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Being in the right place at the right time: wolf spatio-temporal niche in a human-modified environment

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INTRODUCTION

Biodiversity in a crowded world

In 2000 Paul Crutzen and Eugene Stoermer proposed that human modification of the global environment had become significant enough to warrant termination of the current Holocene geological epoch and the formal recognition of a new “Anthropocene” epoch (Crutzen and Stoermer 2000, Crutzen 2002). Anthropocene means human dominance of the earth’s ecosystems. With domestication and agriculture, there was a fundamental shift in the relationship between humans and their environments (Zeder et al. 2006, Smith and Zeder 2013), and ecosystems were basically transformed as human subsistence economies. As a consequence, anthropogenic effects increasingly took precedence over natural climate change as the driving forces behind plant and animal extinctions (Smith and Zeder 2013). With the exponential growth of human population in all but high Arctic landscapes (Woodroffe et al. 2005), wildlife and humans are increasingly competing for space, resources, and places to call home, and highly populated countries currently represents the main playground for conservation challenges. In this context, the capacity of a species to adapt its spatio-temporal niche to human presence and activity becomes a fundamental trait for its persistence in human-dominated environments, and knowledge on such adaptations is essential for effective and long-term conservation planning.

The ecological niche concept

The ecological niche is a cornerstone of ecological theory. The initial definitions of ecological niche stressed the idea of it as an organism’s position in the environment (Grinnell 1917), or its role in the biotic community (Elton 1927). Afterwards, G. Evelyn Hutchinson (1957) proposed a formalization of the niche concept that still remains one of the most used niche definitions (Mcinerny and Etienne 2012). Hutchinson (1957) conceived the “fundamental niche” as a description of where a species would exist within an n-dimensional hypervolume of variables in the absence of competition – “every point in which corresponds to a state in the environment which would permit the species… to exist indefinitely”. In other words, the fundamental niche describes the space in which an organism’s requirements are met. To be noticed, space in this conception is an “ecological space” defined in terms of environmental variables and not in the three dimensions of geographical space. Hutchinson considered this a pre-interactive outcome because competition was not included. The post-interactive “realized niche” was the portion of that hypervolume still occupied by the species in presence of competitive interactions. Soon after Hutchinson (1959) posed his riddle “Why are there so many kinds of animals?”, studies on niche partitioning, i.e., the process by which natural selection drives competing species into different patterns of resource use, have grown exponentially (Schoener 1974). After extensive literature review on community ecology studies, Pianka (1981) found that three major resource-partitioning dimensions may allow for niche differentiation between sympatric species:

food, habitat (or space in general), and time. Spatio-temporal niche partitioning is a result of co-evolutionary processes which lead to species coexistence, and plays a key role in community dynamics, as species are a significant part of one another's environment (Soulé 1985).

A challenging coexistence: humans and large carnivores

Large carnivores are among the most controversial and challenging group of species to manage and conserve, because of the conflict they raise with human interests (Treves and Karanth 2003). As a group, large carnivores exert a strong influence on biological communities via predation and inter-specific competition (Berger et al. 2001, Terborgh et al. 2002), as well as by limiting and often regulating the numbers of their prey (Crooks and Soulé 1999, Ripple et al. 2014). Being charismatic animals, large carnivores are often used as focal species in conservation strategies (Linnell et al. 2000, 2005), and provide emotional, recreational, and cultural benefits to society (Kellert et al. 1996). At the same time, there is a strong hostility to these species in human history and culture, because of perceptions of their negative impacts on human livelihoods (Treves and Karanth 2003). Human-carnivore conflict mainly arises because carnivores' protein-rich diet and large home ranges draw them into recurrent competition with humans for food and space. Indeed, many large carnivore species are specialized for ungulate predation; therefore, some individuals readily kill domesticated ungulates when opportunities arise (Meriggi and Lovari 1996, Peterson and Ciucci 2003). As this competition over food and space show no sign of reduction, an intuitive forecast could be that large carnivores will persist only in protected areas or in some remote and uninhabited wilderness areas (Packer et al. 2013). The alternative scenario follows a landscape-scale conservation approach that aims at human-carnivores coexistence in a shared environment through conflict mitigation programs (Woodroffe et al. 2005, Dickman 2010). This approach seemed to be realistic in Europe, that is succeeding in maintaining, and to some extent restoring, viable large carnivore populations on a continental scale (Chapron et al. 2014). This has recently been reported for four large carnivores species, e.g., wolf (*Canis lupus*), brown bear (*Ursus arctos*), wolverine (*Gulo gulo*) and lynx (*Lynx lynx*), that are persisting in human-dominated landscapes and largely outside protected areas in the European continent (Chapron et al. 2014). Because these carnivores live in human-dominated landscapes, a key to their successful conservation is to better understand the dynamics of human-carnivore coexistence. Accordingly, during the last decades the impact of human activities on large carnivores has received increased attention (Laliberte and Ripple 2004), and the ability of large carnivores to persist in human-dominated landscapes has been extensively debated (Woodroffe 2000, Linnell et al. 2001). In anthropogenic landscapes, the occurrence and persistence of large carnivores seem to be modulated by strong interactions among factors that affect reproductive rates, such as food availability (Fuller and Sievert 2001, Basille et al. 2009), and factors that affect survival such as human activity or landscape context, which can reduce human pressure (Woodroffe 1998).

A succeeding case: the gray wolf

Along these lines, wolves living in human-dominated landscapes are a good model species to investigate this question. Globally, wolf spatial distribution is shaped by food availability and mortality risk (Fuller 1989, Mladenoff et al. 1995, Massolo and Meriggi 1998, Jedrzejewski et al. 2008). However, a lack of knowledge remains about how these factors interact to enable or to limit wolf presence in human-dominated landscapes (Boitani 2000). In Europe, wolves persist in some areas where human densities are considerably higher (Massolo and Meriggi 1998, Blanco and Cortés 2007, Jedrzej et al. 2007) than the upper threshold value reported in North America (Thiel 1985, Mladenoff et al. 1995, Merrill and David Mech 2000). Moreover, these high human and road densities are accompanied by high levels of human activity and settlements (Massolo and Meriggi 1998, Ciucci et al. 2003, Blanco and Cortés 2007). Nevertheless, wolves are adaptable animals, and they proved to be able to live close to humans as long as they are not heavily disturbed (Boitani 2000). Several mechanisms are behind this ability such as the spatio-temporal segregation between wolves and human activities (Ciucci et al. 1997, Theuerkauf et al. 2003), their capacity to use different human-related sources of food (Llaneza and Lopez-Bao 2015), or other behavioral adaptations such as den shifting (Habib and Kumar 2007, Ahmadi et al. 2014). The most severe challenges for wolf conservation are in areas where wolves have previously been extirpated, where the adaptations for coexistence have been lost, or where husbandry practices have evolved toward new production schemes (Chapron et al. 2014). However, even where wolves have a long co-existence history with humans such some areas of southern Europe, their management and conservation arise conflicts and problems to be solved (e.g., Cozza et al. 1996, Gatto et al. 2005), often involving wolf-livestock conflicts that need effective planning of mitigation strategies.

In Europe, wolves were historically present in the entire continent, living in a variety of habitats from tundra to steppes and deserts. They were progressively eradicated throughout Western Europe in the 18th and 19th centuries, surviving only in isolated populations in Spain, Italy and the Balkans (Breitenmoser 1998). Legal protection, socio-ecological changes in mountain and agricultural areas, and the recovery of wild ungulate populations, recreated the conditions for a natural expansion of wolves. In the Italian Peninsula, a remnant wolf population (*Canis lupus italicus*) reached its lowest point in the 1970s, when about 100 wolves were estimated, mostly in the central and southern portion of the peninsula (Zimen and Boitani 1975). After full legal protection was established in 1971 (Boitani and Ciucci 1993), this population started to increase and recolonize most of its historical range, reaching the south-western Italian and French Alps in 1992 (Fabbri et al. 2007), and recently also the eastern Italian Alps (Fabbri et al. 2007). Thus, wolves living in southern-central Italy provide a good opportunity to investigate how wolves adapt their spatio-temporal niche to coexist with human presence and activity, and how humans and landscape attributes, along with their interactions, determine the occurrence of a large predator in a human-modified environment.

