinojo et al. (2022), better connectivity could ensure higher population densities.

BNM study area showed density estimates similar to those of TID study area; in this case, as well, the landscape characteristics allowed for a good applicability of the data collection method. This study area, despite being located in the Po Plain, presents environmental characteristics that make it ideal for the presence of the species. Well-structured broad-leaved woodlands offering continuous and extensive cover along both banks of the Ticino River, indeed represent a highly functional ecological corridor (Dondina et al. 2020). Furthermore, although the area is surrounded by agricultural fields with intensive crops, the ecological corridor allows for the movement of animals in a longitudinal direction, from the Apennines to the Alps (Dondina et al. 2020).

Despite slightly lower population densities, ORB study area still offers suitable habitats for the species. Although agricultural areas predominate over wooded ones, the landscape remains highly heterogeneous and complex, characterized by ecotonal zones and a balanced mix of open and closed habitats. As expected, in this predominantly agricultural landscape where roe deer have thrived, the average group size was higher compared to all other study areas, including the other two predominantly agricultural ones (i.e. MON and COP).

The estimated density for STA study area was rather low. This study area is characterized by a mountainous and predominantly natural landscape, where most of the surface is occupied by woodlands that provide a continuous cover; conversely from LAR study area, within this landscape open habitats (e.g. clearings, meadows, pastures, uncultivated fields) are widespread and scattered and easily visible from the roads used as routes for data collection, thus the detectability of roe deer was relatively high. As a consequence, the obtained low estimate of population density should be reasonable.

Overall, the population density estimates within the seven investigated study areas generally align with the range of values estimated for other roe deer populations studied in Europe. Specifically, densities estimated in studies employing direct counts as data collection method greatly vary ranging between 0.94 and 53.8 ind. per km^2 (Melis et al. 2009).

Population densities offer valuable insights into the status of populations and the factors influencing them. Predatory and hunting pressure are the two key factors affecting population densities. Concerning predatory pressure, in the investigated study areas, it was mainly represented by the stable presence of wolves. In the four areas where wolf packs were present, roe deer was the most consumed species. Based on the results regarding the predator diet, predatory pressure appeared to be particularly high in STA study area, where roe deer represented the staple of the diet of Menconico pack and was even selected compared to its availability. In contrast, in the other study areas, the species was consumed in proportion to its availability (cfr Section 3.3.3). Thus, lower roe deer densities in STA study area, compared to areas where distance sampling was feasible, might be attributed in part to predatory pressure. Hunting pressure should ideally be based on target species population densities. However, hunting quotas often rely on unreliable data, risking overharvesting. Interestingly, among the investigated study areas, the highest densities were observed in two areas where the hunting area was limited (TID hosts large dog training areas) or absent (BNM is included within a Protected Area).

Considering the method used for the data collection for the study of population structure, i.e. camera trapping, it has generally proven adequate as it allowed the gathering of a sufficient number of independent observations to conduct the analyses.

Obtained results regarding population structure have consistently revealed a predominant presence of adult individuals, followed by subadults and then juveniles, in each study area. Mustoni et al. (2005) reported that, theoretically, in a roe deer population, the number of individuals should decrease along the age classes, thus juveniles should be the most represented age class. The high percentage of adults observed in this research is explained by the fact that this age class encompasses all individuals aged 2 years and older, up to even 10-11 years.

Juveniles, the age class represented to a lesser extent across almost all study areas, typically face the highest mortality rates, sometimes reaching up to 35% within their first year (Mustoni et al.

2005). The low number of juveniles, also reported by other researches carried out in Italy (Bontardelli et al. 2003; De Pasquale et al. 2019), may be due to high mortality during the first months of life or low birth rate.

Juveniles are particularly vulnerable to agricultural practices; indeed, during the first weeks of life, newborns often seek refuge within vegetation, cultivated fields included, being exposed to high risk posed by agricultural machinery such as mowers (Kałuziński 1982; Cukor et al. 2019). Also, predation pressure could be particularly high for juveniles; apart from wolves, red foxes and wild boars are capable of preying on newborns (Panzacchi et al. 2008; Van Moorter et al. 2009). Additionally, the presence of free-roaming dogs could contribute significantly to the mortality of juveniles (Pewsner et al. 2017).

The low birth rate might be attributed to heightened stress levels in females caused by anthropogenic disturbances, potentially leading to abortions or birthing complications.

The metrics utilized to depict population structures indicated that certain populations under investigation may be destructured.

While a balanced sex ratio is considered optimal for roe deer populations, it often slightly favours females in natural conditions due to their longer lifespan (Mustoni et al. 2005). Among the investigated study areas, this skewed ratio towards females was particularly evident in ORB study area. On one hand, such a skewed sex ratio towards females typically characterizes populations not subjected to environmental stressors, such as harsh winters or resource scarcity (Gaillard et al. 1993), and potentially indicates high productivity (Focardi et al. 2002). But on the other hand, it could be the result of a destructured population by hunting activity. For instance, the low percentage of males, especially adults, in this area could be due to the selective hunting of this class for the trophies.

The age ratio indicates the proportion of individuals within a population that are not yet capable of reproduction relative to those that are capable of reproduction. Through pairwise comparisons, it emerged that the roe deer population in ORB and BNM study areas had a significant portion of the population consisting of non-reproductive individuals, which could indicate either a recent increase in birth rates or a high survival rate among younger age classes. Conversely, the low age ratio observed in LAR and TID study areas may indicate a larger proportion of reproductive individuals within the population, potentially reflecting stable or declining birth rates or higher mortality rates among younger age classes.

The juvenile/doe ratio provides insights into the reproductive success and recruitment rates within a population. A high juvenile/doe ratio could suggest successful reproduction and recruitment within the population, indicating favourable conditions for reproduction, adequate resources for survival, and potentially a healthy population structure. Conversely, a low juvenile/doe ratio may indicate challenges in reproduction and recruitment. It could suggest lower reproductive success, higher mortality rates among juveniles, or environmental stressors affecting reproductive outcomes. Interestingly, we observed a significant difference between BNM and LAR study areas, with the highest and lowest juvenile/doe ratios, respectively. These two study areas are very different considering their landscape composition and the types of human disturbances. Conversely, predatory pressure was presumed to be low in both areas, especially for adult roe deer, as wolves were either absent or occasional. Therefore, landscape composition and human disturbance, particularly hunting activity, are the factors most likely driving the observed difference. Generally, the juvenile/doe ratio tends to be higher in heterogeneous environmental contexts, such as the one characterizing BNM study area (Panzacchi et al. 2009; OrŁowska and Rembacz 2016). As mentioned, hunting is not allowed in BNM study area, unlike LAR study area. As reported by Sforzi and Lovari (2000), hunting can significantly impact the structure of a population, resulting in an imbalance among different age classes. For example, populations with a low proportion of young individuals, such as that of LAR study area, are probably destructured.

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Chapter 5

Investigating Behavioural Responses of Roe Deer Across Different Landscapes in North-Western Italy

5.1 Introduction

5.1.1 The landscape of fear

Roe deer, like many other prey species, have evolved sophisticated mechanisms to assess and respond to risks in their environment. Their perception of risk is shaped by various factors, including the presence of predators, human activities, and landscape structure (Kuijper et al. 2013). The anthropogenic factors affecting roe deer risk perception encompass both direct threats, like hunting, farming, and urban development, as well as indirect disturbances, such as recreational activities (Hewison et al. 2001; Benhaiem et al. 2008; Bonnot et al. 2013; Scholten et al. 2018). Additionally, landscape structure plays a significant role; dense vegetation and rugged terrain are often perceived as safer due to better cover from predators and humans, while open areas or those with high human activity may be deemed riskier and thus avoided (Lewis and Eby 2002; Ripple and Beschta 2004; Benhaiem et al. 2008; Laundré et al. 2010).

Indeed, the interplay of these factors - predators, human activities, and landscape structure - defines what is termed the “landscape of fear” (Laundré et al. 2001). This concept can be defined as the spatial variation in prey perception of predation risk (Gaynor et al. 2019): a sort of “map” with “peaks” and “valleys” describing an animal perception of the locations as dangerous and safe, respectively (Van Der Merwe and Brown 2008).

5.1.2 The outcomes of fear: antipredator strategies

Perceived risk manifests as fear, which isn't solely felt when imminent danger is present, due to its unpredictable nature, but rather persistently, as a form of anticipatory risk assessment. This fear triggers various antipredator responses aimed at reducing exposure to potential threats. Indeed, the perceived predation risk plays a powerful role in shaping the behaviours of fearful prey, with consequences for individual physiology, population dynamics and demography, and interactions at the community level (Gaynor et al. 2019). The various antipredator responses can generally be attributed to two main strategies:

i) prey may modulate behaviours, such as increased vigilance, reduced feeding activity, or

ii) prey may alter behaviours, such as movement or activity patterns, to avoid or minimize the risk associated with some areas or times (Gaynor et al. 2019).

Among the antipredator responses of deer, several studies have observed changes in habitat selection, aimed at avoiding areas frequented by predators or humans (Bonnot et al. 2013; Kuijper et al. 2013; Scholten et al. 2018; Mols et al. 2022; Marion et al. 2022), and a greater use of woodlands, which offer better protection, in areas and times of higher perceived risk (Hernández and Laundré 2005; Bonnot et al. 2013; Meisingset et al. 2022). Similarly, adjustments in activity patterns have been observed, with a tendency to avoid periods of increased human or predator activity (Gaynor et al. 2019; Bonnot et al. 2020; Rossa et al. 2021; Wiskirchen et al. 2022). Regarding behaviour modulation aimed at limiting the level of risk, ungulates commonly increase vigilance levels in high-risk areas, as this behaviour can help detect the presence of an imminent danger and attempt to flee (Jayakody et al. 2008; Benhaiem et al. 2008; Sönnichsen et al. 2013; Popova et al. 2016; Bergvall et al. 2016). Another possible antipredator response may be the variation in social organization, such as forming larger groups, especially in open environments where vegetation cannot provide adequate cover for hiding; this allows for the dilution of both the level of risk and the time invested in vigilance, reducing the effort required of individual animals in this behaviour (Hewison et al. 2001; Sönnichsen et al. 2013; Olson et al. 2019).

However, these antipredator responses can frequently constrain an individual capacity to engage in other essential survival activities (Lima and Dill 1990), notably impeding the fulfilment of physiological requirements such as sufficient energy intake when habitat selection is influenced more by risk levels than by the quantity and quality of available resources (Hernández and Laundré 2005; Benhaiem et al. 2008). Moreover, increased vigilance levels may detract from time allocated to foraging (Benhaiem et al. 2008; Popova et al. 2016), potentially impacting individual fitness.

The extent to which different antipredator strategies are exhibited is thus a constant balance between the benefits they offer and the associated costs (Lima and Dill 1990), and this varies across both time and space according to the cost-benefit ratio.

5.1.3 Case studies

The following two case studies explore some particular aspects of roe deer behavioural adaptability in a human-dominated environment where its main predator, i.e. the wolf, is rapidly expanding. The interesting point is that these increasing risk levels may, in some cases, necessitate dealing with conflicting strategies.

i) **Assessing roe deer behaviour at the population-level: spatial variations in diel activity patterns** - The behavioural responses of the roe deer to human disturbance and predation risk may include altered activity patterns. Obtained results indicated a general crepuscular behaviour of this species with peaks of activity at dawn and dusk hours. However, significant seasonal deviations from the general pattern were observed, which can be explained by human disturbance and wolf presence.

Human activity is highly predictable and likely generates visible responses in the activity patterns that are less variable compared to those generated by predatory activity of wolves, which is highly unpredictable for roe deer. Interestingly, spring exhibited consistent activity patterns across study areas, likely influenced by biological and ecological factors such as mating and foraging needs. Overall, the study highlights the complex interplay between environmental factors and roe deer behaviour.

ii) **Exploring roe deer behaviour at the individual-level: time-budgeting and factors influencing vigilance** - Animals allocate time to different behaviours based on their biological needs, environmental conditions, and perceived risks. For example, they may spend more time feeding when food resources are abundant and accessible. However, when the perceived risk of predation or human disturbance is high, animals may prioritize vigilance to detect and respond to potential threats quickly. This allocation of time between feeding and vigilance is therefore dynamic. We used camera trapping and direct observations from vantage points to record roe deer videos to be processed and analysed according to an experimental ethogram. Feeding was prevalent on vigilance across all study areas except two; significant differences in the duration and frequency of vigilance behaviour were observed among the different study areas. Landscape structure and associated human-induced disturbances played a significant role, impacting vigilance behaviour more than predator presence.

5.2 Assessing roe deer behaviour at the population-level: variations in diel activity patterns

5.2.1 Introduction

The activity patterns of animals are influenced by a multitude of factors. Endogenous factors encompass physiological states and inherited traits, while exogenous factors such as weather conditions, duration of daylight, and disturbances play crucial roles (Reimoser 2012).

The roe deer, owing to its selective feeding strategy and relatively short bouts of rumination, is known to be a highly active animal (Cederlund 1989). The patterns of species activity mainly reflect various energy intake requirements, thus showing significant variability between seasons and times of day. Furthermore, activity patterns vary based on factors such as sex and age, as well as climate and other environmental conditions (Danilkin and Hewison 1996).

Regarding diel activity patterns, this species typically exhibits two peaks of maximum activity approximately at dusk and dawn, with variations throughout the year, thus adapting to the photoperiod (Danilkin and Hewison 1996; Stache et al. 2013; Pagon et al. 2013; Bonnot et al. 2020). These peaks of activity are characterized by the longest duration of activity for roe deer.

Despite being primarily considered a crepuscular species, the roe deer maintains a certain level of activity throughout the entire 24-hour period (Stache et al. 2013; Pagon et al. 2013; Bonnot et al. 2020) with seasonal variations that may lead, for example, to increased daytime activity during winter and, conversely, nighttime activity during summer, in response to temperature variations as a strategy to facilitate thermoregulation (Pagon et al. 2013).

Circadian rhythms in roe deer can be altered in response to the perceived risk by shifting activity to less risky periods of the day; such temporal antipredator responses seem not to be ubiquitous (Cusack et al. 2020) because several factors influence them. For instance, Bonnot et al. (2020) observed high plasticity in roe deer circadian rhythms, allowing them to adapt to changes in risk levels over time. Roe deer exhibit a strong tendency to shift their activity towards nighttime hours when faced with even minimal anthropogenic disturbance. This adjustment is driven by the concentration of human activities during the day, which is further intensified during hunting seasons when the perceived risk increases (Bonnot et al. 2020). However, if nocturnal predators, such as large carnivores, are present, this behavioural strategy could increase the risk of predation. Consequently, a shift towards reducing nighttime activity and increasing daytime activity is required to mitigate these contrasting risk factors (Bonnot et al. 2020; Rossa et al. 2021). Therefore, the behavioural responses triggered by anthropogenic disturbance and predation risk can be conflicting and thus require careful balancing. Thus, in areas with high levels of human disturbance and predation pressure, we might expect roe deer to be particularly crepuscular, squeezing as much of their activity as possible into dawn and dusk (Bonnot et al. 2020).

Aims - This section aimed to explore the responses of the roe deer to human disturbance and predation risk, by analysing species activity patterns, to assess potential temporal avoidance as an antipredator strategy. The predictions were that (i) in areas where predators were absent but human activity was high, roe deer would exhibit increased nocturnal activity as a response to avoid periods of peak human activity, particularly during the hunting seasons when perceived risk is increased; (ii) conversely, in areas where wolves were present and human activity was low, the response would be primarily determined by predation risk, prompting roe deer to avoid nocturnal hours to reduce potential encounters with predators; (iii) in areas where both wolves and humans contributed to delineate the landscape of fear, roe deer responses would be expected to achieve a balance, resulting in heightened activity during intermediate periods, such as dusk and dawn.

5.2.2 Material and methods

Data collection - Roe deer data were collected using camera trapping. Seasonal camera trapping sessions were conducted over two years from December 2020 to November 2022. In each study area, camera traps were placed using a grid sampling scheme, dividing each study area into sample squares of 1.5 x 1.5 km, following the TSS design. Minimum distances between camera traps were set to prevent capturing the same individual multiple times. Camera traps were deployed simultaneously during each seasonal session and maintained in the field for approximately 120 days, recording 30-second videos with a minimum time delay between consecutive ones. Camera traps were mounted primarily at heights of 50-100 cm on trees and bushes. Captured videos were carefully inspected to identify species and consecutive videos of the same species were considered as separate events only if spaced at least 30 minutes apart to ensure capture independence (for additional information cfr Section 4.3.1 - Data collection).

