University of Sassari
Ph.D. School in Natural Sciences
Via Muroni 25, I-07100 Sassari, Italy

Dissertation for the Degree of Doctor of Philosophy in Environmental Biology
presented at Sassari University in 2013

XXVI cycle

TROPHIC ECOLOGY AND SPATIAL BEHAVIOUR OF WOLF (*Canis lupus)* IN AN APPENNINE AREA

PH.D. CANDIDATE: ***Dr. Elena Bassi***

DIRECTOR OF THE SCHOOL: ***Prof. Marco Curini Galletti***

SUPERVISOR: ***Prof. Marco Apollonio***

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“Se fossi nata uomo in un’altra epoca storica,

sarei sicuramente nata guerriero di Sparta”

…..to the Rock 'n' Roll

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SUMMARY

[ENGLISH] Nowadays the Italian wolf (*Canis lupus*) is expanding its range and increasing in numbers, nevertheless it is still a vulnerable species.

In this work, we have focused on the wolf feeding and spatial behavior, in a region of Northern Apennine.

First, we investigated the relation between wolf and red fox (*Vulpes vulpes*), assessing the extent of their trophic niche overlap. Our findings suggest a very limited trophic competition between them.

Moreover, we analyzed the functional response of wolf to changes in prey availability, and the impact of wolf predation and hunting harvest on ungulate populations.

Wild boar (*Sus scrofa*) was the main and selected prey species for wolves. Moreover, the proportion of roe deer (*Capreolus capreolus*) in wolf diet peaked when boar densities were low, resulting in a functional response dependent on the main prey density fluctuations. Furthermore, even if wolves and hunters focused on targets with different reproductive potential in the population, their combined impact did not exceed the annual recruitment, and thus did not result to limit ungulates population growth.

Finally, analyzing the variables involved in the choice of rendezvous sites locations, we found rendezvous sites typically inside protected areas, and usually distant from human settlements. Over recent years, rendezvous sites have occurred closer to urban areas, thus, our projections of suitability of sites can help planning to minimize possible wolf-human conflicts.

Keywords: wolf predation, interspecific competition, functional response, hunting harvest, rendezvous sites

RIASSUNTO

[ITALIANO] Attualmente in Italia il lupo (*Canis lupus*) sta espandendo il suo range e aumentando in numero; tuttavia è ancora specie vulnerabile.

In questa tesi, abbiamo indagato il comportamento spaziale e alimentare del lupo, in un'area dell'Appennino settentrionale.

Innanzitutto, stimando l'ampiezza di sovrapposizione di nicchia trofica tra lupo e volpe rossa (*Vulpes vulpes*), abbiamo osservato una ridotta competizione alimentare.

Inoltre, abbiamo verificato l'esistenza di una risposta funzionale del lupo, e studiato l'impatto combinato della predazione di lupo e del prelievo venatorio.

Il cinghiale (*Sus scrofa*) è la preda principale e selezionata dal predatore. Il picco di utilizzo del capriolo (*Capreolus capreolus*) nella dieta, corrisponde a basse densità di cinghiale, suggerendo l'esistenza di una risposta funzionale del lupo al variare delle densità della specie preda principale.

Inoltre, anche se lupo e cacciatori utilizzano prede con diversi potenziali riproduttivi, il loro effetto combinato non limita le popolazioni di ungulati.

Infine, analizzando le variabili coinvolte nella scelta dei rendezvous sites abbiamo osservato che risultano essere all'interno di aree protette e lontani dagli insediamenti antropici. A causa della recente espansione, i rendezvous sites risultano essere sempre più vicini alle aree antropizzate. Per questo la nostra proiezione può risultare uno strumento utile per cercare di minimizzare i possibili conflitti tra lupo e uomo.

Parole chiave: predazione del lupo, competizione interspecifica, risposta funzionale, prelievo venatorio, rendezvous sites

INTRODUCTION

INTRODUCTION

Nowadays most of the large carnivores in Europe are expanding their distribution and are increasing in number (Falcucci et al. 2007 and 2008). This happens in response to two main factors: on one hand the conservation actions and the changes in the national and international legislations, and on the other hand the abandonment of mountainous and hills that resulted in a decreased pressure of human activities on large areas. This abandonment triggered re-forestation and re-naturalization, that has led to an increase of several prey species (Boitani 2003, Falcucci et al.2008, Apollonio et al. 2010).

In Italy due to the land-cover changes, from 1960 to 2000 the Alps and the Apennines have shown an increase in forest cover and have experienced a positive trend of large vertebrates populations (Boitani 2003). Wild ungulate populations increased both in numbers and distribution (Apollonio et al. 2010), and consequently wolves (*Canis lupus*) increased from about 100 individuals in the 1970s to more than 1000 individuals in 2010, spreading from the southern Apennines to the central Alps.

At present, the Italian wolf population represents one of the few surviving west European populations, thus it has great conservation importance at both national and European level. Indeed in Italy, even if the populations are experiencing an expansion, wolf is still endangered and threaten (http://www.iucnredlist.org).

On a dietary point of view the wolf is a carnivore specialized in hunting ungulates (Mech 1970, Ballard et. al. 1987, Jedrzejewski et al. 1992 and 2000, Smietana and Klimek 1993, Okarma, 1995), however being quite adaptable, its diet could be extremely various. Indeed wolf can feed on livestock, other carnivores (fox, coyote, or dog), and small mammals (hare, marmot, micro-rodents). Moreover wolf can eat fruit, vegetables, garbage and invertebrates (Meriggi and Lovari 1996, Ciucci et al. 1996, Ciucci and Boitani 1998, Hefner and Geffen 1999).

The same could be observed analyzing the spatial behavior. The predator effectively inhabits most of the habitats of the northern hemisphere (Mech 1970, Carbyn 1987). It is not particularly habitat specific, can move over large areas, and can survive in many different environments (Mech and Boitani 2003). Wolf is habitat generalist and is not dependent on wilderness areas (Mech 1995), and, if it is not in conflict with human activities and has adequate ungulate prey, can disperse in a mixture of managed, human dominated, and semi-wild areas (Mladenoff et al. 1999).

In this scenario, the present work has focused on two main aspects of wolf ecology: trophic habits (**First** and **Second parts**), and spatial behavior (**Third part**).

The wolf represents a keystone species that can drive the ecosystem; as Mech and Boitani (2003) underlined wolves "are probably the single most important predators on large mammals". One of the most fascinating aspect of wolf biology is represented by the coevolution of the wolf and its preys; this process is defined as "an ongoing contest during which the prey must survive in the face of constant threat by the wolf, and the wolf must succeed in overcoming specialized prey defenses often enough to survive" (Mech and Peterson, 2003). Moreover, it is important to take into account that the diet composition depends mainly on the food availability of the area where wolf lives, and this is the reason why it is very important to study its feeding habits even at local scale.

Our study area is represented by a simple system in which wolf coexist with two wild ungulate species, very rich in terms of number of individuals. Wild ungulates are represented by wild boar (*Sus scrofa*) and roe deer (*Capreolus capreolus*). Moreover, in the study area there are no other large carnivores. The only other natural predator is represented by a mesopredator, the red fox (*Vulpes vulpes*). In this scenario we have found the ideal conditions in order to understand the links between wolf and its preys on one hand, and wolf and other predators on the other hand.

According to Schoener (1983), one of the driving forces in community ecology, is represented by inter-specific competition between predators. The effect of interaction between species is different among species, habitats, and density of competitors (Creel 2001). Competition occurs both directly and indirectly. Directly occurs via aggression (Rosenzweig 1966), and in carnivores it may result in intraguild predation (Polis and Holt 1992); indirectly occurs both through differential efficiency in obtain the same food resources, or through kleptoparasitism made by the dominant species. Furthermore, the intensity of competition between carnivores is predicted to be highest at intermediate differences in body size (2-5 times differences) between competitors (Donadio and Buskirk 2006). Advancing the understanding of intraguild interactions could play a very important role for the conservation success of species like large carnivores (Creel et al. 2001). This is the goal of the **Chapter 1** of the present thesis. In this chapter we have investigated seasonal and annual food habits of wolf and red fox, in order to assess the extent of the trophic niche breadth and overlap, and evaluate the differential use of prey species by the two carnivores. This will allow us to understand if inter-specific competition exists between this two canids.

Another important aspect to consider in the study of wolf food habits is the relation between wolf and its preys (**Second Part**). Understanding foraging behavior of wolf may be complex where the density of prey species fluctuates significantly across time and space. In most Eurasian countries, wolf inhabits regions with wide seasonal fluctuations in prey availability (Melis et al. 2006). This phenomenon is more emphasized in those areas where hunting pressure on big game species is stronger. Indeed hunting harvest and disturbance are able to influence population dynamics of wild ungulates, and this could shape the food habits of wolf.

How does wolf choose and use the preys inhabiting its territory? Is there any wolf functional response to a numeric changes in prey populations? Which is the impact of wolf on the prey populations? Is present in the system a top-down regulation by wolf and hunters on the ungulate populations? These are the subjects of the **Chapters 2** and **3**.

Predicting the impact of changing predator numbers on prey species is important for managing populations of both predators and their preys (Wilmers et al. 2007, Berger et al. 2008). It is well known that predators may have multiple effects on prey populations, ranging from density dependence mechanisms to feeding habits modifications. Moreover, predators diet and prey selection can be affected by different factors including: prey and predator densities (Vucetich et al. 2002), functional and numerical responses of predators to changes in prey density (Messier 1994), community composition (Garrott et al. 2007), climatic conditions (Post et al. 1999), vegetation productivity (Melis et al. 2009), and landscape heterogeneity (Kauffman et al. 2007). Mech (1970) has observed that “wolves in each local area become very skilled in hunting prey on which they specialize”, and this is because of elements of learning, traditions, and actual preferences of different packs. Indeed predators tend to specialize on those prey species which they find to be most vulnerable and can be killed most efficiently (Sinclair et al. 2003), regardless of their relative abundance in the available community. Thus because wolf can rely primarily on one prey species (Dale et al. 1995), it may not benefit if another prey species increase. The functional response is defined as the change in the foraging behavior of the predators in response to variation in preys availability (Holling 1959). When wolf coexists with a wild ungulate community consisting of few species only, its foraging behavior may be more significantly shaped by the population dynamics of its preys. In this contest specialization occurs when a given prey is always positively selected and the primary prey is persistently hunted despite low densities in the environment. Due to the peculiar conditions we have in our study area (i.e. only two prey species and one predator), in the **Chapter 2** we have tried to answer if wolf selects for either of the two main prey species available, and if wolf diet is relate to the relative availability of prey species in the area.

Even if wolf represents the only one large predator in the study area, hunters are present as well, and they exploit the same game species. One relevant question is if wolf predation combined with hunting harvest is compensatory or additive to other mortality causes. The understanding of this aspect gives an indication if in a certain ecosystem top-down control is present. Top down control is defined by the "green world hypothesis" (Hairston et al. 1960) and the hypothesis of exploitation ecosystems (Oksanen et al. 1981, Fretwell 1987, Oksanen and Oksanen 2000). These theories predict a strong limitation of herbivore populations by predators. According to these predictions, at a large scale ungulate densities should not change along a habitat productivity gradient. Predation should keep ungulate numbers in check, reduce browsing pressure and, in this way, influence lower trophic levels (e.g. forest regeneration). Several studies have showed that wolves could potentially regulate prey abundance (Eberhardt 1997,Bereguard and Eliot 1998, Peterson 1999, Ripple and Beschta 2003), underlining the existence of a top-down force. Moreover, according to Okarma (1995) it seems that the main driving force for ungulate population dynamics is human hunting, and that the combined effect of predation by both wolves and humans may lead to preys declines (Eberhardt et al. 2003). Actually, it is not very clear if the combined impact of this two predators could limit or just regulate ungulate populations. The aim of the **Chapter 3** is to investigate the combined impact of hunters and wolves on wild boar and roe deer in order to determine if predation, hunting or both can limit or regulate ungulate population in our study area, i.e. if a top-down control is present.

In **Chapter 4**, we move to another important aspect for wolf range expansion and number increase: the spatial behavior. A crucial point in wolf expansion is the opportunity to form new pairs and reproduce (Fuller et al., 2003). Here we have focused on the environmental variables implicated in the choice of rendezvous sites locations. Rendezvous sites are defined as areas used by wolves to raise and leave pups after abandonment of dens

(Mech 1970). The availability of such sites will affect the process of range expansion (Packard 2003), and because pup mortality results to be high during the first six months of life, the choice of dens and rendezvous sites is fundamental for the numerical increasing process (Harrington and Mech 1982a). At present wolf usually lives in a multiple-use landscape that is surrounded by human settlements (Salvatori and Linnell 2005, Ziaei 2008). Nevertheless, wolf suitable habitats are located in areas where forest cover is widespread, human impact is low and wild preys are abundant (Mladenoff et 1998, Karlosson et al. 2007, Jedrzejewski et al. 2008). Very few studies have been carried out on selection of sites for bearing and raising pups, investigating the variables and factors that affect the distribution of home sites, such as temperature, soil composition, vegetation, and canopy cover (Ballard and Dau 1983, Fuller 1989, Norris et al. 2002, Theuerkauf et al. 2003), pack range (Ciucci and Mech 1992), human disturbance (Chapman 1977, Thielet al. 1998) and prey availability (Heard and Williams 1992, Boertje and Stephenson 1992).

Due to the recent expansion of wolf populations in human-dominated areas, wolves have learnt to tolerate various degree of human disturbance (Mech and Boitani, 2003; MacDonald and Sillero-Zubiri, 2004), although anthropogenic disturbance is likely to become an important factor in wolf den site selection. Moreover increasing “range overlap” between wolf and humans could lead to increasing conflicts with hunters as well as with shepherds (Fico et al. 1993, Cozza et al. 1996, Ciucci and Boitani 1998). In this chapter we explore the importance of environmental characteristics related to the rendezvous sites selection in an human-dominated landscape, identifying suitable areas for wolf within the current range and potential areas for further expansion beyond this. In this way we aim to create a tool useful for the conservation of key areas for the current process of wolf expansion.

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FIRST PART

Inter-specific competition

Chapter 1

Trophic niche overlap and wild ungulate consumption by red fox and wolf in a mountain area in Italy

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Original Investigation

Trophic niche overlap and wild ungulate consumption by red fox and wolf in a mountain area in Italy

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Predator–prey system

abstract

Red fox (Vulpes vulpes) and wolf (Canis lupus) are two widespread opportunistic predators living in sympatry in many areas. Nonetheless, scarce information are available on their trophic interactions. We investigated food habits of these two carnivores in a mountain area in Italy and assessed the extent of their trophic niche overlap, focusing on the consumption of wild ungulates. Thereby we analyzed the content of 669 red fox scats and 253 wolf scats collected between May 2008 and April 2009. Red foxes resulted to have a more than three times higher niche breadth than wolves. Vegetables, small mammals, wild ungulates, and invertebrates were major items (altogether 92% of volume) of the red fox annual diet. On the contrary wolf annual diet relied on wild ungulates (94% of volume) with wild boar (Sus scrofa) being the main food item. The degree of trophic niche overlap between the two species was found to be low (Pianka's O = 0.356). Diet variation between the warm and the cold seasons was limited in both species, and higher in red fox than in wolf. The two canids appeared to use wild ungulates unevenly being the former more selective for younger preys, smaller in size (newborn piglets and roe deer Capreolus capreolus fawns), whereas the latter exhibited a preference for medium-sized and large ungulates (10–35 kg wild boar and adult roe deer). Even if wild ungulates represent the main shared food category, the different use of age/weight classes by the two predators, together with their possible consumption as carrions by red fox, suggests a very limited trophic competition between wolf and red fox. This study represents a contribution to the knowledge of trophic interaction in predator–prey systems where sympatric carnivores are present.

Introduction

Red fox and wolf are the most widely distributed carnivores in the world, and this clearly indicates that these species can survive in many different environments (MacDonald and Sillero-Zubiri 2004). The worldwide distribution of red fox ranges from arctic barren areas to temperate deserts, from wilderness to cities (Harris 1981; Jędrzejewski and Jedrzejewska 1992). Some environments are more suitable to support high number of foxes, with heterogeneous habitats usually bearing higher densities than homogeneous ones (Jędrzejewski and Jedrzejewska 1992; Russell and Storch 2004). The wolf original distribution was as wide as fox one but human persecution restricted it to small and fragmented range in the northern hemisphere (Mech 1970; Carbyn 1987). In Europe wolves are mostly restricted to remote, scarcely populated, hilly or mountainous areas (Okarma et al. 1998; Poulle et al. 1999; Corsi et al. 1999; Mech and Boitani 2003). One of the reasons for these species' success and their wide distribution could be their extreme trophic plasticity and adaptability. They are indeed generalist and opportunistic predators as well as efficient scavengers (Reig et al. 1985; Lovari et al. 1994; Peterson and Ciucci 2003; Panzacchi et al. 2008).

In natural habitats, red fox diet mainly consists of small mammals, lagomorphs, birds, insects, fruits, and occasionally wild ungulates (Harris 1981; Jędrzejewski and Jedrzejewska 1992; Sidorovich et al. 2006; Dell'Arte et al. 2007). Wolf feeds mainly on wild ungulates (Jędrzejewski et al. 2002; Capitani et al. 2004; Kojola et al. 2004; Mattioli et al. 2011) but, in limiting conditions, it can also eat small mammals, insects, fruits and vegetables (Meriggi and Lovari 1996; Hefner and Geffen 1999).

In the last years an increasing number of studies have been carried out on these two predators, but very few comparative studies have been conducted on the diet of sympatric populations (MacDonald et al. 1980; Reig et al. 1985; Patalano and Lovari 1993). A number of studies in Italy, where red fox and wolf are the only sympatric wild canids, has focused on the ecology of each species separately (Prigioni and Tacchi 1991; Capitani et al. 2004; Gazzola et al. 2007; Marucco et al. 2008; Mattioli

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et al. 1995, 2011). Just one comparative study addressesed their diets and their trophic niche overlap in a Mediterranean region where the food chain was deeply altered (Patalano and Lovari 1993).

A general lack of information remains on their trophic interaction in natural communities, where wild ungulate populations have recovered and a complex predator–prey system has been restored.

Accordingly, aims of this study are: (i) to investigate, in a qualitative and quantitative way, seasonal and annual food habits of wolf and red fox; (ii) to assess the extent of the trophic niche breadth and overlap; (iii) to evaluate the relative importance of wild ungulate (wild boar and roe deer) in red fox and wolf diet; (iv) to evaluate the use of the different age and weight classes of roe deer and wild boar by the two carnivores.

Material and methods

Study area

The study area is located in the Apennine Mountains, Arezzo province, Italy (43 ° 48^1 N, 11 ° 49^1 E). The study was carried out in an area of 120 km^2, including a strictly protected area of 27 km^2 (Oasi Alpe di Catenaia). In the remaining portion hunting was permitted. Altitude in the area ranges from 400 to 1414 m a.s.l. Forest cover is mainly composed of mixed deciduous woods (76% of the total area), dominated by chestnut (Castanea sativa) and turkey oak (Quercus cerris) in the lower range, and beech woods (Fagus sylvatica) above 900 m a.s.l. Conifer woods (7% of the total area) were composed of black pine (Pinus nigra), white fir (Abies alba), and Douglas fir (Pseudotsuga menziesii). Bush vegetation and pastures are also present (16%).

In addition to wolf and red fox, other carnivores inhabit the study area: badger (Meles meles), weasel (Mustela nivalis), stone and pine marten (Martes foina and Martes martes), and polecat (Mustela putorius). Wild ungulates are represented by roe deer and wild boar, but also red deer (Cervus elaphus) is occasionally observed. The only resident lagomorphs is the brown hare (Lepus europaeus), while rodents are represented by porcupine (Hystrix cristata), red squirrel (Sciurus vulgaris), edible dormouse (Glis glis), garden dormouse (Eliomys quercinus), bank voles (Myodes glareolus), house mouse (Mus musculus), long-tailed field mouse (Apodemus sylvaticus), yellow-necked field mouse (Apodemus flavicollis), black and brown rat (Rattus rattus and R. norvegicus). West European hedgehog (Erinaceus europaeus) is common, while several species of the order Soricomorpha, belonging to the genera Talpa, Sorex, Neomys and Crocidura are recorded (Spagnesi and De Marinis 2002).

The climate is temperate-continental with hot and dry summers and cold and rainy winters. During the study, average month temperatures varied from a minimum of −0.4 ° C in the coldest month (February), to a maximum of 37.2 ° C in the warmest period (August). The amount of precipitation varied from a minimum of 22.7 cm in September to a maximum of 188.7 cm in December. Snowfalls are occasional and occur from October to April, usually above 1000 m a.s.l. Temperature and precipitation data were collected at four weather stations located within and around the study area (Corpo Forestale dello Stato).

Scat analysis

From April 2008 to May 2009 a total of 669 red fox scats and 253 wolf scats were collected along seven 10-km transects walked once per month. Collected faeces were identified by the size, shape and smell, and attributed to a carnivore species following Mattioli et al. (1995). Both red fox and wolf scats were put in polypropilene bags, coded and stored in freezers. At the time of analysis, faeces were broken down in water and washed using a sieve with mesh size of 0.4 mm to separate the macroscopic fraction. This was then oven at a temperature of 65 ° C for 24 h, and identified by comparison with reference material (especially bones, teeth and hairs). Hairs were also analyzed on the basis of microscopical characteristics. To assist food item identification, especially for red fox, the microscopic residuals were examined under a binocular microscope. In the case of ungulates hairs can be used to assess species, age and weight classes (Mattioli et al. 1995). We did not use bones because of their small amount in red fox scats, and because of the different chance to find them in the scats of the two carnivores. We considered three weight classes for wild boar: <10 kg (newborn piglet); between 10 and 35 kg (aged piglet); >35 kg (sub-adult and adult). Two age classes were identified for roe deer, <4 months (hereafter called fawn) and >4 months (hereafter called adult); these age classes distinction was the only possible, because hair of fawns and adults are undistinguishable after the first winter moult (September–October).