Study description

In my PhD thesis, I considered a specific case study of coexistence between humans and the gray wolf (Canis lupus). I focused on the wolf population of the Abruzzo, Lazio and Molise National Park (PNALM), located in the central Apennines (Italy), and representing one of the few historical strongholds of the Italian wolf population. Wolves in the PNALM therefore constitutes one of the source populations that made possible wolf recolonization throughout the Italian peninsula in the last decades (Zimen and Boitani 1975). The PNALM features a landscape that can be considered relatively pristine compared to other parts of Italy, but is still affected by multiple human activities such as livestock grazing, tourism and logging. Despite their protected status and a long co-existence history with humans, wolves are still illegally killed in the park, and show higher levels of stress compared to other protected wolf populations, possibly due to a multitude of direct and indirect anthropogenic effects such as strong presence of sympatric free-ranging dogs (Molnar et al. 2015). From a conservation perspective, it is important to understand how wolves have adapted their spatio-temporal niche to live and persist in proximity to humans, and how this co-adaptation is reflected by their home range behaviour, spatial arrangement, movements, habitat use, and activity patterns.

The general working hypothesis of my PhD research was to find a partitioning in the spatio-temporal niche of wolves leading to a complementary presence and activity of wolves with respect to those of humans in the central Apennines. I first focused on wolf spatial niche, and I structured my investigation on a hierarchical fashion. Specifically, I started with assessing wolf home range behaviour, by analyzing both home range size and configuration. Then, I investigated wolf habitat selection within home ranges, and I concluded my assessment of wolf spatial niche by integrating the aforementioned analysis with the description of wolf diet and selection of kill and scavenging sites. All these steps somehow followed Johnson's orders of habitat selection (Johnson 1980), starting from the establishment of home ranges within the study area (2nd order selection), passing through the selection of habitat patches within home ranges (3rd order selection), and concluding with the fine-scale selection of feeding sites within patches (4th order selection). In this way, I was able to describe how wolves in the PNALM adapt their spatial niche to human presence at different spatial scales. In the second part of my PhD thesis, I focused on wolf temporal niche, and I assessed activity patterns of wolves with respect to both ecological and anthropogenic factors. To address my research questions, I used a Global Positioning System (GPS) data bank, consisting of locations collected from 11 wolves (6 females and 5 males) belonging to 5 packs in the PNALM.

Spatial dimension of wolf ecological niche

From birth to death, individual animals face the challenge of finding their own place to survive and reproduce. When referring to species, the ecological niche finds its spatial expression in the species' range (Hirzel and Le Lay 2008). When referring to single individuals or social groups, however, the ecological niche may be most properly described by means of their home ranges. Home ranges are, by definition, the spatial expressions of the behaviors animals perform to survive and reproduce over some specified period of time (Burt 1943). These patterns consist of routine behaviors associated with basic aspects of the life history such as feeding and resting, that are regulated by predictable variation in the environment (e.g., circadian and seasonal rhythms) as well as responses to unpredictable events that may depend on individual attributes and habitat configuration (Wingfield 2005). In Chapter I, I investigated wolf spatial niche by means of home range analysis. Whereas home range behaviour in wolves has been extensively studied throughout their range (e.g., Okarma et al. 1998, McLoughlin et al. 2004, Rich et al. 2012, Mattisson et al. 2013), increasing attention has been devoted in Europe to the anthropogenic impact on wolf spatial behaviour (Theuerkauf et al. 2003, Kaartinen et al. 2005, Mattisson et al. 2013). However, such studies are still particularly rare in Southern Europe where, due to a relatively higher human density and impact on the landscape, conditions are ideal to investigate spatial and habitat correlates of behavioural responses by wolves. I used state-of-the-art techniques for home range and core areas estimation, i.e., Brownian Bridge Movement Models (Horne et al. 2007) and time-maximizing functions based on exponential regression curves (Vander Wal and Rodgers 2012), respectively. Then, I used Generalized Linear Mixed Models (GLMMs) to assess seasonal, circadian and anthropogenic effects on wolf home range size. Lastly, I used a modified version of the Ecological Niche Factor Analysis (ENFA; Hirzel et al. 2002), to describe the composition of wolf core areas in the human-modified environment of central Apennines.

Once described wolf spatial niche at the home range level, in Chapter II I assessed wolf habitat selection and behavioural responses towards anthropogenic features by means of Habitat Suitability Models (HSMs; Akçakaya 2001). The aim of habitat suitability studies is to reconstruct a realized niche for a species from the key environmental variables measured at the locations it occupies. I used Step Selection Functions (SFFs; Fortin et al. 2005) and Resource Selection Functions (RSFs; Manly et al. 2002) to analyze wolf habitat selection patterns along moving trajectories and non-moving clusters, respectively. I focused my analysis particularly on the behavioural responses of wolves to roads, as these anthropogenic features are well known to affect wolf populations in ambivalent ways, posing a trade-off between direct and indirect negative effects (e.g., increased mortality and disturbance), and the positive effects resulting from increased ease of travel, efficient scent marking and access to prey (Ciucci et al. 2003, Zimmermann et al. 2014).

Wolf feeding ecology

To integrate the analysis of wolf spatial niche with its feeding behaviour, in Chapter III I used data collected from scat samples to describe wolf diet in the PNALM, were livestock represents one of the main economic activities for local people. By using log-linear models, the main goal of wolf diet analysis was to asses to what extent wolves rely on livestock compared to wild ungulates in this human-modified environment. Answering this question may also shed some light on the ecological role played by wolves in the biotic community of the PNALM, which is amongst the richest in the Apennines. Moreover, I used data on prey carcasses collected from GPS cluster checks to complement the dietary investigation by means of scat analysis, and assess selection patterns of wolf kill and scavenging sites.

Temporal dimension of wolf ecological niche

The temporal niche of a species is described by the distribution of its time budgets throughout the 24-h day (i.e., its circadian activity patterns), and is determined by ecological factors that directly affect the adaptive value of the timing of specific behaviors. Studies undertaken across the current wolf range suggest a number of variables that may be correlated with activity patterns of wolves, amongst which the most important are sun periodicity (Theuerkauf 2009), prey activity (Theuerkauf et al. 2007) and human interference (Ciucci et al. 1997, Kusak et al. 2005). Wolf activity patterns in central Apennines have already been investigated in the 1980s by Ciucci et al. (1997), who found that wolves mainly concentrated their activity during night hours. However, at the time when the study was conducted, wild prey communities were virtually absent in the area, and wolves were forced to mainly rely on anthropogenic food sources such as dumps in proximity of settlements. Nowadays, the ecological context of the area has changed, as wild ungulates became widely available for wolves following roe deer (*Capreouls capreolus*) and red deer (*Cervus elaphus*) reintroductions, and wild boar (*Sus scrofa*) introduction in the 70s. In Chapter IV, I investigated the temporal dimension of wolves niche by analyzing their circadian activity patterns. I expressed activity as the average distance travelled by wolves between consecutive GPS locations, and I used these distances to provide a description of wolf activity during the 24-h day. In this way, I first assessed if the renewed presence of wild ungulate populations in the central Apennines produced some detectable changes in wolf temporal niche. Secondly, I used distances travelled by wolves as response variable in Bayesian models to assess the effect of anthropogenic (i.e., roads and settlements) and ecological (i.e., season and social status) factors on wolf activity patterns.

This PhD chapter was carried out within the ERASMUS+ Project, for which I spent 4 months at the Norwegian University of Life Sciences (Ås, Norway). Here, I collaborated with Prof. Solve Sæbø, whose advise was fundamental for the implementation of Bayesian models on wolf activity.

Notes and acknowledgement

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CHAPTER I

DETERMINANTS OF HOME RANGE SIZE AND SPACE USE PATTERNS IN A PROTECTED WOLF (*Canis lupus*) POPULATION IN THE CENTRAL APENNINES, ITALY

INTRODUCTION

Due to their ecological value, large carnivores play a critical role in shaping the evolution, ecology, and behavior of prey species and other carnivores (Miller et al. 2001), as well as the trophic structure of ecosystems (Ripple and Beschta 2012). Moreover, large carnivores are often used as focal species in conservation strategies (Linnell et al. 2000), although they are controversial due to the conflict they generate with human interests and their susceptibility to human interference (Treves and Karanth 2003). Especially in human-modified environments, large carnivores conservation needs to be increasingly based on the promotion of human-carnivores coexistence at the landscape scale (Chapron et al. 2014). In this perspective, a better understanding of large carnivores' behavioural responses and adaptability to varying degrees of human presence and impact is essential for effective conservation planning.