Activity patterns - The activity rhythms were analysed based on data obtained through camera trapping, specifically considering the dates and the times at which roe deer events were recorded. Activity rhythms were estimated for each study area on a seasonal basis, considering that a minimum of 30 events per sample is necessary to obtain reliable results (Lashley et al. 2018). To produce a model of circadian rhythms, the non-parametric method Kernel Density Estimation (Ridout and Linkie 2009) was used, which considers camera trapping events as representative samples of a continuous distribution over 24 hours and employs a probability density function to produce a curve whose peaks are associated with a higher density of roe deer events, which can be interpreted as increased activity at the corresponding time of day. The Watson's test (U^2) was used to evaluate the uniformity of obtained seasonal activity rhythms (Lund et al. 2017).

We compared the different seasons within the same study areas to verify the consistency of species circadian rhythms on a local scale and among the different study areas within the same season. Specifically, a comparison of the activity patterns between areas differing in predator presence (stable vs. not stable) and landscape composition (natural vs. mixed vs. modified) was carried out for the specific purpose of contrasting temporal patterns of the roe deer under different predator pressures and human disturbances (cfr Chapter 2). We tested the significance of observed differences by performing the Mardia-Watson-Wheeler test (W_g) and post-hoc Watson's two-sample test (two-sample U^2), applying the Bonferroni correction to p-values (Pewsey et al. 2013; Torretta et al. 2016); moreover, we quantified the similarities using the coefficient of overlap (Δ), which ranges from 0 (no overlap) to 1 (complete overlap) (Ridout and Linkie 2009). Depending on the sample size (≤ 75 or > 75), we used either Δ_1 or Δ_4 (Meredith et al. 2024). Following Monterroso et al. (2014), we evaluated overlap values from pairwise comparisons categorising overlap as low ($\leq$ 50th percentile), moderate (51st to 75th percentile), or high (> 75th percentile). Differences in the Δ values between seasons, predator pressures and human disturbances were tested using non-parametric tests (i.e. Mann-Whitney test or Kruskal-Wallis test with Dunn test for pairwise comparisons).

We used the "circular" (Lund et al. 2017) and "overlap" (Meredith et al. 2024) packages in R (R Core Team 2024).

Diurnality and nocturnality - Camera trapping events were categorised into four periods of the diel cycle: day (from 1 hour after sunrise to 1 hour before sunset), night (from 1 hour after sunset to 1 hour before sunrise), dawn (1 hour before to 1 hour after sunrise), and dusk (1 hour before to 1 hour after sunset) (Foster et al. 2013; Monterroso et al. 2014; Torretta et al. 2017). The tendency towards diurnality or nocturnality of the roe deer was quantified using indices of diurnal and nocturnal activity (Clauss et al. 2021). These indices were calculated as proportions of the total events recorded during the day and the night compared to the overall events collected during camera trapping sessions at each camera trapping site; therefore, they can range between 0 and 1.

For both indices, a comparison at the seasonal level among the areas differing in predator presence (stable vs. not stable) and landscape composition (natural vs. mixed vs. modified) was carried out using non-parametric tests (i.e. Mann-Whitney test or Kruskal-Wallis test with Dunn test for pairwise comparisons).

Moreover, we performed generalised linear models (GLMs) where diurnality or nocturnality was modelled against the season (spring, summer, autumn, winter), the landscape composition (natural, mixed, modified), predator presence (stable, not stable), and the percentage cover of woodlands, natural open areas (i.e., grasslands, pastures, shrublands), cultivated lands, and protected surface (i.e. no hunting zones) within the sampling square of each camera trapping site. We first formulated global models considering all the predictors; then we selected the best models based on AICc scores (models with Δ AICc ≤ 2; Burnham and Anderson 2002), ignoring redundant models (e.g. more complex versions of any simpler model).

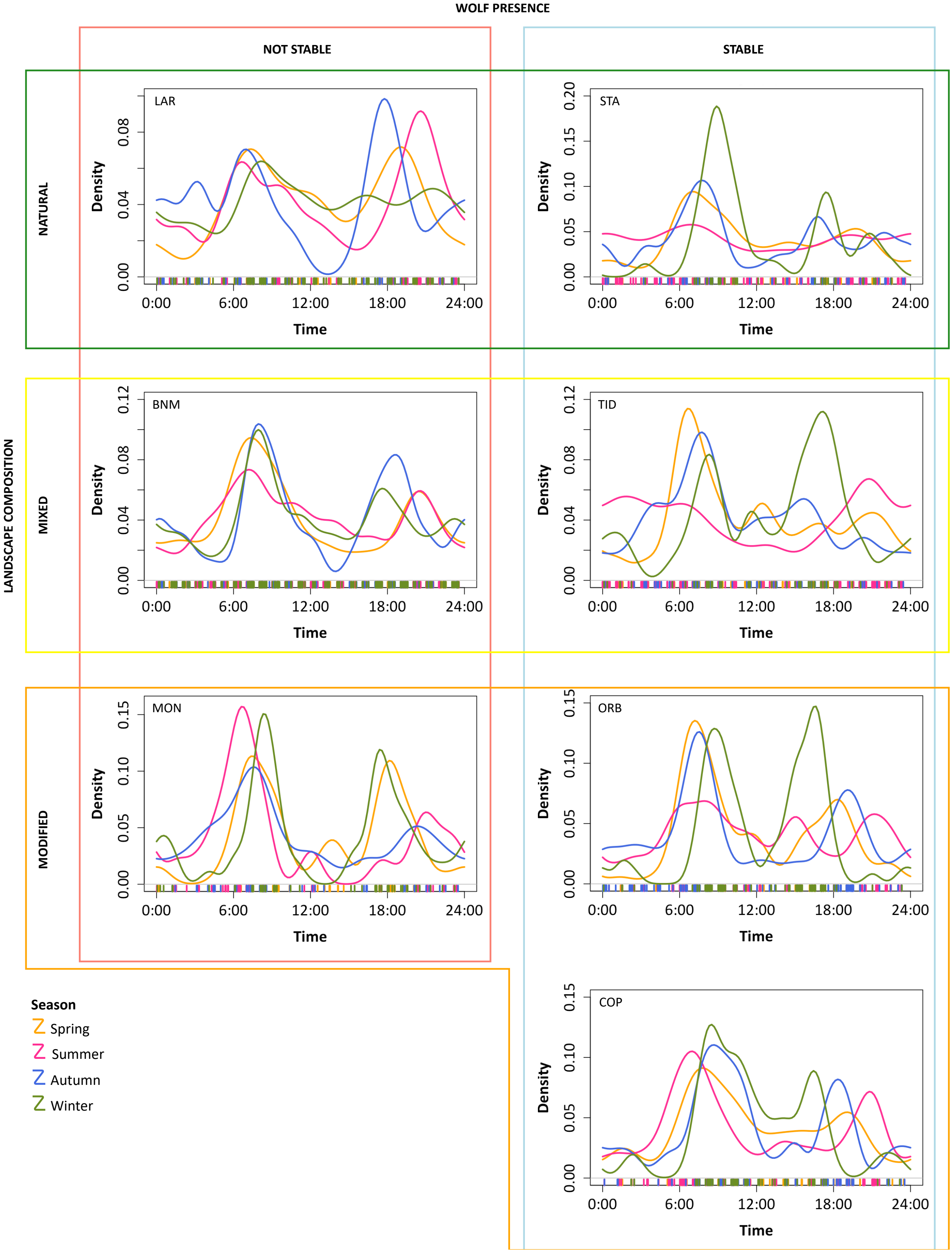
We performed GLMs using the "stats", "MuMIn" (Bartoń 2023), and "visreg" (Breheny and Burchett 2017) packages in R (R Core Team 2023).

5.2.3 Results

Collected data - Across all study areas and combining data from all eight sampling seasons, a total of 2749 roe deer events were recorded. The number of roe deer events recorded was sufficient to conduct the analyses (n ≥ 30) for each season in each study area (cfr Table 4.3.10).

Activity patterns - The diel activity patterns of the roe deer showed a significant deviation from uniform distribution in almost all seasons in every study area, with a few exceptions (Supplementary Material 5.2: S.1). Thus, generally, the roe deer showed non-uniform diel activity patterns with two main peaks of activity, at dawn and dusk hours, in every study area with seasonal differences (Figure 5.2.1 and Supplementary Material 5.2: S.2).

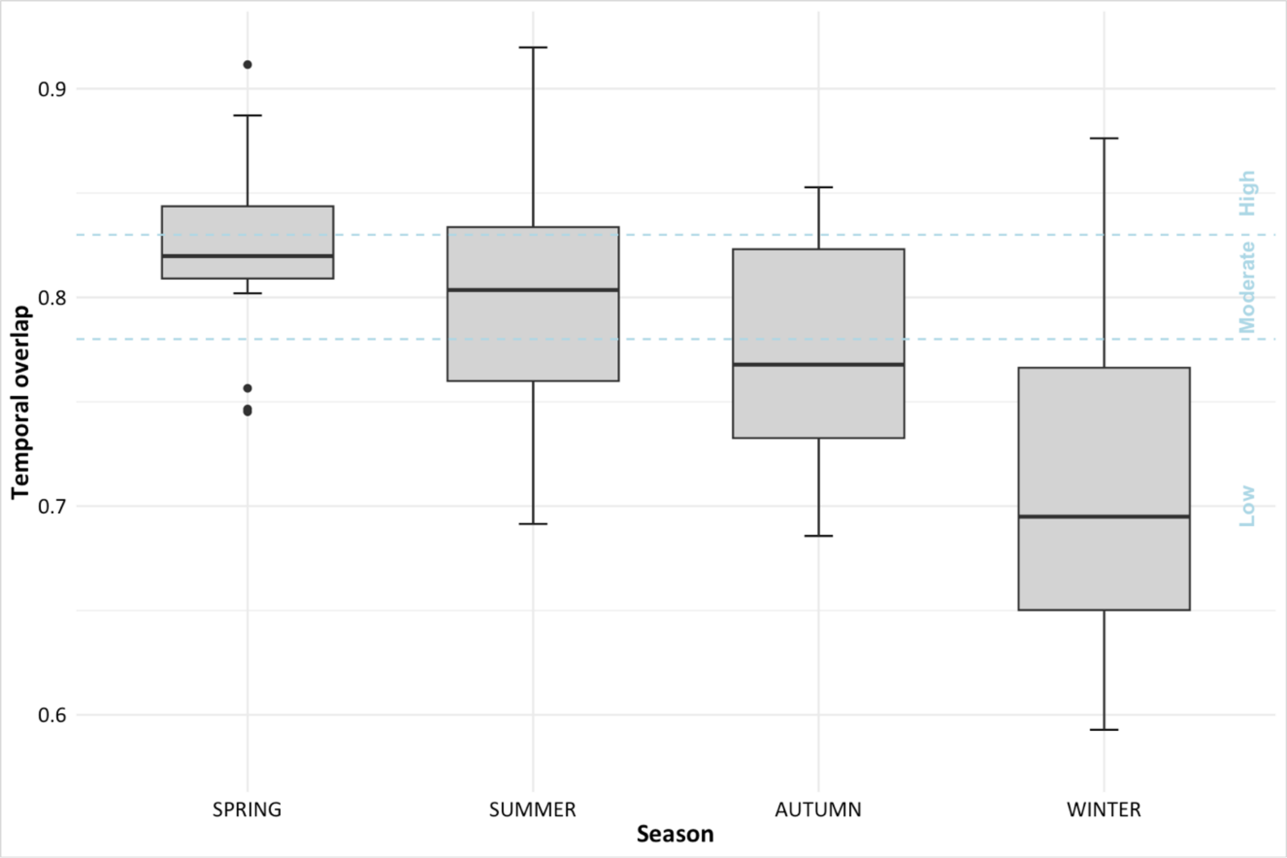
Figure 5.2.1 - The circadian activity rhythms of the roe deer in north-western Italy estimated for each season using the data collected from 2020 to 2022



The comparison of the activity rhythms on a seasonal basis revealed notable differences in all seasons except spring, where roe deer activity remained consistent and the Δ values showed moderate-to-high overlap. In contrast, summer and winter showed high variability in activity rhythms, with the Δ values indicating moderate overlap in summer and low overlap in winter (Figure 5.2.2 and Supplementary Material 5.2: S.3).

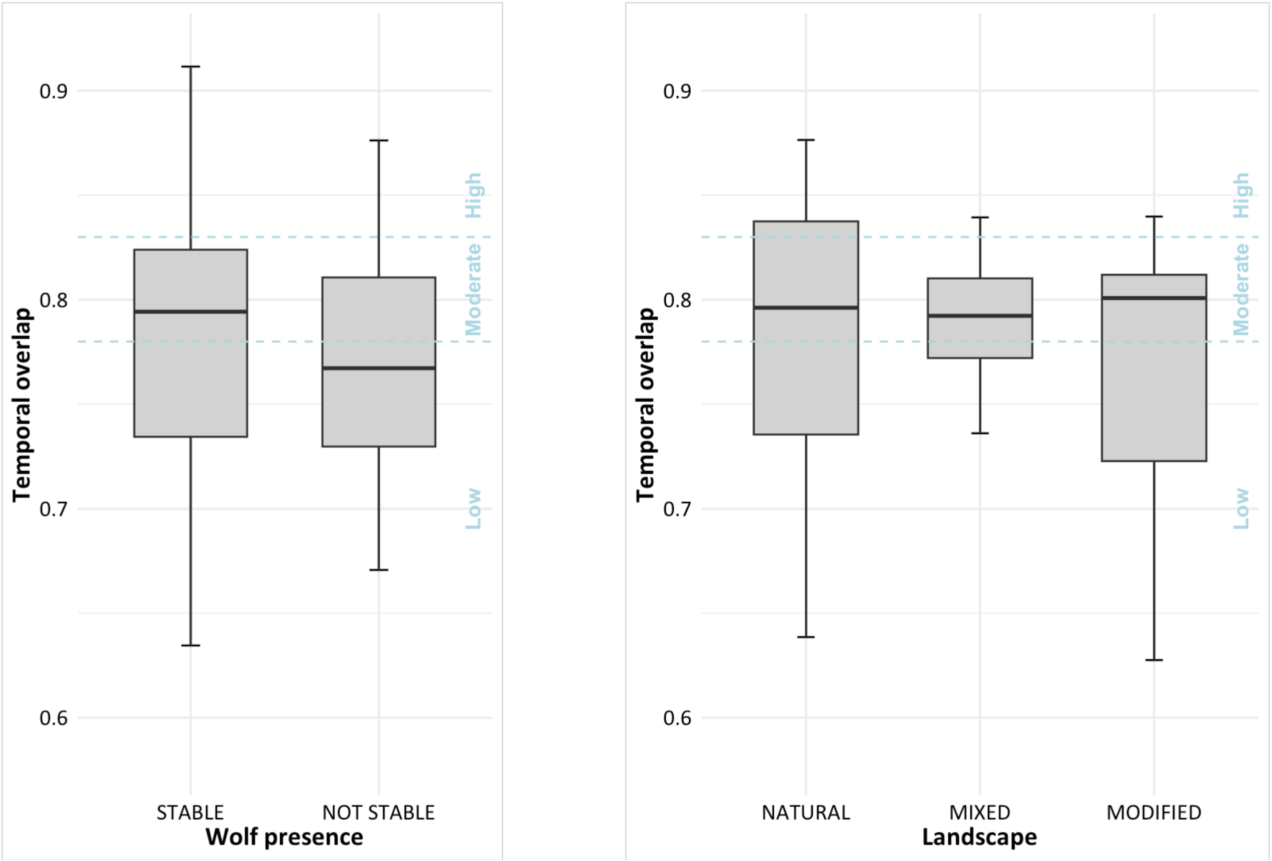
The values of the coefficient of overlap were found to be different between seasons (H = 27.80, df = 3, p < 0.001); in particular, the temporal overlap was significantly higher and, therefore, the activity patterns were more uniform during spring and summer compared to winter (SP vs. WI: p < 0.001 and SU vs. WI: p = 0.001).

Figure 5.2.2 - Seasonal similarity of roe deer activity rhythms in north-western Italy quantified by the coefficient of overlap (Δ)



Conversely, no significant differences emerged in the comparisons of the activity patterns between areas differing in predator presence (stable vs. not stable) and landscape composition (natural vs. mixed vs. modified) (Figure 5.2.3).