Before carrying out scat analysis, the capacity to identify prey species weight and age classes of wild ungulates, from the hairs therein found, was tested by means of a blind test performed on 50 artificial scats containing 13 mammal prey items in different combinations. Two of the authors (E.B. and E.D.), who did never fail to recognize the species, as well as weight/age classes, made the assessment for all the faecal samples.

In order to estimate the contribution of each species in the diet, in terms of volume, we categorized each food item in a scat by 5% steps (i.e. <5%; 6–10%; 11–15%) as described by Russell and Storch (2004). We calculated the frequency of occurrence (FO%) and the average volume (AV%) for each food item. The FO% was calculated as $n_i/N \times 100$ where n_i is number of scats containing a given item i and N is the total number of scats; AV% was defined as $V_i/N \times 100$ where V_i is the total volume of a given food item i, and N is the total number of scats.

The trophic niche breadth and overlap between red fox and wolf were evaluated through Levins' index (1968) and Pianka's index (1973), applied to the volume of food categories in their respective diet. Levins' formula is: $B = 1/ \quad p_i^2$, where p_i is the contribution of each item in the total diet of red fox. Eleven food items were considered: (1) wild ungulates, (2) cat, (3) hare, (4) porcupine, (5) small mammals, (6) invertebrates, (7) reptiles, (8) birds, (9) livestock, (10) vegetables, and (11) other. Thus, index B could achieve value from 1 (strong specialization on one category) to 11 (extremely opportunistic foraging behaviour). Pianka's formula is: $O_{fw} = \quad p_{if} p_{iw} / \sqrt{\quad p_{if}^2 \quad p_{iw}^2}$ where O_{fw} is Pianka's measure of niche overlap between red fox and wolf; p_{if} is the proportion of the resource i out of the total resources used by red fox, while p_{iw} is the proportion of the resource i out of the total resources used by wolf, and i could range from1 to n, where n is the total number of food items considered. The value of index O could range from 0 (no overlap) to 1 (full overlap).

In data analyses, the year was partitioned into two periods: a warm season (hereafter called summer), lasting from May to October, and a cold period (winter) lasting from November to April.

The x^2-test was used to test for differences between summer and winter diet in the two species and between their respective use of wild ungulate age/weight classes. Two-sample randomization tests (1000 iterations) were used in PopTools 3.2.3 (Hood 2010) to test for differences between items.

Wolf and red fox selectivity was evaluated among age and weight classes by comparing their frequency in the population with their frequency in the predators' diet (estimated as FO). Density estimates for wild boar and roe deer were obtained by drive censuses completed in May 2008, in both the protected and non-protected parts of the study area (methods described in Mattioli et al. 1995). For roe deer, these surveys provide pre-reproductive counts, as births in this species take place from late May to early June (Andersen and Linnel 1997). Hence, to calculate post-reproductive proportions between age classes, we estimated the number of newborns from the overall number of females and from fertility data (i.e. percent of pregnant females and mean number of embryos/adult female – winter 2007–2008 – Fish and Wildlife Office, Provincial Administration of Arezzo). For wild boar, the annual drive census provided an estimate of the post-reproductive population size, partitioned into adults (>1 year) and piglets (<1 year). To convert these proportions into weight classes, we assumed that all adults were >35 kg (according to Mattioli et al. 2011) and splitted piglets into the other two classes, considering that 33% of a sample of 252 piglets, captured in summer in a 9-year period, weighed less than 10 kg.

Manly's selectivity index (Manly et al. 1972) was used to determine the carnivore hunting preference: ¸**i** = ri/ni (1/�(rj/nj)) where ¸**i** = preference index for prey class i, ri and rj = proportion of prey type i and j respectively in the diet (i and j = 1, 2, ... m), ni and nj = proportion of prey class i or j, respectively, in the area, and m = number of prey classes (2 for roe deer and 3 for wild boar). According to Manly et al. (1972), the following interpretation keys were adopted: if ¸**i** > 1/m class i was selected for by the predator, if ¸**i** < 1/m class i was avoided, if ¸**i** = 1/m class i was not selected for. We assessed carnivore selectivity only in summer (May–October), as this is the period when all the adopted classes of both species occur in the population.

Results

Annual diet

Between May 2008 and April 2009, 669 red fox scats (449 in summer and 220 in winter) and 253 wolf scats (112 in summer and 141 in winter) were collected in the study area. Overall, red fox scats contained 11 different food categories, whereas wolf scats contained 5 categories.

Vegetables, small mammals, wild ungulates, and invertebrates were prevalent in the red fox diet (representing in total ~92% AV% and ~87% FO%, Table 1). Among ungulates, wild boar represented the main item (AV% = 10.65%; FO% = 10.22%), followed by roe deer (AV% = 5.78%; FO% = 4.34%). Cat, hare, porcupine, reptiles and birds were accessory food items (Table 1), amounting to 3.88% AV% and 10.31% FO% in total. Niche breadth of red fox indicated a specialization on the four main items: vegetables, small mammals, wild ungulates and invertebrates (B = 3.826).

Wild ungulates were the bulk of the wolf diet, representing 93.91% of the total food volume consumed and 88.96% FO% (Table 1): in particular, wild boar was the main prey used by wolf (AV% = 64.64%; FO% = 60.71%), while roe deer represented a secondary resource (AV% = 25.25%; FO% = 24.68%). Red deer was a marginal food item (AV% = 4.03%; FO% = 3.57%), like small mammals, livestock, and vegetables (Table 1). According to these patterns, Levins' index (B = 1.132) indicated a very pronounced specialization. Pianka's index, describing the trophic niche overlap between red fox and wolf diets, revealed a low overlap ($O_{fw} = 0.356$).

Taking into account the cumulative volume represented by wild ungulates (Fig. 1), in the red fox diet young wild boars represented the most used classes (10–35 kg: AV% = 32.54%; piglets <10 kg: AV% = 25.44%), followed by roe deer fawns (AV% = 20.08%). On the other hand, wolf used mostly 10–35 kg wild boars and adult roe deer (AV% = 47.44% and AV% = 23.21%, respectively, Fig. 1). There was no significant difference between the two predators in the use of roe deer ($x^2 = 2.39$, $P > 0.05$), but there was in the use of wild boar, that was more used by wolf ($x^2 = 49.46$, $P < 0.01$).

Seasonal diet

Vegetables, small-mammals, wild ungulates and invertebrates were the most consumed items by red fox both in winter and summer (Table 1), although the overall proportions were significantly different ($x^2 = 372.6$, $P < 0.01$). In particular, a significant difference in the consumption of invertebrates was found between the two seasons ($P_{randomization} < 0.001$). Levin's index was 3.930 in winter and 3.712 in summer. Wolf seasonal diet was based on wild ungulates as resulted in the annual diet (winter: AV% = 94.04%; summer: AV% = 93.75%, Table 1), and wild boar was the most consumed item in both seasons (winter: AV% = 69.30%; summer: AV% = 58.71%, Table 1). No significant difference between overall seasonal wolf diets was observed ($x^2 = 1.19$, $P > 0.05$). Levin's index was 1.129 in winter and 1.136 in summer.

The use by the two predators of the 10–35 kg wild boar weight class was relevant both in winter and in summer (Fig. 2a and b), but they showed significant differences in the seasonal use of the other two classes ($x^2_{boar\_summer} = 221.99$, $P < 0.001$; $x^2_{boar\_winter} = 29.03$, $P < 0.01$). In summer, red fox used more small piglets and wolf used more >35 kg wild boar (Fig. 2a). Actually, while wolf showed a selection for piglets of the intermediate class (Manly's selectivity index: ¸$_{boar\ 10–35} = 0.71 > 1/m$), the lightest class was selected by red fox ($a_{boar\_<10} = 0.51 > 1/m$). For both predators we observed a peak of piglets consumption in May followed by a progressive decline up to July: this category disappeared in wolf diet, while it remained in red fox diet (Fig. 3a and b). In winter, wolf did not use the lightest class at all and increased the use of 10–35 kg wild boars (Fig. 2b). Roe deer use by the two predators was significantly different in summer ($x^2_{roe\_summer} = 9.51$, $P < 0.01$, Fig. 2a). Indeed in this season a selection for fawns was observed in red fox (¸$_{roe\ fawn} = 0.77 > 1/m$), whereas adult roe deer were selected for by wolf (¸$_{roe\ adult} = 0.55 > 1/m$). Nonetheless, both predators showed a peak in the use of fawns during the beginning of the summer period (May–June), followed by a decline (Fig. 4a and b).

Diet overlap between the two predators was $O_{fw} = 0.390$ in winter and $O_{fw} = 0.337$ in summer.

Discussion

Red fox and wolf are both common predators in the Italian Apennines (Spagnesi and De Marinis 2002), with different roles in mammal communities. The wolf is a top predator naturally relying on wild ungulate populations (Mech 1970), whereas the red fox is a mesocarnivore with a regulatory action on small mammal populations (Jędrzejewski and Jedrzejewska 1992). Nonetheless they show extremely variable and flexible food habits, with the capacity to shift their diet within and between years following temporal variation in prey abundance (Huggard 1993; Cavallini and Volpi 1996). Given this flexibility we are aware that the results of our study may change in relation to the annual variations in food availability. However we consider important to compare the diet of these two canids in face of resource disposal year round.

In our study area, small mammals and vegetables represented the bulk of the red fox diet year round while invertebrates were an accessory food item, with a relevant consumption during the warm season. Such food habits are comparable with those observed in other studies (Patalano and Lovari 1993; Russell and Storch 2004; Dell'Arte et al. 2007). However, in addition, wild ungulates were found to be an important food source during the year, as occasionally reported elsewhere (Lanszki 2005; Sidorovich et al. 2006; Panzacchi et al. 2008).

Wolf's diet was characterized by one main food category only, namely wild ungulates. Several studies have demonstrated that, where natural conditions are present, wild ungulates are staple

Table 1
Red fox and wolf annual and seasonal diets in Alpe di Catenaia, Italian Apennines. Data are expressed as average volume (AV%) and frequency of occurrence (FO% in brackets). N is the sample size (i.e. number of analyzed scats).

Food item	Red fox			Wolf		
	Annual N = 669	Summer N = 449	Winter N = 220	Annual N = 253	Summer N = 112	Winter N = 141
Wild ungulates	16.43 (14.56)	15.69 (13.37)	17.93 (17.36)	93.91 (88.96)	93.75 (94.57)	94.04 (87.86)
Cat	0.20 (0.15)	0.30 (0.21)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Hare	1.56 (1.31)	1.24 (3.15)	2.23 (3.67)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Marten spp.	0.21 (0.15)	0.11 (0.11)	0.41 (0.24)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Porcupine	0.52 (0.58)	0.30 (0.42)	0.98 (0.98)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Small mammals	25.87 (22.15)	25.40 (21.47)	26.81 (23.72)	0.49 (0.65)	0.89 (0.78)	0.18 (0.58)
Invertebrates	9.91 (16.99)	13.20 (21.26)	3.20 (7.09)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Reptiles	0.55 (1.48)	0.76 (2.11)	0.14 (0.49)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Birds	2.32 (4.64)	1.28 (3.66)	4.43 (6.84)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Livestock	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.20 (0.32)	0.00 (0.00)	0.35 (0.58)
Vegetables	39.63 (33.91)	40.33 (33.05)	38.20 (33.98)	3.83 (8.44)	3.75 (3.10)	4.04 (9.25)
Other	2.80 (2.50)	1.39 (1.16)	5.66 (6.62)	1.57 (1.62)	1.79 (1.55)	1.40 (1.73)
Total	100.00	100.00	100.00	100.00	100.00	100.00

prey for wolves (Jędrzejewski et al. 1992; Capitani et al. 2004; Gazzola et al. 2005; Valdmann et al. 2005). Although studies conducted in Central European regions showed that, among wild ungulates, wolves preferred cervids (Jędrzejewska et al. 1994; Okarma et al. 1998), several studies in Italy (Meriggi et al. 1996; Mattioli et al. 1995, 2011) pointed out that wild boar was the most important prey for wolf. Our data are in agreement with the latter finding, because in our study area wild boar was by far the main prey for wolves in terms of occurrence in scats.

Seasonal variation was observed in the red fox's feeding behaviour but not in the wolf. The former tended to use young ungulates and invertebrates in summer. This is because of their increased availability and abundance and because they are more profitable items compared to adult ungulates or small mammals (Panzacchi et al. 2008). On the contrary, wild boar was the main food item for wolves in both seasons with no apparent variation in the selection of weight class. Roe deer consumption is higher in summer, probably due to the temporary availability of the more profitable fawns (Fig. 4b). These results are in agreement with those obtained by Mattioli et al. (2004, 2011) in a nearby area.

Overall, the degree of food niche overlap between red fox and wolf was low, as shown by the low value of Pianka's index. This indicates that the food items are different between the two predators with the red fox trophic niche being more than three times broader than the wolf one. The result are in general agreement with previous studies conducted both in similar (Patalano and Lovari 1993) and very different conditions (Zhang et al. 2009), where wolves and red foxes preyed on animals with different body size, thus limiting diet overlap.

Comparing red fox and wolf diets, in our study area, wild ungulates represented the most shared food item. They sometimes appeared to consume similar age classes of ungulates with the tendency of red fox to consume younger preys. Concerning wild boar, both predators consumed primarily the same weight class (10–35 kg) in both seasons (Fig. 2a and b), although a selection in summer was observed only by wolf. On the contrary, fawns younger

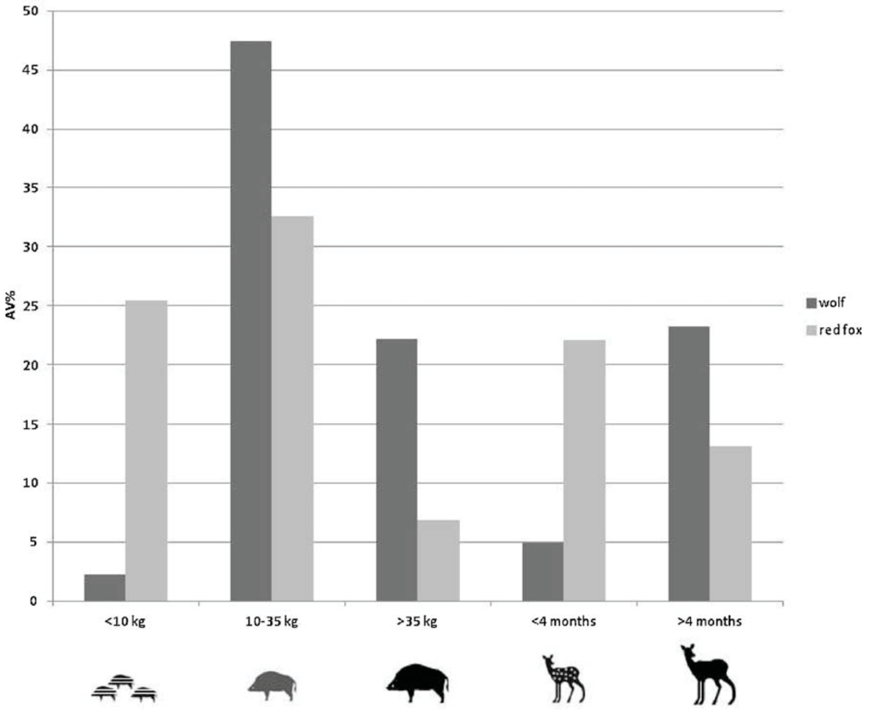

Fig. 1. Annual use of wild boar weight classes and roe deer age classes by red fox and wolf in Alpe di Catenaia, Italian Apennines (2008–2009). Data are expressed as average volume percentage (AV%), setting the cumulative volume of wild boar and roe deer to 100%.

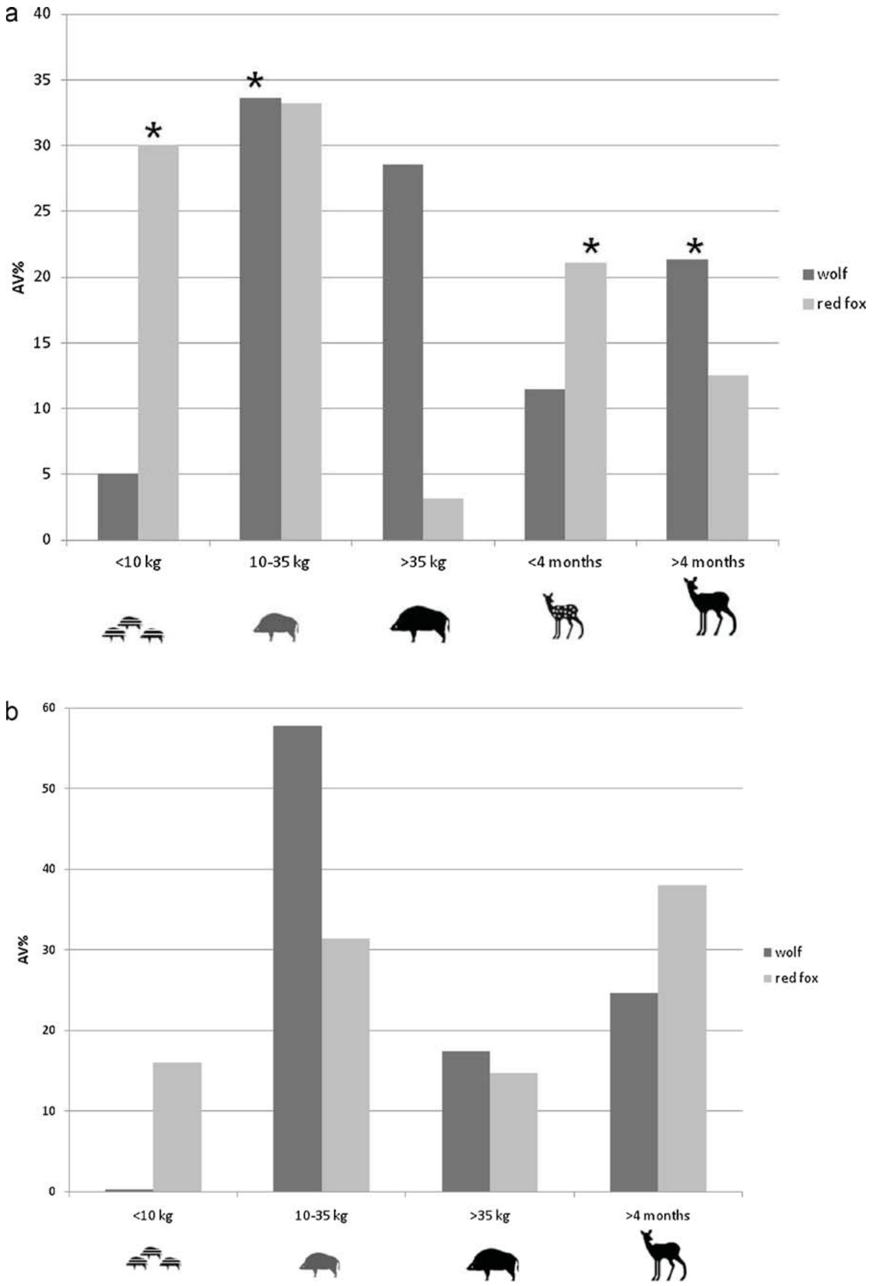

Fig. 2. Seasonal use of wild boar weight classes and roe deer age classes by red fox and wolf: (a) summer 2008, (b) winter 2008–2009. Data are expressed as average volume percentage (AV%), setting wild boar and roe deer total volume to 100%. Stars indicate the prey classes selected by the two canids (Manly's selectivity index).

than 4 months were the most used roe deer class by red fox and they were strongly selected in summer (i.e. when available), when a slight selection for adults was observed in wolf. Interestingly, the consumption of young wild boars decreased from May to August in both species, but while it then disappeared in wolves, it was maintained through the year in the red fox diet (Fig. 3a and b). This trend could reflect the possibility of long-lasting birth distribution in the wild boar population, partially contrasting with the assumption of a concentration of births in February–May (Dardaillon 1988; Fernàndez-Llario and Carranza 2000).

Similar to wild boar, the monthly use of fawns decreased during summer (Fig. 4a and b), like it was reported in a long-term study on red fox predation on roe deer in Sweden (Jarnemo et al. 2004). However, contrary to the reported evidence of a predation limited to 2–6 weeks after their birth (Linnell et al. 1995; Aanes and Andersen 1996), in our study fawns were found in the red fox diet until September.

The habit to hunt juvenile wild ungulates is a well known aspect of feeding behaviour both in red fox (Panzacchi et al. 2008) and wolf (Mattioli et al. 2011). The birth season of roe deer peaks between late May and early June (Andersen et al. 1998) and, contrary to wild boar, it is synchronized, with more than 80% of births taking place in less then 30 days (Linnell et al. 1995). So, roe deer fawns are available to predators during a more restricted period than piglets.