Behavioural responses towards human interference have been reported for a variety of large carnivore species. For example, in North America mountain lions (*Puma concolor*) show nocturnal activity in areas with high human presence (Van Dyke et al. 1986), and coyotes (*Canis latrans*) living in urban landscapes avoid land-use types associated with human activity (Gehrt et al. 2009); in Europe, brown bears (*Ursus arctos*) and Eurasian lynx (*Lynx lynx*) show spatio-temporal patterns shaped by avoidance of humans (Ordiz et al. 2014; Bouyer et al. 2015). Amongst large carnivores in Europe, the gray wolf (*Canis lupus*) proved to be the most successful in persisting in human-modified environments (Chapron et al. 2014). Coexistence tactics by wolves may involve the adoption of a primarily nocturnal activity pattern (Vila et al. 1993, Ciucci et al. 1997, Kusak et al. 2005, but see Theuerkauf et al. 2007), as well as the spatial avoidance of areas heavily used by humans (e.g., Theuerkauf et al. 2003a, Kaartinen et al. 2005, Kusak et al. 2005). Wolves in Scandinavia settle their home ranges in high-quality habitat characterized by a low impact of human presence (Karlsson et al. 2007), and also tend to avoid anthropogenic structures when moving within them (Kaartinen e al. 2005, Kojola et al. 2016). This pattern is even more apparent when referring to the selection of critical sites such as dens, rendezvous sites, and diurnal retreat areas (Theuerkauf et al. 2003b, Capitani et al. 2006, Iliopoulos et al. 2014).

Whereas home range behaviour in wolves has been extensively studied throughout their range (e.g., Fuller 1989, Ballard et al. 1998, Okarma et al. 1998, Walton et al. 2001, Rich et al. 2012, Mattisson et al. 2013), increasing attention has been devoted in Europe to the anthropogenic impact on wolf spatial behaviour (e.g., Theuerkauf et al. 2003a, Kaartinen e al. 2005, Mattisson et al. 2013). However, such studies are still particularly rare in Southern Europe where, due to a relatively higher human density and impact on the landscape, conditions are ideal to investigate spatial and habitat correlates of behavioural responses by wolves. In Spain, wolves strongly select forest cover during daytime as a mean to segregate from humans (Vila et al. 1993), and similar patterns have been observed in Croatia (Kusak et al. 2005). Also wolves living in the open and agricultural landscapes of northern Greece have been recently reported to locate core areas in the more forested portions of their home ranges (Karamanlidis et al. 2016). In human-modified landscapes, the spatial behaviour of wolves may involve a complex integration between home range configuration and activity, revealing quite predictable spatio-temporal patterns complementary to human interference (Ciucci et al. 1997). Although critical, however, our knowledge in this respect is still limited to a paucity of studies, all of which are based on particularly low sample sizes (i.e., 1-2 instrumented packs per study; Vila et al. 1993, Ciucci et al. 1997, Kusak et al. 2005, Karamanlidis et al. 2016).

The home range is one of the most fundamental space use descriptors by animals, as it reveals the spatial patterns of behaviours performed to survive and reproduce (Burt 1943). Global Positioning Systems (GPS) today allow researchers to collect a large amount of location data from continuously tracked individuals to estimate their home ranges, even though this presents new challenges that are hardly met by traditional home range estimators (Kie et al. 2010). In particular, the lack of spatial and temporal independence between GPS location data may lead to bias in home range estimates if estimators, including traditional kernels, assume temporal independence between observations (Hansteen et al. 1997). Complementary to the home range concept, core areas have also been traditionally used to represent areas of intense (Samuel et al. 1985) or most concentrated (Seaman and Powell 1990) use within the home range. The most frequently adopted criterion to delineate core areas is using the 30% or 50% isopleth of the utilization distribution (i.e., the contour encompassing 30% or 50% of the utilization distribution volume), even though this choice is arbitrary and irrespective of any specific behavior (Laver and Kelly 2008). Accordingly, biologically-based methods have been advocated to more adequately delineate and interpret internal home range use by animals (Powell 2000).

From 2008-2010, based on 8 GPS-collared wolves, comprising 7 permanently or temporarily resident in 5 packs and 6 floaters, we studied home ranges, core areas, spatial interactions, extra-territorial movements, and dispersal in one of the few historical strongholds of the Italian wolf's population (Zimen and Boitani 1975), living in the Abruzzo Lazio and Molise National Park (PNALM). Investigating space-use patterns by wolves in the human-modified landscape of the central Apennines in Italy might reveal spatio-temporal adaptations by wolves in an area of long-established coexistence with humans (Zimen and Boitani 1975). In addition, thriving on an abundant and diversified wild prey community, wolves in the PNALM currently experience enhanced ecological conditions compared to the situation revealed a few decades ago in the central Apennines, when wolves relied on anthropogenic food sources such as dumps in proximity to settlements (McDonald et al. 1980, Ciucci et al. 1997).

By adopting the Brownian Bridge movement model (BBMM; Horne et al. 2007) to estimate GPS-revealed home ranges, and a time-maximizing function to delineate individually-based core areas (Glenn et al. 2004, Vander Wal and Rodgers 2012), the aims of our work were: (i) to describe annual and seasonal space use patterns by wolves in the PNALM, including resident pack members and floaters; (ii) to assess seasonal and circadian effects on home range size, as well as the effect of the density of anthropogenic features (i.e., roads and settlements) within the home range; (iii) to contrast landscape composition between core areas used by resident pack members and their entire home ranges, as this might reveal habitat-mediated behavioral adaptations by wolves living in human-altered landscapes. We finally report cases of extraterritorial forays, pre-dispersal, and dispersal movements of wolves from the PNALM.

MATERIALS AND METHODS

Study area

Our 1,505 km^2 study area is centered in the Abruzzo, Lazio and Molise National Park (PNALM) and its external buffer zone in the Central Apennines, Italy (Fig. 1). The area is typically mountainous, with elevations ranging from 400 to 2285 m, and is characterized by a Mediterranean montane climate, with dry summers and cold, snowy winters (Piovesan et al. 2003). Agricultural grasslands, pastures, and mixed forests, mainly comprised by oak (*Quercus* spp.) and maple (*Acer* spp.), predominate along valley bottoms. Higher elevations are mostly covered by deciduous forests, composed primarily of beech (*Fagus sylvatica*), whereas sub-alpine grasslands prevail above 1800 m. Human population density averages 14.6 inhabitants/km^2, even though residents live aggregated in a few mountain villages and many areas are virtually void of people (Ciucci et al. 2015). Tourism and livestock grazing are important economic activities, whereas agriculture is scarce and mostly take places along the valley bottoms near human settlements. Prey populations include wild boar (*Sus scrofa*), red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*) and Apennine chamois (*Rupicapra pirenaica ornata*), whose densities, albeit formally unknown, are relatively high based on sightings and pellet group counts (R. Latini, PNALM Scientific Service, pers. comm.).

Based on repeated snow-tracking in winter and wolf howling in summer (Ciucci and Boitani 2009), supplemented by non-invasive genetic sampling during winter (Boggiano et al. 2013), we estimated 7-9 wolf packs in the study area from 2005-2010. The relatively high wolf density we revealed (>5 wolves/100 km^2), and a tight territory mosaic, are consistent with a wolf population about saturation levels in the PNALM (Ciucci and Boitani 2009, Ciucci et al. unpubl. data). Despite positive attitudes towards wolves and bears by local residents (Glikman et al. 2012), a minimum of 33 wolves were found dead in the study area in the years of our work; of these, excluding 8 cases whose causes of death could not be ascertained, 20 died from human-induced causes, comprising 14 illegally killed (poison, shooting) and 6 that died from collision with vehicles (L. Gentile, pers. comm.). Compared to other protected wolf populations elsewhere, during the year of our study wolves in the PNALM suffered higher levels of stress, possibly as a consequence of direct and indirect anthropogenic effects (Molnar et al. 2015).

Wolf capture and GPS telemetry

Between December 2007 and October 2010, we equipped 11 wolves with GPS collars (Televilt-Tellus, Followit AB, Lindesberg, Sweden; and Vectronic Pro Light-1, Vectronic Aerospace GmbH, Berlin, Germany). Wolves were live-captured using dragged, modified Aldrich foot-snares (M. Collinge, Animal Damage Control, Idaho, U.S.A., pers. comm.) activated along trails and dirt roads and lured with fresh wolf scats. Using VHF trap-transmitters (Mod. 6, ATS, Isanti, Minnesota, U.S.A), we remotely monitored activated traps 24-hr a day and were able to retrieve captured wolves and tranquilize them within an average of 37±21 min (range: 14-90 min) since caught. Using a blowpipe (Telinject, Agua Dulce, California, U.S.A.), we administered to captured wolves the Hellabrunner mixture of ketamine hydrochloride (3.5 mg/kg) and xylazine hydrochloride (2.7 mg/kg), the latter being antagonized, upon release, with 0.25 mg/kg of atipamezole (Antisedan). Captured wolves were sexed, weighed, and classified as pups, yearlings, and adults based on tooth eruption and wear patterns (Gipson et al. 2000). We equipped with GPS collars only wolves ≥ 8 months old. Permit for wolf capture was obtained by the Ministry of the Environment, and capture and handling procedures reflected international guidelines (Sikes et al. 2011). We configured GPS collars according to 30-day cycles, during which fix attempts were programmed every 0.5-1 hours for 10 days, and every 3 hours for the successive 20 days. We downloaded GPS locations remotely through the Global System for Mobile Communications.