Figure 5.2.3 - Seasonal and spatial variations of the coefficient of overlap (Δ) of roe deer activity rhythms in north-western Italy



Diurnality and nocturnality - In the comparison of the diurnality index among the different landscapes within the same season, we observed overall uniformity in diurnality levels across all seasons except for autumn (H = 7.73; p = 0.021). Specifically, higher diurnality levels were observed in modified landscapes (MON, ORB, and COP) compared to natural ones (LAR and STA) (p = 0.026). Significant variations emerged when comparing the diurnality index across seasons within the same landscape, except for modified landscapes (MON, ORB, and COP). In natural landscapes (LAR and STA) (H = 15.76; p = 0.001), higher diurnality levels were observed during spring compared to autumn (p = 0.001). In mixed landscapes (BNM and TID) (H = 14.64; p = 0.002), higher diurnality levels were observed during spring compared to autumn (p = 0.002) and winter (p = 0.021) (Figure 5.2.4).

In the comparison of the diurnality index between areas with a stable presence of wolves versus areas with an absence or an occasional presence of wolves within the same season, we observed significant higher diurnality levels in wolf areas (ORB, COP, TID, and STA) during spring (H = 4.52; p = 0.034) and winter (H = 5.47; p = 0.019).

Moreover, in areas where wolves are absent or occasionally present (LAR, BNM, and MON) (H = 21.77; $p < 0.001$), diurnality levels were higher during spring compared to autumn ($p = 0.015$) and winter ($p = 0.003$) and also during summer compared to autumn ($p = 0.010$) and winter ($p = 0.002$). In areas where wolves are stable (ORB, COP, TID, and STA) (H = 18.74; $p < 0.001$), diurnality levels were higher during spring compared to autumn ($p < 0.001$) (Figure 5.2.5).

Figure 5.2.4 - Seasonal variation in the diurnality index of the roe deer across landscapes with different composition

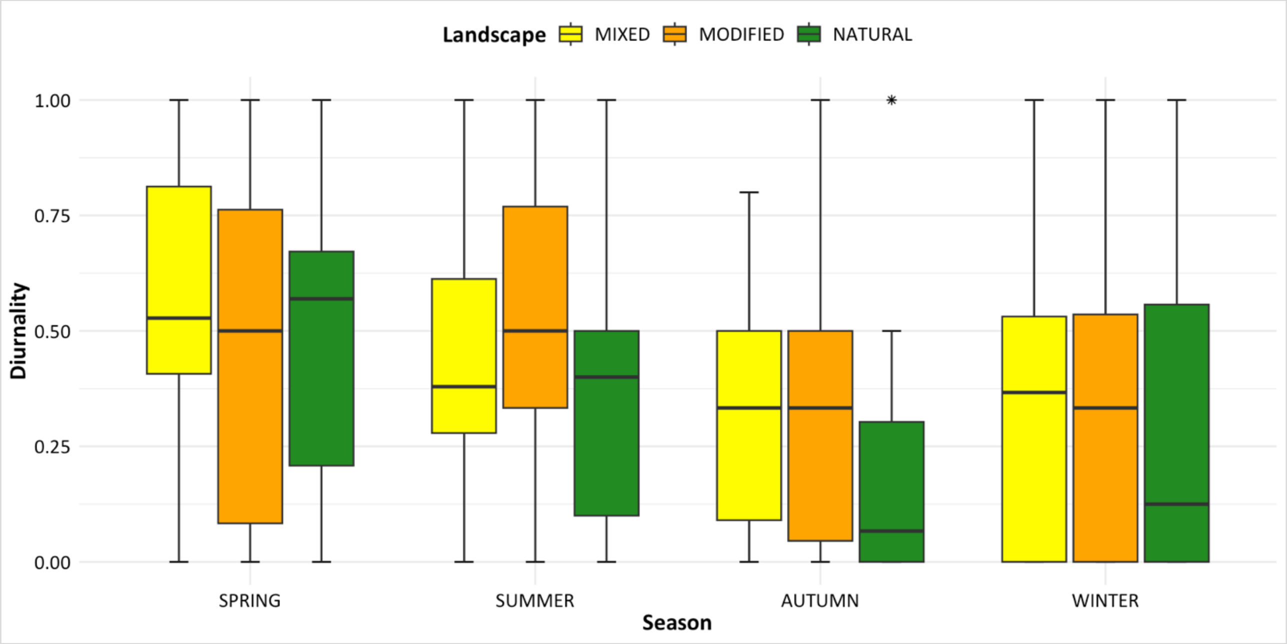
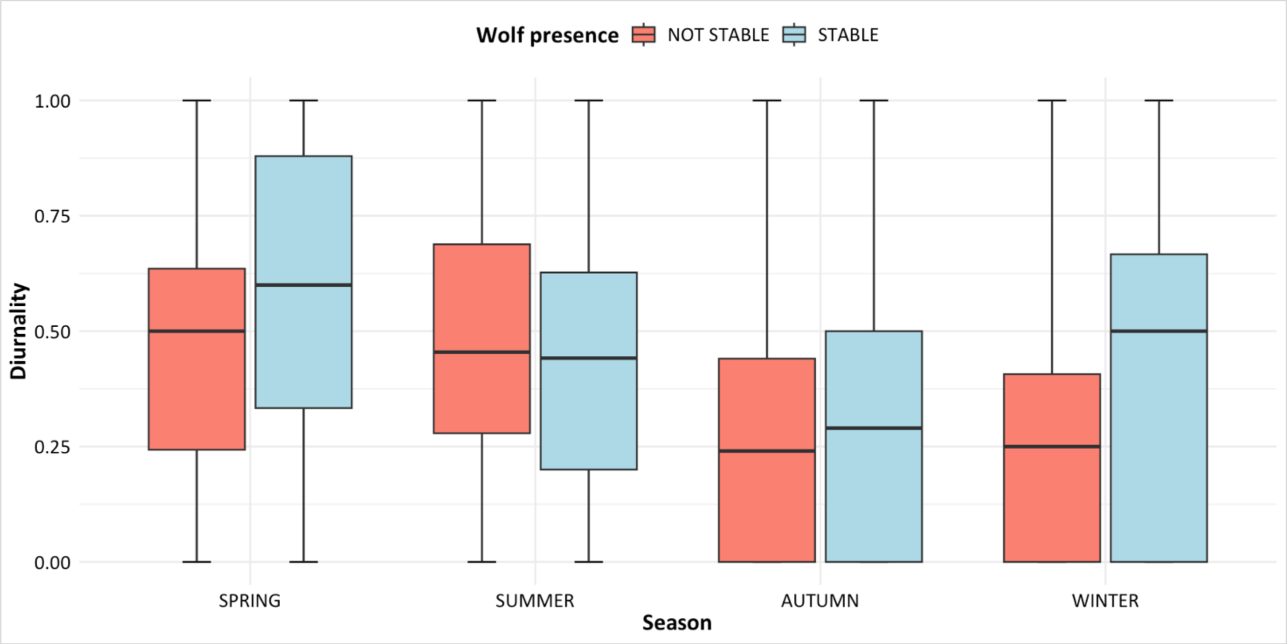


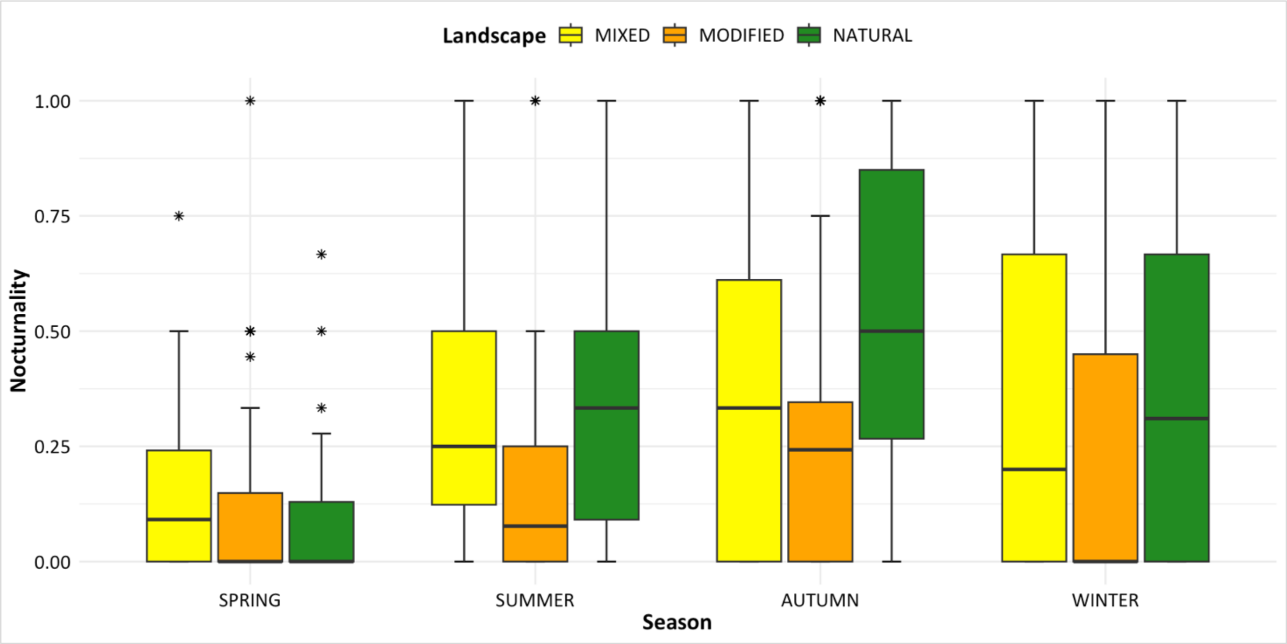
Figure 5.2.5 - Seasonal variation in the diurnality index of the roe deer across areas with different predator presence



Considering the nocturnality index among the different landscapes within the same season, we observed significant seasonal variations during summer (H = 14.30; p = 0.001) and autumn (H = 8.49; p = 0.014). Specifically, during summer, lower nocturnality levels were observed in modified landscapes (MON, ORB, and COP) compared to natural (LAR and STA) (p = 0.004) and mixed landscapes (BNM and TID) (p = 0.005). Similarly, during autumn, lower nocturnality levels were observed in modified landscapes (MON, ORB, and COP) compared to natural ones (LAR and STA) (p = 0.011).

Moreover, significant variations also emerged when comparing the index across seasons within the same landscape. In natural landscapes (LAR and STA) (H = 22.64; p < 0.001), lower nocturnality levels were observed during spring compared to summer (p = 0.008) and autumn (p < 0.001). Similarly, in mixed landscapes (BNM and TID) (H = 12.56; p = 0.006), lower nocturnality levels were observed during spring compared to summer (p = 0.019) and autumn (p = 0.011). In modified landscapes (MON, ORB, and COP) (H = 8.82; p = 0.032), lower nocturnality levels were observed during spring compared to autumn (p = 0.019) (Figure 5.2.6).

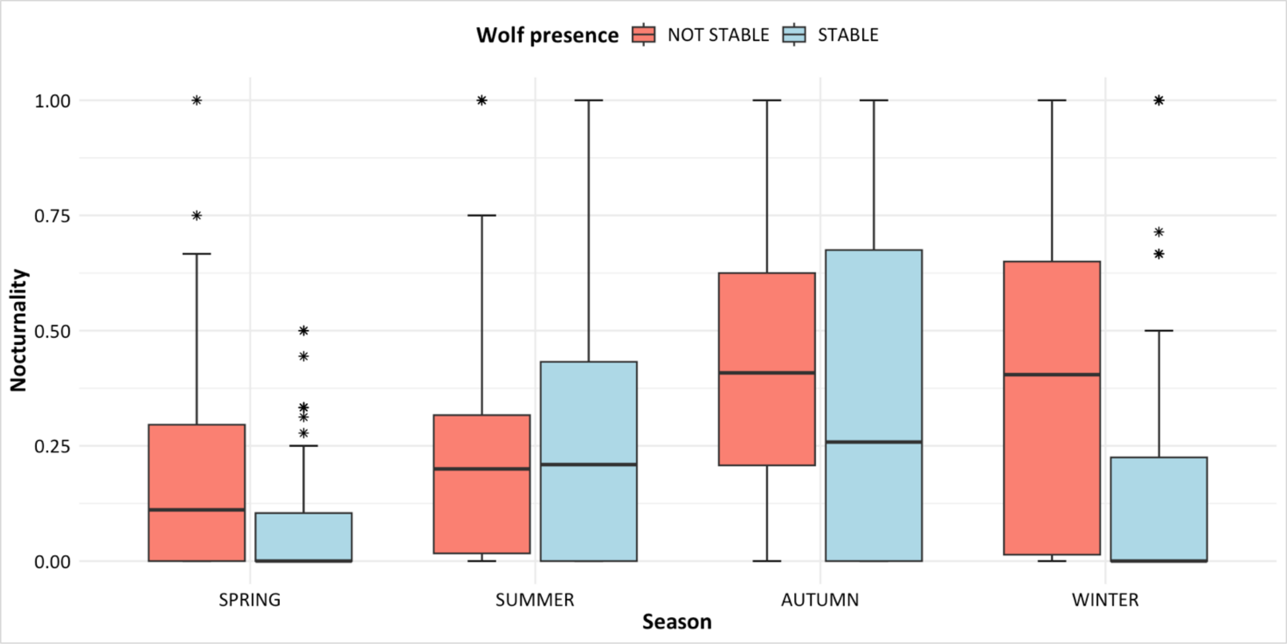
Figure 5.2.6 - Seasonal variation in the nocturnality index of the roe deer across landscapes with different composition



In the comparison of the nocturnality index between areas with a stable presence of wolves versus areas with an absence or an occasional presence of wolves within the same season, we observed significant higher nocturnality levels in wolf-free areas (LAR, BNM, and MON) during spring (H = 10.89; p = 0.001) and winter (H = 11.02; p = 0.001).

In areas where wolves are absent or occasionally present (LAR, BNM, and MON) (H = 18.04; p < 0.001), nocturnality levels were lower during spring compared to autumn (p = 0.001) and winter (p = 0.015). In areas where wolves are stable (ORB, COP, TID, and STA) (H = 30.43; p < 0.001), nocturnality levels were lower during spring compared to summer (p = 0.001) and autumn (p < 0.001); moreover, nocturnality levels were lower during winter compared to summer (p = 0.039) and autumn (p = 0.011) (Figure 5.2.7).

Figure 5.2.7 - Seasonal variation in the nocturnality index of the roe deer across areas with different predator presence

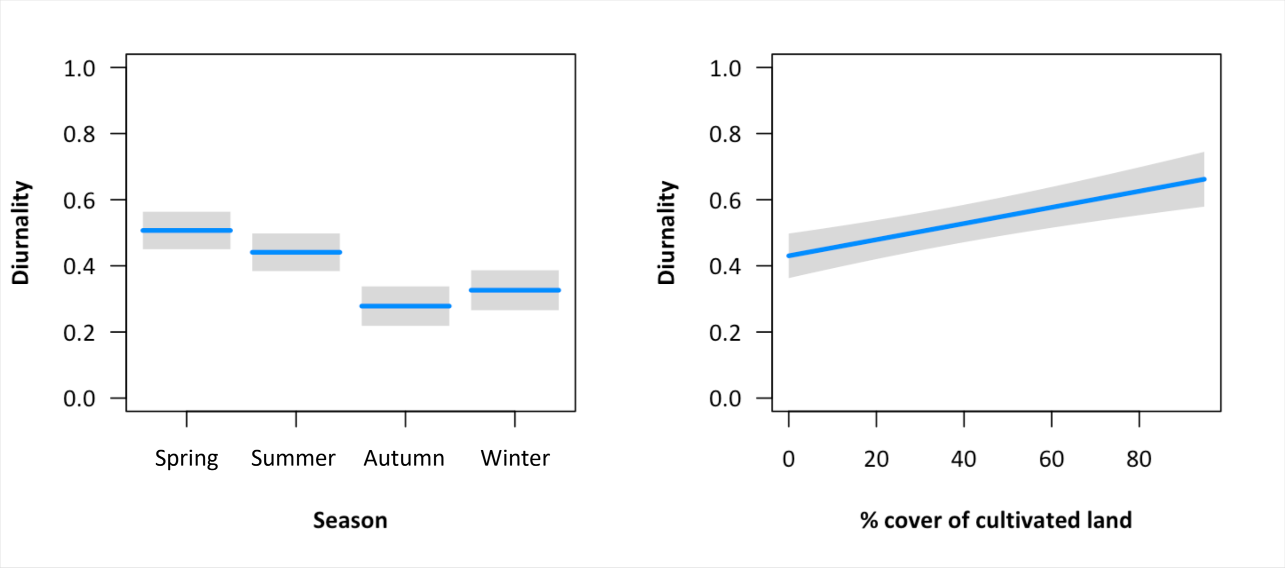


Among the obtained models describing diurnality with a Δ AICc ≤ 2 (n = 5), we considered only the first model (Supplementary Material 5.2: S.4). Diurnality levels increased with the increasing cover of cultivated lands and peaked during spring (Table 5.2.1 and Figure 5.2.8).