The increased availability of fawns in June can explain their strong use by both canids in this period, and the consequent predation pressure can justify the selection of dense habitats by lactating does (Bongi et al. 2008). Red fox seems to use roe deer, when fawns are available, switching then to adults which are mainly used as carrions (Panzacchi et al. 2008). Roe deer fawns seem to be

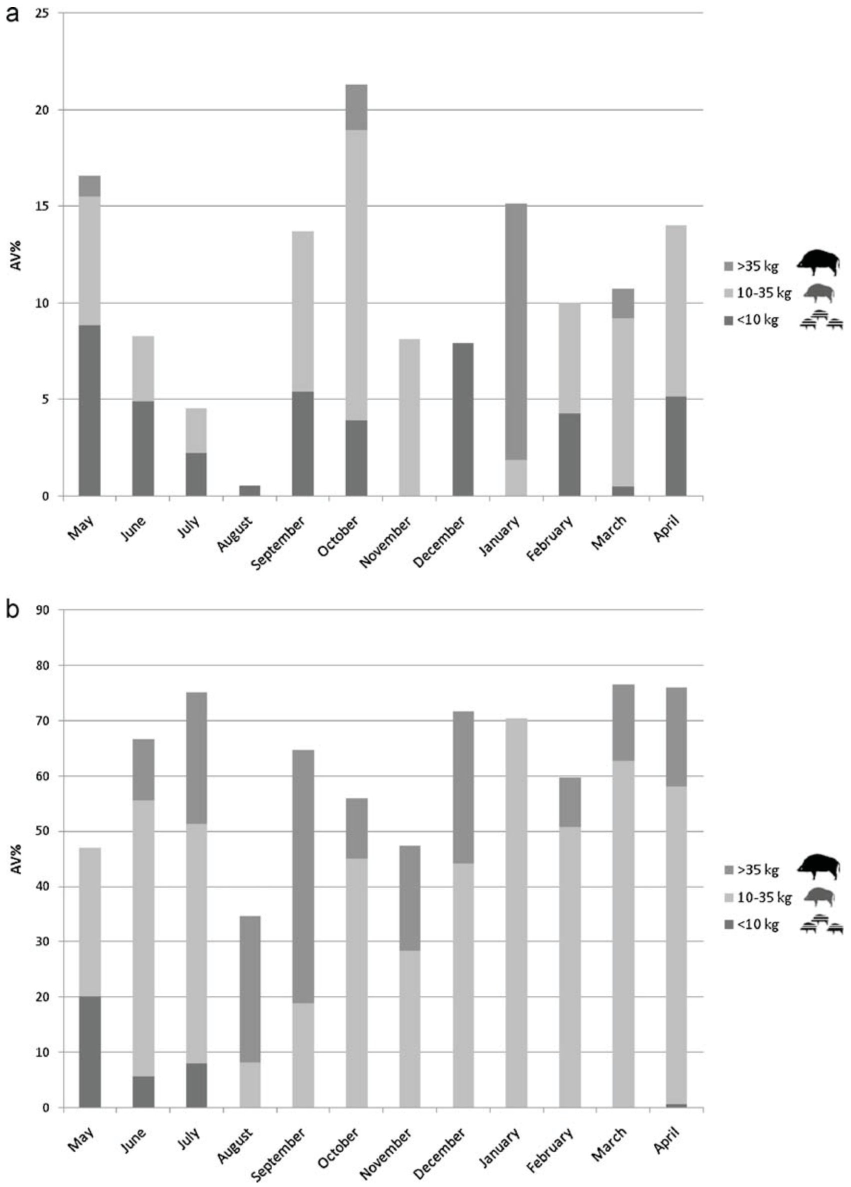

Fig. 3. Monthly use of wild boar weight classes by red fox (a) and wolf (b) year round (2008–2009). Data are expressed as average volume percentage (AV%).

important in red fox diet during the first months of summer, when red fox mothers need a lot of energy for lactation. As observed by Panzacchi et al. (2008), the rapid appearance of fawns in red fox diet at the start of the birth season and their disappearance at the end of that period, together with the high mortality of fawns due to red fox, can indicate that this carnivore does actively hunt them. Accordingly, in forest habitats the cost/benefit trade-off may induce wolves to prefer fawns for a very limited period (June–July). The same could be said to explain the reason why wolf prefers 10–35 kg wild boars (high energy gain, low handling time and slight risk of injury) than the heavier ones. Concerning red fox, <10 kg piglets might represent useful and convenient preys: given the size of a red fox, the predation of a such small piglet is possible with limited risk and can give a substantial amount of food. These findings, besides confirming the tendency of wolf to prey upon ungulates, give support to the possibility that even a mesopredator like red fox can actively prey on the lightest and youngest classes of ungulates.

On the other hand, red fox is a very effective scavenger, as demonstrated by several studies (Cagnacci et al. 2003; Sidorovich et al. 2006; Kidawa and Kowalczyk 2011). In regions where large carnivores are present, remains of their kills represent an important food resource for mesocarnivores, especially when other food is scarce (Jędrzejewski and Jedrzejewska 1992). In our study area, red foxes may profit from wolf predation and this can partially explain the occurrence of adult ungulates in its diet. This can be helpful to interpret the overlap of wolf and red fox in the use of 10–35 kg piglets possibly hunted by wolves and scavenged by red foxes. Nevertheless, scavenging activity can also be linked to ungulate mortality due to other causes, like starvation, disease or hunting (in case of severely wounded animals).

Our data suggest that in a forested habitat and in presence of roe deer and wild boar, the red fox shows an opportunistic feeding behaviour with a wide spectrum of food sources and uses small and medium-sized mammals as preys or carrions, while the wolf selects opportunistically medium-sized and large mammals as preys. A very slight overlap of their trophic niche was observed, mainly represented by wild ungulates, although their different use (as prey or carrions) by the two carnivores would suggest not to interpret this overlap as direct competition.

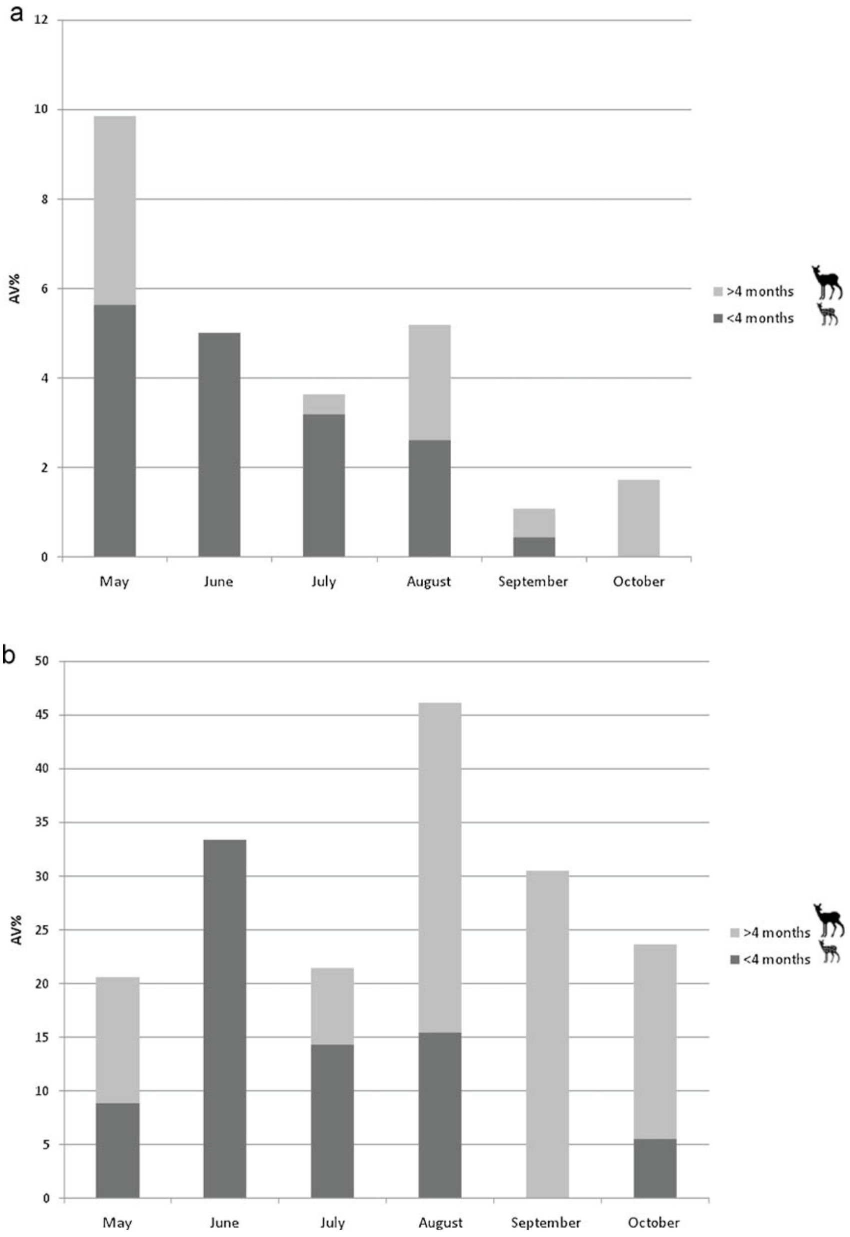

Fig. 4. Monthly use of fawns (roe deer <4 months) by red fox (a) and wolf (b) in summer 2008. Data are expressed as average volume percentage (AV%).

The assessment of predator food habits is necessary in order to evaluate possible long-term effects of predation on prey populations. In this context, it is important to investigate the respective role of these two carnivores on the ungulate communities, in order to assess the overall impact of predation on large prey populations. This represents a crucial step to better understand the causes of demographic fluctuations in ungulate communities and to verify possible functional responses of predators. All these information will contribute to preserve species of conservation interest, like wolf, and to manage exploited prey populations.

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SECOND PART

Preys-Predator interaction

Chapter 2

Prey selection by an apex predator: the importance of sampling uncertainty

Prey Selection by an Apex Predator: The Importance of Sampling Uncertainty

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Abstract

The impact of predation on prey populations has long been a focus of ecologists, but a firm understanding of the factors influencing prey selection, a key predictor of that impact, remains elusive. High levels of variability observed in prey selection may reflect true differences in the ecology of different communities but might also reflect a failure to deal adequately with uncertainties in the underlying data. Indeed, our review showed that less than 10% of studies of European wolf predation accounted for sampling uncertainty. Here, we relate annual variability in wolf diet to prey availability and examine temporal patterns in prey selection; in particular, we identify how considering uncertainty alters conclusions regarding prey selection. Over nine years, we collected 1,974 wolf scats and conducted drive censuses of ungulates in Alpe di Catenaia, Italy. We bootstrapped scat and census data within years to construct confidence intervals around estimates of prey use, availability and selection. Wolf diet was dominated by boar (61.563.90 [SE] % of biomass eaten) and roe deer (33.763.61%). Temporal patterns of prey densities revealed that the proportion of roe deer in wolf diet peaked when boar densities were low, not when roe deer densities were highest. Considering only the two dominant prey types, Manly's standardized selection index using all data across years indicated selection for boar (mean = 0.7360.023). However, sampling error resulted in wide confidence intervals around estimates of prey selection. Thus, despite considerable variation in yearly estimates, confidence intervals for all years overlapped. Failing to consider such uncertainty could lead erroneously to the assumption of differences in prey selection among years. This study highlights the importance of considering temporal variation in relative prey availability and accounting for sampling uncertainty when interpreting the results of dietary studies.

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Competing Interests: The authors have read the journal's policy and have the following conflicts: one of the authors, S.G. Willis is an editor at PLOS ONE. This has in no way affected the integrity of the authors' submission. This does not alter the authors' adherence to all the PLOS ONE policies on sharing data and materials.

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Introduction

Predator populations that have long been subjected to persecution are receiving increased conservation attention and are recovering in both North America and Europe [1–3]. Predicting the impact of changing predator numbers on prey species is important for managing populations of both predators and their prey [4–6]. Accurate predictions require a thorough understanding of predator diets and prey selection, which can be affected by a multitude of factors including: prey and predator densities [7]; the functional and numerical responses of predators to changes in prey density [8–9]; community composition (particularly the presence of alternative prey [10–11]); climatic conditions [12]; vegetation productivity [13–14]; and landscape heterogeneity [15]. These drivers can result in considerable temporal and spatial variation in patterns of predation. For this reason, studies of predation often require large sample sizes and high quality data to overcome uncertainty. However, because large predators are generally elusive and exist at low densities, they are expensive and time-consuming to study, meaning that large sample sizes are rare and results must usually be interpreted with caution. Failure to describe adequately the uncertainty in a dataset can promote misleading conclusions about predator feeding habits.

In Europe, the wolf (Canis lupus) is recovering from centuries of persecution. The expansion of wolf populations in many European countries [3] has the potential to change fundamentally the ecology of communities by exposing large ungulates to natural predation after decades (and in some cases, centuries) of predator absence. In North America, wolves limit ungulates in some areas [8,16] and predation by recovering wolf populations has triggered complex trophic cascades, altering prey distribution and plant recruitment [2,17]. Studies of ungulate dynamics and distributions in Europe indirectly suggest that wolves might play a similar role by limiting prey [11,14,18] but the intricacies of wolf-prey relationships and the potential for trophic cascades in European communities is poorly understood [19–20]. Dietary studies that accurately describe wolf prey selection are a necessary first step toward understanding wolf predation impacts on European wildlife.

Over the past three decades, scat analysis has been used to describe the dietary composition and prey selection of wolves, and to estimate their potential impact on prey communities [18,21–27]. Scat-based dietary studies in Europe have highlighted the flexibility of the wolf as a predator. This variability is especially evident from reports of wild boar (Sus scrofa) utilisation among sites. Based on a review of results from the Bialowieza Primeval Forest (BPF), Poland, and other literature, Okarma [11] concluded that wild boar are generally avoided, while red deer (Cervus elaphus) are the prey of choice. However, BPF has a diverse ungulate community comprising 5 species (Cervus elaphus, Sus scrofa, Capreolus capreolus, Alces alces, Bison bonasus), some of which are no longer common elsewhere in modern-day Europe. By contrast, studies in southern and Mediterranean areas of Europe indicate that boar are sometimes preferred as prey [22–23,25,28–29]. Some of these southern sites are dominated by only two species, wild boar and roe deer (Capreolus capreolus), and could be considered more representative of communities throughout much of Europe [30]. Selection between these two prey appears to vary both among and within sites. This has been attributed to a variety factors including differences in community composition and in the vulnerability of individuals (as influenced by age, body size, grouping behaviour and season); unfortunately, the data required to distinguish between these alternatives are lacking [23,25,28,31].

Some of the apparent variability in wolf diet may be a result of the scat analysis methods that are widely used to determine diet. Several papers have highlighted potential pitfalls in the scat analysis process, including those which may arise from the analysis of small datasets [32–36]. The potential for sampling error to arise is particularly high when the number of scats collected is small relative to the number produced by the study population. Such samples might not be representative and can lead to incorrect conclusions about diet, especially when the uncertainty in estimates based on small samples is not reported. Reynolds and Aebischer [33] advocated the use of re-sampling techniques (e.g. bootstrapping) to produce confidence intervals around estimates of dietary composition. While some recent studies (e.g. [35]) have used re-sampling techniques, much of the existing literature on European wolf diet does not account for uncertainty due to sampling error in results (20 out of 22 studies examined; Text S1, Table S1). In addition, studies of prey selection require estimates of prey availability, which are themselves subject to error. Failure to consider uncertainty in both prey use and prey availability can result in inappropriate conclusions.

Predation patterns may be further obscured by neglecting variation in prey selection among years, within a site. Many studies of wolf diet are either relatively short or pool scat samples across years (to increase sample size), thereby obscuring inter-annual variation (Text S1, Table S1). Mattioli et al. [29] found that prey use can vary substantially among years and that much of this variation is unaccounted for by the changing abundance of prey. Environmental factors affecting prey vulnerability (e.g. weather conditions, land use) may vary substantially from one year to the next, creating variability that could underlie some of the inconsistencies observed in wolf predation among sites. Long term studies that explicitly incorporate this variability will facilitate comparisons of wolf diet among sites and enable the identification of potential drivers of predation patterns across the continent.

In this study, we combine re-sampling techniques with nine years' scat sampling and drive census data to address the following questions regarding the dietary habits of wolves in Alpe di Catenaia: 1) do the wolves select for either of the two main prey species available, roe deer and wild boar? 2) how might an explicit consideration of uncertainty affect our conclusions about wolf dietary selection? and 3) how does wolf diet relate to the relative availability of prey species in the area?

Methods

Study area

The 120 km^2 Alpe di Catenaia study area is in the Apennine mountains in the north-east of Tuscany, Italy (Arezzo province, 43u489N, 11u499E). A 27 km^2 area within this site is a protected area where hunting is banned (Fig. 1). Altitude within Alpe di Catenaia ranges from 300 to 1414 m above sea level. Vegetation cover is mainly composed of mixed deciduous hardwoods (76% of total area), dominated by oak (Quercus spp.), chestnut (Castanea sativa) and beech (Fagus sylvatica). The climate is temperate and seasonal with hot, dry summers, and cold, wet winters. Snowfall usually starts in October and may continue through April. There are a number of farms surrounding the study area which raise livestock (mostly sheep) that are a potential additional source of prey for wolves.

Prey density and biomass estimation

The wild ungulate community included only wild boar and roe deer for the first seven years of the study; red deer have been occasionally recorded in the study area since 2007. Densities of wild boar and roe deer were estimated from drive censuses completed every May (2000–2005, and 2007–2008; method also described by Mattioli et al. [28]) by the Provincial Administration of Arezzo; the 2006 census excluded a large portion of the study area, so was excluded from our analyses. Census work was undertaken following accepted guidelines for monitoring wild ungulates, with permission granted by the Regional Government of Tuscany and Provincial Government of Arezzo. Censuses took place in both the protected and non-protected parts of the study area each year, encompassing about 80% wooded area and 20% other cover types. Government employees, researchers, and volunteers encircled an area of forest (each 0.14–0.52 km^2 in size) then moved inwards and counted wild boar and roe deer observed in the contained area. Between 9 and 15 such forest blocks were sampled each year. The average density of observers during these surveys was approximately 110 persons per km^2 [24]. In order to extrapolate from the surveyed areas to estimates of overall density at the site, we corrected for the differences in block area and the forest cover surrounding each block. The latter is necessary because wooded areas surrounded by more open habitat could appear to have higher densities of animals because during drives animals congregate in the more sheltered, forested areas [37]. The percentage area covered by forest within a 1 km buffer surrounding each forest block was extracted using GIS (ArcGIS version 10 [38]). The corrected density of animals within each surveyed block was thus calculated as number of individuals counted divided by block area and multiplied by percentage forest cover of the surrounding area (median value 81%, range 41–96.1% across blocks). The overall density of wild boar and roe deer at the site was then estimated as the mean across the different blocks. Drive census are a widely used technique and, while some animals are not seen during a census, it has been found that such drive census generally give higher density estimates than alternative methods [39]. To convert densities to biomass densities (kg per km^2) we

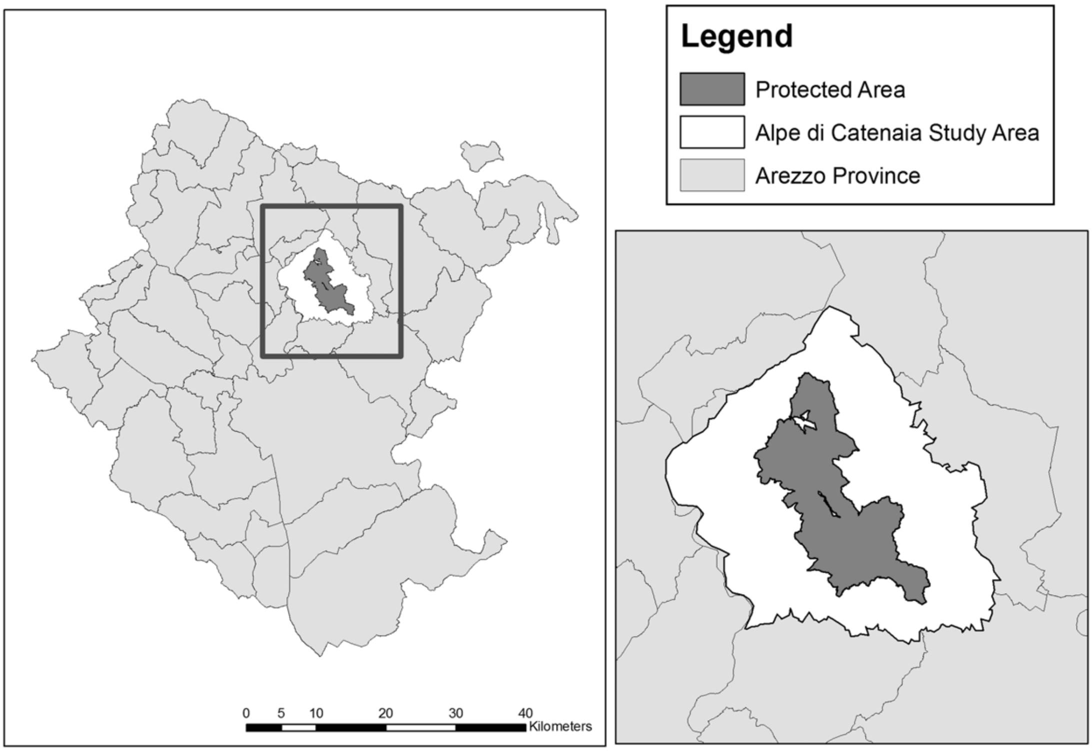

Figure 1. Alpe di Catenaia, Italy. The Alpe di Catenaia study site is located in the Arezzo province in Northern Tuscany, Italy. The study site includes a central protected area, where hunting is prohibited.
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used the average body mass of boar (43.260.33 [SE] kg, n = 5003) and roe deer (21.160.12 [SE] kg, n = 2355) hunted in the districts that immediately surround the protected area (all age classes included).

Scat collection and assessment of wolf diet

During the study period the area supported a single wolf pack which contained 3–6 individuals. This was confirmed using genetic analysis of scats (unpublished data), snow-tracking [18] and wolf-howling surveys [40]. Similar to the drive censuses, this work was undertaken with permission from the governments of Arezzo and Tuscany. Wolf scats were collected monthly between May 2000 and April 2009 from seven transects distributed throughout the study area (total length: 73 km per month). Years were defined as extending from May to the following April (i.e. scats collected between May 2000 and April 2001 were assigned to the year 2000–01). Scats were washed and the recovered prey remains were oven-dried at 68uC for 24 hours. Prey categories included wild boar, roe deer, red deer, hare (Lepus europaeus), small rodents, goats, sheep and cattle. Prey remains were identified through comparison to a reference collection of mammal hair, bones, and teeth collected from within the study area. Specimens were identified to species and age-class (for ungulates only) when possible. This identification was based on the macroscopic characteristics of hairs and bones following Mattioli et al. [28–29]. Boar remains were divided into three age-weight classes: newborn piglet (,10 kg), piglet (10–35 kg), and adult (.35 kg). Roe deer remains were classified into two classes: fawn (,1 year) and adult (.1 year). The ability of researchers to discriminate among samples from different species and age-classes was verified by means of a blind test using artificial ''scat samples'' containing prey remains from a variety of species and age-classes. A total of 200 samples were stored in plastic bags, each consisting of remains from one potential prey item. All potential prey in the area were represented in these samples, including hair samples from animals during both summer and winter. Each researcher was assigned 50 of these bags, chosen at random, and was assessed on their ability to correctly identify the age-class and species represented by the sample. Ability to discriminate among wild boar weight classes was additionally assessed using a further 25 samples per researcher. Only researchers who correctly identified all test samples went on to analyse true scat samples.