Each year, we used intensive snow-tracking during winter to determine the minimum number of wolves in each instrumented pack, and specifically to assess the social status (i.e., resident pack member vs. floater) of GPS-collared wolves. We revealed reproduction in each instrumented pack by means of wolf howling surveys (Harrington and Mech 1982), and successively verified pup presence using remote cameras and field investigations of dens and rendezvous sites once these were vacated. We also confirmed a posteriori the status of instrumented wolves using information derived from their GPS locations, with resident pack members sharing with other wolves the same exclusive area for ≥ 1 season, and floaters using larger areas across multiple wolf territories (Gehrt et al. 2009). Seven of these wolves were permanently or temporarily members of 5 packs (Table 1), including M22 who became a floater after losing his alpha status in the Villa pack, and F24 who dispersed from the Villa pack and eventually pair-bonded and established a new pack (Bisegna pack) in spring 2010. Three other instrumented wolves, all females, were floaters when first captured (Table 1); two of them maintained this status for the reminder of the study, whereas F23 pair-bonded with M26 establishing a new pack (Canneto pack) in fall 2009. To approximate the location of other, non-instrumented packs in the study area, we used a 5-km radius (i.e., the mean radius of annual wolf territories in the PNALM, estimated using 95% BBMMs; see below), centered in the centermost point of all indirect signs of presence (e.g., scats, tracks in the snow, wolf-howling replies) of a given pack (Fig. 1).

Spatial analysis

We used the Brownian bridge movement model (BBMM; Horne et al. 2007) to estimate home range size, and we also report home range size using 100% Minimum Convex Polygons (MCP 100%; White and Garrott 1990) to facilitate comparison with studies reported in the literature. The BBMM estimator is particularly suited to large set of autocorrelated GPS location data as it avoids the assumption of independence among relocations by explicitly incorporating the time lag between them (Horne et al. 2007, Kittle et al. 2015). Two smoothing parameters have to be set into the BBMM: the Brownian motion variance (σ1), that is related to the animal's mobility, and the location imprecision (σ2). We estimated σ1 following the maximum likelihood approach (Horne et al. 2007) using the R function *liker* (*adehabitatHR* R package, Calenge 2006), whereas we set σ2=20 m as 93.4% of our GPS locations were 3-dimensional, which generally have an error < 20 m (Di Orio et al. 2003). To avoid overrepresentation of areas used during periods of higher fix rate (Fieberg 2007), we systematically subsampled GPS locations to obtain a comparable acquisition rate (i.e., 1 location/3 hrs) throughout the sampling period. As only 6.6% of the GPS locations that we obtained were 2-dimensional, and because estimates of animal home ranges are robust to expected levels of GPS measurement error (Frair et al. 2010), we did not use a data quality filter thus avoiding to further subsample our dataset. Excluding extraterritorial forays (see below), we therefore estimated home ranges as that area encompassed by the 95% isopleth of the BBMM utilization distribution (UDs; Laver and Kelly 2008) using a 20x20m grid size. As we distributed trapping effort across packs, we rarely had >1 instrumented wolf per pack and therefore assumed that GPS locations from 1 pack member adequately represented the entire pack's home range (Ciucci et al. 1997, Benson and Patterson 2014). In just one pack we had 2 instrumented pack members, and we selected for the analyses the one with the longer tracking period and the higher acquisition rate. We also used 95% Brownian bridges to depict space use by floaters but, as these wolves did not necessarily remain stationary in a defined area, we used net squared displacement curves (NSD; Turchin 1998) to aid interpretation of their movements. Using the 3D Analyst Tools for ArcGIS (version 10.2.2), and a digital elevation model provided by the Italian Military Geographic Institute, we performed a surface area correction to provide an orographically corrected measure (i.e., 3D) of the home range size in a mountainous environment (Jenness 2004).

We delineated core areas of resident wolf packs' home ranges using a time-maximizing function based on exponential regression analysis (Vander Wal and Rodgers 2012). Using BBMM UDs, we plotted UD area against volume, the latter indicating the likelihood of the animal spending time within different portions of its home range. As this relationship is approximated by an exponential regression, the point at which the slope (i.e., the tangent) of the exponential curve fitted to the data equals 1 represents the threshold beyond which the home range area begins to increase proportionally faster than the probability of use, thus delineating the core area boundary (Vander Wal and Rodgers 2012).

For resident wolves whose sampling period was long enough (based on the relation between the cumulative proportion of annual home range size [MCP 100%] and the number of months included in the sampling period; Mattisson et al. 2013), we estimated home ranges on an annual basis. We also estimated seasonal home ranges according to three periods: summer (May-September), corresponding to reproduction, pup attendance at dens and rendezvous sites, as well as to increased availability of livestock and human activity throughout the study area; fall (October-December), when packs are expected to travel together throughout their territory at their maximum size; winter (January-April), comprising the mating period, the peak of dispersal, and increased vulnerability of prey species due to food stress, clustered dispersion, and the presence of snow (e.g., Kunkel and Pletscher 2001, Smith et al. 2004). We assessed seasonal home range fidelity using an UD overlap index (UDOI; Fieberg and Kochanny 2005), as implemented in the *adehabitatHR* R package (Calenge 2006). For each season, we also estimated diurnal vs nocturnal home ranges by assigning GPS locations to daylight or night periods using the R function *solarpos* (*maptools* R package, Bivand and Lewin-Koh 2015).

We used Linear Mixed Models (LMMs; *nlme* R package, Pinheiro et al. 2016), with pack as a random factor, to assess seasonal and circadian effects on home range size, as well as the effect of the density of paved roads (km/km^2) and settlements (km^2/km^2) within wolf home ranges. The roads layer included all paved roads within the study area, and was obtained by a combination of the De Agostini, GeoNext, and TeleAtlas databases, integrated with field surveys by the National Forest Service; the settlements layer comprised all types of human constructions in the study area, including isolated houses, and was provided by the Italian Institute of Statistics at the sub-township level (updated at 2001; www.istat.it). We included in these models only resident wolves whose sampling period was ≥ 50% of the season length. To investigate seasonal and circadian effects on the orographically corrected, log-transformed home range size (response variable) we used season and circadian periods as fixed factors, with fall and daylight as the reference levels, respectively. Given the correlation between the density of paved roads (km/km^2) and settlements (km^2/km^2) at the home range level (Pearson's r =0.7), we used only road density as a proxy of anthropogenic disturbance. As the number of GPS locations differed slightly among individual wolves due to variations in the acquisition rate and the effective sampling period, we accounted for the potential effect of sample size on home range estimates by including a fixed variance structure in LMMs (Zuur et al. 2009:71-84) proportional to the number of GPS locations. Starting with a saturated model, including all fixed factors and their interactions, we compared all possible models through dredging and finally performed model selection using the Akaike Information Criterion with small-sample size correction (AICc; Burnham and Anderson 2002). We than performed model averaging (*MuMIn* R package, Barton 2016), according to the natural average method and retaining models within ΔAIC ≤ 10 (Burnham and Anderson 2002). To assess goodness-of-fit of selected models, we computed their marginal and conditional coefficients of determination (R^2; Nakagawa and Schielzeth 2013) using the *r.squaredGLMM* function in the *MuMIn* R package (Barton 2016); in addition, we also regressed observed vs predicted values of home range size (Piñeiro et al. 2008).

We further investigated responses by resident wolves towards roads and settlements at the study area level (i.e., when establishing home ranges, or 2nd order selection; Johnson 1980) by contrasting (Wilcoxon test) road and settlement densities within annual home ranges with those within 20 synthetic, randomly located wolf territories; these were built as circular buffers of 5-km radius, equal to the mean radius of observed wolf packs' home ranges (see results).