Table 5.2.1 - Factors influencing diurnality levels of the roe deer in north-western Italy. Estimates of model coefficients (B), standard errors (SE), 95% confidence intervals (95% CIs), and p-values are shown

Variable	**B**	**SE**	**95% CIs**	**p**
(Intercept)	0.430	0.034	0.36; 0.50	< 0.001
Season (summer)	-0.066	0.041	-0.15; 0.01	0.107
Season (autumn)	-0.229	0.042	-0.31; -0.15	< 0.001
Season (winter)	-0.181	0.042	-0.26; -0.10	< 0.001
% cover of cultivated lands	0.002	0.001	0.001; 0.003	< 0.001

Figure 5.2.8 - Factors influencing diurnality levels of the roe deer in north-western Italy. Response curves and 95% confidence intervals estimated through generalized linear models

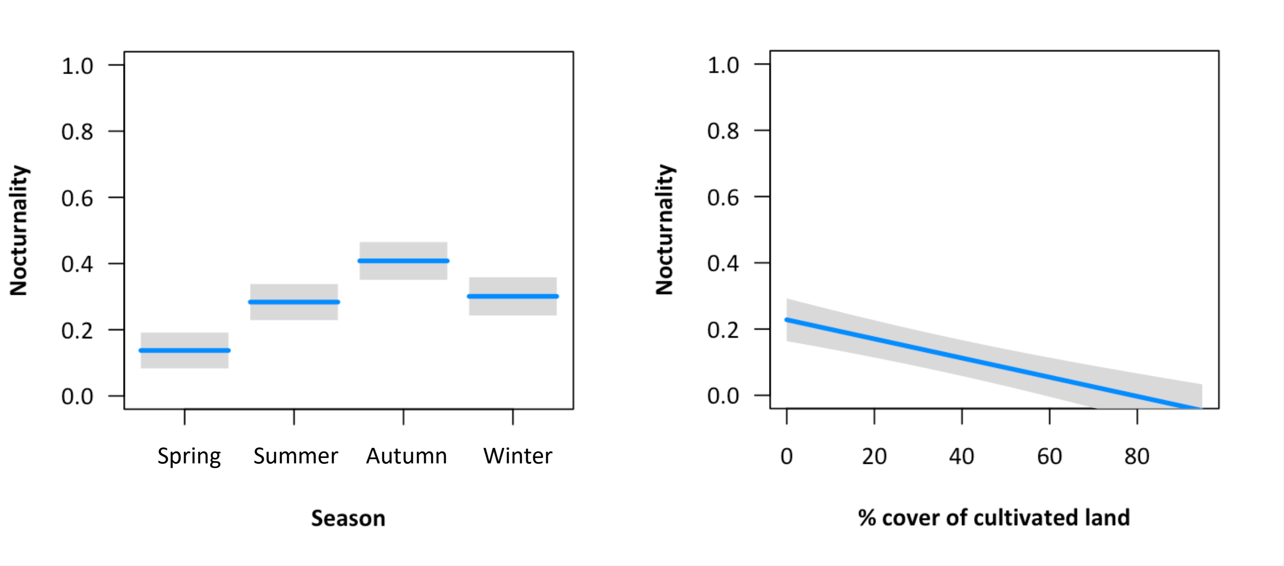


Considering top-ranked models describing nocturnality (n = 4), we considered only the first model, as the others were more complex versions of the best one (Supplementary Material 5.2: S.5). Nocturnality levels decreased with the increasing cover of cultivated lands and peaked during autumn (Table 5.2.2 and Figure 5.2.9).

Table 5.2.2 - Factors influencing nocturnality levels of the roe deer in north-western Italy. Estimates of model coefficients (B), standard errors (SE), 95% confidence intervals (95% CIs), and p-values are shown

Variable	**B**	**SE**	**95% CIs**	**p**
(Intercept)	0.228	0.033	0.16; 0.29	< 0.001
Season (summer)	0.146	0.039	0.07; 0.22	< 0.001
Season (autumn)	0.270	0.040	0.19; 0.35	< 0.001
Season (winter)	0.163	0.040	0.08; 0.24	< 0.001
% cover of cultivated lands	-0.003	0.001	-0.004; -0.002	< 0.001

Figure 5.2.9 - Factors influencing nocturnality levels of the roe deer in north-western Italy. Response curves and 95% confidence intervals estimated through generalized linear models



5.2.4 Discussion

The results obtained from this study underscored the general tendency towards bimodality of roe deer circadian activity rhythms, characterized by two peaks in activity around dusk and dawn periods. These findings confirmed the crepuscular behaviour of the species (Danilkin and Hewison 1996; Stache et al. 2013; Pagon et al. 2013; Bonnot et al. 2020).

Nevertheless, activity patterns exhibited notable variations at both local and seasonal scales.

Interestingly, in two study areas (LAR and BNM), roe deer activity rhythms exhibited uniformity, with no discernible seasonal differences detected. Assuming that variations in the circadian activity of roe deer reflect responses to changes in perceived risk levels, this condition of substantial uniformity throughout the year should therefore show a general lack of fluctuations in risk levels, which are mainly represented by human activities. In particular, in LAR study area the landscape is uniformly occupied by woodlands, providing plentiful shelter sites, and the main sources of disturbance are the recreational activities represented by outdoor activities (e.g. hikers and climbers) and hunting; both activities have a strong, but opposite, seasonal variation. Outdoor activities are mainly engaged during late spring and summer or winter seasons, whereas hunting is mainly done in autumn, but selective hunting of ungulates is also carried out during the other seasons with specific time frames allocated for different sexes and age classes. Therefore, while acknowledging that the two forms of human activity have different impacts (disturbance vs. hunting), they both entail a consistent presence of humans in the area throughout the year. In BNM study area, conversely, hunting is prohibited as it falls within a Protected Area. Therefore, the main sources of disturbance are the recreational activities represented by hiking (often with unleashed dogs) and biking, which are common throughout all seasons. In addition, the presence of extensive agricultural areas implies intensive fieldwork, which varies depending on the crop, but generally occurs within a few days of concentrated activity. Moreover, human activities related to agricultural areas are concentrated in the surroundings of the area, while cyclists, hikers, and walkers with dogs tend to traverse the area extensively, facilitated by the spreading network of pathways.

Among the other study areas, there was greater variability in roe deer activity rhythms during the summer season. Summer is the season when anthropogenic disturbance from outdoor recreational activities, such as hiking and motocross, reaches its peak. These activities, especially motocross and similar off-road activities, can be a significant source of disturbance for wildlife because, being highly unpredictable, they can increase individual stress levels and disrupt normal behaviour patterns and, thus, trigger antipredator responses. Interestingly, the results regarding summer activity rhythms indicated a shift of the second peak of activity towards the nighttime hours, typically occurring between 9:00 PM and midnight, and an overall increase in nocturnal activity between the two peaks of higher activity, i.e. at dusk and dawn. This trend towards a more pronounced nocturnal activity was also confirmed by the results of the nocturnality index, which, notably for mixed and natural landscapes, showed a significantly higher value. In fact, in some of the areas within these landscapes (e.g. TID and STA), the aforementioned activities are widely practised and not subject to any control or regulation.

Pagon et al. (2013) related the pronounced nocturnal activity observed in summer to the need for thermoregulation. Although thermoregulation is a highly plausible explanation, if it were the primary factor influencing roe deer activity rhythms, we would have expected to observe more pronounced differences in the plain study areas, where summer temperatures are warmer.

Even during winter significant variability in activity rhythms was observed. In some study areas, for example ORB and TID, the second peak of activity, occurring at dusk and encompassing the preceding hours leading up to it, was notably pronounced. Human presence during winter is generally minimal, both in terms of recreational activities and agricultural practices. However, besides human disturbance, climate and weather conditions also play a role in influencing roe deer activity rhythms during the adverse season (Danilkin and Hewison 1996; Pagon et al. 2013). Decreased nighttime activity during winter has previously been observed and was believed to be associated with environmental factors (Pagon et al. 2013). In colder months, roe deer may increase daytime activity to search for food when temperatures are slightly warmer, although they still tend to exhibit crepuscular behaviour.

Concerning autumn, a noteworthy observation is the heightened nocturnal activity detected in LAR, ORB, TID, and STA study areas. These are all characterized by the beginning of the hunting season occurring in the same period. Indeed, hunting has been shown to modify the behaviour of deer species in several ways, including the increase in nocturnal activity (Kilgo et al. 1998). Bonnot et al. (2020), in particular, observed that nocturnality can be adaptative and reflects roe deer response to the perceived risk of predation, even if is associated with human disturbance (especially hunting).

Considering the study areas where these seasonal differences in activity rhythms were most pronounced, it is notable that the four areas exhibiting the greatest variability (i.e. ORB, COP, TID, and STA) are those where the presence of wolves is stable (cf. Section 3.3). Human activities create a well-defined landscape of fear, as they are easily predictable for animals (for example, agricultural disturbance is linked to cultivated fields and, thus, is spatially predictable), prompting proactive responses from prey, whereas the landscape of fear due to large carnivores may be more difficult for prey to anticipate (Bonnot et al. 2020). Interestingly, Crawford et al. (2021) observed increased heterogeneity in prey behaviour, which was the white-tailed deer (*Odocoileus virginianus*), as a response to predator presence. Therefore, the antipredator responses to predation risk enacted by deer may be more temporally variable.

Interestingly, during spring the activity rhythms of the roe deer remained consistent and uniform across the study areas. During this season, the rhythms clearly exhibited the two typical peaks of activity, at dawn and dusk, along with moderate daytime activity and reduced nighttime activity. This pattern, observed in all areas, is linked to the biological and ecological characteristics of the species and is likely minimally influenced by landscape structure, human activities associated with it and other recreational activities, as well as wolf predation activity. Spring provides abundant easily digestible vegetation, which is essential for roe deer to regain body condition after the winter months. As rumination takes up less time than grazing, they spend a significant amount of time foraging for fresh grasses, herbs, and other plant matter (Danilkin and Hewison 1996). Moreover, roe deer engage in various activities related to mating and reproduction; bucks, in particular, engage in rutting behaviour, which involves marking territory, vocalizing, and fighting with rival males to establish dominance and, consequently, access to females for mating (Danilkin and Hewison 1996). Therefore, regarding the spring season, it seems that roe deer activity rhythms are more regulated by endogenous factors rather than exogenous ones.

In general, the diurnality and nocturnality levels of roe deer appear to be closely linked to seasonal variations, as previously discussed, as well as habitat characteristics. Our findings underscore a significant relationship between diurnality or nocturnality levels and the abundance of cultivated lands, exhibiting opposite trends. Cultivated areas not only offer plentiful and concentrated food resources but also provide suitable spaces conducive to daytime activities, potentially reducing predation risk for roe deer. Indeed, these spaces may offer both visibility and freedom of movement and adequate cover, allowing roe deer to efficiently scan their surroundings for potential threats or predators while engaging in daytime activities such as feeding, grooming, and social interactions.

The alternation between open and closed habitats represents a dynamic response of roe deer to changing environmental conditions and ecological pressures, which exhibit marked seasonal variations (De Groeve et al. 2023). The observed pattern, with diurnality levels, especially during spring and summer, positively related to the abundance of cultivated lands, and nocturnality levels, especially during autumn and winter, negatively related to the abundance of cultivated lands, aligns with findings from other studies. For instance, Bonnot et al. (2013) demonstrated that roe deer may access open areas during the daytime, both as a source of food and cover, particularly in summer when crops are abundant in the fields and can also provide hiding cover.

Supplementary Material 5.2

S.1 - Deviation from the uniform distribution in the 24-hour of seasonal roe deer activity rhythms in north-western Italy

The diel activity patterns of the roe deer showed a significant deviation from uniform distribution in almost all samples, except for the winter season in LAR study area, the summer season in ORB study area, and the summer and autumn seasons in STA study area.

Results of Watson's test (U^2) used to evaluate the uniformity of obtained seasonal activity rhythms

Study area	**Spring**		**Summer**		**Autumn**		**Winter**	
	U^2	**p**	**U^2**	**p**	**U^2**	**p**	**U^2**	**p**
LAR	0.28	< 0.010	0.22	< 0.025	0.22	< 0.050	0.09	> 0.100
BNM	0.73	< 0.010	0.45	< 0.010	0.32	< 0.010	0.36	< 0.010
MON	0.42	< 0.010	0.80	< 0.010	0.26	< 0.025	0.29	< 0.010
ORB	0.68	< 0.010	0.11	> 0.100	0.58	< 0.010	1.19	< 0.010
COP	0.49	< 0.010	0.41	< 0.010	0.40	< 0.010	0.78	< 0.010
TID	0.54	< 0.010	0.54	< 0.010	0.27	< 0.010	0.35	< 0.010
STA	0.41	< 0.010	0.12	> 0.100	0.18	> 0.050	0.51	< 0.010

S.2 - Consistency of roe deer activity rhythms across seasons within the study areas located in north-western Italy

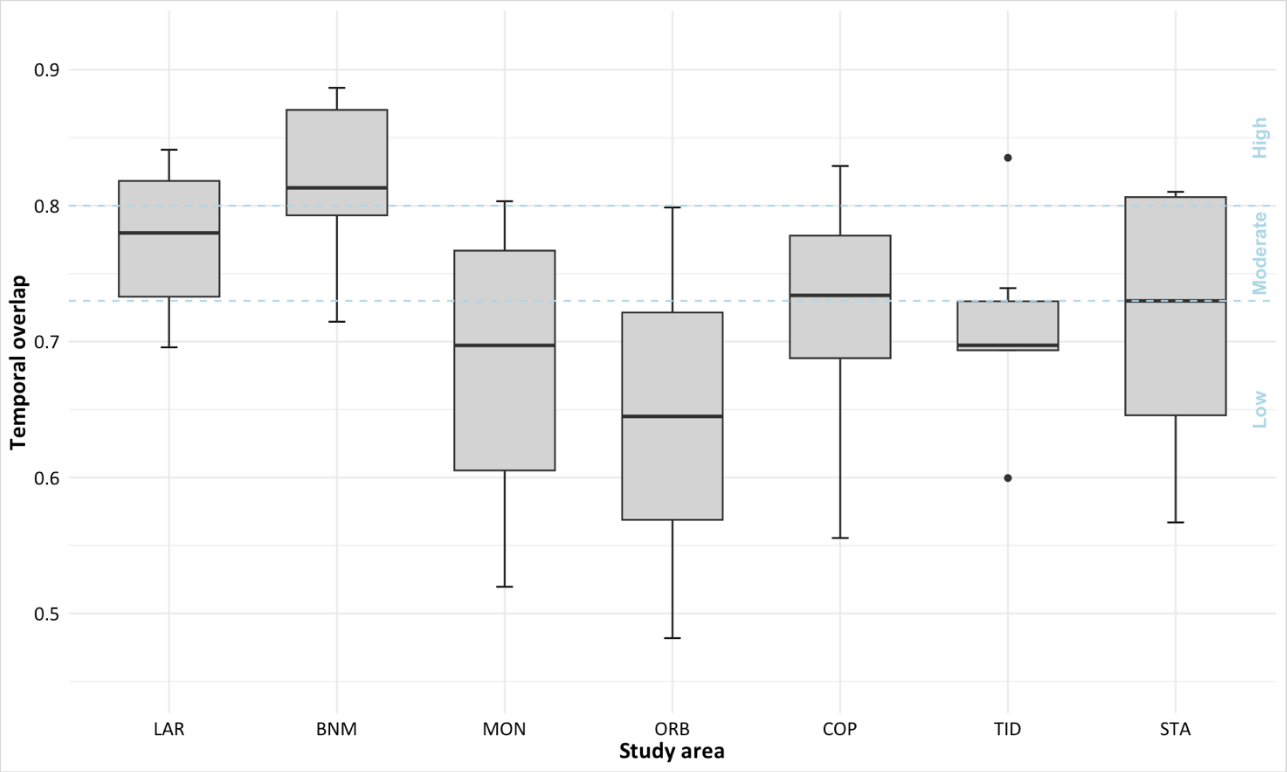
Comparing activity rhythms across different seasons revealed notable differences in most study areas, except for LAR and BNM, where the activity rhythms remained consistent and uniform. The coefficient of overlap (Δ) showed moderate-to-high overlap between seasonal pairwise comparisons in these areas. In contrast, the other study areas exhibited high seasonal variability, as indicated by many significant pairwise comparisons and moderate-to-low seasonal overlap.