Most scats were entirely composed of just one prey item; the relative volume of these scats amounted to 100% of the same prey type. When more than one prey type was evident in a single scat, the relative volume of each was estimated as approximately 25, 50 or 75% of the scat's total volume. When the age class of ungulate remains could not be identified, the relative volume of the unidentified material was redistributed according to the proportions of the age-groups observed among other scats collected during the relevant period. The biomass of prey consumed to produce the collected scats was estimated using Weaver's [32] biomass model. In this model the live weight (w_i) of an individual of prey type i is converted into c, an estimate of the biomass (kg) of that prey type that must have been consumed to produce one scat, according to the following equation:

$$c\sim0{:}439z0{:}008 * w_i$$

Multiplying c by the summed relative volumes of scats attributable to each prey species gave the inferred total biomass of each prey species consumed (hereafter, the 'biomass consumed'), as indicated by the sample of scats collected. The weights of different age classes (obtained from data on hunted individuals in each age class) were accounted for in this calculation. The general composition of wolf diet each year was described as the percentage of total biomass consumed attributable to each prey group. These calculations were completed for the entire set of scat samples collected each year.

Wolf dietary response and prey selection within the main, two-ungulate community

Wild boar and roe deer dominated the prey community in Alpe di Catenaia and were the main prey items of importance. To estimate selection by wolves, we focused on boar but, obviously, the complement of our estimated parameters applies to roe deer. Based on the scat analysis, we inferred the biomass consumed of boar (C_B) and roe deer (C_R), calculating the relative use of boar as $U_B = C_B/(C_B+C_R)$. U_B was calculated for each of the nine years and is hereafter referred to simply as boar use. The relative availability of wild boar for eight years of the study (the 2006–07 census was excluded, see above) was given by $A_B = B_B/(B_B+B_R)$, where B_B and B_R are, respectively, the biomass densities of boar and roe deer in the area.

We used linear regression to model relative boar use as a function of boar availability. Consistency with the assumptions of linear regression was checked using diagnostic plots. Several studies have found seasonal differences in the absolute consumption of wild boar (percent of diet) by wolves [18,23,25,41], so we initially developed models that included a seasonal component. However, season was not significant in these models so was not considered further (Text S2, Table S2).

Wolf selection for wild boar (within the wild boar-roe deer community) was assessed using Manly's standardized selection ratio, a [42–43]:

$$a\sim\frac{U_B{=}A_B}{\text{ð}U_B{=}A_B\ \text{Þzðð}1\{U_B\text{Þ}{=}1\{A_B\ \text{Þ}}$$

Here, a is the probability that wild boar would be selected when offered in equal biomass to roe deer. An estimate of a_i<0.5 indicates use of boar in proportion to boar availability. a_i.0.5 indicates selection for wild boar, while a_i,0.5 indicates selection against boar. We calculated Manly's selectivity index for boar for all eight years with availability estimates.

Uncertainty estimation

Uncertainty in our estimates of wild boar use, availability, and selection by wolves within years was determined by bootstrapping [44]. For estimating boar use, all scat samples for a year were randomly sampled with replacement to produce a new estimate of the biomass consumed of both wild boar and roe deer. Similarly, for estimating boar availability, densities based on drives in separate areas of the study site were randomly sampled with replacement to produce a new estimate of density for both ungulate species. As drives in some areas each year failed to find any individuals of a given species (resulting in a density of 0 for that drive) the possibility existed for bootstrap estimates of site densities to be zero (causing analytical problems when dividing use by availability); we controlled for this by assuming a minimum possible density equal to the total number of individuals observed divided by the total area sampled that year in all drives. We used this approach to generate 4,000 bootstrap samples within each year. The relative use and relative availability of wild boar and Manly's selectivity ratio were calculated for each bootstrap sample, using the 2.5% and 97.5% quantiles to construct 95% confidence intervals around these estimates for each year. All analyses presented here were performed in R 2.13.0 [45].

Results

Ungulate community composition

Wild boar density estimates ranged from 4.7 to 26 km^{22} during the nine year study period (mean = 14.362.57). Roe deer density was less variable than boar density and ranged from 32.8 to 47.7 deer km^{22} (mean = 39.661.64; Fig. 2). Confidence intervals, representing the uncertainty surrounding yearly density estimates due to potential sampling error, were wide for both species and made it difficult to say with confidence that densities differed among years. In fact, only the low boar density observed in 2004–05 was significantly different from other years, with 95% confidence limits that excluded the mean density observed across years. Bootstrapping simulations resulted in an exceptionally wide confidence interval for the boar density estimate for 2007–08 (Fig. 2), which reflects the high variation observed among different drives in that year (boar densities ranged from 0 to 304 km^{22} across the 15 areas surveyed). Due to the combined uncertainty surrounding density estimates of both species, the confidence intervals surrounding our estimates of the relative availability of wild boar (based on biomass density) within this two species community were also wide and overlapped among years (Fig. 3a).

Wolf diet and relative use of wild boar

A total of 1,974 wolf scats were collected and analyzed during the study. The diet of wolves in Alpe di Catenaia was consistently dominated by the consumption of wild boar and roe deer, which together made up 95.261.29% of the annual diet (Table 1). Wild boar was the primary prey, being found in the majority of scats collected, and accounting for 61.563.90% of biomass eaten. Roe deer, the second most prevalent prey species, accounted for 33.763.61% of total prey biomass. Other prey, including livestock, represented only a very small proportion of the diet (Table 1).

Although boar and roe deer consistently accounted for over 90% of biomass eaten, the percent of diet individually attributable to either species was variable across the nine year study period (Table 1); this is reflected in our estimates of boar use by wolves (Fig. 3a). Boar use (mean over the entire period: 0.6560.039; Fig. 3a) was generally higher than that of roe deer and, for five of the years analyzed, the percent of wolf diet made up of wild boar was more than twice that of roe deer. Confidence intervals surrounding estimates of boar use were narrow in comparison to those calculated for boar availability (Fig. 3a), reflecting the large number of scats collected each year during the study (.140 scats each year compared to only 9–15 drives per year that were used to estimate availability).

Inter-annual fluctuations in boar use, the proportional biomass of wild boar in wolf diet relative to that of roe deer and wild boar combined, reflected changes in the proportional availability of wild boar as a prey item. Based on the regression of boar use as a function of availability, boar availability accounted for 62% of the variation in boar use across years (b_{BA} = 0.78460.2222, R^2 = 0.621, t_6 = 3.529, P = 0.012; Fig. 3b). The years of comparably low boar use (2001–02, 2004–05, and 2005–06; Fig. 3a) coincided with years of low boar density, rather than years of high roe deer density (Table 1, Fig. 2).

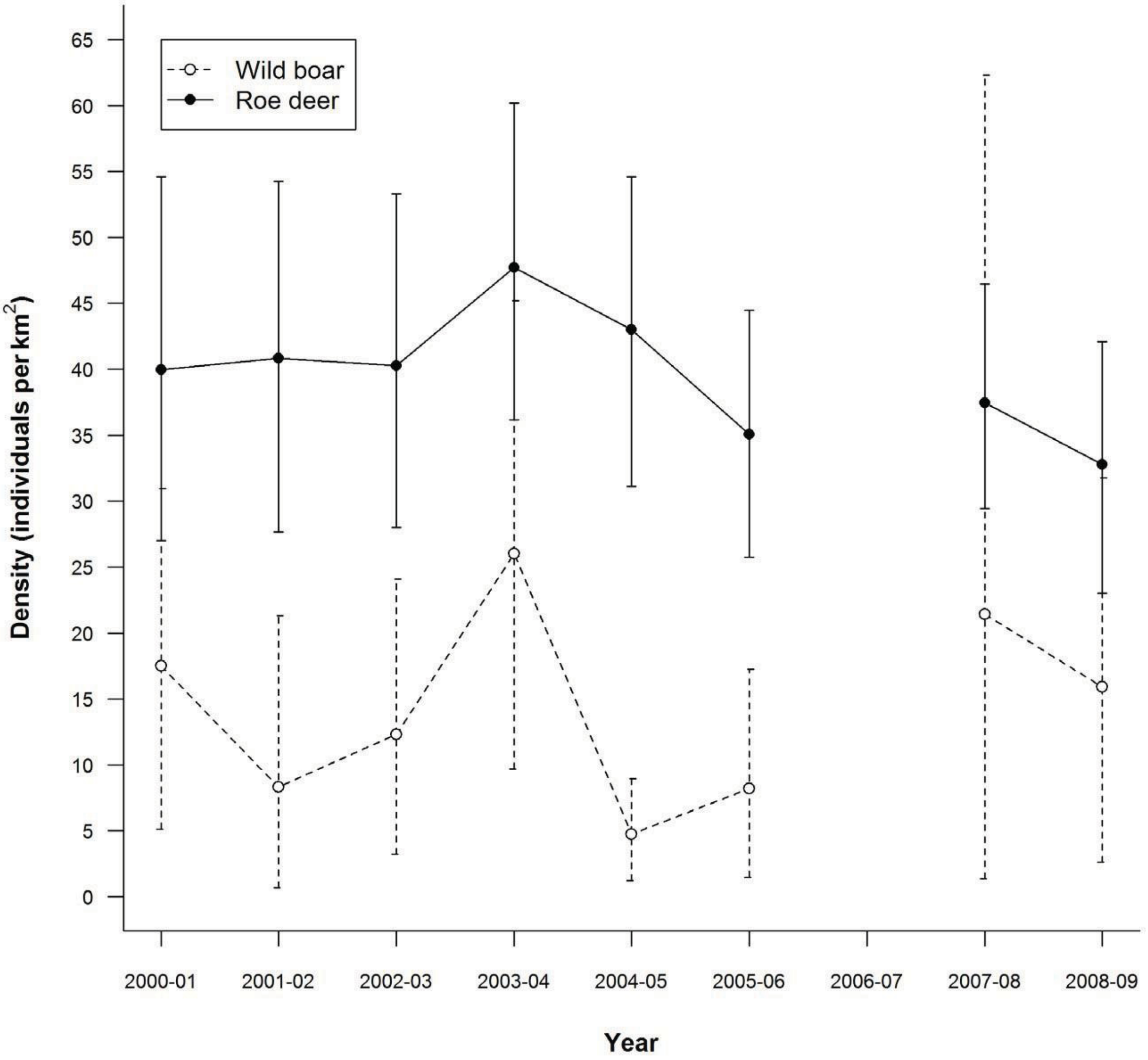

Figure 2. Wild boar and roe deer density in Alpe di Catenaia. The densities of the two main wolf prey items, wild boar (open circles) and roe deer (solid circles), from drive counts conducted each April in Alpe di Catenaia. Error bars represent 95% confidence intervals. Density estimates for the year 2006–07 were unavailable.
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Prey Selection

Estimates of Manly's selectivity index ranged between 0.60 and 1.82 across eight years with a mean of 0.7360.023 indicating a strong tendency for selection for boar and against roe deer by the wolves in Alpe di Catenaia (Table 2). Estimates of Manly's index indicated selection for boar (a_{Boar}.0.5) in five out of the eight years examined (Table 2). This reflects the fact that boar use was generally high relative to its availability (Fig. 3a). The confidence intervals for the yearly estimates of Manly's index were wide, representing a high level of uncertainty due to sampling variation among individual scats and drive censuses. The overlap of confidence intervals among years cautions against the temptation to infer variation in selection for boar during the study period (Fig. 4).

Discussion

We found that the consumption of wild boar dominated wolf diet and the use of boar as prey (relative to the use of roe deer) is strongly related to the relative availability of wild boar within the study area. Wolves in Alpe di Catenaia selected wild boar over roe deer as prey and there is little evidence of variation in the strength of this selection among years. Had we not recognized the uncertainty inherent in our data we may have erroneously interpreted variation in our estimates of prey selection as indicative of differential selection among years. The length of our study combined with our large sample size of scats (1,974 over the nine year study period) allowed us not only to examine inter-annual variation in wolf predation, but also to consider the potential impacts of sampling error on our results. The amalgamation of uncertainty from multiple sources (i.e. the estimation of both prey availability and use) means that the uncertainty surrounding final estimates of prey selection is very large. Accounting for this uncertainty limited the conclusions we were able to make but ensured that our interpretation of inter-annual variability in prey selection by wolves in Alpe di Catenaia was fully supported by the data.

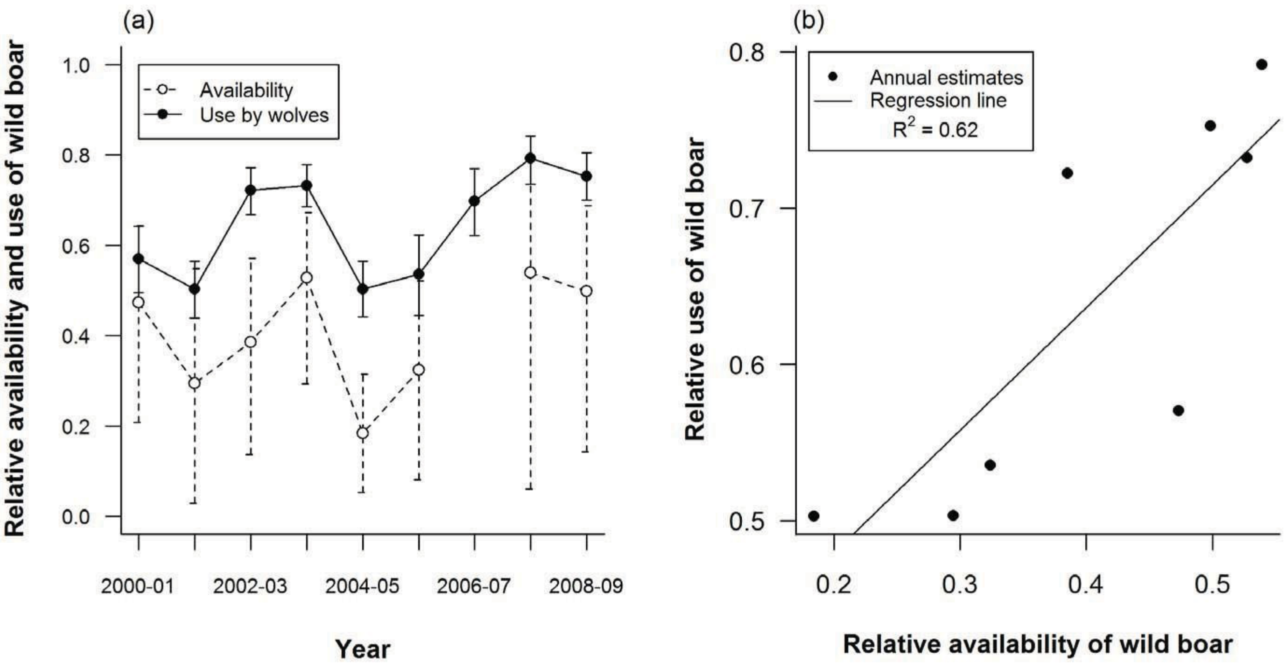

Figure 3. Wild boar use and availability. The relationship between the availability and use of boar (relative to ungulate community including wild boar and roe deer only) is shown. a) Relative availability (grey line, open circles) and relative use (black line, solid circles) was estimated each year from 2000–01 to 2008–09 excluding the year 2006–07. Error bars represent 95% confidence intervals. b) Linear regression analysis was used to illustrate the relationship between the relative availability and the relative use of wild boar across the eight years (solid circles) for which availability was estimated (black line, $y = 0.323 + 0.784x$, $R^2 = 0.621$, $P = 0.0124$).
doi:10.1371/journal.pone.0047894.g003

Wolf diet in Alpe di Catenaia

As in other areas with an abundance of wild prey [11,18,22,28,46], the wolves inhabiting Alpe di Catenaia site subsist mainly on wild ungulates, with a very low frequency of livestock predation. It is the selection of prey species within the wild ungulate community that appears somewhat unusual. In contrast to wolves in other parts of Europe which often avoid boar as prey [11], wolves in Alpe di Catenaia appear to rely heavily on wild boar. Despite the wide confidence intervals surrounding our annual estimates of boar selection we found that boar were selected (over roe deer) in six of the eight years examined. Boar made up the majority of biomass eaten throughout most of the study period. While we cannot be certain of a causal relationship, the strength of boar availability as a predictor of boar use suggests that wolf diet was tracking the fluctuations in boar densities. Roe deer, while an important prey item, usually made up a smaller

Table 1. Composition of wolf diet was assessed based on scat samples collected in Alpe di Catenaia, Italy.

Wolf diet composition from 2000 through 2009: percentage of biomass consumed per prey item

Prey item	Scat samples[a]	178	242	262	293	232	143	144	208	272	Mean ± SE (n = 9)
Wild boar	1284	55.9	48.2	68.5	71.2	48.8	46.1	68.7	76.5	69.6	61.563.90
Roe deer	804	42.1	47.6	26.3	26.1	48.2	39.9	29.8	20.1	22.9	33.763.61
Red deer	12	0	0	0	0	0	0	0	0.4	6.1	0.760.67
Hare	26	0	0	0.6	1.8	1.1	4.5	0.6	1.0	0	1.160.47
Small rodents	18	0.4	0.2	0.3	0.4	1.2	0	0	0	0.3	0.360.12
Sheep	29	1.6	3.7	4.3	0.5	0.8	8.5	0	0	0.3	2.260.95
Goat	3	0	0.4	0	0	0	1.0	1.0	0	0	0.360.14
Cattle	3	0	0	0	0	0	0	0	2.0	0.8	0.360.22

[a] Scat samples per year sum to the total number of samples used in all analysis over 9 years (1,974). Scat samples per prey item are defined as the total number of scats found containing that prey item in any proportion and may, therefore, sum to more than the total number of scat samples collected.

[b] For analysis purposes our data years began in May and ended in April; the 2000–01 year represents all scats collected between 1 May 2000 and 30 April 2001.

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Table 2. Selection of wild boar as a prey species based on estimates of boar use by wolves and relative availability within Alpe di Catenaia, Italy.

Year[a]	Scat samples collected	Relative wild boar availability[b]	Relative wild boar use	Manly's standardized selection ratio, calculated for wild boar use in wolf diet[c]	Bootstrapped 95% confidence intervals on Manly's standardized selection ratio	
					Lower limit	Upper limit
2000–01	178	0.47	0.57	0.60	0.41	0.84
2001–02	242	0.30	0.50	0.71	0.45	0.97
2002–03	262	0.39	0.72	0.81	0.66	0.94
2003–04	293	0.53	0.73	0.71	0.56	0.87
2004–05	232	0.18	0.50	0.82	0.68	0.95
2005–06	143	0.32	0.54	0.71	0.50	0.93
2006–07	144	Not available	0.70	NA	NA	NA
2007–08	208	0.54	0.79	0.77	0.49	0.99
2008–09	272	0.50	0.75	0.75	0.58	0.95
Mean 6 SE		0.4060.043	0.6560.039	0.7360.023		

[a] Data years began in May and ended in April; the 2000–01 year represents all scats collected between 1 May 2000 and 30 April 2001.

[b] Wild boar availability and use in wolf diet are calculated based on biomass (kg per km^2) relative to the availability and use of the main ungulate community in Alpe di Catenaia consisting of wild boar and roe deer only. See methods for more detail.

[c] For Manly's standardized selection ratio, values approximately equal to 0.5 indicate prey use in proportion to availability in a two-prey system while selection for and against the prey type of focus would be indicated by higher and lower values respectively.

doi:10.1371/journal.pone.0047894.t002

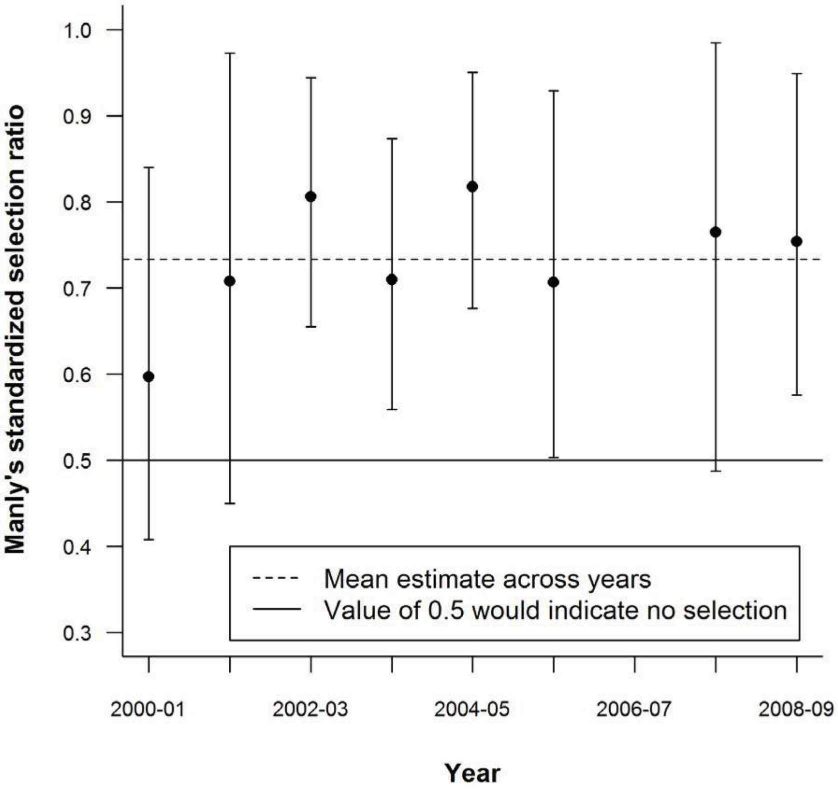

Figure 4. Uncertainty and variation in the selection of wild boar across years. Manly's standardized selection ratio for wild boar (in wolf diet) was calculated for eight years from 2000–01 to 2008–09. This index is based on the relative availability and use of boar within the main two-prey community composed only of wild boar and roe deer. Error bars representing bootstrapped 95% confidence intervals are displayed. Values approximately equal to 0.5 (black line) indicate prey use in proportion to availability in a two-prey system while selection for and against wild boar are indicated by higher and lower values respectively. The mean value of Manly's selection ratio for boar during the study period was 0.73360.0234 (dashed line).