As composition of core areas may reflect habitat-mediated behavioral responses by wolves, we used a modified version of the Ecological-Niche Factor Analysis (Hirzel et al. 2002) to contrast the ecological conditions within the BBMM-defined core areas with those within the whole home range. Similarly to traditional applications of ENFA to investigate habitat selection (e.g., Xuezhi et al. 2012, Galparsoro et al. 2009), our aim was to identify the ecological conditions that most differentiate the core areas (i.e., marginality), and the degree of environmental variation therein represented (i.e., sensitivity), with respect to the entire home range. To do this, we contrasted topographic, environmental, and anthropogenic variables (Supplementary Table S1) of each 20 x 20 m pixel within the core area with those from the entire home range. We conducted the ENFA for each pack and season separately, and used average marginality and sensitivity values to report the results at the population level. Spatial analysis were performed using ArcGIS (version 10.2.2), whereas the *adehabitatHR* R package (Calenge 2006) was used to run the ENFA.

We investigated spatial interactions between wolves within the same pack (n = 1 wolf pair in 1 pack), in adjacent packs (n = 1 pairwise interactions between 2 packs), and between resident pack members and floaters (n = 4 pairwise interactions between 2 floaters and 3 packs). To this aim, we integrated the UDOI index (Fieberg and Kochanny 2005; see above) with a proximity analysis implemented in the R package *wildlifeDI* (Long 2014) and based on the proportion of simultaneous (i.e., ±5 min) locations in which wolves were separated by increasing distances (i.e., <100 m, 100–500 m, 0.5–1 km, and >1 km; Benson and Patterson 2014).

On a seasonal basis, we described extraterritorial forays of resident wolves as GPS trajectories of ≥3 successive locations extending beyond the home range boundaries and traversing an area exclusively used in that particular occasion. We characterized extraterritorial forays, including pre-dispersal movements, based on: (i) frequency and duration; (ii) maximum net displacement (i.e., the straight-line distance between the arithmetic center of the home range and the farthest location reached); (iii) minimum distances traveled (i.e., the sum of the Euclidean distances traveled between successive locations recorded at 3-hr intervals, including the first and last locations of the trajectory both within the home range). Similarly, we quantified minimum and net distances travelled by dispersing wolves (i.e., the distance from the arithmetic center of the natal home range to the arithmetic center of the final one or the farthest location while dispersing; Blanco and Cortés 2007). We defined as dispersers those wolves performing directional movements away from their natal home range and that either settled in a new home range, or did not return to it until the date duration of the study.

RESULTS

From December 2007 to October 2010, we live-trapped and equipped with GPS collars 11 wolves, 6 females and 5 males (Table 1). Excluding 3 wolves whose GPS data and tracking period were considered inadequate (Table 1), we GPS-tracked the remaining collared wolves for 3.5-19.3 months, with an average acquisition rate of 87.2 (±5.1)% (range: 80.7-93.8%; Table 1). We estimated that a minimum of 7 months was necessary to approximate annual home ranges of resident pack members (Fig. 2). Limited to such packs (n=3; Fig. 1), annual, orographically-collected (i.e., 3D) home range size averaged 104 (± 24 SD) km^2 (100% MCP, planar size: = 151±61 km^2), 9–15% larger compared to planar sizes (Table 2). Road and settlement densities within annual home ranges averaged 29.4 (±11.3 SD) km/100 km^2 and 1.4 (±1.0 SD) km^2/100 km^2, respectively, and both did not differ compared to those within the study area (W=230, p=0.20, and W=180, p=0.70, respectively; Table 4).

Mean seasonal (3D) home range size ranged from 63.9 (±18.4 SD) km^2 in summer (n=5 packs) to 113.4 (±39.9 SD) km^2 in fall (n=3 packs; Supplementary Table S2, Supplementary Fig. S1). However, according to model selection, the seasonal effect was much less marked compared to circadian and anthropogenic effects. The parameter estimates of the top 7 models (Supplementary Table S3) were averaged to obtain the final one, according to which wolves used larger home ranges both during the night (Fig. 3) and when faced with higher road densities (Table 3). Season itself did not result to affect wolf home range size, but when considered in interaction with the circadian period, we found that wolves used larger home ranges during the night compared to daylight hours especially during summer months (Table 3). Marginal and conditional R^2 of the top model, including the circadian and road density effects only, were 0.79 and 0.99, respectively, whereas R^2 of the other selected models ranged 0.66-0.89 and 0.98-0.99 for marginal and conditional value, respectively (Supplementary Table S3). Averaged models' predicted values also indicated a good model fit (R^2=0.88, slope =0.87, Supplementary Fig. S2).

Seasonal core areas were delineated by a mean isopleth value ranging 70.7 (±1.1 SD)%-72.9 (±2.8 SD)%, during summer and fall, respectively, and their mean size (3D-corrected) ranged 22.6 (±8.0 SD)-41.8 (±12.1 SD) km^2, in summer and fall, respectively (Supplementary Table S2). Based on the ENFA marginality values (Supplementary Table S4), core areas during summer were established in the more forested and inaccessible (i.e., higher elevation, steeper and orographically complex terrain) portions of home ranges, with a tendency towards prevailing north-easterly slopes compared to the rest of the home range (Fig. 4); on the other hand, roads, settlements, agricultural areas and shrublands were markedly avoided (Fig. 4). Conversely, during fall and winter we did not reveal marked differences in environmental variables between home ranges and core area , with the exception of a positive selection of settlements by wolves during fall (Supplementary Table S4). Based on one pack only (Canneto pack), a single core area was used in summer, whereas up to three disjointed core areas were used in fall and winter. In reproducing packs, known dens and rendezvous sites were always located within summer core areas (Supplementary Fig. S3).

We tracked floaters (n=4) for 5.5-15 months (Table 1), and the area (3D-corrected) they used was larger than the annual home range size of wolf pack members (Supplementary Fig. S4; range: 293.8-408.7 km^2, Table 2). NSD curves revealed different space use patterns employed by floaters (Supplementary Fig. S5). Whereas wolf F21 moved centripetally to her natal home range (Villa pack), periodically revisiting this area, wolf M22 (Villa pack), following social rejection, also moved centripetally to his previous home range by gradually enlarged its range. Wolf F23 displayed a gradual reduction of the area visited to finally settle, after pair-bonding, in a new territory. Wolf F29 increased the distance traveled and continuously shifted the area used to finally stabilize in the Mainarde region of the PNALM, at least up to the duration of the tracking period.

Excluding F21 that considerably overlapped the area used with its former natal home range, the other floaters only slightly overlapped the territories of adjacent, GPS-instrumented packs (i.e., M22, F24, M26), and were found at >1 km apart 95.0% of the time (Supplementary Table S5). Similarly, two adjacent packs (i.e., Canneto and Orsara packs) slightly overlapped their home ranges, whereas the two GPS-collared wolves belonging to the same pack (i.e., F23 and M26; Canneto pack) extensively overlapped their seasonal home ranges, and were found at <100 m from each other 73.5% of the time (Supplementary Table S5).

We detected 27 extraterritorial forays performed by 6 wolves in 5 packs, including 3 pre-dispersal movements (Supplementary Table S6). Wolves engaged in extraterritorial forays on average every 13 days, ranging 1–84 days, almost exclusively during winter (50%) and fall (46%). Extraterritorial movements lasted on average slightly more than one day (i.e., 26.3 ±21.0 hrs), for a mean net displacement of 10.3 (±2.5 SD) km. Two out of 9 wolves that were GPS-tracked for ≥ 2 months dispersed (F24 and M28, Table 1). Pre-dispersal movements (Supplementary Table S6) occurred on average 21 (± 9 SD) days prior to dispersal and lasted 1-6 days, corresponding to 24.2 (±17.7 SD) km of net displacement from the natal home range. Wolf F24 dispersed for a net displacement distance of 9.7 km in 33 hours, carving out a new territory after pair-bonding (Supplementary Fig. S6). Wolf M28 dispersed for a net displacement distance of 79.7 km in 41 days, travelling from the PNALM to the adjacent Majella National Park, even though these are to be interpreted as minimum figures as the collar failed during dispersal (Supplementary Fig. S6).

DISCUSSION

We investigated the home range use and spatial arrangement of wolves in the human-modified environment of central Apennines, Italy, where wolves are long coexisting with humans (Zimen and Boitani 1975). Since the late 80s, wolves in the Apennines experienced ecologically renewed conditions and, after decades of marked synanthropy (Macdonald et al. 1980, Boitani 1982, Ciucci et al. 1997), they increasingly relied on re-flourishing prey populations. Wolves in the PNALM currently live at high densities and thrive on a rich and diversified wild prey community; although protected, they live in a human-affected landscape and suffer non-trivial levels of human-caused mortality. Under these conditions, we expected space-use patterns by wolves in the PNALM to be different compared to those revealed a few decades ago, though still revealing behavioral adaptations to a human-modified landscape.