Results of Mardia-Watson-Wheeler test (W_g) and post-hoc Watson's two-sample test (two-sample U^2), with the Bonferroni correction to p-values, used to evaluate the uniformity of obtained activity rhythms

Study area	**W_g**	**p**	**Pairwise comparisons**	**U^2**	**p**
LAR	10.74	0.097			
BNM	9.57	0.144			
MON	37.33	< 0.001	SU vs. SP	0.71	< 0.001
			SU vs. WI	0.70	< 0.001

Study area	W_g	p	Pairwise comparisons	U^2	p
ORB	56.90	< 0.001	WI vs. SP	0.63	< 0.001
			WI vs. SU	0.45	< 0.001
			WI vs. AU	1.60	< 0.001
COP	32.98	< 0.001	SU vs. SP	0.31	< 0.01
			SU vs. AU	0.38	< 0.01
			SU vs. WI	0.72	< 0.001
TID	53.96	< 0.001	SU vs. SP	0.74	< 0.001
			SU vs. AU	0.51	< 0.001
			SU vs. WI	0.77	< 0.001
			SP vs. WI	0.32	< 0.01
STA	22.65	< 0.001	SU vs. SP	0.28	< 0.01
			SU vs. WI	0.53	< 0.001
			AU vs. WI	0.32	< 0.01

Results of the coefficient of temporal overlap (Δ) used to quantify the seasonal similarity of obtained activity rhythms



S.3 - Consistency of roe deer activity rhythms across the study areas located in north-western Italy within seasons

Results of Mardia-Watson-Wheeler test (W_g) and post-hoc Watson's two-sample test (two-sample U^2), with the Bonferroni correction to p-values, used to evaluate the seasonal uniformity of obtained activity rhythms

Season	W_g	p	Pairwise comparisons	U^2	p
Spring	20.07	0.066			
Summer	48.72	< 0.001	LAR vs. MON	0.41	< 0.001
			BMM vs. TID	0.65	< 0.001
			BNM vs. MON	0.48	< 0.001
			MON vs. TID	0.42	< 0.001
			COP vs. TID	0.39	< 0.001
Autumn	31.20	0.002	ORB vs. COP	0.51	< 0.001
Winter	57.84	< 0.001	LAR vs. ORB	0.54	< 0.001
			LAR vs. COP	0.41	< 0.001
			BNM vs. ORB	0.84	< 0.001
			BNM vs. COP	0.53	< 0.001
			MON vs. ORB	0.67	< 0.001
			MON vs. COP	0.49	< 0.001

S.4 - Model selection for factors influencing diurnality levels of the roe deer in north-western Italy

Models were estimated through generalised linear models (GLMs). The top-ranked models are shown (Δ AICc ≤ 2); for each model, the variables, the number of parameters, AICc, ΔAICc and standardized weight are reported

Model	Variables	K	logLik	AICc	Δ AICc	Weight
Best	**Season + % cover of cultivated lands**	**6**	**-89.37**	**190.95**	**0.00**	**0.36**
2nd	Landscape + Season + % cover of cultivated lands	8	-87.97	192.30	1.35	0.18
3rd	Season + % cover of cultivated lands + % cover of woodlands	7	-89.16	192.60	1.65	0.16
4th	Wolf presence + Season + % cover of cultivated lands	7	-89.23	192.73	1.78	0.15
5th	Season + % cover of cultivated lands + % cover of protected areas	7	-89.25	192.77	1.82	0.15

S.5 - Model selection for factors influencing nocturnality levels of the roe deer in north-western Italy

Models were estimated through generalised linear models (GLMs). The top-ranked models are shown (Δ AICc ≤ 2); for each model, the variables, the number of parameters, AICc, ΔAICc and standardized weight are reported

Model	Variables	K	logLik	AICc	Δ AICc	Weight
Best	**Season + % cover of cultivated lands**	**6**	**-70.71**	**153.62**	**0.00**	**0.45**
2nd	Landscape + Season + % cover of cultivated lands + % cover of natural open areas	7	-70.55	155.38	1.76	0.19
3rd	Wolf presence + Season + % cover of cultivated lands	7	-70.58	155.44	1.82	0.18
4th	Season + % cover of cultivated lands + % cover of protected areas	7	-70.58	155.44	1.82	0.18

5.3 Exploring roe deer behaviour at individual-level: time-budget and factors influencing vigilance

5.3.1 Introduction

When observing how individuals behave during periods of activity, they should mostly engage in feeding or vigilance (Benhaiem et al. 2008; Sönnichsen et al. 2013)

Foraging, i.e. the act of searching for food, and food intake, i.e. the consumption of food, are crucial for sustenance and require a significant time investment dependent on both the quantity and quality of available resources (Danilkin and Hewison 1996).

Vigilance refers to a state of heightened alertness which entails carefully monitoring the surrounding environment (Quenette 1990) through sight, hearing, and/or smell to detect and, consequently, respond to potential threats quickly, enhancing the chances of survival (Beauchamp 2015).

For the roe deer, which is a prey species (see Section 3.1.3), vigilance is crucial. The time allocated to this behaviour increases with the perceived risk level (Benhaiem et al. 2008, Sönnichsen et al. 2013, Popova et al. 2016). Consequently, the time spent vigilant may vary both seasonally (Benhaiem et al. 2008; Sönnichsen et al. 2013; Bonnot et al. 2013) and daily (Sönnichsen et al. 2013). The average number of times per day that roe deer exhibit behaviours in response to a perceived risk seems to be directly correlated with the level of disturbance; where disturbance is very marked, especially during the hunting season, the normal daily activity breaks down (Danilkin and Hewison 1996). In particular, hunting leads to increased vigilance not only during the day, when hunters are active, but also during the night (Popova et al. 2016). However, this increased vigilance comes at the expense of feeding time, as these activities are mutually exclusive or cannot be performed simultaneously without compromising efficiency (Lima 1987).

To optimise the balance between feeding and vigilance, a possible strategy is to process food (i.e. chew) during vigilance bouts. This cost-free vigilance increases with increasing food density because of the decreasing of time used to forage. However, when the perceived risk level is particularly high, animals need to perform exclusive vigilance (Benhaiem et al. 2008).

Usually under these circumstances, the behavioural antipredator responses of the roe deer are manifested through an extended vigilance behaviour (Benhaiem et al. 2008, Sönnichsen et al. 2013, Popova et al. 2016, Bonnot et al. 2020). Some common changes in vigilance behaviour may include, for example, an increase in frequency and intensity of scanning the surroundings, frequent movements between different locations, seeking areas perceived as safe or avoiding areas perceived as risky and general heightened alertness.

Another strategy is to form larger groups. This allows for the distribution of vigilance among a greater number of individuals, thus reducing individual time spent being vigilant (Sönnichsen et al. 2013). Indeed, with increasing group size, a general decrease in vigilance behaviour for each individual, combined with time gained for forage intake, has been observed (Lark and Slade 2008).

Aims - In light of the intricate balance between feeding and vigilance behaviour in response to perceived risk, this section aims to investigate how human disturbance, represented by landscape structure, and predator presence influence individual behaviour in the roe deer. In particular, high human disturbance and predator presence are expected (i) to lead to a shift in roe deer time-budget, prioritizing vigilance over feeding, and (ii) to induce roe deer to allocate more time to vigilance.

5.3.2 Material and methods

Data collection - Data regarding roe deer individual behaviour were collected using two non-invasive sampling methods, which were i) camera trapping and ii) direct observation from vantage points. Camera trapping was carried out following the sampling protocol already explained (cfr Section 4.3.1 - Data collection). Among the recorded camera trapping events, only those, regarding the same individual, with a minimum duration of 1 min. 30 sec. were considered in further analyses. Direct observations represent the most used method of collecting data aimed at studying animal behaviour, both in studies conducted on animals in captivity and in the wild.

In each study area, during a preliminary field investigation phase, suitable sites for data collection were identified and mapped using QGIS software. These sites were selected based on their visibility on the surrounding open areas, such as grasslands or cultivated fields, and therefore the possibility of observing animals without being seen and without causing disturbance (Mayle et al. 1999). A total of 57 vantage points were randomly chosen (Table 5.3.1).

The vantage points within each study area were visited seasonally, similar to camera trapping sampling. To avoid as much as possible registering the same individual multiple times in a single sampling season, it was chosen not to return to the same site during the same season and not to visit neighbouring sites on consecutive days. Thus, varying as much as possible the observation sites within the study areas and considering that roe deer have relatively stable home ranges (Danilkin and Hewison, 1996), we considered our observations to be statistically independent.

Table 5.3.1 - Details on the sampling sites (i.e. vantage points) used for roe deer direct observations in north-western Italy from 2020 to 2022

Study area	N° of sites	Mean (± SD) Distance between sites (m)
LAR	5	671 ± 613
BNM	8	893 ± 527
MON	3	3124 ± 2844
ORB	12	953 ± 598
COP	3	2326 ± 1827
TID	17	487 ± 164
STA	9	789 ± 764

Direct observations from vantage points were primarily, but not exclusively, conducted during the periods of dawn and dusk. In addition to these time slots, other particularly suitable moments for spotting the species were utilized, such as after the end of summer or spring rains (Perco 2011).

The focal sampling method (Altmann 1974; Martin and Bateson 2010) was employed, which involves observing an individual (or a pair or a group) for a predetermined period to standardize the duration of observations. Specifically, in this study, only one individual at a time was recorded on video for 5 minutes, using the continuous recording method, which allows for documenting all occurrences of different behavioural patterns during the observation period. The selected observational method poses the risk of subjects being obstructed by vegetation or moving out of the observer's field of view. In such cases, video recording was paused until the subject reappeared, and then resumed until the predetermined duration elapsed. If the subject permanently disappeared, attempts were made to locate a new observation subject. In cases where groups of two or more roe deer were observed, only one individual was selected for video recording to avoid correlation between observations. In these situations, the subject was generally chosen randomly or, if visibility conditions were not optimal, the most easily observable individual was selected.

Whenever possible, recorded individuals were identified for sex (male, female) and age (juvenile, sub-adult, adult).

The recordings were made using equipment suitable for observing individuals located even at distances of several hundred meters from the observer, specifically a camera with a 1000X magnification capability. Recorded data, i.e. the locations of observer and observed roe deer, were georeferred with GPS (WGS 84 UTM zone 32N).

Ethogram - The collected videos, obtained both from camera trapping and direct observations from vantage points, were analysed using the BORIS software (Friard and Gamba 2016) to identify the various behaviours exhibited by the species. Observed behaviours were classified according to an experimental ethogram (Table 5.3.2) based on several ethological researches (Turner 1979; Danilkin and Hewison 1996; Gottardi 2011; Reimoser 2012; Kröschel et al. 2017).

An ethogram is a comprehensive inventory of behaviours exhibited by a species; these behaviours are commonly displayed by individuals in their natural environment. Ethograms serve as a standardized reference for researchers to classify and analyse behaviours during observational studies, allowing for consistent interpretation and comparison of behaviour across different individuals, populations, and studies.

Table 5.3.2 - Experimental ethogram of the roe deer

Behaviours	Modifiers	Description
Moving	Walking Trotting Galloping	The roe deer moves through space using various gaits
Resting	Lying Standing	The deer lies on the ground or stands (without vigilance behaviour)
Feeding	On the ground On branches Drinking	The deer is browsing the ground or higher vegetation (trees or shrubs) or the deer is drinking
Elimination	Urination Defecation	The deer is urinating or defecating
Grooming	-	The deer uses either its hindlegs or its mouth to scratch its body
Alarm	Vigilance	The deer stands, the head held high above the ground, ears pricked up and oriented in the same direction of the eyes and sniffs the air. The deer walks while looking around, twitching the ears or walking tip-toe (carefully placing the feet)
	Sudden rush	The roe deer suddenly flees
	Barking	The roe deer emits an alarm vocalization in response to an external disturbance
Marking	Scraping	The buck scrapes the ground with the forelegs
	Rubbing	The buck rubs with his foreheads against the tree, freys the bark with antlers and sniffs the frayed spot
	Barking	The buck barks during marking
Exploration	Sniffing	The deer sniffs the air, the ground or an object

Social interaction	Agonistic	e.g. Parallel walking, Fighting, … [between bucks];
	Mating	e.g. Anogenital inspection (buck sniffs and/or licks the genital area of a female; often followed by flehmen); Chasing (buck is directly following a female within 1–10 m distance in fast walk, trot or gallop); Mounting
	Affiliative	e.g. Nose contact (one individual approaches another one and makes nose contact, either to the head or torso region); Grooming (one individual is nibbling the fur of another one with its mouth)
Interspecific interaction		The deer interacts with an individual of another species

Vigilance - Among the identified behaviours, particular attention was given to vigilance, which is an alarm behaviour representing an important antipredator response for the roe deer; its expression should therefore be conditioned by the perceived level of risk. When exhibiting vigilance, the animal remains watchful, scanning its surroundings for any signs of predators or other disturbances. This behaviour typically includes frequent head movements, scanning movements with the eyes, and sometimes freezing in place to better assess the situation (Danilkin and Hewison 1996). Vigilance allows the animal to detect and respond quickly to potential threats, helping to enhance its chances of survival.

For the vigilance behaviour, the mean duration (in seconds), the total duration (as a percentage of the total video duration), and the inter-event interval (in seconds) were calculated for each collected video. These three metrics provide different information regarding the vigilance behaviour contributing to a more detailed characterization of the roe deer time budget:

i) the mean duration provides information about the average length of time spent on the specific behaviour by each individual; in other words, it helps understand the intensity of the behaviour within the observation period;

ii) the total duration gives an overall measure of the proportion of time spent on the specific behaviour relative to the total observation period; it offers insight into the relative importance or prevalence of the behaviour compared to other behaviours observed;

iii) the inter-event interval reflects the time elapsed between consecutive occurrences of the behaviour and indicates how frequently the behaviour is interrupted by other activities or events.

These metrics were compared, using non-parametric tests (i.e. Mann-Whitney test or Kruskal-Wallis test with Dunn test for pairwise comparisons), among the different seasons, the age classes and sexes.

Moreover, the study areas were grouped according to landscape composition into three groups (natural, mixed, and modified) and the stable presence of the wolf into two groups (cfr Chapter 2).

Thus, to better assess the influence of human disturbance and predator presence, the same metrics were compared, using non-parametric tests (i.e. Mann-Whitney test or Kruskal-Wallis test with Dunn test for pairwise comparisons), among the reported groups.

We used generalised linear models (GLMs) to examine the relationship between the three metrics and several environmental variables. These variables included season (spring, summer, autumn, winter), landscape composition (natural, mixed, modified), predator presence (stable, not stable), protected area (i.e. no hunting zone), and the distance (in meters) of each observation to woodlands, natural open areas (e.g. grasslands, pastures, shrublands), cultivated lands, and urban areas. Additionally, we incorporated the method used for data collection (direct observation or camera trapping) as a proxy for habitat type (open or closed, respectively). We chose not to incorporate group size as a variable in the model due to incomplete data from camera trapping, where it's uncertain whether the entire group was captured. Additionally, group sizes observed directly did not vary between study areas. We first formulated global models considering all the predictors; then we selected the best models based on AICc scores (models with Δ AICc ≤ 2; Burnham and Anderson 2002), ignoring redundant models (e.g. more complex versions of any simpler model).

We performed GLMs using the “stats”, “MuMIn” (Bartoń 2023), and “visreg” (Breheny and Burchett 2017) packages in R (R Core Team 2023).

5.3.3 Results

Collected data - Direct observations from vantage points yielded 112 direct videos of roe deer, whereas camera trapping yielded 156 indirect videos scattered among the study areas (Table 5.3.3).

Table 5.3.3 - Number of direct and indirect (i.e. camera trapping events) videos collected and analysed to study roe deer behaviour in north-western Italy from 2020 to 2022

Study area	N° of direct videos	N° of indirect videos	N° of total videos	Total recording time
LAR	10	17	27	83 min. 56 sec.
BNM	17	35	52	195 min. 0 sec.
MON	0	20	20	47 min. 57 sec.
ORB	21	23	44	136 min. 20 sec.
COP	3	16	19	56 min. 32 sec.
TID	34	24	58	204 min. 9 sec.
STA	27	21	48	154 min. 19 sec.

The recorded videos featured 268 roe deer individuals: 80 adult males, 27 sub-adult males, 20 juvenile males, 2 males of undetermined age, 57 adult females, 46 sub-adult females, 29 juvenile females, 3 females of undetermined age, and 4 juveniles of undetermined sex, distributed across the different study areas.

Time-budget - The analysis of collected videos provided insight into how roe deer allocated their time among the various behaviours outlined in the ethogram.