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portion of wolf diet. The percentage biomass of roe deer in wolf diet appeared to peak when boar densities were low, not when roe deer densities were highest. In Alpe di Catenaia, the relatively stable roe deer population may represent an alternative prey source which suffers higher predation when wild boar densities decline. That the extent of wolf predation on roe deer can fluctuate widely, even when roe deer are relatively stable, underlines the importance of taking a community perspective to investigate and predict predation impacts on any given species [10,47].

The strength of selection for boar in Alpe di Catenaia raises the question of why similar selectivity is not seen throughout Europe. There could be three reasons for this. Firstly, many European ungulate communities include red deer, which appear to be a favoured prey of wolves in many sites (reviewed by Okarma [11]). The scarcity of red deer (completely absent until 2007) in Alpe di Catenaia could lead to stronger selection for wild boar and could drive the dietary response of wolves to changes in boar availability observed in this study. A study of wolves in another region of the same Italian Province but with a more diverse prey community (including red and fallow deer in addition to roe deer and wild boar [29]), found that while wolves relied heavily on boar consumption, the composition of wolf diet was unrelated to boar availability. Secondly, wild boar in Mediterranean areas are relatively small; for example, adult boar in Alpe di Catenaia, weighed 66.560.48 kg (based on mass data for 1,286 adult boar carcasses collated by the Province of Arezzo). In central Europe, where adult male boar can exceed 300 kg in size [48], their active defence behaviour can, reportedly, make them dangerous prey for wolves [49]. This small size of adults in Alpe di Catenaia may make boar less threatening as prey and, in combination with their large litter sizes (often exceeding 5 piglets per litter [50]) and grouping behaviour, may encourage wolves to select boar over roe deer [22,28–29]. Finally, this study included only a small number of wolves, believed to belong to a single pack, and therefore it is possible that the preference for boar reflects the habits of this particular pack or the individuals within it. However, similar studies in the region have also identified a preference of wolves for wild boar over roe deer [29,51]. Individual preferences could lead to variation in selection for prey among years but we found no evidence of significant interannual variation in this study (see below for further discussion).

The importance of intra-annual uncertainty when considering variation in prey selection

Variation in wolf predation patterns (e.g. disparate prey selection among sites with similar prey communities) may reflect underlying differences in the ecology of distinct sites or a failure to assess accurately the uncertainty inherent in estimates of wolf feeding habits. Our final estimates of prey selection indices had very wide confidence intervals, suggesting high levels of uncertainty in the data on boar use (from wolf scats) and, in particular, the data on boar availability (from drive censuses). Sampling error is difficult to avoid and is present in all datasets, to some extent. Uncertainty in this study arose particularly from the estimation of annual prey densities, because of the low number of 'density samples' (drive censuses from different areas of the study site) in each year. This is a common situation in European ungulate research and many datasets will incorporate similar levels of uncertainty in their density estimates.

Without considering uncertainty, our results would suggest substantial variation among years in the strength of selection for boar by the wolves in Alpe di Catenaia. However, when we put the observed variation into the context of within-year uncertainty it is not possible to say with any confidence that prey selection in our site differed from one year to the next. This finding also compels caution when comparing selectivity estimates between different sites. For example, comparing the point estimates of Manly's a from this study to those observed in other areas could suggest geographic variation in selection (especially if the studies being compared were of short duration or if results had been pooled across years). We do not suggest that such variation does not exist but, in some cases, reported differences in wolf predatory habits among sites (or time periods within sites) might disappear when uncertainty in estimated metrics (such as selection indices) is accounted for.

Caveats and considerations for future research

Our findings should be considered in light of several important caveats. The first two relate to the fact that only one census of prey was possible each year. While the prey selection observed in this study could arise for the reasons described above (relating to community composition and boar body size) it could also be partially driven by variation in prey vulnerability due to temporal fluctuations in population age structure. In particular, because wild boar can produce two litters within a single year and boar piglets are likely to be more vulnerable as prey, there is a high potential for both inter- and intra-annual variation in the overall vulnerability of wild boar [52]. Estimating the age structure of prey populations multiple times each year would help isolate the influence of changing prey vulnerability on selection by wolves. Additionally, seasonal movement of prey species could affect their relative availability, and such intra-annual variation will not be reflected by annual drive censuses. However, telemetry studies at the site suggest that the mean home range areas (Minimum Convex Polygons) of the prey species (roe deer: 4.064.43 km^2, n = 69 individuals; wild boar: 7.569.50 km^2) were substantially smaller than the study site (120 km^2), suggesting that such intra-annual migration was unlikely to be a major factor.

Three further caveats suggest general lessons for studies of dietary selectivity.

Firstly, we do not know how much of the prey consumption we observed could be due to scavenging upon carcasses rather than direct predation. In the future, closer observation of individual wolves, using radio-telemetry, may provide estimates of scavenging frequency and allow us to adjust our estimates of predation accordingly. Secondly, all density estimation methods incorporate some degree of error due to unobserved individuals and the drive censuses used in this study are no exception. McCullough [53] estimated that errors in drive census estimates can be as large as 20–30% of the true population size. Estimates of wild boar densities are particularly challenging due to their wide-ranging behavior and aggregated distributions [54]. Capture-mark-recapture estimates might provide more accuracy but can be more resource intensive (in terms of time, equipment and labor). When mark-capture-recapture estimates are not possible, researchers can form more robust conclusions from studies requiring density estimates by acknowledging the uncertainties associated with chosen methods and, when possible, by comparing estimates based on a variety of methods (e.g. pellet counts, camera surveys etc.) simultaneously. Finally, on a related note, our spring density estimates took place before the birth of new roe deer fawns but after the initial pulse of boar births. This means that we might be over-estimating the relative availability of boar within this two-prey system and therefore under-estimating the strength of selection for boar as prey. Our conservative estimates of boar selection would most likely be strengthened if we were able to use post-reproductive roe deer densities. In the future, this bias could be avoided by either using estimates of roe deer reproduction to estimate post-reproductive densities or by surveying ungulate densities later in the spring.

Conclusion

Wild boar are the primary prey of wolves in Alpe di Catenaia, Italy. For the wolves in this area, roe deer represent an alternative prey source which increases in dietary importance when boar densities decline. While accounting for sampling uncertainty in our data, we were able to show that boar were significantly selected for during the majority of the years studied. Boar use throughout the study period was strongly related to the relative availability of wild boar within this predominantly two-prey community, a finding which suggests a dietary response by wolves to the availability of wild boar. The high natural variability of wild boar populations [52,55] thus could have important ramifications for predator impacts on roe deer.

Our findings demonstrate that failing to account for uncertainty when interpreting inter-annual variation in studies of predator diet might lead to conclusions that are not fully supported by the data. In addition to presenting multi-year datasets without pooling data across years, when possible, future studies of prey selection should strive to account for possible sources of uncertainty due to sampling procedures. While the comparison of a predator's dietary composition and prey selection across years and sites can yield important information about large-scale patterns of predation, such analyses often incorporate uncertainty from multiple sources. Caution must be taken to describe such uncertainty before drawing ecological conclusions, so that the nature of complex predator-prey relationships is properly represented.

Supporting Information

Text S1 Literature reviewed on European wolf diet (see Table S1).
(DOC)

Text S2 The analysis of boar use in response to season (methods and results).
(DOC)

Table S1 Published studies of wolf diet in Europe surveyed for analysis of uncertainty and inter-annual variability in estimates of dietary composition and prey selection.
(DOC)

Table S2 Repeated measure ANOVA of the effects of boar availability, season and their interaction on seasonal use of boar by wolves (relative to the wild ungulate community including boar and roe deer). Data were collected from 2000–2009 in the Alpe di Catenaia study site inItaly.
(DOC)

Contract S1 Contract for ungulate work, 2005.
(PDF)

Contract S2 Contract for ungulate work, 2009.
(PDF)

Contract S3 Contract for wolf work, 2000.
(PDF)

Contract S4 Contract 1 for wolf work, 2001–03.
(PDF)

Contract S5 Contract 2 for wolf work, 2001–03.
(PDF)

Contract S6 Contract for wolf work, 2004.
(PDF)

Contract S7 Contract for wolf work, 2005–07.
(PDF)

Contract S8 Contract for wolf work, 2008–09.
(PDF)

Contract S9 Contract for wolf work, 2009–2013.
(PDF)

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Author Contributions

Conceived and designed the experiments: MA MD PS. Performed the experiments: CC EB AM ED. Analyzed the data: MD PS EB AM. Contributed reagents/materials/analysis tools: PS CC EB AM ED. Wrote the paper: MD PS MA SW.

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Chapter 3

Combined effect of predation by wolves (Canis lupus) and culling by hunters on an ungulate community if central Italy

Combined effect of predation by wolves (*Canis lupus*) and culling by hunters on a ungulate community of central Italy

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ABSTRACT

We use data from 2000 to 2010 to investigate the predator-prey system of Oasi Alpe di Catenaia (OAC, eastern Tuscany, central Italy). Here a pack of wolves lives together with dense populations of wild boar and roe deer. We evaluate the combined impact of wolves and hunters, and investigate the age/weight classes preferences of the two predators to understand if they can limit or regulate ungulate community. Analyzing data we took into account the uncertainty of the ungulates density estimations, calculating the 95% CI. For both prey species removal by hunters resulted to be higher than wolves. Wild boar was the main prey species for wolves and the most harvested one by hunters. For both prey species, the wolves focused on the intermediate weight class, while the hunters focused on the largest. The combined impact of hunters cull and wolves predation did not exceed the recruitment of ungulate populations, and thus did not result to limit wild ungulates population. We can therefore confirm that a bottom up regulation takes place in this species in our study site: in particular we supported our previous finding on the importance of forest productivity for wild boar population dynamic.

Keywords: wolf predation, hunting, feeding habits, wild boar, roe deer, ungulate recruitment, forest productivity

INTRODUCTION

Nowadays wolf (*Canis lupus*) is one of the most widespread carnivores in the world and its population is both expanding its range and growing in numbers. Wolves represent a keystone species that can drive the ecosystem they are part; as Mech and Boitani (2003) underlined wolves “are probably the single most important predators on large mammals”.

The ways in which wolf and preys interact are manifold and can operate at very different levels. The effect of wolf predation on prey population is the result of the combination between the reproductive potential of the prey (i.e. the annual increment), and the prey-killing potential of wolf-population (i.e. numerical and functional responses of wolves to the changing prey population size). The possible effects of the prey-predator interaction could be i) sanitation effect in the prey population, ii) control or limitation of prey numbers, iii) stimulation of prey productivity, iv) increase in food for scavengers, and v) shift in predation on unusual prey species (Mech and Peterson 2003). Moreover, the predation of wolves can change not only the size of the prey population, but also its population structure, its habitat selection and space use, and finally its behavior (Mech and Peterson 2003).

As ungulates have reached densities close to carrying capacity in many areas, the interest of wildlife managers has grown in the last decades, focusing on the factors that could affect and regulate the ungulate population (Okarma 1995, Jędrzejewska and Jewdrzejeski 2005, Nores et al. 2006, Melis et al. 2009). Many authors focused on the relation between predators and preys in order to evaluate if predation had a regulatory or a limiting effect on prey populations (Boyd et al. 1994, Eberhardt and Peterson 1999, Jędrzejewski et al. 2000, Kojola et al. 2004, Vucetich et al. 2005, Nores et al. 2006, Melis et al. 2009, Mattioli et al. 2011, Gervasi et al. 2012) . Some of these authors found a limiting effect of wolf population, suggesting a top-down regulation of the prey species (Bereguard and Elliot 1998, Peterson 1999, Ripple and Beschta 2003), while others did not, finding a bottom-up regulation (Mech 1986, Okarma 1995, Peterson et al. 1998, Nelson and Mech 1986a, 2000, Melis et al. 2006). Moreover, several recent studies support the view of complex ecosystems in which both top-down and bottom-up control are at work simultaneously; long-term data from Bialowieza Primeval Forest suggests that ungulates were affected by both climate-related food availability and predation (Jędrzejewska and Jędrzejewski 2005).

In this very broad and complex scenario, large-scale studies may reveal the patterns of top-down and bottom-up processes, while small-scale ones can provide insight into the mechanisms of these processes (Mech and Peterson 2003). Moreover, it is easy to understand that the effect of wolf on the prey population strongly depends on the different ecosystems, characterized by its unique predator and prey composition, and by differences in habitat productivity and climate. All the above listed factors can influence the degree to which preys are limited by wolf predation (Mech et al. 1998).

As demonstrating by several studies (Mech and Karns 1977, Gasaway et al. 1992, Gervasi et al. 2012) one of the most critical aspect in this topic is the presence of other predators. Indeed, in its very wide range, wolf coexists with others predator species (brown bear -*Ursus arctos*-, grizzly bear –*Ursus arctos horribilis*-, black bear –*Ursus americanus*-, lynx -*Lynx lynx*-, mountain lion -*Felis concolor*-, etc..) or at least with human hunters. While in North America numerous areas exist where the human influence on predator-prey relationships is low, on the contrary in Europe most forest ecosystems have been drastically managed by humans. In fact, several authors agreed in defining hunting harvest as the major source of ungulate mortality and as a stronger limiting factor than predation, habitat quality, disease, winter severity, or accidents (Okarma 1995, Jędrzejewska and Jędrzejewski 1998, Wright et al. 2006, Gazzola et al. 2007, Nores et al. 2008, Jędrzejewski et al. 2011). Thus natural predation could generally regulate herbivore abundance (Hairston and Hairston 1993, Eberhardt 1997, Krebs et al. 1999), but Eberhardt et al. (2003) shown that the combined effects of predation by both wolves and humans may lead to prey decline.

It is therefore important to understand if in a given ecosystem the role of the predation and hunting is compensatory or if it has an additive effect, in order to understand if and how much their combined effect could regulate or limit the prey population, and which of the two factors exerts the greater impact. The potential conflicts between large predators and hunters can arise from their similar role of predators of ungulate populations (Okarma 1995, Berger 2005, Jędrzejewski et al. 2011), even if they could focus on different prey target and their impact could have different extent on the ungulate community (Boyd et al. 1994, Wright et al. 2006, Proffitt et al. 2009). However in spite of the fact that in human dominated areas hunting harvest had a greater impact on ungulate populations, hunters perceived the predators as strong competitors for the same resources. Thus, it is fundamental for the maintenance of a rich animal community to find a balance between the increase of ungulates and their removal by predators: the new ecosystems that were created in western Europe as an outcome of the deep socio economical modifications in human society are a challenge for this task.

Our study has been carried in an area that represents a simple ecosystem in which a single pack of wolves coexists with no other large predators, except for the hunters. Ungulate community consists of only two common species : wild boar (*Sus scrofa*) and roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*) is recently appeared with limited densities. Thus our study area represented a simple system in which it would be easier to study the interaction between predators and their preys.

Thus, looking at wolves and hunters share in ungulate population we aim:

- To investigate the combined impact of hunters and wolves on wild boar and roe deer
- To evaluate the differential share in age and weight classes of both prey species
- To determine if predation, hunting or both can limit ungulate population.

MATERIALS AND METHODS

2.1 Study area

Wolf-prey relationships were investigated in an area located in a mountainous region of the Apennines, in the North-Eastern part of Tuscany (Arezzo province, Italy, 43°48’ N, 11°49’ E). The total surface was 120 km^2, of which the central portion of 27 km^2 represented a strictly protected area called Oasi Alpe di Catenaia (OAC), while the remaining surrounding 93 km^2 represented a portion in which hunting was permitted (Figure 1). The altitude in the area ranged from 300 to 1414 m a.s.l. The vegetation cover was mainly composed of mixed deciduous woods (76% of total area), dominated by oak (*Quercus* spp), chestnut (*Castanea sativa*), and beech (*Fagus sylvatica*). Coniferous forest (7%) and open areas (16%) were also present.

The climate is temperate with hot and dry summers and cold and rainy winters. Snow fall usually begins in October and lasts until April.

The wild ungulate community in the study area was composed of two species: roe deer and wild boar. Roe deer was the more abundant species and its density was relatively stable throughout the study period. By contrast, wild boar population size showed wide fluctuations. Moreover, red deer (*Cervus elaphus*) have been occasionally recorded in the study area and in the wolf’s diet since 2007. A pack of wolves inhabited the study area throughout the whole study period (2000-2010), red fox (*Vulpes vulpes*) was present but no numerical data are available.

Hunting was forbidden inside OAC and regulated outside OAC. We can discern two kinds of hunting: (i) roe deer stalking with rifles and without hounds (hunting from high seats within areas assigned to individual hunters; hunting was permitted all days excluding Tuesdays and Fridays); (ii) hunting with hounds (target species being wild boar) began on the third Sunday of September and ended on January 31st. Wild boar hunting was permitted on Wednesdays, Saturdays and Sundays, and included battues (i.e. dog drives) with 30–50 hunters (no less than 25 hunters by law) and many hounds.

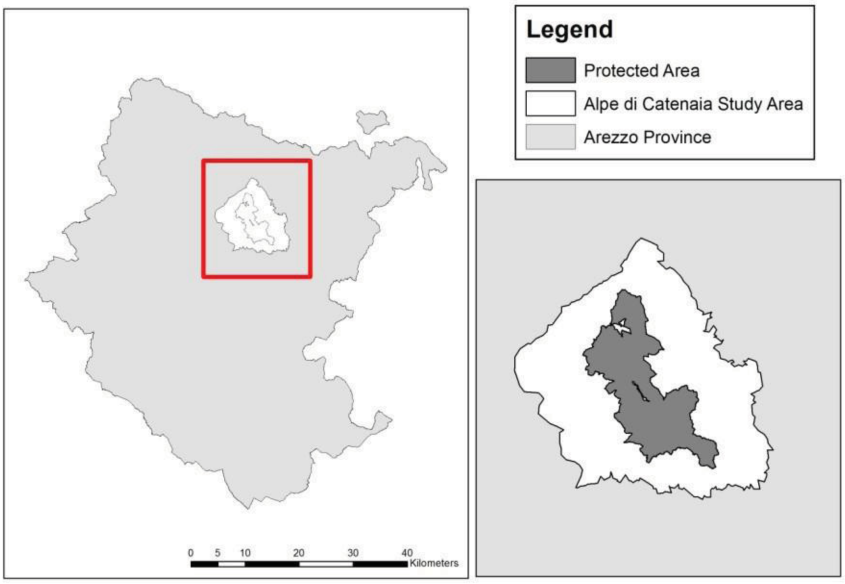


Figure 1. Study area. On the left it is represented the whole province and the study area is in the red rectangle. On the right: enlargement of the study area divided in protected area (dark grey) and hunting area (white).

2.2 Ungulate abundance and annual recruitment

Densities of wild boar and roe deer in the study area were estimated with drive censuses every May (2000–2005, and 2007–2010; method also described by Mattioli et al. 2004) by the Provincial Administration of Arezzo; the data relative to the 2006 census was not used because they were incomplete. Censuses took place in both the protected and non-protected parts of the study area each year, encompassing about 80% wooded area and 20% other cover types; between 9 and 15 forest blocks were sampled each year. The density of animals within each surveyed block was calculated as described in Davis et al. (2012). Summer abundance of roe deer was calculated on the basis of their spring counts, the percentage of adult females in the population and the female fertility. The percentage of female in the population was derived from the direct observations during the drive census, and the female fertility was obtained by counts of fetuses found in females shot by hunters (Hunting Plan Provincial Administration of Arezzo). Parameters on population structure for wild boar and roe deer were reported in Table 1.

	Parameter	Wild boar	Roe deer
Population structure	% juvenille	62.6	38.7
	% adult	37.4	61.3
	Sex ratio	1:1	1:1
Population growth	Weight of reproductive female	≥ 35 kg	≥ 19 kg
	% reproductive female	79.4	83.5
	Average number of fetuses per female	5.02	1.91

Table 1. Wild boar and roe deer population structure and population growth parameters.

2.3 Wolf diet and energy requirement

Wolf pack size in the area were monitored by integrating data obtained every year from snow-tracking during winter (Jędrzejewski et al. 2000), wolf-howling during summer (Gazzola et al. 2002), molecular analysis and direct observations all year round, from 2000 to 2010; following Jędrzejewski et al. 2000, we assumed that the largest recorded number of wolves in the wolf pack represented the size of the wolf pack in that given year. To evaluate wolf pack's diet, we walked monthly seven standard scat-trails (total length: 68732 m; mean length: 9819 m), covering homogeneously the area. Scats were collected between May 2000 and April 2010; according to the biological cycle of wolves, years were defined starting in May and ending in the following April (i.e. scats collected between May 2000 and April 2001 were assigned to the year 2000–01). Wolf diet was determined by means of scat analysis, determining the relative volume of the food item presented in the scats. Scats were washed in a sieve of 0.5 mm and the prey remains (hairs and bones), fruit and grasses found in every scat were dried at 68°C for 24 h. Prey categories included wild boar, roe deer, red deer, hare (*Lepus europaeus*), small rodents, and livestock (goats, sheep and cattle). Prey remains were identified through comparison to a reference collection of mammal hair, bones, and teeth. We identified the prey species and age or weight class (for ungulates only) when possible. This identification was based on the macroscopic characteristics of hairs and bones following Mattioli et al. (2004, and 2011). Boar remains were divided into three weight classes: newborn piglet (<10 kg), piglet (10–35 kg), and adult (>35 kg). Roe deer remains were classified into two classes: juvenile (<1 year) and adult (>1 year). The ability of researchers to discriminate among samples from different species and classes was verified by means of a blind test using artificial ‘‘scat samples’’ prepared from a collection of 200 bags containing prey remains from a variety of species (all potential prey of the area), and from a variety of weight and age classes. Each researcher was assigned 50 bags, chosen at random, and was assessed on their ability to correctly identify the class and species represented by the sample. Ability to discriminate among wild boar weight classes was additionally assessed using a further 25 samples per researcher. Only researchers who correctly identified all test samples went on to analyze the collected scat samples.