For 3 of the 5 packs we studied, we were able to estimate their annual home ranges, whose average size (MCP 100%: = 152 ±60 km^2, planar size) falls within the range for established wolf populations in Southern and Central Europe (MCP 100%: 82-243 km^2; Okarma et al. 1998), as well as in North America (e.g., MCP 100%: 116.3±30.6 km^2; Fuller 1989; 219.6±160.6 km^2; Fritts and Mech 1981). However, much larger home ranges have been reported at higher latitudes both in North America (e.g., MCP 100%: 1.868 ±249 km^2; Ballard et al 1997; 3.375±1.973 km^2; Ballard et al. 1998; 42.847±6.542 km^2; Walton et al. 2001) and in northern Europe (e.g., MCP 100%: 1.372.4±514.4 km^2; Kaartinen et al. 2005; 1.017±73 km^2; Mattisson et al. 2013). Home range size in wolves has been shown to relate negatively to prey abundance (Wydeven et al. 1995, Fuller et al. 2003, Jedrzejewski et al. 2007) and wolf density (Peterson et al. 1984, Ballard et al. 1987), but positively to latitude (a proxy of dietary productivity; Okarma et al. 1998, Mattisson et al. 2013), and human density (Rich et al. 2012). Unfortunately, due to lack of accurate data on prey density and distribution in the PNALM, we could not assess the effect of prey abundance on wolf packs' home range size. However, following wild prey reintroductions in the PNALM since the mid 70s (Apollonio and Lovari 2001), local prey availability and diversity are thought to be amongst the highest in the central Apennines, corresponding to a relatively high density of wolves in the area. Whereas this should correspond to relatively smaller home ranges (Fuller et al. 2003), the PNALM wolves had home ranges on average larger compared to wolves thriving exclusively on wild boar in the southern Apennines (MCP 100%: = 99.6 ±43.8 km^2, n = 5 packs; P. Ciucci unpubl. data), and this difference may be related to pack size, on average larger in the PNALM.

In addition to wild prey and wolf density, other factors may act as determinants of home range size in wolves, especially in human-modified landscapes (e.g., Rich et al. 2012, Mattisson et al. 2013). Among these, anthropogenic and circadian effects appear to be relevant in our study area. Larger nocturnal compared to diurnal home ranges, as we revealed, tends to be a common feature of wolves living in human-dominated areas (e.g., Poland: Theuerkauf at al. 2003a), reinforced by the fact that it is not observed elsewhere (e.g., northeastern Minnesota: Demma and Mech 2011). Where human presence and activity during daylight hours exceeds a minimum threshold, wolves may display spatial avoidance by restricting their movements and activity during daytime (Ciucci et al. 1997, Hebblewhite and Merrill 2008, Kojola et al. 2016). However, the impact due to increased human activity during daylight could also act indirectly, for example mediated by the spatial behaviour and activity patterns of prey species that also try to avoid contacts with humans (Bonnot et al. 2013, Theuerkauf and Rouys 2008), including wild boar (Boitani et al. 1994) which is among the most frequently consumed prey by wolves in the PNALM (P. Ciucci, pers. comm.). Home range size of wolf packs in the PNALM also tended to increase at higher road density (also highly correlated with settlements in our study area), and this can be interpreted as a response by wolves to enhance spatial avoidance of humans while possibly compensating for adequate levels of prey availability (e.g., Murray et al.

2010, Rich et al. 2012). However, this relationship could be inverse (i.e., smaller wolf packs' home ranges) where prey species concentrate in areas of high human footprint, as revealed for wolves feeding on roe deer in southern Sweden and Norway (Mattisson et al. 2013). Habitat fragmentation caused by roads may also force wolves to increase home range size to reach a minimum threshold of undisturbed area to meet their ecological needs, such as the presence of refuge areas for resting and attending pups. The positive relationship between wolf home range size and the density of anthropogenic features is consistent with this argument, and has been shown for other carnivore species living in human-dominated landscapes (e.g., Canis latrans; Gehrt et al. 2009).

We did not observe a seasonal effect on home range size, whereas we expected to detect smaller home ranges during summer as a response adopted by wolves to avoid areas of higher human presence during the touristic and grazing season, when they are more likely to be affected by human disturbance at their homesites (Theuerkauf et al. 2003b, Iliopoulos et al. 2014) or, concurrently, as a consequence of centripetal movements around dens and rendezvous sites (Jedrzejewski et al. 2001, Packard 2003). Although seasonal variation in wolf home range size have been reported in areas characterized by large differences in prey density and distribution throughout the year (e.g., Alaska, Ballard et al. 1997), differences in summer vs winter home range size have often not been reported in areas where wolves mainly prey on non-migratory ungulates (e.g., Minnesota: Fuller 1989; Yukon Territory: Hayes et al. 1991), as in the PNALM. However, season resulted to be a secondary determinant of wolf home range size when considered in interaction with the circadian period. In particular, we detected smaller home ranges during summer days compared to night hours, indicating that this season likely represent a period during which wolves actually restrict their diurnal movements to avoid the higher levels of human activity found in the study area compared to fall and winter. More studies with a larger number of packs are needed to strengthen these conclusions.

Wolf packs' home range size and configuration that we revealed in the PNALM are likely part of a more complex behavioral response by wolves in human-modified landscapes that also entails habitat-mediated adaptations (Glenz et al. 2001). At the home range scale, and especially during summer, we revealed that wolves selected core areas whose environmental, topographic, and anthropogenic features ensured proper concealment from humans and where human accessibility is particularly limited. Accordingly, we did not observe a marked avoidance of anthropogenic features within wolf core areas during fall and winter, reflecting lower susceptibility by wolves in these seasons and a reduced human impact within the PNALM. Conversely, wolves seemed to positively select human settlements during fall, likely indicating a corresponding preference for low elevation areas, that may reflect higher prey abundance compared to the rest of the wolf pack's home range (Ausband et al. 2010). However, in addition to anthropogenic impact, other factors may as well drive selection of core areas by wolves within their home ranges. For example, the selection of north-easterly facing slopes within core areas in summer may account for microclimate selection of home sites (i.e., to facilitate thermoregulation of pups), and/or increased seasonal quality and availability of forage for wolves' prey (Albon and Langvatn 1992).

In addition to ecological factors which may act as drivers of home range size, methodological caveats are of paramount importance when estimating and comparing home ranges across studies (Laver and Kelly 2008). In this study, we attempted to address several weaknesses found in home range estimation, namely the autocorrelation among successive GPS locations, the adoption of a priori criteria to define core areas within the home range, and the use of planar measures when estimating home ranges in typically mountainous areas. As a consequence, the estimates of home ranges and core areas we report are hardly comparable with other studies on purely methodological grounds, if not by recurring to more traditional methods (i.e., MCP), and by using the correction factors we provided to account for orography. By using BBMMs to estimate home ranges (Horne et al. 2007) we formally accounted for the large amounts of autocorrelated GPS relocations while giving relevance to movement paths, instead of simple relocations, in estimating utilization distributions. Moreover, although BBMMs still require the user to specify two basic parameters (see Methods), these can be directly estimated from the study population, as opposed to the choice of the kernel method and the corresponding smoothing parameter (Horne and Garton 2006), which are often defined based on the subjective choice of the researcher. Unfortunately, BBMMs are still little used by biologists, probably due to the wide availability of commercial software solutions implementing traditional home ranges estimators, e.g., kernel estimators. Similarly, instead of setting arbitrary thresholds to define core areas, we used an individual-based procedure that reflects the within home range intensity of space use based on a time-maximizing function (Vander Wal and Rodgers 2012). This has the advantage to account for the expected individual variability in home range behavior due to the spatial distribution of critical resources (Vander Wal and Rodgers 2012). Interestingly, none of our wolf core area was outlined by the 50% isopleth traditionally reported in the literature. The use of that threshold could have led to core areas underestimation, potentially leading to erroneous conclusions on wolf spatial and ecological requirements.