All behaviours and relative modifiers outlined in the ethogram were observed at least once in the collected videos, except barking. Overall, feeding and alarm behaviours were the predominant behaviours in which roe deer invested the majority of their time across all study areas. Specifically, feeding emerged as the most frequent behaviour, constituting 43.0% of the observed time, while alarm behaviours accounted for approximately half of that time, at 21.8%.

Even when considering the study areas separately, feeding generally outweighs alarm behaviour. However, exceptions are observed in MON and COP study areas, where alarm behaviours marginally exceed feeding ones (Table 5.3.4).

Table 5.3.4 - Frequencies of occurrence (%) of roe deer behaviours observed in north-western Italy from 2020 to 2022

	Behaviour										
Study area	**Moving**	**Resting**	**Feeding**	**Elimination**	**Grooming**	**Alarm**	**Marking**	**Exploration**	**Social interaction**	**Interspecific interaction**	**Not visible**
LAR	7.4	1.5	47.0	0.0	12.0	15.0	0.2	8.4	0.0	0.0	8.4
BNM	2.6	8.5	47.5	0.5	4.8	21.8	0.1	8.7	0.7	0.0	4.8
MON	4.6	8.2	28.2	0.6	4.1	29.9	3.0	15.5	0.0	0.0	5.9
ORB	10.7	6.9	36.4	0.6	5.8	24.1	0.2	8.3	1.9	0.0	5.2
COP	3.3	6.3	26.3	0.0	3.7	30.8	0.6	16.9	4.9	0.0	7.2
TID	6.2	4.5	44.8	0.2	7.0	22.8	0.3	8.9	0.2	0.1	5.0
STA	7.6	3.9	49.4	0.0	6.1	16.3	1.6	7.0	0.9	0.0	7.3
Total	**6.2**	**5.7**	**43.0**	**0.3**	**6.3**	**21.8**	**0.6**	**9.3**	**1.0**	**0.0**	**5.9**

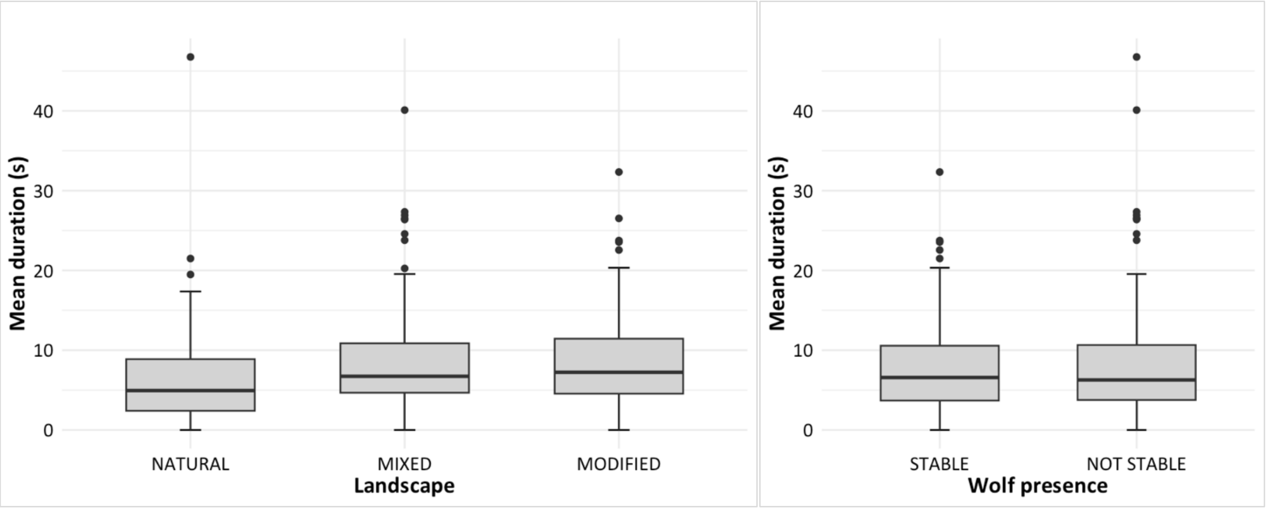
Vigilance - Vigilance behaviour was observed in all study areas, but not in all sampled individuals; the total number of individuals showing vigilance was 246 out of 268 deer. Specifically, it was observed in 22 out of 27 deer in LAR (81.5%), 47 out of 52 in BNM (90.4%), 19 out of 20 in MON (95.0%), 43 out of 44 in ORB (97.7%), 18 out of 19 in COP (94.7%), 56 out of 58 in TID (96.6%), and 41 out of 48 in STA (85.4%).

The mean duration of vigilance behaviour, considering the total number of observed individuals, was 7.98 seconds (± 0.41 SE), and the time allocated to this behaviour accounted for an average of 20.4% (± 1.1 SE) of the individual total recording time. The mean inter-event interval between vigilant events was 24.13 seconds (± 1.56 SE) (Supplementary Material 5.3: S.1).

All three metrics showed no variation between seasons, age classes or sexes.

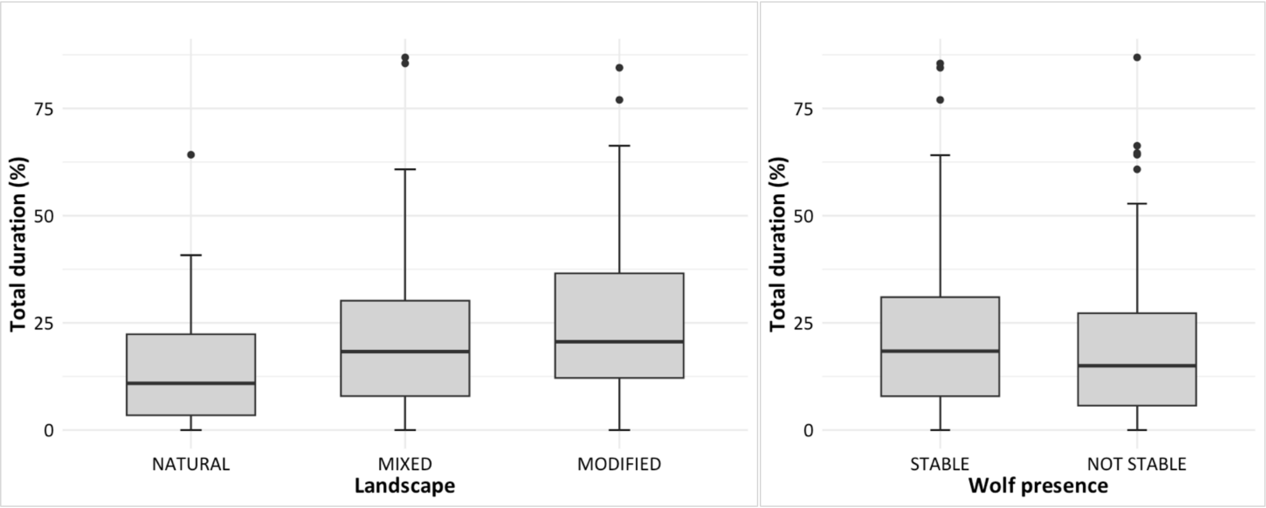
The mean duration (s) of vigilance behaviour varied significantly among the different landscapes ($H = 11.12$; $p = 0.004$); in particular, it was significantly lower in more natural landscapes (LAR and STA) compared to highly modified landscapes (MON, ORB, and COP) ($Z = 3.17$; $p = 0.005$) and also compared to mixed landscapes (BNM and TID) ($Z = 2.63$; $p = 0.025$). Conversely, this metric showed no significant difference between wolf presence groups (Figure 5.3.1).

Figure 5.3.1 - Mean duration (s) of vigilance behaviour exhibited by roe deer in the different study area groups (landscape and wolf presence) in north-western Italy



Similarly, the total duration (%) of vigilance behaviour varied significantly among the different landscape groups ($H = 21.80$; $p < 0.0001$); in particular, it was significantly lower in more natural landscapes (LAR and STA) compared to highly modified landscapes (MON, ORB, and COP) ($Z = 6.63$; $p < 0.0001$) and also compared to mixed landscapes (BNM and TID) ($Z = 3.09$; $p = 0.006$). This metric showed no significant difference between wolf presence groups (Figure 5.3.2).

Figure 5.3.2 - Total duration (%) of vigilance behaviour exhibited by roe deer in the different study area groups (landscape and wolf presence) in north-western Italy



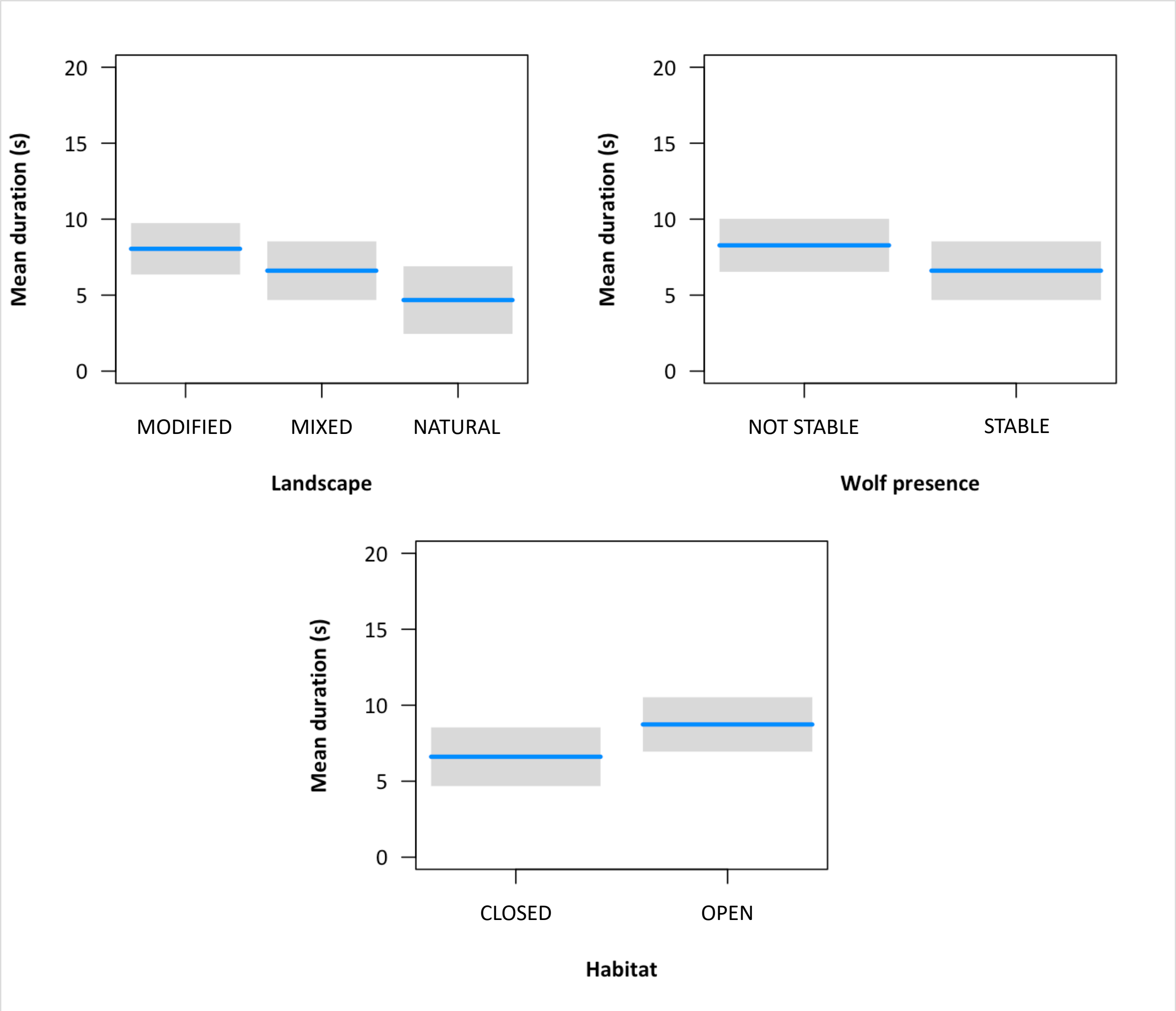
The inter-event interval (s) of vigilance behaviour did not vary according to landscapes or wolf presence.

Among the models obtained to describe the mean duration (s) of roe deer vigilance behaviour (Supplementary Material 5.3: S.2), the best model revealed variations in the average time allocated to this behaviour based on landscape type, predator presence, and habitat type. Specifically, durations decreased from modified to natural landscapes. Additionally, lower durations were observed in areas characterized by stable wolf presence. Furthermore, vigilance durations were higher in open habitats compared to closed ones (Table 5.3.5 and Figure 5.3.3).

Table 5.3.5 - Factors influencing the mean duration of vigilance behaviour of the roe deer in north-western Italy. Estimates of model coefficients (B), standard errors (SE), 95% confidence intervals (95% CIs), and p-values are shown

Variable	**B**	**SE**	**95% CIs**	**p**
(Intercept)	9.719	1.043	7.67; 11.76	< 0.001
Landscape (mixed)	-1.439	1.083	-3.56; 0.68	0.185
Landscape (natural)	-3.373	1.246	-5.82; -0.93	0.007
Wolf presence (stable)	-1.669	0.951	-3.53; 0.19	0.081
Habitat (open)	2.126	0.969	0.23; 4.02	0.029

Figure 5.3.3 - Factors influencing the mean duration of vigilance behaviour of the roe deer in north-western Italy. Response curves and 95% confidence intervals estimated through generalized linear models

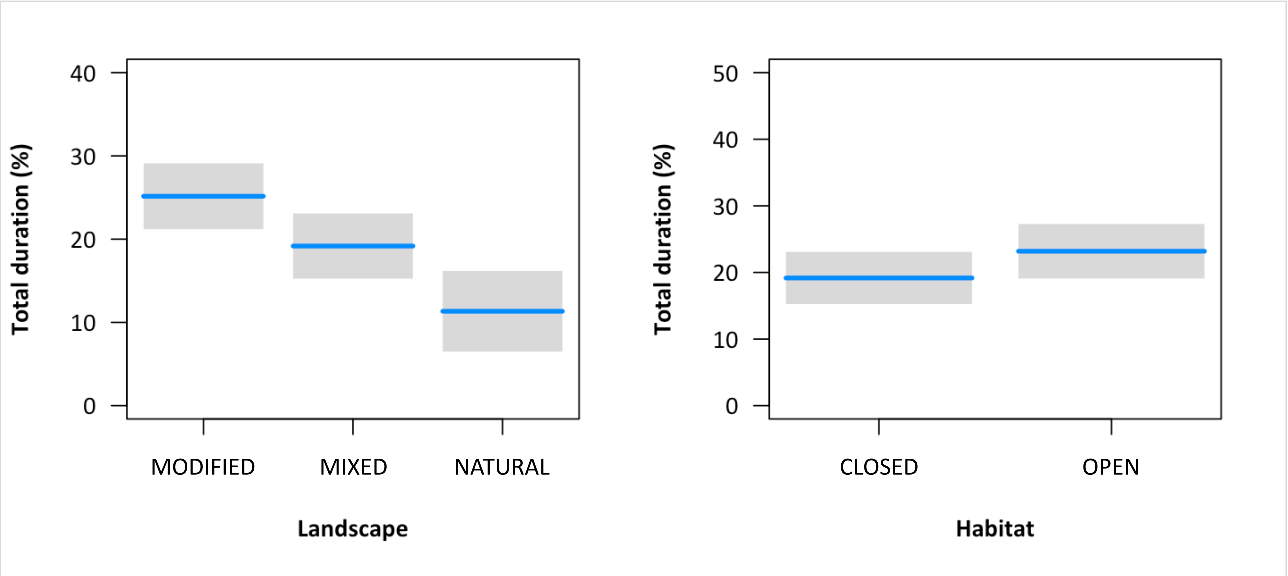


Similarly, among the models obtained to describe the total duration (%) of vigilance behaviour (Supplementary Material 5.3: S.3), the best-performing model revealed that the proportion of time allocated to this behaviour, relative to the total observation period, was influenced by landscape and habitat type. Specifically, this metric decreased from modified to natural landscapes and was higher in open habitats compared to closed ones (Table 5.3.6 and Figure 5.3.4).