The relative biomass of ungulate species and other mammals was calculated using the relative volume values. We applied the biomass model of Weaver (1993) $y= 0.439 + 0.008x$, where y represents the biomass (kg) of prey for each collectable scat and x is the live weight of prey. The weights of different prey’s classes (obtained from data on hunted individuals in each class), and also the summer and winter weights were accounted for in this calculation.

Predation impact on wild boar and roe deer were investigated. The total number of prey killed by wolves was defined as the product of wolf number and their per capita killing rate. Kill rate was estimated by a theoretical approach using the daily food consumption. Daily food consumption by wolves was calculated through the field metabolic rate (FMR) for all eutherian mammals (Głowaciński and Profus 1997). The equation, derived from Nagy’s formula (1987), is closely correlated with body weight of animal: FMR (kJ/d)= 52.58 W0.862, where W is body weight in grams. This allows indirect estimates of total daily energy expenditure of a free-living animal. Data from Italy give an average body weight of 32 kg for an adult wolf (> 1 year old) (Gazzola et al. 2007). Calculations based on FMR yielded an estimate of 2.6 kg of meat per day for an adult wolf. Thus it was possible to calculate the amount of kilograms of meat needed by the pack each year, and it was also possible to divide this amount of meat needed between the different age and weight classes, according to the proportion found in the diet. Thus, dividing the kilograms of meat needed for the average weight of the prey species (taking into account both the difference of weight between the season and, of course, the different age and weight classes), we evaluated the number of individuals removed by wolves each year for each class.

2.4 Hunting harvest and ungulates mortality causes

We collected age and body weights of all ungulates killed in the area from hunters bags every year from 2000. Both deer and boar were aged on the basis of tooth eruption and wear.

The average number of roe deer hunters was 158, while the wild boar hunters was on average 300.

Shooting plans are mandatory for roe deer hunting: they must be issued by hunting districts (ATC), must be linked to any small hunting areas and must be approved by the provincial government. Shooting plans prescribe not only the overall number of individuals to be culled but also the age and sex class of animals to kill. Individual hunters are assigned an exact number of animals for each sex and age class.

In the case of wild boar, there is only an overall hunting quota on the whole province, without any prescribed cull of sex and age class. There is no assignment of an exact number of heads neither to individual hunting teams nor single hunters.

Moreover, we use data derived from a long-term study on collared wild boars to obtain data about death causes for the wild boar populations. Study was conducted from 2002 to 2010. Animals were captured by means of vertical drop nets or capture cages, hand caught, blindfolded, fitted with Televilt radiocollars (Televilt, Sweden) and released. We used Televilt receivers and 4-element Yagi antennas to triangulate the position of deer 8–12 times per month. Once we received the mortality signal, we looked for the animals and we established the cause of death.

2.5 Uncertainty estimation and role of predators' impact on ungulate abundance

According to the outcomes by Davis et al. (2012), we calculate the 95% confidence intervals around the estimations of ungulates' density and around ungulates' use by wolves, following the same procedure; we used bootstrap resampling technique (Efron 2000), performing 4000 replications for each sample, each years. For estimating ungulates use, all scat samples for a year were randomly sampled with replacement to produce a new estimate of the relative use of both wild boar and roe deer. Similarly, densities based on drives in separate areas of the study site were randomly sampled with replacement to produce a new estimate of density for both ungulate species (for details see Davis et al., 2012).

The $\chi2$-test was used to test for differences between the respective use of wild ungulate age and weight classes by the two predators. We also calculated Manly's selectivity index (Manly et al. 1972) to determine the wolves and hunters preference for the different age and weight classes.

The formula used was the followed:

$\alpha i= ri/ni\ (1/\sum(rj/nj))$

where αi represented preference index for prey type i, ri, rj the proportion of prey type i or j in the diet (i and j = 1, 2, 3, … m), ni, nj the proportion of prey type i or j in the environment, and m number of possible prey types.

When αi = 1/m, feeding is not selective. When αi is greater than 1/m, prey species i is preferred in and when is smaller than 1/m, prey species i is avoided. Moreover 95% bootstrap confidence limits were generated for the Manly's selectivity index. We simulated 1000 sets for each bootstrap simulation.

We used linear regression analysis to compare wolf kills and hunting bags data with ungulate abundance.

Moreover, to investigated the additive effects of hunters' and wolves' pressure on prey densities and thus to understand if the predation could regulate the dynamic of ungulate populations, we performed several linear regressions linking data on yearly hunting bags with recorded hunting bag and wolves predation impact of the previous ones. In order to take into account the uncertainty around the density estimations, we performed three linear regressions using the three values of density (estimated value, the lower and upper values calculated by the confidence intervals.

All the analyses presented here were performed in R 2.15.2 (http://cran.r-project.org/).

RESULTS

3.1 Wolf status and ungulate abundance

Between 2000 and 2010, we monitored a single wolf pack in the study area; the size of the pack ranged from 3 to 6 individuals (average value ± standard deviation: 4.9 ± 1.5; Figure 2).

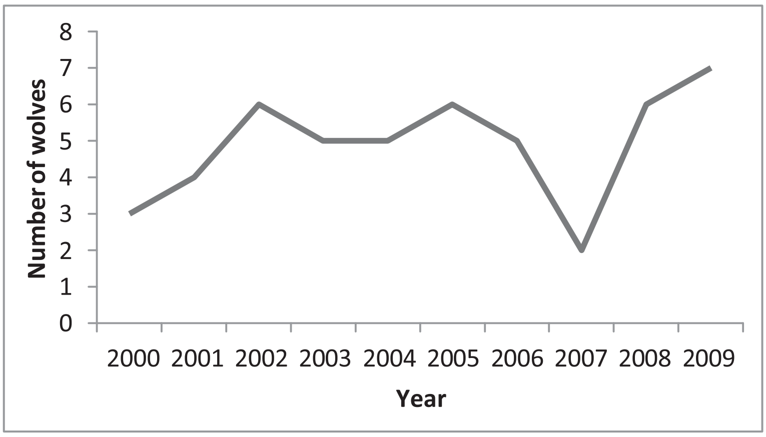


Figure 2. Number of wolves censused in the study area in the 2000-2010 period.

Roe deer resulted to be the most abundant prey species in the study area with an average value that ranged between 32.7 and 47.7 individuals/Km^2 (post reproductive densities; Figure 3 a); during the study period roe deer population resulted relatively stable. On the contrary wild boar, the less abundant prey species, showed large fluctuations; indeed its density ranged between a minimum of 4.3 and a maximum of 26.0 boars/ Km2 (Figure 3 b).

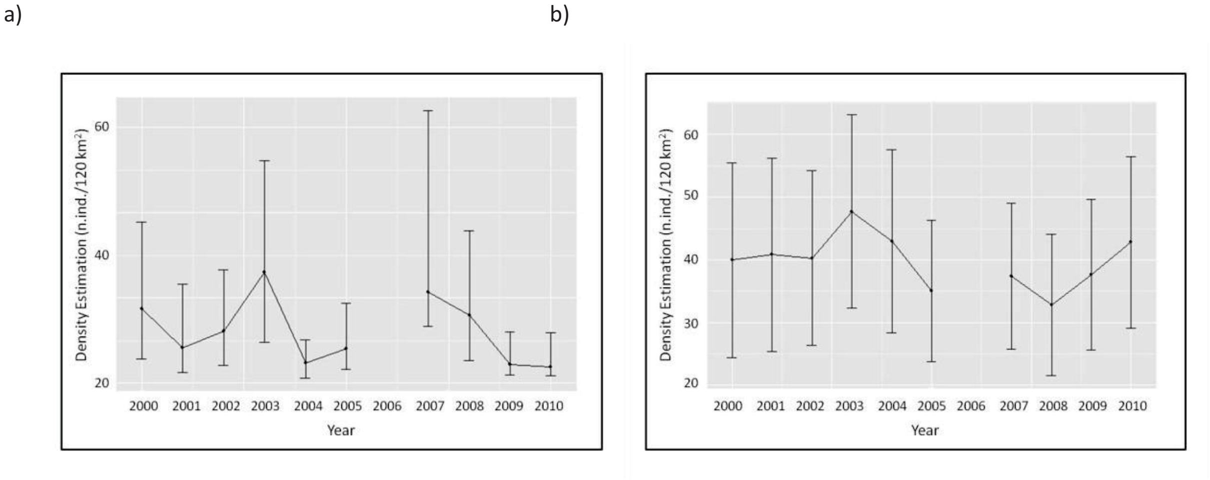


Figure 3. Uncertainty evaluation around density estimation for wild boar (a) and roe deer (b).

3.2 Wolf diet and hunting harvest

The diet of wolves have been studied analyzing a total of 2150 wolf scats. Wild ungulates accounted for 96.3 ± 3.91% of the annual diet, representing the main food item used by the pack in the Alpe di Catenaia (Table 2). Wild boar was the primary prey, and accounting for 63.2 ± 3.90% of biomass eaten. Roe deer, the second most prevalent prey species, accounted for 32.4 ± 3.61% of total prey biomass.

N	178	242	262	293	232	143	144	208	272	176
Category	2000-01	2001-02	2002-03	2003-04	2004-05	2005-06	2006-07	2007-08	2008-09	2009-10
Wild boar	55.9	48.2	68.5	71.2	48.8	46.1	68.7	76.5	69.6	78.1
Roe deer	42.1	47.6	26.3	26.1	48.2	39.9	29.8	20.1	22.9	20.8
Red deer	0	0	0	0	0	0	0	0.4	6.1	1.1
Hare	0	0	0.6	1.8	1.1	4.5	0.6	1.0	0	0
Rodents	0.4	0.2	0.3	0.4	1.2	0	0	0	0.3	0
Sheep	1.6	3.7	4.3	0.5	0.8	8.5	0	0	0.3	0
Goat	0	0.4	0	0	0	1.0	1.0	0	0	0
Cattle	0	0	0	0	0	0	0	2.0	0.8	0

Table 2. Wolf diet assessed thought scat analysis in Alpe di Catenaia from 2000-01 to 2009-10. Data are expressed as percentage of biomass. N represent the number of scats analyzed every year.

Concerning wild boar, we found that wolves' pack consumed more wild boar of the intermediate weight class (10-35 kg), followed by the <10 kg, and then the >35 kg (average percentage of use ± standard deviation: 65.4 ± 9.7, 25.9 ± 12.7, and 8.6 ± 4.9 respectively). On the contrary, during the study period, the two age classes of roe deer were used in a more similar way: on average 51.3% (± 23.6) of the deer category were composed by juvenile ones, and 48.8% (± 23.4) by the adult ones.

On the basis of the field metabolic rate (FMR) an adult wolf (32 kg) needs 2.56 kg of meat per day. Concerning the relative importance of the staple prey items in wolf diet, and of the age classes preyed upon, we estimated the annual quotas of ungulate species consumed by wolf pack. From 2000 to 2010, wolves take off between 72 and 278 wild boars per 120 km^2 (mean ± standard deviation: 147 ± 59), and between 20 and 189 roe deer per 120 km^2 (mean ± standard deviation: 102 ± 47) (Table 3 a, and b).

a)

		2000/01	2001/02	2002/03	2003/04	2004/05	2005/06	2006/07	2007/08	2008/09	2009/10
WOLVES	<10	20	35	35	13	40	62	63	8	12	117
	10-35	45	48	124	108	83	93	111	47	109	143
	>35	7	10	17	15	3	7	5	7	29	18
	Total	72	92	177	136	126	163	178	94	150	278
HUNTERS	<10	15	20	7	36	4	2	19	9	24	17
	10-35	149	197	204	352	109	159	185	384	421	243
	>35	233	309	341	443	183	260	312	505	601	345
	Total	397	526	552	831	296	421	516	898	1046	605

b)

		2000/01	2001/02	2002/03	2003/04	2004/05	2005/06	2006/07	2007/08	2008/09	2009/10
VES	Adult	25	24	33	29	43	67	46	8	43	37
	Juvenile	53	108	65	49	109	122	39	12	45	58
	Total	77	132	98	78	153	189	85	20	88	96
	Adult	135	75	195	138	209	141	215	212	230	297

Table 3. Number of wild boar (a) and roe deer (b) removed from the wolves' pack and hunted by hunters in the study area between 2000 and 2010. We reported the total number and the numbers divided in the different age and weight classes.

Wolf predation impact on prey species, expressed as the percentage of animals consumed out of the total numbers of individuals estimated in summer, constituted 14.7 ± 6.7% for wild boars, and 2.2 ± 1.1% for roe deer (Table 4). Wolves pack resulted to removed 13.4% of the boars annual recruitment, and 4.0% of the roe deer one (Table 5).

a)

Density value	Wolves	Hunters
Lower	41.7 ± 37.6	81.4 ± 27.1
Estimated density	14.7 ± 6.7	63.3 ±28.0
Upper	6.0 ± 5.8	19.9 ± 9.8

b)

Density value	Wolves	Hunters
Lower	3.4 ± 1.7	10.8 ± 4.4
Estimated density	2.2 ± 1.1	7.2 ± 3.0
Upper	1.7 ± 0.9	5.4 ± 2.3

Table 4. Average percentage (± standard deviation) of wild boar (a) and roe deer (b) removed by hunters and wolves. Percentage of removal were calculated for the estimated spring counts, and for the lower and upper values of density derived from the calculation of the confidence intervals.

In the same period, hunters harvested 296-1046 wild boars (mean ± standard deviation: 609 ± 241), while the number of roe deer harvested annually ranged from 114 and 533 individuals (mean ± standard deviation: 339 ± 119) (Table 3 a, and b). Hunting harvest accounted for 63.3 ± 28.0% of the estimated summer density of wild boar, a value much higher than that for roe deer (7.2 ± 3.0%, Table 4). Looking at the annual recruitment, hunters removed 55.5% of the boars and 13.5% of the deer population (Table 5).

a)

Density value	Wolves	Hunters
Lower	33,7	140,2
Estimated density	13,4	55,5
Upper	5,6	23,3

b)

Density value	Wolves	Hunters
Lower	6,1	20,4
Estimated density	4,0	13,5
Upper	3,0	10,1

Table 5. Percentage of recruitment of wild boar (a) and roe deer (b) removed by hunters and wolves. Percentage of removal were calculated for the estimated spring counts, and for the lower and upper values of density derived from the calculation of the confidence intervals.

Hunting pressure appeared to be by far a more important factor in wild boar mortality than wolf predation. Moreover, looking at the data collected from collared animals, it was possible to notice that hunting harvest and poaching accounted for 92.2% of the total mortality, while wolf predation represented only the 1.11% (Table 6).

Cause of death	Number of animals	Percentage
Hunting	127	70,9
Poaching	38	21,2
Wolf predation	2	1,1
Others	12	6,7
Total	179	100

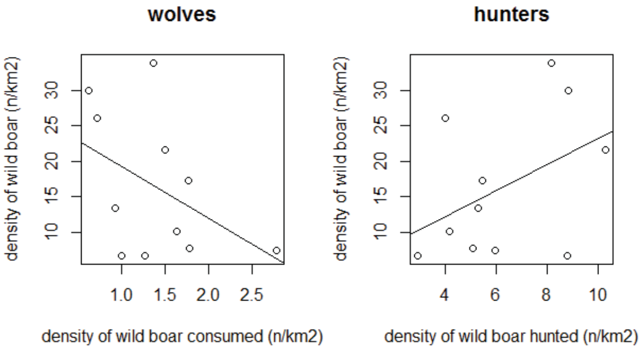
Table 6. Mortality causes of 179 collared wild boars found dead in the study areas. Data were derived from a long-term telemetry study conducted from 2000 to 2010 on 357 collared individuals.

Wolves and hunters showed a different use both of wild boar age classes (χ2 wolf-hunter = 1022, P < 0.01), and of roe deer age classes (χ2 wolf-hunter = 359, P < 0.01). Wild boar between 10 to 35 kg were preferred by wolves (χ2 wolf-census = 120, P < 0.01; Manly's selectivity index: α = 0.83; 95% confidence limits: 0.800-0.923), whereas hunters selected adult individuals (χ2 hunter-census = 522, P < 0.01; α adult = 0.66; 95% confidence limits: 0.594–0.787).

Similarly, juveniles of roe deer were preferred by wolves (χ2 wolf-census = 44, P < 1.1 ; α juvenile = 0.71; 95% confidence limits: 0.661–0.845), while hunters positively selected adult roe deer (χ2 hunter-census = 28, P < 0.01; α juvenile = 0.70; 95% confidence limits: 0.659–0.731).

Wild boar and roe deer taken by wolves and their availability did not show a statistically significant linear relationship (wild boar: R^2 = 0.20, F = 2.24, P = 0.17; roe deer: R^2 = 0.03, F = 0.37, P = 0.56). Moreover we found that hunting bags was not related to densities (wild boar: R^2 = 0.19, F = 2.12, P = 0.18; roe deer: R^2 = 0.07, F = 0.06, P = 0.81, Figure 4 a, and b).

a)



b)

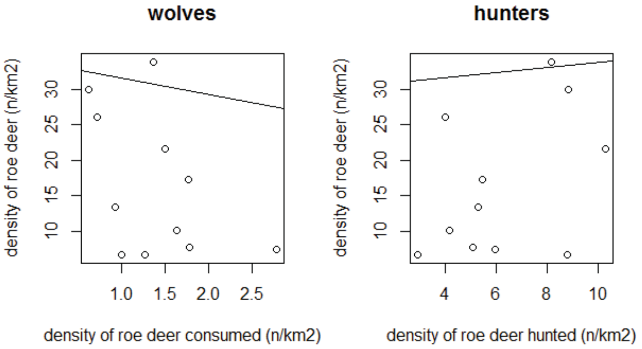


Figure 4. Relation between wild boar and roe deer availability and number of prey taken by wolves and hunters. Wild boar (a) and roe deer (b).

Furthermore, looking at the linear regressions performed, we found that the number of wild boar culled by hunters and killed by wolves in the previous year did not influence the wild boar abundance in the following year for any value of density (Figure 5 a). The same result was also found for roe deer (Figure 5 b).

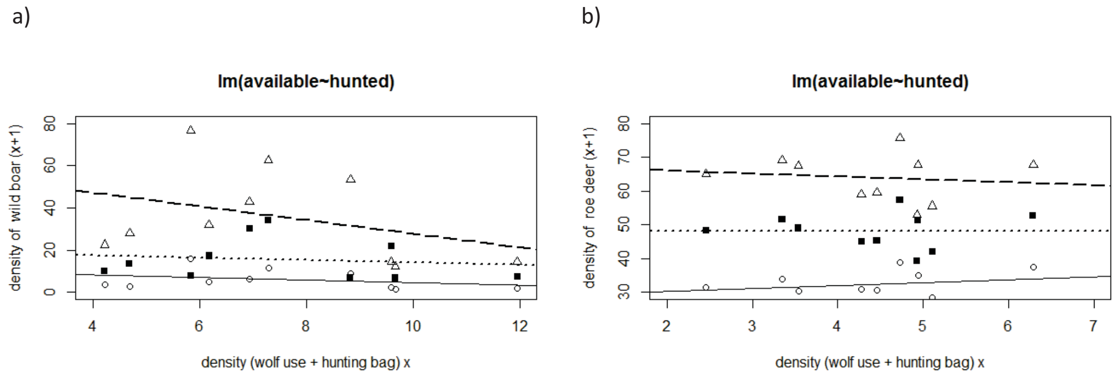


Figure 5. Relation between wolf kills and hunting harvest and wild boar (a) and roe deer (b) estimate of the next year. Dotted, solid and dashed lines represent the relation between wolf and hunting harvest and respectively the estimated, lower and upper values of density derived from the calculation of the confidence intervals.

DISCUSSION

4.1 Wolf diet and hunting harvest: role of predators' impact on ungulate abundance

The study was focused on a predator-prey system of the Alpe di Catenaia, a simple system consisting in two main predators (hunters and wolves) and two main prey species (roe deer and wild boar). The predators were represented by circa 450 ungulate hunters and a wolves pack of an average size of 4.9 individuals (average values for the study period).

As already shown by Bassi et al. (2012) and Davis et al. (2012), the diet of the pack focused on wild ungulates, and among them wild boar was the most abundant food item in the pack diet; the same could be said for the hunters that killed more wild boars than roe deer.

Even if both predators insists on the same prey species, their impact differ in some aspects such as the timing of predation, the age/weight classes removed, and the numbers of individuals removed. The results of χ2-test shown that the two predators effectively selected different age/weight classes. According to the optimal foraging theory (Stephens and Krebs 1987), wolves focused on the most vulnerable individuals in the population, i.e. the intermediate weight class of wild boar (10-35 kg class), and on the youngest roe deer. Indeed, as shown by several studies (Salvador and Abad 1987, Jędrzejewski et al. 1992, Okarma 1995, Jędrzejewski et al. 2002, Mattioli et al. 2004, and 2011), young ungulates represented usually the prey preferred by wolves because they are generally slower, less dangerous, and more inexperienced with predators than adults (Mech 1970).

Conversely, hunters focus more on larger animals from reproductive age classes, both for wild boar and roe deer (Table 2 a, and b). Nevertheless, for roe deer, hunters should killed animals belonging to the different age classes according to their relative frequency in the population, following the hunting plan; however not always the hunters reached the hunting quotas and often hunters focused on adults individuals. In the case of wild boar, the hunting plan did not depend on the relative frequency of the different weigh classes in the population.

Moreover, the difference between the two predators was not only in qualitative but also quantitative; indeed hunting harvest in our study area removed a much higher percentage from ungulate populations than wolf predation. Every year hunters removed on average 63.3% (± 28.0) of the wild boar population and 7.2% (± 3.0) of roe deer population, while the wolf pack 14.7% (± 6.7) and 2.2% (± 1.1) respectively. The ratio between hunters and wolves share in wild boar population varied between 1: 0.24 and 1: 0.27, while for roe deer ranged between 1: 0.17 and 1: 0.35. This is the outcome of the numerical prevalence of hunters, (they were on average, 64 times more abundant than wolves), that was obviously not compensated by the year long "hunting season" of wolves.