Lastly, estimating home ranges of species living in areas with rough topography using planar measures can be quite misleading, whereas correcting planar measures to account for orographic relief is quite customary using any GIS platform and accurate enough Digital Elevation Terrain model (Jenness 2004). Not only neglecting orography-corrected measures of home range size impedes meaningful comparison across studies, but it may also limit interpretation of the home range as an informative parameter of space-use patterns. Orography affects the spatial behaviour of a species, and it is correlated with energetic constraints (Stamps 1995) and resources acquisition (Powell and Mitchell 1998). This aspect can be particularly important for territorial predators such as wolves, as the energetic costs of monitoring and defending territories are directly related to territory size (Rich et al. 2012), an association expected to be strongest in mountainous environments. In our case, for example, rough topography within wolf packs' home ranges can potentially represent an additional factor affecting home range size, and this is the reason why we used 3D-corrected estimates as a response variable in our GLMMs models.

Lack of overlap and proximity among adjacent wolf territories, coupled with a tight territorial mosaic with little or no territorial interstices, tend to confirm that wolves in the PNALM live at high density and are likely approaching saturation levels; these conditions are possibly acting through significant intra-specific constraints in space use and home range location (Fritts and Mech 1981, Zubiri et al. 1995). Six of the 8 GPS-collared wolves we tracked were permanently or temporarily members of 5 resident packs during the study, with the proportion of floaters ranging 16.7-75% (= 58.3±52.0% SD) each year. Non-territorial wolves float more or less temporarily over the mosaic of territorial packs (Mech and Boitani 2003) and may include older animals that leave their former packs, as well as younger wolves that eventually disperse in search of a new territory (Fuller et al. 2003). Floaters represent an important demographic and reproductive buffer, and they can readily replace breeders when they die (Fuller et al. 2003). We hypothesize this mechanism ensuring the high density and reproductive output of wolves in the PNALM despite the relatively high levels of known anthropogenic mortality (Molnar et al. 2015). For example, wolf F23, a floater when first captured, in 2008 readily pair-bonded and settled in a territory formerly occupied by another pack that abruptly vanished in 2009 possibly due to a poisoning event (P. Ciucci, pers. comm.).

Extraterritorial movements by resident wolves represent one aspect of home range behavior that has rarely been addressed, both in Southern Europe (Vila et al. 1993, Blanco and Cortés 2007) and elsewhere (e.g., Fritts and Mech 1981, van Ballenberghe 1983, Messier et al. 1985, Merrill and Mech 2000). Extraterritorial movements by PNALM wolves were quite frequent, relatively short, and of brief duration, and occurred essentially in fall and winter. However, being GPS-revealed, unlikely these movements would have been detected and classified as such in previous VHF-based telemetry studies. Conversely, during our study we did not detect any long-distance extraterritorial movement as reported elsewhere (e.g., > 400 km; Merrill and Mech 2000). Whereas the latter cases, also preferentially observed during fall and winter, were possibly related to a lower prey accessibility compared to summer (Fritts and Mech 1981, van Ballenberghe 1983, Messier 1985), we do not believe this was the case in our study area. The length and duration of the extraterritorial movements we reported appeared more of an exploratory nature, and were likely related to an increase in social interactions accompanying the onset of the mating period. We also recorded two cases of dispersal in our study population. Although anecdotal, the former (wolf F24) confirmed the occurrence of budding (Mech and Boitani 2003) as a mechanism of pack formation and territory establishment, whereas the latter (wolf M28) suggests that functional connectivity in wolves do occur in human-dominated landscapes, further supporting accumulating evidence of dispersal elsewhere in Europe (e.g., Blanco and Cortés 2007, Ciucci et al. 2009, Kojola et al. 2009). Indeed, wolf M28 performed a long-distance dispersal that ended up in the proximity of the Majella National Park, that also hosts a stable wolf population. Although based on a single event, this observation provides evidence that gene flow is maintained among wolf populations inhabiting protected areas along the Apennines ridge, supporting evidence already provided by genetic studies (Fabbri et al. 2007).

Although the ecological context of the PNALM differs from more pristine environments in North America and Northern Europe, habitat conditions of our study area can be considered as relatively ideal for wolves compared to other, more densely populated areas, both in Italy and elsewhere in southern Europe.

Wolves in the PNALM live at high density and display a tight territorial mosaic, and both their reproductive performance and the proportion of floaters seem capable of ensuring both the replacement of pack breeders lost due to natural or human-induced mortality, and the availability of dispersers outside the study area. Accordingly, with respect to the whole Apennine range, we would expect that areas of comparable size or larger, featuring similar habitat conditions with similar or lower extent of anthropogenic impact, are critical for the stable occupancy of wolves. Although space-use patterns and habitat-mediated behavioral adaptations of wolves in the PNALM are apparently not sufficient to prevent a non-trivial share of human-caused mortality, they nevertheless account for the stable occupancy of wolves in the area. Further research dealing with wolf presence in human-dominated landscapes needs to address space-use patterns and behavioral adaptations by wolves in areas featuring higher levels of human presence, as this might reveal the functional response by wolves to anthropogenic pressure and provide indications useful to define habitat suitability at the landscape scale in human-dominated landscapes.

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Table 1. Capture chronology and social status of wolves equipped with GPS-collars in the Abruzzo, Lazio and Molise National Park, Central Apennines, Italy, 2007-2010.

Wolf ID [a]	**Age (years)**	**Tracking period**				**Social status** [d]
		From–to	**Months**	**GPS locations** [b]	**Acquisition rate (%)** [c]	
F19 [e]	<1	2 Dec 2007–5 Feb 2008	2.2	282	62.6	Orsara pack (6)
F21	3-4	19 May 2008–19 Aug 2009	15.2	1,691	84.4	Floater
M22	6-7	18 May 2008–30 Sep 2008	4.5	441	89.5	Villa pack (6)
		1 Oct 2008–14 Mar 2009	5.5	486		Floater
F23	3-4	7 Dec 2008–26 Oct 2009	10.8	1,390	80.7	Floater
		27 Oct 2009–10 Jul 2010	8.5	1,545		Canneto pack (2)
F24	2-3	13 May 2009–12 Jan 2010	7.9	1,094	91.3	Villa pack (7)
		13 Jan 2010–14 Jan 2010	-	11		Disperser
		15 Jan 2010–16 Jun 2010	5.1	1,150		Bisegna pack (2)
F25	2-3	5 Oct 2009–19 May 2010	7.5	1,342	92.7	Orsara pack (6)
M26	2-3	27 Oct 2009–26 Aug 2010	10.1	2,137	93.8	Canneto pack (2)
M27 [e]	3-4	17 Nov 2009–4 Jan 2010	1.6	306	82.4	Iorio pack (6)
M28	1-2	25 May 2010–21 Oct 2010	2.1	880	81.0	Collelongo pack (n.a.)
		22 Oct 2010–2 Dec 2010	1.4	149		Disperser
F29	1	7 Jun 2010–4 Mar 2011	9	1,754	89.2	Floater
M30 [e]	2-3	27 Oct 2010–12 Jan 2011	2.6	343	57.2	Orsara pack (n.a.)

[a] F = female, M =male.
[b] excluding those acquired at high rates (i.e., 0.5–1 location/hour), subsampled at a rate of 1 location/3 hrs.
[c] including GPS locations acquired at high rates.
[d] name of pack if pack member, or floater otherwise. Note that some wolves changed their social status during the study period. In parenthesis, the maximum number of wolves detected in each instrumented pack during the tracking period.
[e] GPS data obtained from these wolves were not used in the spatial analysis due to a low acquisition rate (F19) or insufficient tracking period (M27).

Table 2. Annual home range size (BBMM: 95% Brownian Bridge Movement Model; MCP: 100% Minimum Convex Polygon) and anthropogenic descriptors as estimated in a protected wolf population in the Abruzzo Lazio and Molise National Park (central Apennines, Italy, 2008-2010). GPS-tracked wolves are distinguished based on their social status (i.e., resident pack members vs floaters); for floaters, home ranges are intended as areas used during the whole tracking period.

Social status [a]	Wolf ID	Tracking period		GPS locations (no.)	Home range [b]				
		From–to	Months		BBMM (km^2)	2D	MCP [c] (km^2)	2D	Elevation [d]
Canneto	M26 [f]	27 Oct 2009–26 Aug 2010	10.1	2,135	130.1	0.87	254.1	0.87	1,440 (717–2,228)
Orsara	F25	5 Oct 2009–16 May 2010	7.5	1,467	83.1	0.90	129.7	0.89	1,397 (974–2,256)
Villa	F24	13 May 2009–22 Dec 2009	7.4	1,093	98.7	0.92	126.5	0.92	1,287 (659–1,963)
Floater	F21	19 May 2008–19 Aug 2009	15.2	2,402	297.3	0.90	724.7	0.89	1,354 (659–2,096)
Floater	F23	7 Dec 2008–26 Oct 2009	10.7	1,389	293.8	0.88	643.2	0.89	1,433 (417–2,256)
Floater	F29	7 Jun 2010–4 Mar 2011	9.0	3,170	408.7	0.87	772.9	0.88	1,365 (390–2,256)
Floater	M22	1 Oct 2008–14 Mar 2009	5.5	486	406.3	0.93	434.7	0.87	1,148 (630–1,988)

[a] Name of pack if pack member, or Floater otherwise.