Table 5.3.6 - Factors influencing the total duration of vigilance behaviour of the roe deer in north-western Italy. Estimates of model coefficients (B), standard errors (SE), 95% confidence intervals (95% CIs), and p-values are shown

Variable	**B**	**SE**	**95% CIs**	**p**
(Intercept)	25.148	2.019	21.19; 29.11	< 0.001
Landscape (mixed)	-5.979	2.609	-11.09; -0.87	0.023
Landscape (natural)	-13.812	3.037	-19.76; -7.86	< 0.001
Habitat (open)	4.013	2.352	-0.60; 8.62	0.089

Figure 5.3.4 - Factors influencing the total duration of vigilance behaviour of the roe deer in north-western Italy. Response curves and 95% confidence intervals estimated through generalized linear models

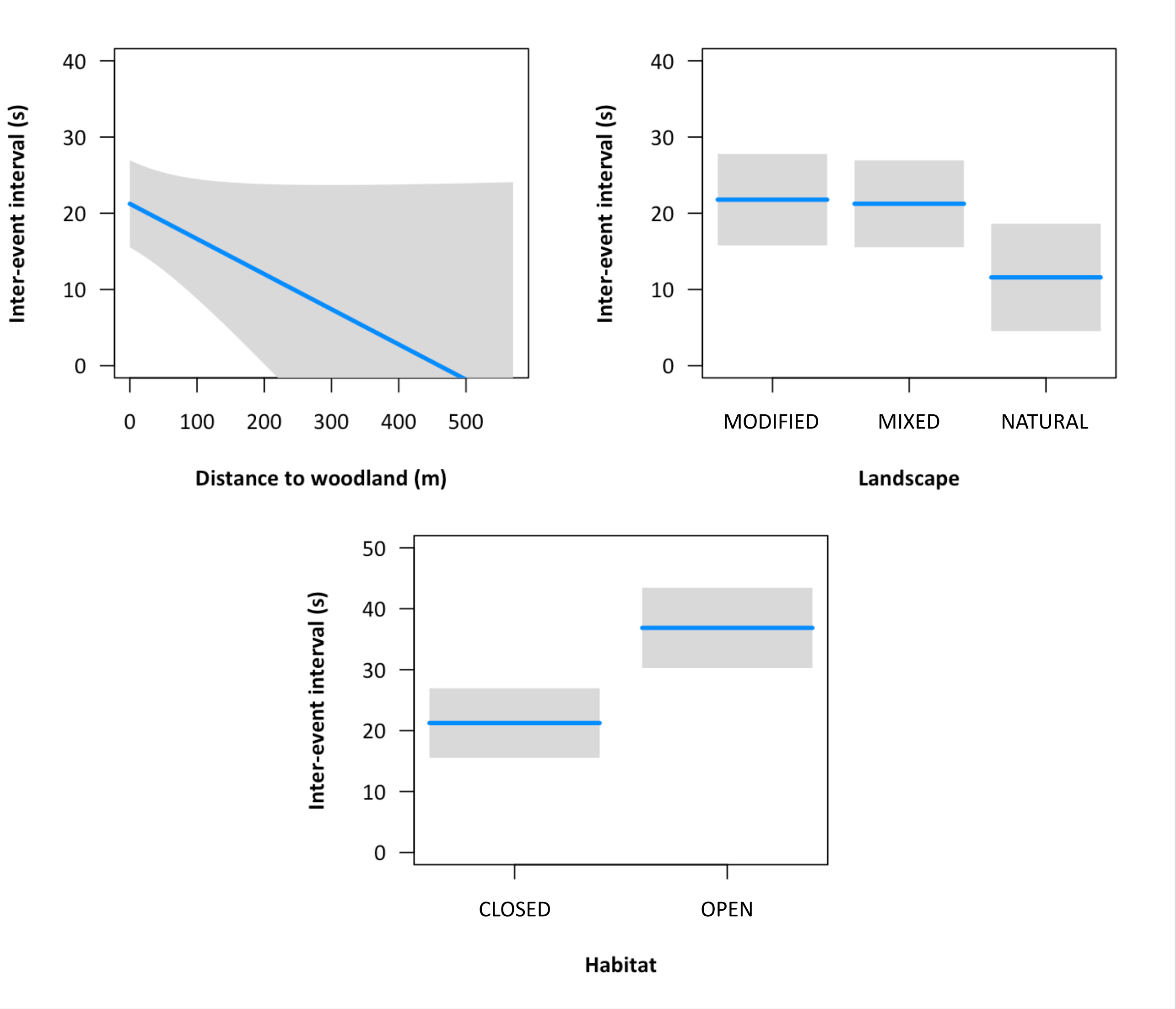


Among the models obtained to describe the inter-event interval (s) of vigilance behaviour (Supplementary Material 5.3: S.4), the first and best model showed that the time interval between successive vigilance events was influenced by the distance to woodlands, landscape composition, and habitat type. Specifically, the inter-event interval showed a negative correlation with the distance to woodlands and was lower in more natural landscapes and closed habitat types (Table 5.3.7 and Figure 5.3.5).

Table 5.3.7 - Factors influencing the inter-event interval of vigilance behaviour of the roe deer in north-western Italy. Estimates of model coefficients (B), standard errors (SE), 95% confidence intervals (95% CIs), and p-values are shown

Variable	**B**	**SE**	**95% CIs**	**p**
(Intercept)	21.780	3.058	15.79; 27.77	< 0.001
Distance to woodlands (m)	-0.046	0.025	-0.09; 0.00	0.068
Landscape (mixed)	-0.539	3.951	-8.28; 7.21	0.891
Landscape (natural)	-10.196	4.541	-19.10; -1.29	0.026
Habitat (open)	15.619	3.820	8.13; 23.11	< 0.001

Figure 5.3.5 - Factors influencing the inter-event interval of vigilance behaviour of the roe deer in north-western Italy. Response curves and 95% confidence intervals estimated through generalized linear models



5.3.4 Discussion

As expected from published literature (Benhaiem et al. 2008; Sönnichsen et al. 2013), our results confirmed that roe deer use to spend most of their time feeding or vigilant, with feeding being predominant in almost all areas except for MON and COP study areas; both these areas are predominantly characterized by extended and intensive agricultural lands. In these areas, the prevalence of vigilance behaviour over feeding could be linked to a higher perceived risk associated with human activity.

Comparisons between landscapes revealed a significant difference in the mean duration of individual vigilance and the total percentage of time dedicated to this behaviour between more natural landscapes, with lower values, compared to highly modified and mixed landscapes. Both these metrics can be considered indicators of the perceived risk by roe deer, as anti-predator strategy generally involves increased time devoted to vigilance behaviour, which can manifest as either longer bouts or higher frequency of vigilance leading to an overall increase in time spent in such behaviour. These findings can thus be interpreted as a signal of lower perceived risk in more natural landscapes, with extended woodlands, compared to the other areas characterized by higher levels of anthropogenic disturbance.

As predicted by the risk allocation hypothesis (Lima and Bednekoff 1999), the roe deer should allocate high levels of vigilance to situations of perceived high risk. In this regard, the observed differences seem to be more related to anthropogenic disturbance across the different areas rather than the presence of the large predator; indeed, comparing groups of study areas according to the type of landscape and the presence of the wolf, significant results emerged only for landscapes. Both the mean duration of individual vigilance and the total percentage of time dedicated to this behaviour showed increasing values along the gradient from the least disturbed to the most modified landscapes.

These findings may be associated with (i) the structures of the landscapes, (ii) the heightened anthropogenic disturbances in more modified landscapes, or (iii) the increased predictability of risk within these landscapes.

Human-modified landscapes typically consist of open habitats, primarily extensive agricultural areas where vegetation, whether seasonal crops or permanent cultivation, only seasonally offers adequate cover for roe deer. Indeed, in the case of certain seasonal crops, the cover provided is adequate only for a few weeks before harvesting. Habitat plays a crucial role in influencing vigilant behaviour: in open habitats, where visibility is high and cover is scarce, animals may need to be more vigilant to detect and respond to potential threats. This has been extensively studied, for example, in the red deer. The individuals not only showed an increased vigilance according to vegetation cover, but also modified their posture (lying vs. standing) and movements (moving vs. resting) while vigilant (Jayakody et al. 2008).

Human-modified landscapes are, by definition, constantly frequented by humans. Vehicle traffic, agricultural practices, and recreational activities represent a continuous source of disturbance, at least during daylight hours, for animals. For example, vehicular traffic, including cars, trucks, and motorcycles, extends along the roads and highways that traverse these landscapes. The noise generated by traffic, along with the movement of vehicles, can startle and disturb wildlife. Agricultural practices represent a diversified source of disturbance including loud noises, vibrations from machinery, and changes in vegetation cover, which can affect the behaviour of animals. Lastly, recreational activities, such as hiking and biking, also contribute to disturbances being caused by direct encounters with humans.

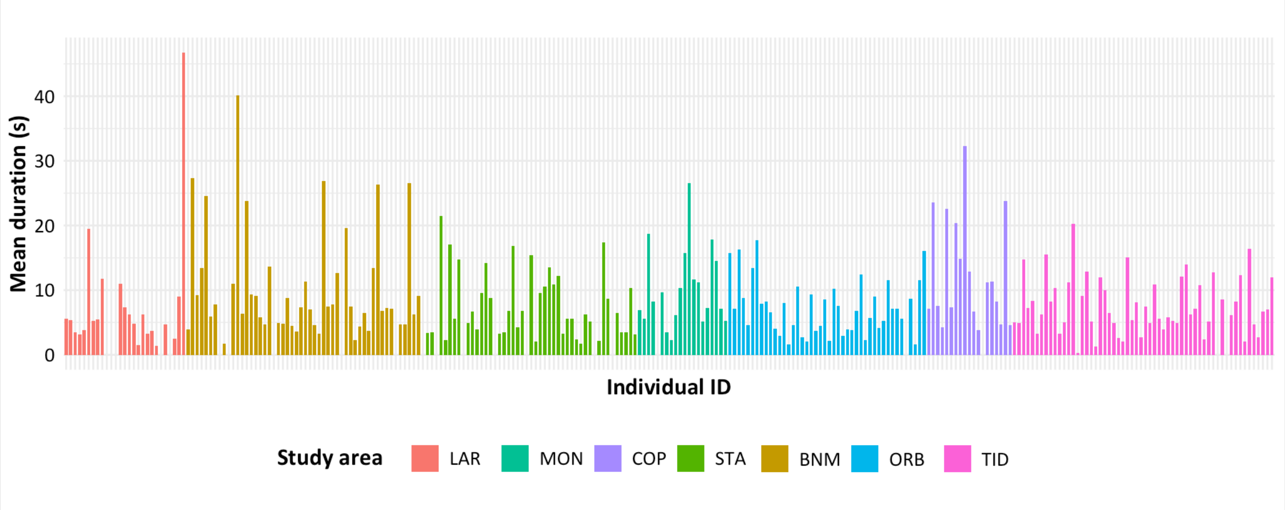
Human-induced risk is highly predictable within the landscape, both at temporal and spatial scales (Proudman et al. 2021). Indeed, human activities often follow regular daily and seasonal patterns and tend to concentrate in certain areas within the landscape, making it easier for wildlife to anticipate when human-induced risk is highest. On the contrary, predatory risk is highly unpredictable because encounters with predators can occur sporadically and unexpectedly. Thus, antipredator responses should be proactive, i.e. in response to an a priori assessment of the level of risk, and reactive, i.e. in response to an immediate threat (Creel 2018), respectively.

Interestingly, we did not observe differences in vigilance between sexes or age classes; even if results were not consistent across previous studies, some authors observed sex- or age-related differences in deer species (Pecorella et al. 2019 and references therein).

Supplementary Material 5.3

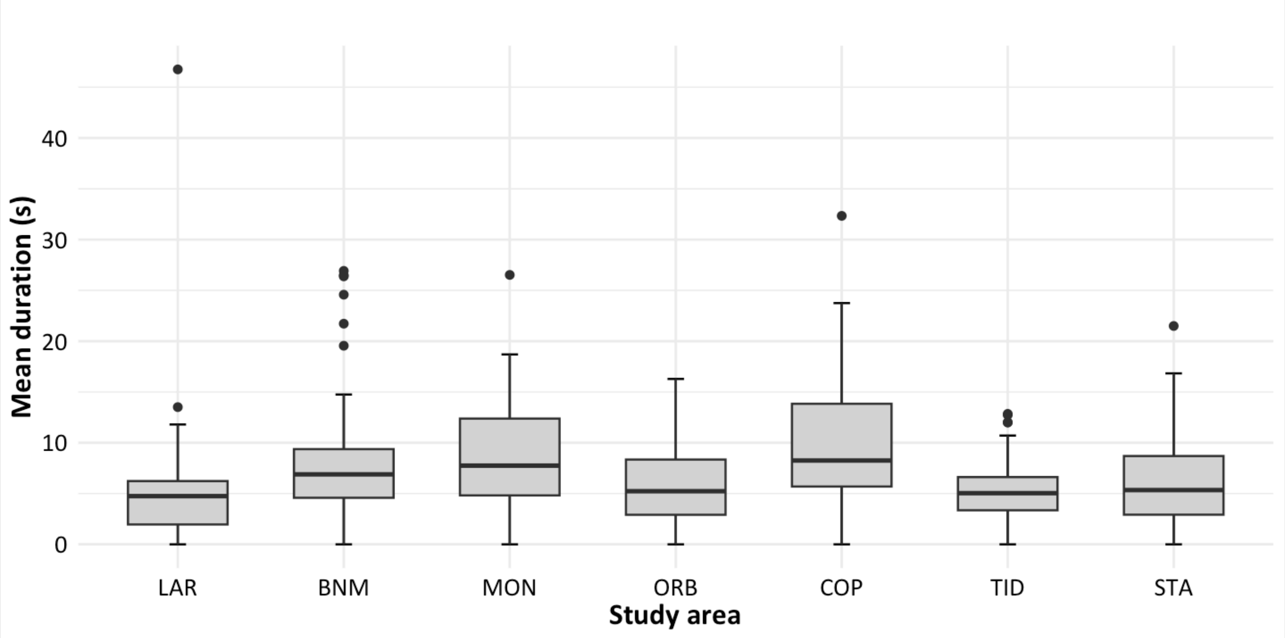
S.1 - Vigilance behaviour exhibited by roe deer in the different study areas in north-western Italy

Mean duration (s) of vigilance behaviour exhibited by observed individuals (n = 268) in north-western Italy

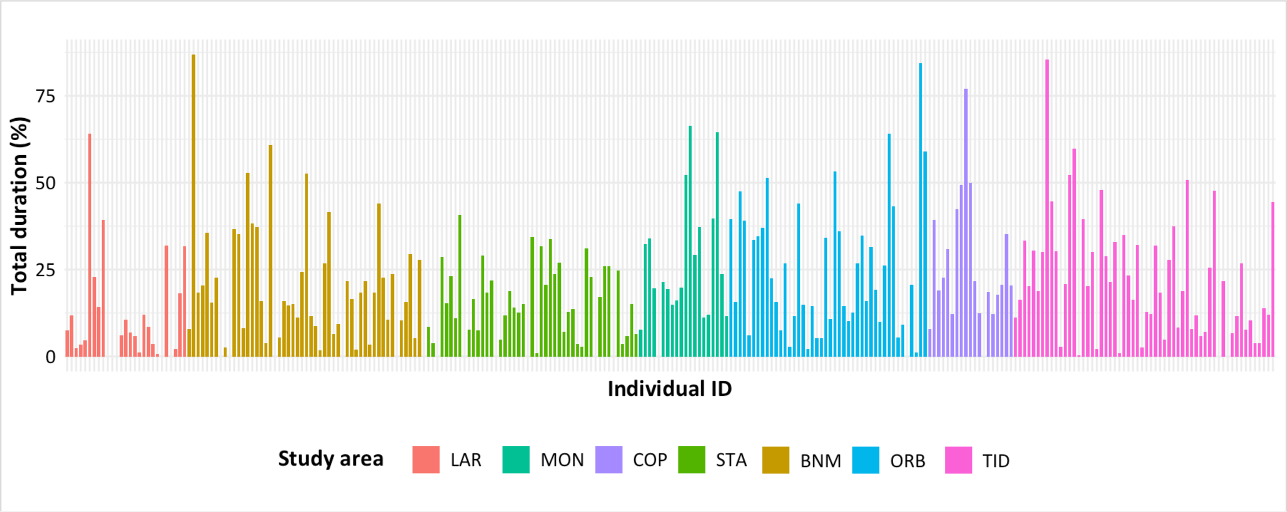


The mean duration (s) of vigilance behaviour varied significantly among the different study areas (H = 20.72; P = 0.002); notably, it was significantly greater in COP compared to LAR (Z = -3.20; P = 0.028).