In fact the times and the ways in which the two predators hunted, were different. Hunters chased for a limited time of the year that varied on the basis of the game species, whether wolves are active all year long with seasonal changes in the food habits. Moreover, linear regressions showed that both in the case of wolves and hunters, there were no significant linear relationship between the number of animals killed and their availability. Concerning the relation between hunting harvest and deer availability, this latter result was quite unexpected. Deer hunting quota was planned on the basis of roe deer abundance, then we would expect positive relation. Nevertheless, in the protected area (OAC) animals faced suitable conditions which allowed them to reach high densities. In this scenario, we can suppose that OAC with its high deer density values could represented a source and a refuge for the animals, who consequently resulted to be not affected by the overall hunting dynamics that occurred in our study area.

The question if wolves had a limitating effect on ungulate population is still open. Some studies suggested the presence of a limiting effect of predation on ungulate population (Bergerud and Eliot 1986, Gasaway et al. 1992, Jędrzejewska and Jędrzejewski 1998, Kojola et al. 2004), while others do not (Mech et al. 1998, Peterson et al. 1998, Melis et al. 2006, Mech and Nelson 2000, Nores et al. 2008). Moreover the coexistence of both bottom-up and top-down forces are also suggested (Okarma 1995, Vucetich and Peterson 2004, Jędrzejewska and Jędrzejewski 2005, Melis et al. 2006). The conclusion is that the effect of wolves predation could vary from locally extirpation of prey species (Mech and Karns 1977), to simply compensation of other mortality sources (Ballard et al. 1987).

In our study area in order to better understand the role of wolf predation on a ungulate community, we necessary had to take into account the presence of hunters. Human hunting was generally an additional mortality factor to natural predation (Aanes and Andersen 1996, Melis et al. 2010). Furthermore, according to Okarma (1995), human hunting dictated populations dynamics of ungulates over most of Europe. Indeed many studies identified hunting harvest as the major source of ungulate mortality and as a stronger limiting factor than predation, habitat quality, disease, winter severity, or accidents (Okarma 1995; Jędrzejewska and Jędrzejewski 1998; Wright et al. 2006; Gazzola et al. 2007; Nores et al. 2008). In addition it resulted important to analyze the patterns of hunting harvest between wolves and hunters, because different patterns might lead to different effect on wild ungulate population dynamics. Indeed selected prey individuals can contribute in different ways to the annual growth of the prey population because their reproductive values depends on age and sex (Gillard et al. 2000).

Wolf focused primarily on the youngest prey, thus its strongest influence on the demography of ungulate populations should lie in the increase of juvenile mortality (Pimlott 1967, Mech 1970, Sand et al. 2012). On the other hands, hunters focused on older animals, and therefore they resulted as mortality factor for individuals with high reproductive values. If wolf predation and hunting harvest were additive, the combination of hunters removing adult animals and wolves preying heavily on young of the year might negatively affect recruitment (Wright et al. 2006).

In our study area removal by wolves and hunters did not exceed annual increase of ungulate populations. This could imply that the two combined mortality factors could be compensatory to other causes of mortality and may limit but did not regulate ungulate densities. Due to the strong reduction of large predator populations, modern game management and alteration of habitat by forestry and agriculture, ungulate populations have reached high densities in many regions of Europe (Apollonio et al. 2010). Actually, as already showed by Apollonio et al. (2004), in our study area we recorded an ungulate biomass higher than in other European areas. This high value of biomass meant that the population of ungulates inhabiting the study area had so high recruitment that could compensate the impact of the two predations togheter.

As shown in figure 2 (b), in our study area the density of wild boar showed a large fluctuations. If hunting harvest and wolf predation were not responsible of these fluctuation, which other cause could explain them?

Melis et al. (2006) demonstrated that wolf predation had a non-significant effect on wild boar abundance at biogeographic scale. Many factors could have a role in the ungulates' population dynamic, as, for example, acorn crops, starvation, diseases, winter condition (i.e. snow depth, Okarma 1995, Jonas et al. 2008), and climate (Hebblewhite 2005, Jonas et al. 2008). Furthermore it seemed that seed productivity was the most important factor explaining fluctuations in wild boar population dynamics (Groot Bruinderink Groot et al. 1994, Okarma 1995, Jędrzejewska et al. 1997, Jędrzejewska and Jędrzejewska 1998, Kruger 1998).

Another study, conducted in AOC, confirmed this hypothesis. This work shown that turkey oak and chestnut showed high levels of seed mass production, and it seemed that these pulsed resources of chestnut and oak positively affected wild boar piglet (<10 kg) density; moreover, these analyses highlighted the influence of snow cover on wild boar population dynamics (Cutini et al 2013).

Our study area took place in an area with relatively mild climate with winter in which permanent snow cover rarely occurs, and where annual recruitment was not nullify either by wolf predation, nor by hunting harvest due to the high densities of ungulates. Thus according to the outcomes of this work it is reasonable that in our study area predation by wolves and harvest by hunters have a minor importance in determining the inter- summer period) could play an important role in causing preys population fluctuations.

AKNOLEGMENT

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THIRD PART

Spatial behavior: environmental variables implied in rendezvous sites selection

Chapter 4

Predicting potential spatial distribution of wolf (Canis lupus) breeding areas in a mountainous region of central Italy

Predicting the spatial distribution of wolf (*Canis lupus*) breeding areas in a mountainous region of central Italy

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ABSTRACT

Wolves (*Canis lupus*) in Italy, which represents a relict west Europe population, are currently vulnerable, though have increased in number and expanded their range in recent decades. Here we use 17 years of monitoring data (from 1993 to 2010) collected in a mountainous region of central Italy (Arezzo, Tuscany) in an ecological niche-based model (MaxEnt) specifically to characterize breeding sites (i.e. the areas where pups were raised) within home ranges, as detected from play-back responses. From a suite of variables related to topography, habitat and human disturbance we found that elevation and distance to protected areas were most important in explaining the locality of wolf responses. Rendezvous sites were typically 800 to 1200 m a.s.l., inside protected areas, and were usually located along mountain chains distant from human settlements. In these areas human disturbance is low and the densities of ungulates are high. Rendezvous sites were also negatively associated with roads. Over recent years, rendezvous sites have occurred closer to urban areas as the wolf population has continued to expand, despite the consequent human disturbance. This suggest that undisturbed landscapes may be becoming filled, leading to less favorable sites being colonized.

Applying our model, both within and beyond the species' current range, we identify sites both within the current range and also further afield, that the species could occupy in future. Our work underlines the importance of the present protected areas network in facilitating the recolonisation by wolves. Our projections of suitability of sites for future establishment as the population continued to expand can inform planning to minimize future wolf-human conflicts.

Key words: ecological-niche based model, elevation, human disturbance, MaxEnt, rendezvous sites, wolf, wolves-human conflicts.

INTRODUCTION

The wolf (*Canis lupus*) is an adaptable and generalist species. It is not especially habitat specific, can move over large areas, and can survive in many different environments, tolerating various degree of human disturbance (Mech and Boitani, 2003; MacDonald and Sillero-Zubiri, 2004). Historically, it was widely distributed across the northern hemisphere but human persecution has reduced it recently to a small and fragmented range (Mech, 1970; Carbyn, 1987). In Europe, after 1980, some remnant populations expanded into novel areas where human density was low and wild prey abundant (Promberger and Schroder, 1993; Boitani, 2000). Till the last decade in Europe wolves were largely restricted to remote, scarcely populated, hilly or mountainous areas (Okarma et al., 1998; Corsi et al., 1999; Mech and Boitani, 2003) with some exception with Spain (Salvador and Abad 1987).

The Italian wolf population represents one of the few surviving west European populations. Thus, it has great conservation importance at both a national and European level. The IUCN red list of threatened species classified the Italian peninsula population as vulnerable (category D1), and populations in the Alps as endangered (category D) (http://www.iucnredlist.org). Therefore, restoration and recovery of this top predator is a conservation priority. In recent decades, both population size and the range extent of wolves have increased in Italy. Wolves are recolonising their historical range, moving from the Apennines to the western part of the Italian Alps (Scandura et al 2001; Fabbri et al., 2007), and they are predicted to expand into the eastern Alps in the next ten years (Marucco and McIntire, 2010). The Italian wolf population (easily identified by a unique MtDNA haplotype [Lucchini et al. 2002]) is fundamental for wolf restoration in Western Europe. Nevertheless, the small current populations remain susceptible to effects of demographic stochasticity (Genovesi 2002).

As wolves are social carnivores and live in social units (packs), their density and territory configuration are a reflection not only of reproduction and mortality but also of group behaviour (Mech and Boitani, 2003). Moreover, social and physical factors influence individuals and their reproductive fitness relative to the population in which they live and reproduce (Packard, 2003). All these factors together can affect the opportunity for new pairs to form and also their reproductive success (Fuller et al., 2003). Pup mortality is typically high during the first six months of life, and is related to the choice of home sites

(Harrington and Mech 1982a). Home sites are defined as the combination of dens and rendezvous sites; rendezvous sites being the areas used by wolves to raise and leave pups after abandonment of dens.

The locality of home sites can therefore be considered the focal point of a pack's home range and the availability of such sites will affect the process of range expansion. Recently, several studies have related the choice of the home sites by wolves to variables such as climate, soil type, vegetation type, tree cover, human disturbance, and prey availability (Ballard, 1983; Fuller, 1989; Boertje e Stephenson, 1992; Thiel et al., 1998; Norris et al., 2002; Theuerkauf et al., 2003; Capitani et al., 2006). However, most of these studies have been on North American wolf populations with few comparable analyses from Europe.

Many modelling approaches are available to relate species presence-absence data to environmental variables (Cowley et al., 2000; Jedrzejewski et al. 2008; Elith et al., 2009; Monterroso et al., 2009; Marucco and McIntire, 2010). However, some species (particularly elusive species) can be overlooked during monitoring. Additionally, expanding populations are not at equilibrium with respect to potential explanatory variables rendering absence data problematic. Both of these situations are relevant to expanding wolf populations in Italy. In such a situation an approach that uses only recoded presences, such as maximum entropy modelling (Phillips et al. 2006), provide a more appropriate modelling framework.

Here we explore the importance of the environmental variables in determining rendezvous sites in the Northern Apennines; an area of circa 4 million hectares in the zone of wolf colonisation in Italy. We model the suitability of the wider landscape of Italy for wolves, identifying suitable areas for wolves within the current range and potential areas for further expansion beyond this.

MATERIALS AND METHODS

STUDY AREA

Arezzo province is an area of circa 3,235 km^2, located in Tuscany, Italy (Figure 1). 57% of the province is above 400 m a.s.l. with 7.4% being more than 1000 m a.s.l.. The northern portion of Arezzo is mostly montane, including the Apennine chain and other secondary chains with altitudes ranging from 300 to 1654 m a.s.l., and 66% of the area is forested. The southern portion comprises the lower course of the Arno River and Chiana Valley, the Chianti Hills and some low mountains; the altitude here ranges from 120 to 1081 m a.s.l., with only 32% of the area forested and approximately 50% comprising cultivated fields.

Forests in the province are predominantly deciduous with oaks (*Quercus cerris, Q. pubescens*) being the dominant species, along with beech (*Fagus sylvatica*) and sweet chestnut (*Castanea sativa*). Conifers comprise only a small component of forests (6.5%), represented principally by *Abies alba, Picea excelsior, Pseudotzuga menziei* and *Pinus* spp.. The climate in the province is temperate-continental, with mean temperature ranging from 1.4°C in January to 24.9°C in July. The province supports a rich wild ungulate community including wild boar (*Sus scrofa*), roe deer (*Capreolus capreolus*), fallow deer (*Dama dama*), red deer (*Cervus elaphus*) and mouflon (*Ovis orientalis musimon*). Wild boar and roe deer are widely distributed throughout the study area, whereas the latter three species are more localised. Wild boar and roe deer represent the main prey species for wolves in the region (Capitani et al., 2004; Mattioli et al., 2004) and they do not represent a limiting sources of food for wolves, occurring at an average density of 9.79 and 18.24 heads/100 ha respectively; their distribution coinciding with hills or mountain above 400 m a.s.l.

Hunting of ungulates is forbidden in numerous localities within the study area (the mean size of no-hunting areas being 8.25 km^2 and totalling 404 km^2, Figure 1).

The province is divided into 39 municipalities with a human population of circa 350,000 (107 people/ km^2, ISTAT census of 2010). Urban settlements are restricted to lower altitudes and account for only 4.1% of the province. The road density (included paved roads, highways and forested roads) is 3.3 km/km^2, with two-thirds of the roads concentrated in the southern portion. Cultivated areas are centred around urban settlements and represent 42.3% of the province, comprising mostly plantations, pastures, and other cultivations (7%, 9.5%, and 25.8% by area respectively). Wolves remained in the highest mountain ranges of the northern portion of Arezzo, throughout recent decades, though the first sign of wolves in the southern portion did not occur until 2003 (Figure 3).

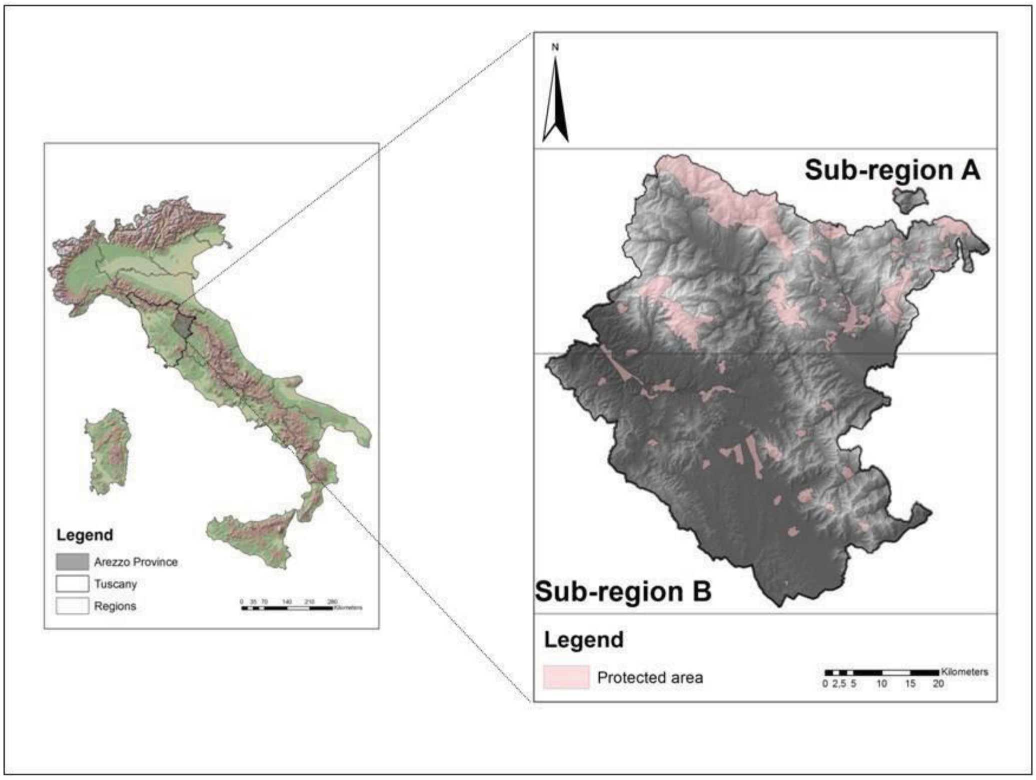

Figure 1. Map of Arezzo province, Tuscany, Italy. Pink shapes in the right part of the figure represent the protected areas. The grey shading represents the hill shade of the Province; light grey represents the highest altitudes, while dark grey represents the lower altitudes. The two sub-regions interested by the monitoring effort. The sub-region A was monitored from 1998 to 2004; sub-region B was occasionally monitored from 1998 to 2005, when it started to be regularly monitored.

WOLF MONITORING

The occurrence of wolves in Arezzo were monitored all year round, integrating results obtained from snow-tracking, wolf-howling, molecular analyses of biological samples (scats, hairs, tissues, blood), and direct observation (Apollonio et al., 2004). Home site locations were monitored from 1993 onwards. Between 1993 and 1998, we collected data only in the Casentinesi Forests National Park (where wolf evidence was focused at that time). From 1998 to 2004, as wolves spread from the national park, we extended the research to the northern portion of the province sub-region A, Figure 1), monitoring the southern portion only occasionally, and then from 2005 monitoring the whole province (sub-region A, and sub-region B, Figure 1). Rendezvous sites were located using howling playback surveys, and following the saturation approach described by Harrington and Mech (1982b), and the protocol described by Gazzola et al. (2002). During a single night, two or more teams performed wolf howling surveys in adjacent areas of the province concurrently. We assumed that responding wolf pack were different if: (i) groups with pups were detected (see explanatory text below) by the same team in the same night in different valleys; (ii) groups were located by different teams in the same night in areas >5 km apart. In addition, if groups replied on different nights >5 km apart and had been identified as different packs (i.e. based on criteria (i) or (ii) above) during the previous year, then they were recorded as different packs again in the current year.

Every chorus and single response obtained during wolf howling surveys was recorded and the sonograms of registrations were analyzed using the software Raven Lite 1.0 (Passilongo et al., 2010). By analyzing fundamental harmonics it was possible to count the minimum number of wolves that joined the chorus and, in the case of good-quality recordings, the presence of pups could be detected by their howl structure (Harrington and Asa, 2003). After September in their year of birth, pup calls could not be reliably separated from adult calls. As we were interested in determining the environmental factors relating to rendezvous sites we utilised in our analyses only those chorus replies in which we could discriminate the presence of pups.

As a single pack can use the same rendezvous site for several year (Capitani et al., 2006), to avoid overestimating the environment characteristics of a point recorded on numerous occasions (and hence minimize pseudoreplication), we excluded from our analyses repeat records from any sites (assuming that any records within 500 m constitute the same site). Rendezvous site locations were pinpointed by triangulation and were overlaid onto 1:10000 scale digital maps (using ArcMap 9.3).

ENVIROMENTAL PREDICTOR VARIABLES

We used seven classes of environmental variables, both categorical and continuous, as potential predictors of wolf habitat suitability. These variables were chosen based on their ecological relevance from other studies on den and habitat selection in wolves (Mladenoff et al., 1995; Corsi et al.,1999; Norris et al., 2002; Jedrzejewski et al., 2004; Capitani et al., 2006; Trapp et al., 2008). The variables were: 1) habitat composition, divided into eight land-use categories (deciduous forest, coniferous forest, coppice forest, shrubbery, cultivated fields, urban settlements, paved roads, and unpaved roads); 2) distance from protected areas; 3) distance from water sources; 4) distance from road (both paved and unpaved); 5) elevation; 6) aspect, divided into four temperature-related classes (the coldest, NE; the warmest, SW; and the other two intermediate, SE and NW) and 7) slope. The first four variables were extracted from a geographic information database of the Fish and Wildlife Office, Provincial Administration of Arezzo. The latter three variables were computed from a digital terrain model (available in the Tuscany Forest Inventory Map: http://web.rete.toscana.it/sgr/webgis/consulta/viewer.jsp). These data were represented as raster layers in a grid of 100x100 m resolution (1 ha) with an area of 6305 square km, to cover the whole province.

HABITAT SUITABILITY MODELLING

To relate the occurrence of rendezvous sites (from the vocalization censuses) to the landscape of the Arezzo region we used the maximum entropy based machine learning program, MaxEnt (version 3.3.3; http://www.cs.princeton.edu/~schapire/maxent, Phillips et al., 2006). We used MaxEnt for our modelling framework for two principal reasons. Firstly, it is a presence-only model and demands only species presence data and not absences, which are less reliably recorded for secretive and wide-ranging species such as wolves. Secondly, it is less sensitive than other approaches to the number of locations required to develop an accurate model (Hernandez et al., 2006; Phillips et al., 2006; Wisz et al., 2008); in some studies the added benefit of additional locations has been found to plateau at circa 50 records (Hernandez et al., 2006).

For the model evaluation, MaxEnt produces both a threshold-dependent test, termed the “equalized predicted area” test, and a threshold-independent test, a ROC analysis (Phillips et al., 2006). The first test is based both on the omission rate, defined as the proportion of test localities that fall into cells predicted as unsuitable for the species, and on the “proportional predicted area”, defined as the proportion of cells that are predicted as suitable for the species (Phillips et al., 2006). The AUC (area under the curve) for a ROC (receiver operating characteristic) plot of sensitivity versus 1-specificity is used as a threshold-independent test of model performance; AUC being 0.5 when the model predictions were no better than random (for presence only data).

Jackknife tests in MaxEnt were used to assess the relative contribution of individual variables to simulating the observed distribution and to identify the most informative variables in the final model. For the most informative variables we produced response curves to depict their relationship to habitat suitability.

We undertook cross-validation in MaxEnt, with 10,000 replications, which produced error estimates for ROC curves and average AUC values across models.

We used default parameters for MaxEnt and we selected logistic output format, as generally recommended. MaxEnt’s logistic output transforms the model from an exponential family model to a logistic model to avoid the possibility of probabilities of >1 (Elith et al. 2011).

RESULTS

MODEL EVALUATION AND VARIABLE CONTRIBUTIONS

Over the sampling period we recorded a total of 147 rendezvous locations(Figure 3).

Model performance, as indicated by the area under the receiver operating characteristic curve (AUC) value, ranged between 0.853 and 0.899 (mean value 0.876, SD 0.023), indicating that the environmental variables were very good descriptors in predicting rendezvous sites.

Elevation and the distance to protected areas were the two most important variables in the models; the former explaining 65.8%, and the latter 17.8% of explained variance (Table 1). The variable with the highest gain when used in isolation is elevation; moreover it is also the variable that decreases the gain the most when it is omitted, meaning that it contains the most information not present in the other variables. Distance to protected areas immediately follow it. On the other hand aspect represents the variable that less contributes to the model building.

Variable	Variable importance (%)	Permutation importance (%)	Jackknife of regularized training gain Without variable	 With only variable
			(average log probability of the presence samples)	
Aspect	1.2	1.7	13.18	0.01
Distance to protected areas	17.8	26	11.44	0.46
Distance to rivers	1.5	3	12.97	0.02
Distance to roads	7.8	8.4	12.39	0.38
Elevation	65.8	55.7	0.99	0.96
Land use	4.6	3.4	13.08	0.33
Slope	1.4	1.9	13.09	0.31

Table 1. Summary variable importance and evaluation statistics for variables included in the MaxEnt model. Regularized training gain, and represents the gain of the training data, regularized using the iterations performed by the model (n=5,000,000), calculated both without one variable at time, and with only the variable of interest. The gain is a measure closely related to deviance. It starts at 0 and increases towards an asymptote during the run. At the end of the run, the gain indicates how closely the model is concentrated around the presence samples.