Mean duration (s) of vigilance behaviour exhibited by roe deer in the different study areas

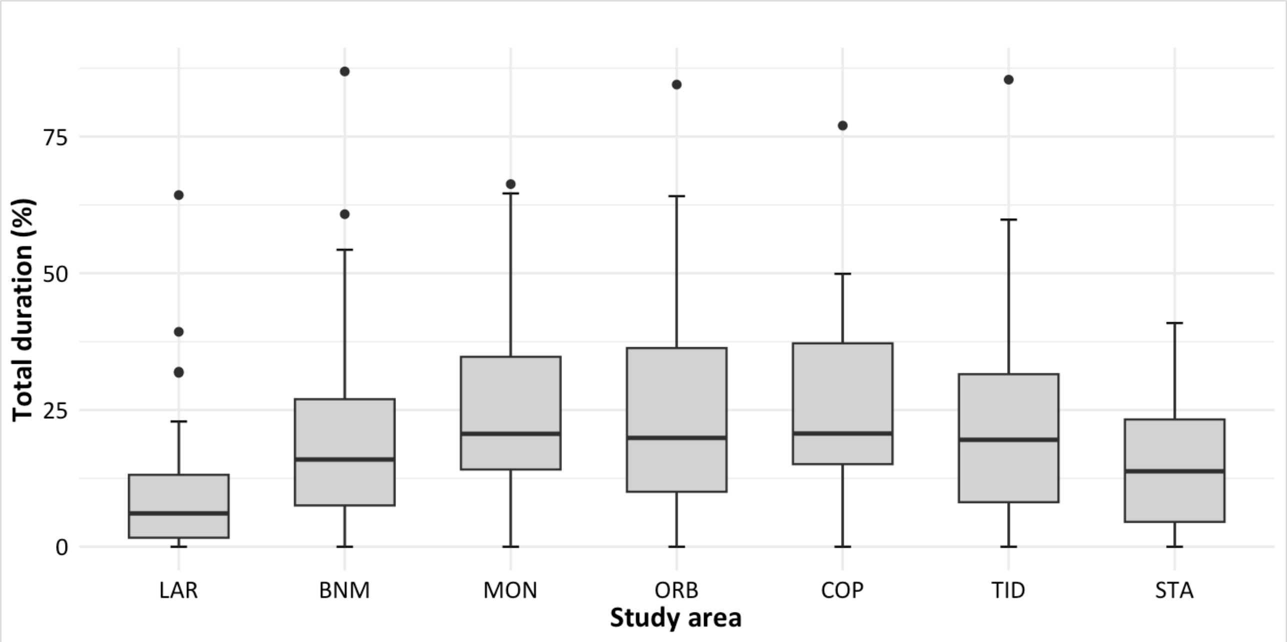


Total duration (%) of vigilance behaviour exhibited by observed individuals (n = 268) in north-western Italy

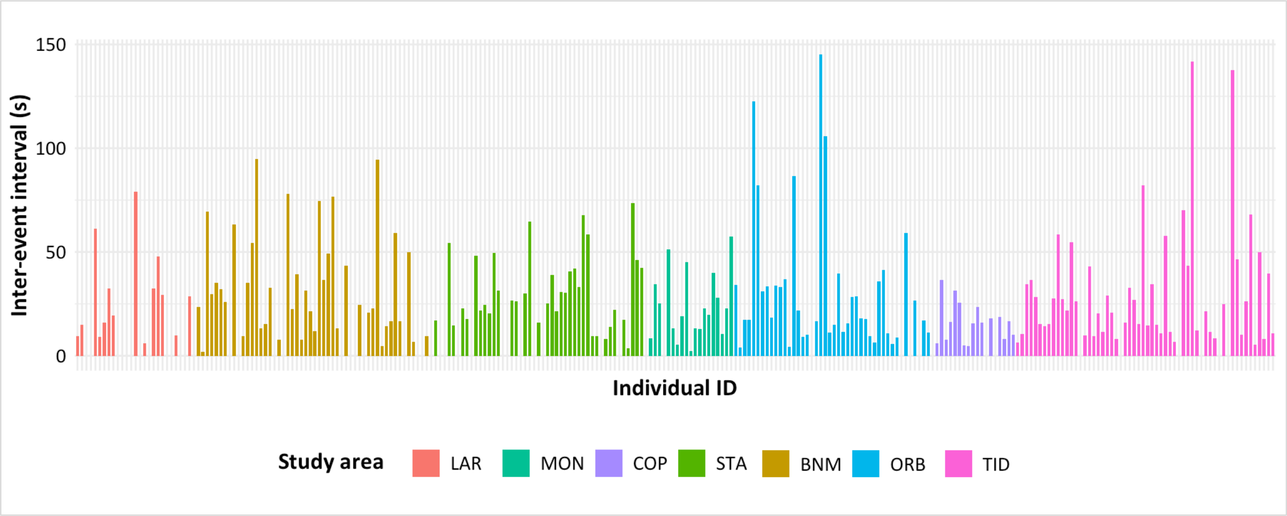


Even when considering the total duration (%) of the behaviour, significant differences were found among the various areas (H = 25.13; P < 0.001); values recorded in LAR were lower compared to those in MON (Z = -3.54; P = 0.008), ORB (Z = -3.68; P = 0.005), COP (Z = -3.54; P = 0.009), and TID (Z = -3.31; P = 0.019).

Total duration (%) of vigilance behaviour exhibited by roe deer in the different study areas



Total duration (%) of vigilance behaviour exhibited by observed individuals (n = 268) in north-western Italy



Conversely from the previous metrics, the inter-event interval (s) of vigilance behaviour did not vary among the different study areas.

S.2 - Model selection for factors influencing the mean duration of vigilance behaviour of the roe deer in north-western Italy

Models were estimated through generalised linear models (GLMs). The top-ranked models are shown (Δ AICc ≤ 2); for each model, the variables, the number of parameters, AICc, ΔAICc and standardized weight are reported

Model	Variables	K	logLik	AICc	Δ AICc	Weight
Best	**Landscape + Wolf presence + Habitat**	**6**	**-790.38**	**1593.12**	**0.00**	**0.15**
2nd	Protected area + Habitat	4	-792.73	1593.63	0.51	0.11
3rd	Distance to urban areas + Landscape + Wolf presence + Habitat	7	-789.71	1593.91	0.78	0.10
4th	Protected area + Landscape + Habitat	6	-790.82	1594.01	0.89	0.09
5th	Distance to woodlands + Landscape + Wolf presence + Habitat	7	-789.81	1594.11	0.98	0.09
6th	Landscape + Habitat	5	-791.94	1594.14	1.02	0.09
7th	Distance to urban areas + Landscape + Habitat	6	-791.02	1594.41	1.28	0.08
8th	Distance to woodlands + Landscape + Habitat	6	-791.20	1594.76	1.63	0.06
9th	Distance to woodlands + Distance to urban areas + Landscape + Wolf presence + Habitat	8	-789.10	1594.84	1.71	0.06
10th	Distance to woodlands + Distance to urban areas + Landscape + Habitat	7	-790.25	1594.99	1.86	0.06
11th	Distance to urban areas + Protected area + Landscape + Habitat	7	-790.31	1595.11	1.98	0.05
12th	Protected area + Landscape + Wolf presence + Habitat	7	-790.31	1595.11	1.99	0.05

S.3 - Model selection for factors influencing the total duration of vigilance behaviour of the roe deer in north-western Italy

Models were estimated through generalised linear models (GLMs). The top-ranked models are shown (Δ AICc ≤ 2); for each model, the variables, the number of parameters, AICc, ΔAICc and standardized weight are reported

Model	Variables	K	logLik	AICc	Δ AICc	Weight
Best	Landscape + Habitat	5	-1007.09	2024.45	0.00	0.24
2nd	Landscape	4	-1008.56	2025.30	0.85	0.16
3rd	Distance to urban areas + Landscape + Habitat	6	-1006.60	2025.57	1.12	0.14
4th	Distance to cultivated lands + Landscape + Habitat	6	-1006.95	2026.27	1.82	0.10
5th	Distance to cultivated lands + Habitat	5	-1008.01	2026.27	1.82	0.10
6th	Protected areas + Landscape + Habitat	6	-1007.02	2026.41	1.97	0.09
7th	Distance to natural open areas + Landscape + Habitat	6	-1007.04	2026.44	1.99	0.09
8th	Distance to woodlands + Landscape + Habitat	6	-1007.04	2026.45	2.00	0.09

S.4 - Model selection for factors influencing the inter-event interval of vigilance behaviour of the roe deer in north-western Italy

Models were estimated through generalised linear models (GLMs). The top-ranked models are shown (Δ AICc ≤ 2); for each model, the variables, the number of parameters, AICc, ΔAICc and standardized weight are reported

Model	Variables	K	logLik	AICc	Δ AICc	Weight
Best	**Distance to woodlands + Landscape + Habitat**	**6**	**-1094.86**	**2202.08**	**0.00**	**0.19**
2nd	Distance to woodlands + Distance to cultivated lands + Habitat	5	-1095.96	2202.19	0.11	0.18
3rd	Distance to cultivated lands + Habitat	4	-1097.36	2202.88	0.81	0.13
4th	Landscape + Habitat	5	-1096.56	2203.39	1.31	0.10
5th	Distance to woodlands + Distance to cultivated lands + Wolf presence + Habitat	6	-1095.65	2203.66	1.59	0.09
6th	Distance to cultivated lands + Wolf presence + Habitat	5	-1096.79	2203.84	1.77	0.08
7th	Distance to woodlands + Distance to cultivated lands + Landscape + Habitat	7	-1094.70	2203.88	1.81	0.08
8th	Distance to woodlands + Distance to cultivated lands + Distance to urban areas + Habitat	6	-1095.81	2203.99	1.91	0.07
9th	Distance to woodlands + Protected areas + Landscape + Habitat	7	-1094.78	2204.06	1.98	0.07

5.4 Conclusions

The discussed case studies provide valuable insights into roe deer behaviour, examining both population-level and individual-level dynamics.

In Section 5.2, the focus was on understanding roe deer activity rhythms across different seasons and landscapes. Our findings confirmed the crepuscular behaviour of roe deer, with distinct activity peaks around dawn and dusk. However, notable variations were observed, particularly in response to human-induced disturbances and predator presence. Human activity is highly predictable and likely generates visible responses in the activity patterns that are less variable compared to those generated by predatory activity of wolves, which is highly unpredictable for roe deer. Our findings underscored the adaptability of roe deer populations in adjusting their activity rhythms to mitigate perceived risks.

Conversely, in Section 5.3, we delved into the vigilance behaviour of roe deer in relation to human-induced and predatory risks. Our analysis revealed that roe deer allocate significant time to vigilance, especially in areas characterized by heightened anthropogenic disturbances.

Overall, these two case studies complement each other by providing a comprehensive understanding of roe deer behaviour at both population and individual levels. By examining behaviour from these dual perspectives, we gain a more holistic view of how roe deer interact with their environment and respond to ecological pressures.

In conclusion, our study emphasizes the adaptive nature of roe deer behaviour: from adjusting activity rhythms to mitigate human disturbances to allocating time for vigilant behaviour in response to perceived risks, roe deer exhibit remarkable behavioural plasticity.

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Chapter 6

Key Findings and Implications

This research aimed to investigate the relationship between the landscape, meant both as spatial structure of the land cover and perception of risk, and the local responses, in terms of demography and behaviour, of the roe deer in different study areas in north-western Italy. In this context, the specific objectives of the study were:

i) to define the importance of the roe deer as prey species for the wolf;

ii) to analyse the demography of roe deer populations inhabiting different study areas located along a gradient, in landscape composition, from natural to modified and with different predator pressure;

iii) to analyse the anti-predator responses of the roe deer within the same study areas and, consequently, identify the main factors influencing roe deer behaviours.

The first specific objective, addressed in Chapter 3, was evaluated within the framework of a long-term research project carried out in the Lombard Apennines focused on predator-prey dynamics involving wolves and wild ungulates. The following main conclusions could be drawn from the results obtained from this project:

i) the expansion of the wolf population from the mountains to the hills, first, and towards the plain, then, was most likely supported by prey availability. In particular, the probability of colonization was positively influenced by roe deer abundance;

ii) conversely to the general pattern observed in recent years in Italy, with the wild boar as the most consumed species, we found that the roe deer was the staple of the wolf diet. We observed a gradual shift from the prevalent consumption of wild boar (2007-2008 and 2011-2012) to the prevalent consumption of roe deer, which increased from 2015 onward;

iii) in accordance with these general results, obtained from an analysis at the population level, even the diets of the wolf packs settled in the Lombard Apennines and the Po Plain were based on roe deer consumption.

These conclusions suggest that wolf expansion would probably follow that of the roe deer across the Po Plain with the settlement of wolf packs in roe deer high-density areas.

The second and third specific objectives were evaluated within the framework of a specific research project carried out with a species-oriented approach in seven different study areas located in north-western Italy. These were characterized by different levels of landscape modifications and predation risk. Two study areas (LAR and STA) were characterized by a predominant natural landscape with abundant woodlands. The other two study areas (BNM and TID) were characterized by a mixed landscape with an alternation of woodland and cultivated patches. Finally, the last three study sites (MON, ORB, and COP) were characterized by a predominantly agricultural landscape with few patches of woodland. Three study areas (LAR, BNM, and MON) were located in areas where the wolf could be considered absent or sporadic; three study areas (COP, TID, and STA) were located along the Lombard Apennines, where the wolf was present with reproductive packs since the ‘90s; finally, in the last study area (ORB) the wolf became a stable presence only during the last four years.

The second specific objective was addressed in Chapter 4 and the following main conclusions could be drawn regarding the demography of roe deer populations inhabiting the mentioned study areas:

iv) population densities provide crucial information about the health and status of populations, offering insights into the interplay between the various factors influencing them. Concerning the potential key factors affecting deer population densities in north-western Italy, predatory pressure might have been particularly impacting in STA study area. Here low roe deer densities were estimated (2.4 ± 0.04 (SE) ind. per km^2), despite the general habitat suitability for the species; on the other hand, wolf consumption of the roe deer was particularly high (VM% ± SE: 88.3 ± 3.0) being selective, considering species relative abundance in the area. In addition, even hunting pressure could have affected this population. For instance, among the investigated study areas, the highest densities were observed in the two areas where hunting pressure was mild or negligible. These study areas were TID, with an estimated roe deer density of 12.5 ± 3.0 (SE) ind. per km^2, and BNM, with an estimated roe deer density of 11.5 ± 1.2 (SE) ind. per km^2. TID study area hosts large dog training areas, where hunting is not allowed, whereas in BNM study area hunting is prohibited, because it is included within a Protected Area;

v) the analysis of population structures revealed potential destructuring in some populations. For example, the roe deer population in LAR study area showed signs of destructuring, as indicated by the low juvenile/doe ratio, which suggested challenges in reproduction and recruitment. Given the absence of large predators in the area, the obtained results were probably influenced by hunting. Furthermore, the skewed sex ratio towards females in ORB study area could also indicate potential population destructuring, possibly due to selective hunting of males, especially adults, leading to an imbalance in sex ratios within the population.

These conclusions suggested that hunting, which ideally aims to achieve and maintain optimal population densities and structures, might have exerted a detrimental effect on certain populations. This impact was observed through two main mechanisms: excessive harvesting, which directly affected population densities alongside wolf predation, and targeted removal of specific individuals, resulting in population destructuring.

The results also brought attention to certain issues associated with the methods employed for data collection. Therefore, another significant conclusion, pertaining to methodology, emerged from this study:

vi) limitations associated with the spatial structure of the landscape cannot be mitigated without a substantial increase in sampling efforts. This was particularly evident in study areas characterized by open spaces with restricted surface area or limited accessibility (e.g., LAR, MON, and COP study areas).

The last specific objective was addressed in Chapter 5 and the following main conclusions could be drawn regarding the behavioural responses of the roe deer to perceived risk:

vii) a general tendency towards bimodality in diel activity patterns, with two peaks of daily activity around dawn and dusk, respectively, was observed within the study areas. This result confirmed the crepuscular behaviour of the roe deer;

viii) nevertheless, some local and seasonal variations in roe deer diel activity patterns have been observed. These temporal adjustments along the diel cycle could be interpreted as a response to fluctuations in the perceived level of risk by the animals. On one side, anthropogenic disturbance, which could be evidenced by heightened nocturnal activity during seasons with increased human presence; on the other side, the presence of the predator, which appeared to have led to more noticeable variations in activity rhythms across the different seasons;

ix) the roe deer allocated significant time to vigilance, especially in areas characterized by heightened anthropogenic disturbances (e.g. MON and COP study areas);

x) vigilance behaviour, measured using some behavioural metrics, showed increasing values along the gradient from the least disturbed to the most modified landscapes, whereas wolf stable presence did not cause such an evident outcome.

These conclusions confirmed the adaptive nature of roe deer behaviour: from adjusting activity rhythms to mitigate human disturbances to allocating time for vigilant behaviour in response to perceived risks, roe deer exhibited remarkable behavioural plasticity.