Rendezvous sites were associated with elevations between 800 m and circa 1200 m a.s.l. and sites within protected areas (Figure 2). Roads were negatively correlated with rendezvous sites though distance to roads accounted for only 7.8% of explained variance (Figure 2).

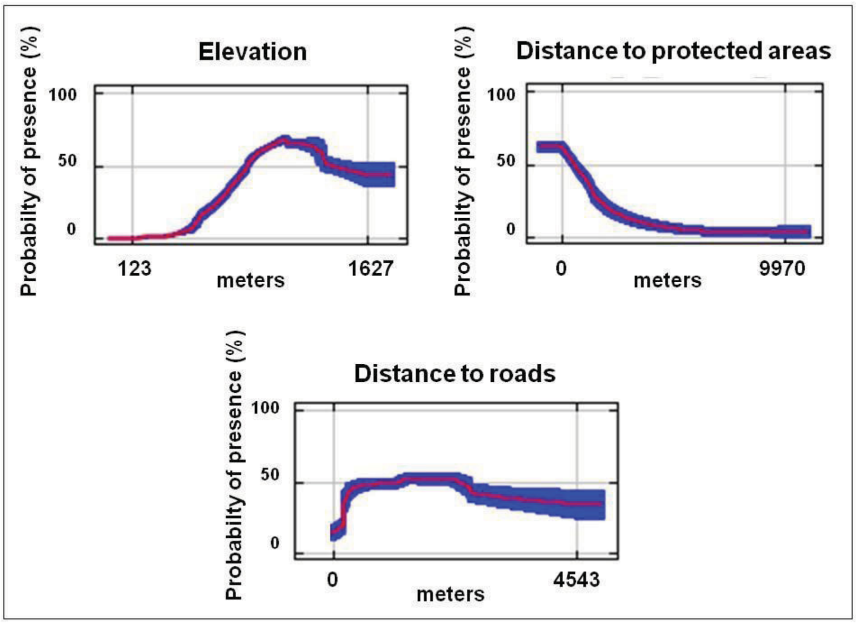

Figure 2. Response curves for the three most important environmental variables of the rendezvous sites recorded during the study period. The curves produced by Maxent show how the logistic prediction changes as each environmental variable is varied, keeping all other environmental variables at their average sample value. Red lines represent the average trend for the variable considered, while the blue shading represent the standard deviation from 10,000 replications made by bootstrapping technique.

SIMULATED SUITABILITY FOR WOLVES

The most suitable areas (e.g. suitability values >0.6) for rendezvous sites from the models were located predominantly in the northern section of the province, mainly along the mountain chains, consistent with the known species distribution (Figure 3). In the southern section of the province the suitable areas were fewer in number and extent and tend to contain smaller areas of the highest suitability.

Looking at figures 3 and 5 simultaneously, it is possible to notice that the areas with the highest suitability values are those included in parks or other protection institutes. Furthermore the map created by MaxEnt highlights the presence of some areas along the secondary chain with suitability values between 0.3 and 0.6 in which wolves have not been recorded yet; even in this case, it is possible to notice that these not-yet occupied areas largely overlap the shape of the protected areas, or alternatively are located between two of them.

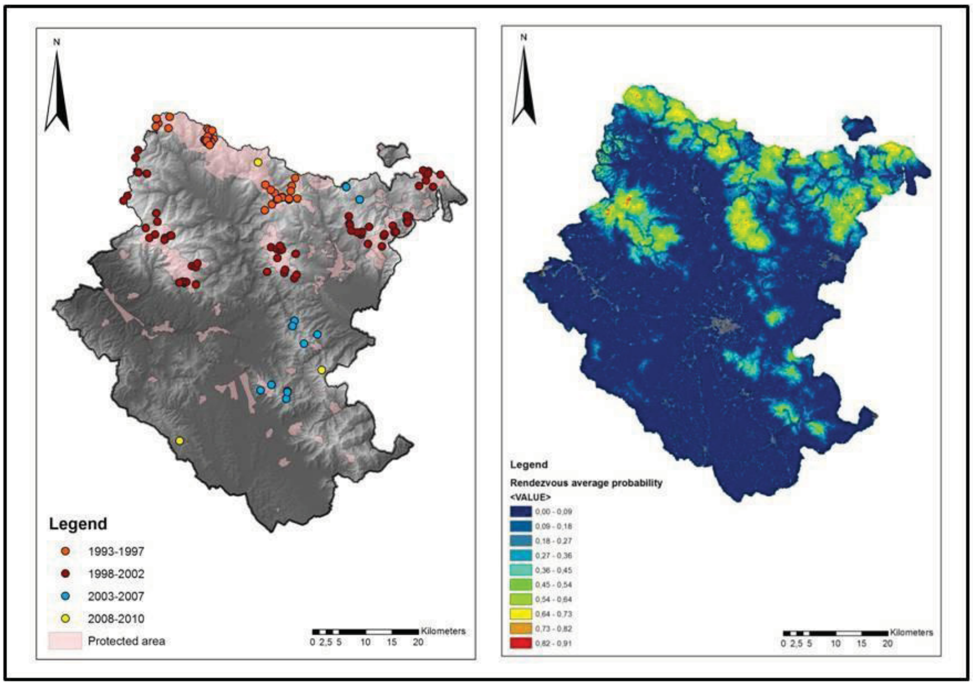

Figure 3. Left part: Locations of all rendezvous sites collected during the study period (147 localities). Red and orange dots represent the rendezvous that have been recorded in the period that ranges between 1993 and 2003, while light blue and yellow dots represent the ones that have been recorded between 2003 and 2010. Pink shapes represents the protected areas. Right part: Modelled mean suitability for rendezvous sites from the 10,000 model replications. Red indicating highest probability of occurrence (or highest standard deviation), green indicating intermediate probability and blues low probability.

DISCUSSION

MODEL EVALUATION AND VARIABLES' CONTRIBUTION

The model of habitat suitability for rendezvous sites performed well in predicting recorded wolf distributions, with elevation and distance to protected areas being the two most important predictors of wolf breeding areas. Elevations associated with high probability of presence ranged between 800 m and 1200 m. These altitudinal range could represent a combination of human avoidance (Capitani et al., 2006) and protection against high summer temperature, particularly for the pups (minimum daily mean temperature and maximum daily mean temperature are 20-22 °C and 25-32 °C respectively, during the last 10 years in the July-September period, http://www.arsia.toscana.it). Moreover, this elevation range is also suitable as it is characterized by largely natural forested environment far from urban settlements and this favour high density of roe deer and wild boars (i.e. the most important prey species for wolves in the province of Arezzo, [Mattioli et al. 2004, 2011, Davis et al. 2012]) also avoiding conflicts with agriculture.

In this study, wolves chose to stay within, or very close to, protected areas during the pup raising period (Figure 2). Protected areas provide protection from direct persecution (as hunting is forbidden), human disturbance is low (a limited number of tourists and mushrooms or chestnut collectors in the summer period), and the presence of food resources are high. In the protected areas the access is controlled and human activity is limited to daylight hours. The tendency to locate rendezvous sites or dens far away from humans and close to food resources is a well known aspect of wolf behaviour (Ciucci and Mech, 1992; Heard and Williams, 1992; Theuerkauf et al., 2003; Capitani et al., 2006). Indeed, as suggested by Capitani et al. (2006), the location of rendezvous inside or at the border of protected areas could represent a strategy for providing both reasonable protection to the pups and also high prey availability. This tendency of wolves to avoid areas with high human densities seems to be substantiated by the principal recorded causes of wolf mortality in Arezzo province, which are most commonly related to human activities (68% of recorded mortalities between 1990-2012, unpublished data).

Roads had little influence on the location of rendezvous sites. However, it should be noted that we performed an analysis combining paved and unpaved roads. Whereas the former may represent a major source of mortality due to traffic accident (Capitani, 2005), the latter are regularly used by wolves in their movements. Nonetheless, suitability for rendezvous sites did increase with increasing distance from roads (Figure 2), as noted in other studies (Theuerkauf et al., 2003; Capitani et al., 2006). The density of roads has also been found to be an important predictor of wolf habitat avoidance (Massolo and Meriggi, 1998; Mladenoff and Sickley, 1998; Corsi et al., 1999; Kohn et al., 2000). Ghering (1995) found that wolves preferred areas with low density of roads, but that they frequently travelled close to trails and forest roads. In a study conducted in north America, wolf dens tended to be located in roadless or in low road density areas, and were generally located more than 1 km away from paved roads (Unger, 1999). The distance of our rendez-vous sites from roads was similar, with a mean distance of1138 metres (SD 816 metres) to the nearest road.

We found the proximity of rivers to be unimportant in the locality of rendezvous sites in our study, despite other studies finding that natal dens were often located close to water (Joslin, 1967; Mech, 1970). This is probably due to water not being limiting in our study area, occurring widely and relatively homogeneously.

The suitability maps presented here represent the first step in predicting the locality of rendezvous sites and, as a consequence, are informative in understanding habitat selection and the potential for future spread of the wolf across Italy. It remains to be seen whether the continued expansion of wolves in the region will occur predominantly in the areas we model as unoccupied but suitable. However, we should be cautious in using our suitability maps to strictly interpret the potential future range extent of wolves in the region, as models built using earlier rendez-vous sites may not perform well in predicting the later colonised sites.

In our study area for instance initially wolves were using only a subset of suitable habitat, perhaps the most suitable or isolated, but that since filling such areas they have expanded their niche into additional, perhaps less suitable habitats. This highlights the caution that is needed in interpreting habitat suitability models for species distributions that are not at equilibrium, and that suitable wolf habitat could be more widespread than the areas the currently occupy.

It is notable that areas with moderate modelled habitat suitability were much more widespread than the highly suitable areas. Before the widespread wolves loss across Italy (pre-1900), wolf populations were found in a much wider wide range of habitats, from sea level to the highest elevations (Cagnolaro et al., 1974). That the northern portion of the province, especially the mountain chains, are simulated as more suitable from our model of rendezvous sites, than the southern lower elevations probably reflects the tendency of the relict Italian wolves populations to be restricted to, and hence recolonise from, mountainous, densely forested, and scarcely urbanized areas (Cagnolaro 1974; Zimen and Boitani, 1975).

If we take into account that the size of wolf home ranges in southern and central Europe varied between 82-243 km^2 (Okarma et al. 1998), being 150 km^2 in Dalmatia (Kusak et al. 2005), and 197 km^2 in Italy (Ciucci et al. 1997). and that about half of the province territory is still available for wolves colonization we could expect the possible creation of at least 10 new packs.. These new packs will occupy the still free territories, i.e. the ones closer to the human settlements.

Several studies have demonstrated that wolves can tolerate human presence and they can live close to humans (Vilà et al., 1995; Ciucci et al., 1997; Mech et al., 1998; Thiel et al., 1998; Merril, 2000), but in these situations they tend to adopt a spatiotemporal segregation to avoid human presence and activities (Theuerkauf et al., 2003).

To survive wolves need both protection from man and a healthy prey-base (Mech, 1995). Maintaining stable ungulates population is necessary to minimise conflicts between wolves and other stakeholders, such as hunters and livestock owner. Hunters sometimes consider wolves as competitors for the same resource and tend to overestimate the predation of wolves on ungulates, despite many studies identifying hunting as the major source of ungulate mortality and the most limiting factor for ungulate populations (Okarma 1995; Jędrzejewska and Jędrzejewski 1998; Gazzola et al. 2007; Nores et al. 2008). In this context only dense ungulate populations can reduce the competition perceived. Moreover having rich and differentiated ungulate communities could also decrease the conflict with livestock breeders because stable ungulate populations can reduce depredation risk on livestock (Okarma, 1995; Sidorovich et al., 2003; Peterson e Ciucci 2003), but see also Meriggi and Lovari (1996).

In this contest the approach we used, can find a meaningful application in order to facilitate social and economic acceptance of the predator, predicting wolves arrival and settlements in advance, and allowing to enforce proper management initiatives. To have wolves too close to humans could lead to conflicts in relation to the negative attitude that people have towards the predator for both social and economic reasons (Mishra, 1997; Fritts et al., 2003; Treves et al., 2003; Treves and Karanth, 2003; Bisi et al., 2007). The tolerance of humans towards wolves depends mostly on their familiarity with their presence (Zimen and Boitani, 1975), and the reverse is also true with wolves and humans (Fritts et al., 2003): i.e. more wolves and humans have coexisted togheter, more they are able to tolerate each others.

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CONCLUSIONS

CONCLUSIONS

The aim of the **First Part** of the present thesis (**Chapter 1**) was to investigate the respective role of the two carnivores (wolf and red fox) on the ungulate community, in order to assess the overall impact of predation on large prey populations. This represents a crucial step to better understand the causes of demographic fluctuations in ungulate community and to verify possible functional responses of predators. Results about annual diet have showed that red fox has wide trophic niche, relaying on four different food items (i.e. vegetables, small mammals, wild ungulates, and invertebrates). Concerning wolf, its diet consists mainly of one food item, represented by wild ungulates. These results have also been confirmed assessing seasonal diets. The food habits we described are in agreement with the findings of other studies, in which wolf relies mainly on wild ungulates, while red fox has wider food niche (Jedrzejewski et al. 1992, Capitani et al. 2004, Russell and Storch 2004, Gazzola et al. 2005, Lanszki 2005, Sidorovich et al. 2006, Dell'Arte et al. 2007,Panzacchi et al. 2008, Mattioli et al. 2011).

The calculation of the Pianka index confirmed a very little diet overlap between the two predators, both for the entire year and for each of the two seasons. The only shared food item by the two canids was represented by wild ungulates. Among the latter, wolf and red fox focused on different age and weight classes. Our results suggest that wolf selects wild boar of intermediate weight class (10-35 kg) and adult roe deer, while red fox selects wild boar piglets and roe deer fawns. Our analysis have showed diet overlap in terms of species but not in terms of age/weight of the individuals killed; indeed both wolf and red fox feed on wild ungulate, but among them they select different targets. This suggests that in our study area there is not direct competition between the two canids. Moreover, the little diet overlap observed for preys that are too big in size to be hunted by red fox (i.e. wild boar that weight between 10 to 35 kg and more than 35 kg, and adult roe deer) suggests that in the area wolf provides carcasses for red fox, who is known to be an efficient scavenger (Cagnacci et al. 2003, Sidorovich et al. 2006, Kidawa and Kowalczyk 2011).

The trophic habits of wolf have been confirmed even in the **Second Part**, where we have investigated the relation between the predator and its preys through the analysis of scat samples collected across 10 years. Wild boar and roe deer represent the staple food item for wolves, accounting for more that 90% (each year) of the entire diet. Even if the roe deer population is more abundant and stable in the study area, wild boar resulted to be the main food item in the wolf diet, and it resulted to be positively selected by wolf.

In the **Chapter 2** we observed that wild boar was positively selected in 6 of the 8 years of study. Moreover our results have showed that boar availability explained 62% of the variation in boar use across years; this means that low use of wild boar by wolf coincides with low density of wild boar in the population. Typically, one prey species shapes the food composition of predators, i.e. variations in the primary prey availability influence the predators' consumption of secondary prey types (Angelstam et al. 1984, Jedrzejewski et al. 2000). This is what we found in our research. For the wolves in this area, roe deer represents an alternative prey source which increases in dietary importance when boar (primary prey species) density declines. In a more complex prey-predator system situated along the Tuscany Northern Apennines, very close to our study area (Foreste Casentinesi National Park), Mattioli et al. (2011) did not observe any functional dietary response of wolves to density variations of either the main (wild boar) or of the secondary prey species (red deer, roe deer and fallow deer). In contrast, in our study area wolves are able to track wild boars availability, and switch their diet to roe deer when density of wild boar resulted to be low. This finding suggests a dietary response by wolves to the availability of wild boar when a simple ungulate community exists and just one more prey species is available. This is a finding to take seriously into account, because the high natural fluctuations of wild boar populations (Bieber and Ruf 2005, Sabrina et al. 2009) could have important ramifications for predator impact on roe deer. Moreover in this chapter we analyze the importance to estimate the uncertainty around the data collected. Our findings demonstrate that failing to account for uncertainty might lead to conclusions that are not fully supported by the data. Thus, it is necessary to be careful in describing such uncertainty before drawing ecological conclusions, in order to properly represent and interpret the different ecological issues.

Another aspect we have to consider to better understand the dynamics between wolves and their preys, is the contemporaneous presence of hunters in the same area (**Chapter 3**). They both relay on the same game species but it is important to understand the patterns of hunting harvest between wolves and hunters, because different patterns might lead to different effects on wild ungulate population dynamics. Moreover it is fundamental to evaluate the hunting harvest impact because it seems to be one of the main forces that drive ungulate population dynamics (Okarma et al. 1995, Gazzola et al. 2007, Nores et al. 2008).

Wild boar results to be the main prey species for both predators, while roe deer is the secondary one. The most effective predators for the ungulate population are the hunters, who remove a much higher percentage of the population, both for roe deer and for wild boar. Nevertheless the two predators focused on different age and weight classes among preys. This is a crucial point in understanding the role of predation on the prey population. Indeed selected prey individuals can contribute in different ways to the annual growth of the prey population because of their different reproductive value, which depends on age and sex (Gillard et al. 2000). Wolf focuses primarily on the youngest preys, thus its strongest influence is in term of juvenile mortality (Pimlott 1967, Mech 1970, Sand et al. 2012). On the other hand, hunters focus on older animals, and therefore they result as mortality factor for individuals with high reproductive value. Nevertheless our results show no relationship between the harvest of one year and the prey density in the following year. Several studies provide evidence of predation as an important limiting factor of ungulate densities (e.g. Bergerud and Eliot 1986; Gasaway et al. 1992; Jędrzejewska and Jędrzejewski 1998; Rettie and Messier 2000; Kojola et al. 2004), while others do not (Okarma 1995, Peterson et al. 1998, Oksanen and Oksanen 2000, Bieber and Ruf 2005). If wolf predation and hunting harvest were additive, the combination of hunters and wolves might negatively affect recruitment (Wright et al. 2006), but this is not the case of our study area. This could imply that the two combined mortality factors could be compensatory to other causes of mortality and may limit but do not regulate ungulate densities. This finding confirms the hypothesis that in our study area bottom-up regulation is operating, according also to Cutini et al. (2013). Thus it is reasonable that in our study area predation by wolves and harvest by hunters have a minor importance in determining the inter-annual fluctuation of the game species population, while forest productivity probably has the main effect. This is in agreement with Melis et al. (2009), who found out that top-down control by predation was relatively weak in highly productive environments but increased markedly in regions with low productivity.

The **Third Part** of this thesis is about spatial behavior. In **Chapter 4** we analyzed the environmental variables related to the choice of rendezvous sites locations. The most important variable resulted to be the elevation, with suitable elevation values ranging from

800 to 1200 m a.s.l.. Moreover the suitability increases in those areas inside or close to the protected areas, and far from roads. These outcomes are in agreement with findings of other studies (Ciucci and Mech 1992, Heard and Williams 1992, Theuerkauf et al. 2003, Capitani et al. 2006). Indeed wolves choose high elevations in order to avoid human disturbance and in order to protect pups against high summer temperatures. Again to avoid human disturbance, they prefer to locate rendezvous sites close (or inside) protected areas, and far away from roads. Protected areas provide protection from direct persecution (as hunting is forbidden), human disturbance is low (a limited number of tourists and mushrooms or chestnut collectors in the summer period), and the presence of food resources are high. Moreover in the protected areas the access is controlled and human activity is limited to daylight hours. As suggested by Capitani et al. (2006), the location of rendezvous sites inside or at the border of protected areas could represent a strategy for providing both reasonable protection to the pups and also high prey availability. Paved roads may represent a major source of mortality due to traffic accident, while unpaved roads are regularly used by wolves in their movements. In this study road presence has little influence on suitability for rendezvous sites, whose value increases with increasing distance from roads, as noted in other studies (Theuerkauf et al., 2003; Capitani et al., 2006).

It is also possible to notice that the main part of the free territory has a medium value of suitability, and that the best areas are already occupied. In this scenario of expanding wolf population, this suggest us that this process of recolonisation started from the very suitable areas (mountainous, densely forested, and scarcely urbanized areas [Cagnolaro et al. 1974, Zimen and Boitani 1975]), and then moved to the less suitable ones.

The outcomes of all these studies have confirmed that wolf needs two main issues to survive: i) protection from man and ii) an healthy prey-base (Mech 1995). These two points result extremely important in the current scenario of expanding wolf population in a human dominated landscape. Indeed it is necessary to take into account the human dimension as two remarkable categories of stakeholders are present in the territory: the hunters and the livestock breeders. A good tool to avoid conflicts between wolf and these stakeholders would be maintaining stable ungulates population. This could help because on one hand hunters consider wolf as competitor for the same resources, and most of the time they overestimate the impact of wolf predation. On the other hand livestock owners suffer the depredation of the herds, that may be limited in presence of a wide and diversified ungulate community (Meriggi and Lovari 1996).

All the information collected are crucial to preserve species of conservation interest, like wolf, and to manage exploited prey populations. However it is still necessary to enhance some aspects of wolf biology. At the moment, due to the expansion and the saturation of the suitable areas, wolves are arriving closer to the villages and cities. In this contest we are assisting to the phenomenon of “urban wolves”, and it is known that low tolerance towards wolf could be important in shaping the distribution of the species (Boitani 2003). Thus it would be interesting to study the population dynamics of the predator, and collect data about its relation with human related aspects (i.e. influence of humans in population dynamics [especially mortality and dispersion events], influence of wolf on human activities, and hybridization phenomena). All these data would help us to: i) understand the real magnitude of the present increasing and expansion, ii) predict the direction of the future expansion, and iii) to critically analyze the consequences of the expansion in relation to humans. These additional information would allow the implementation of better conservation strategies.

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