Orographically (i.e., 3D) corrected estimates are reported; to obtain the planar size of the home range, multiply BBMM size by the correction factor (2D).

[c] Orographically (i.e., 3D) corrected home range size; to obtain the planar size of the home range, multiply MCP size by the correction factor (2D).

[d] Mean (min-max) within the home range (BBMM).

[e] Primary and secondary paved roads.

[f] Although M26 pair-bonded with F23, we selected the former due to its longer tracking period and higher acquisition rate.

Table 3. Selected models' averaged coefficents to investigate seasonal, circadian and road density (km/km2) effects on wolf (3D-corrected) home range size in the central Apennines, Italy (2008-2010). In all 7 selected models (see Supplementary Table S3), fall and daylight hours were used as reference levels at the seasonal and circadian scale, respectively. Pack ID was added as random effect. We also added a fixed variance structure proportional to the number of GPS locations used to estimate home ranges.

Variable	**Regression coefficient**	**SD**	**P-value**
Night	0.517	0.236	*0.037*
Road density	0.021	0.005	*< 0.01*
Night*road density	-0.011	0.007	0.134
Summer	-0.318	0.203	0.147
Winter	-0.062	0.151	0.713
Night*summer	0.433	0.176	*0.030*
Night*winter	0.058	0.227	0.822

Table 4. Estimates of settlement and paved road density within the study area (as defined by locations obtained from GPS-collared wolves) and annual home ranges of 3 wolf packs in the Abruzzo, Lazio and Molise National Park (central Apennines, Italy; 2008-2010). Home ranges were estimated using 95% Brownian Bridge Movement Models.

	Area (km^2)	**Anthropogenic features**	
		Settlements (km^2/100 km^2)	**Roads** (km/100 km^2)
Study area	1,505	1.87	34.7
Canneto	113.39	0.40	32.3
Orsara	74.79	1.39	38.9
Villa	91.19	2.46	16.9

Figure 1. Location of the study area in Italy (inset) and spatial distribution of home ranges of resident wolf packs (95% BBMMs) in the central Apennines, Italy, 2008-2010. Approximate territories of non-radioed packs are reported with circular, dashed buffers of 5-km radius (equal to the average annual home range calculated using 95% BBMMs).

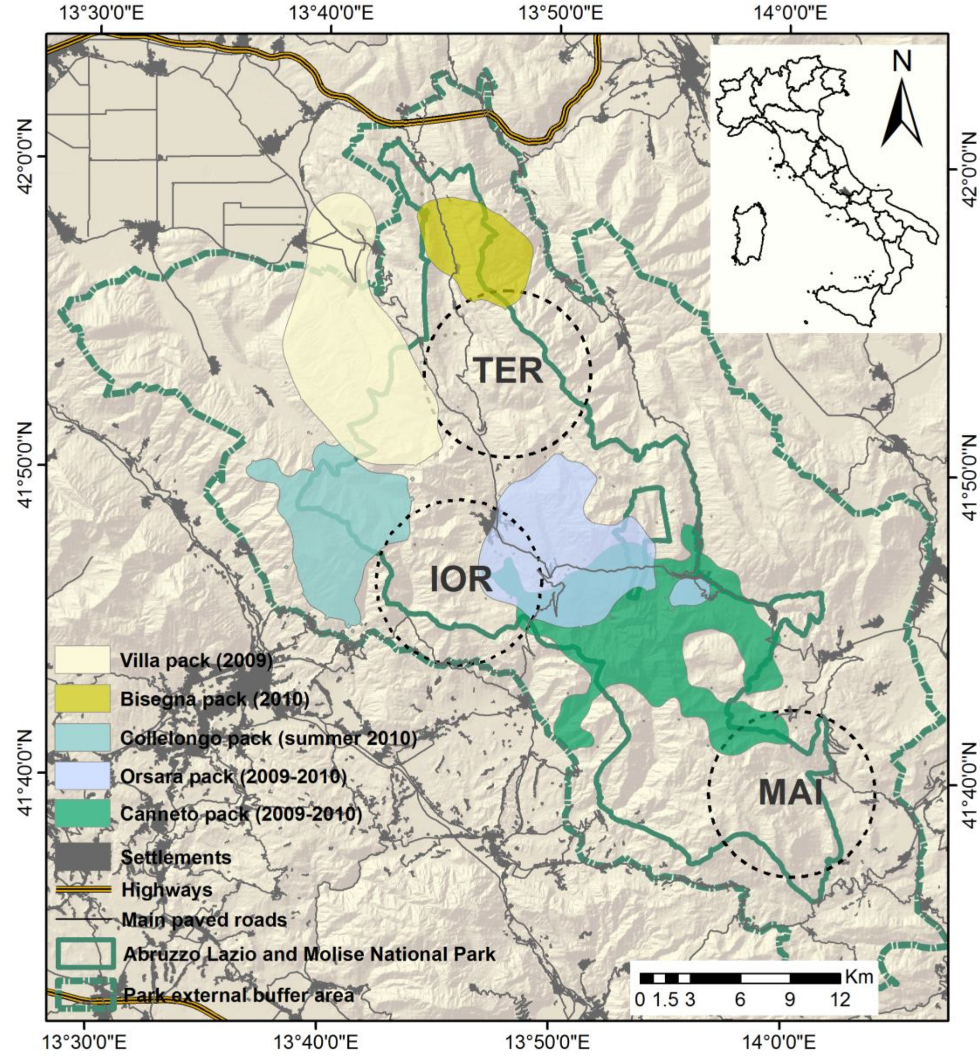


Figure 2. Cumulative proportion (box-plots) of annual home range size in relation to sampling period (months) for 4 wolf packs GPS-tracked up to 10 months; home range size was calibrated using MCP 100%.

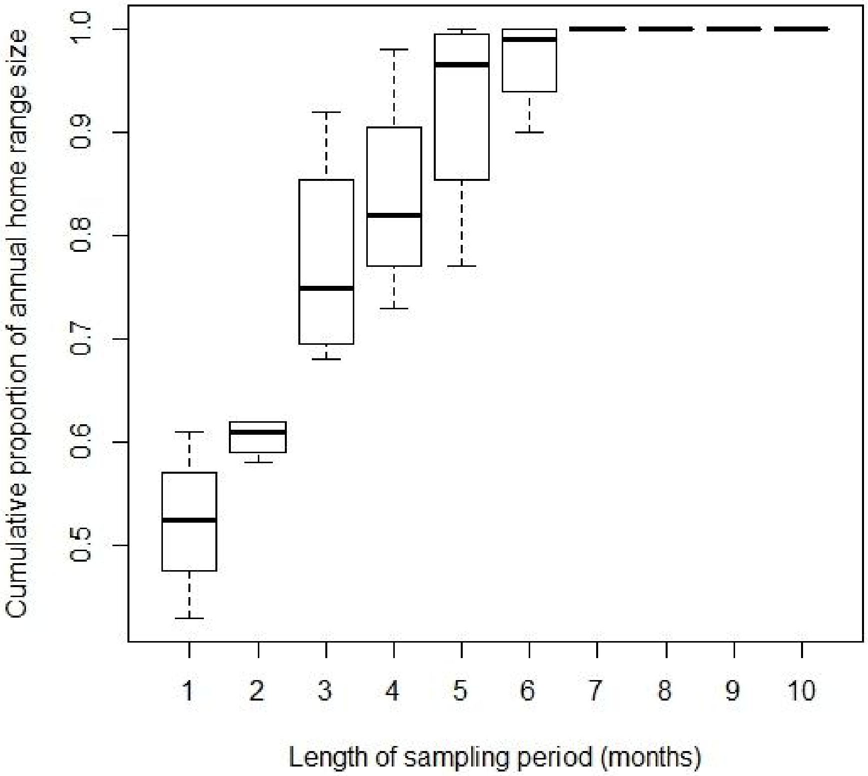


Figure 3. Diurnal (dashed line) and nocturnal (solid line) BBMM-home ranges of 4 wolf packs (A=Orsara, B=Villa, C=Collelongo, D=Canneto) in the central Apennines, Italy, 2008-2010.

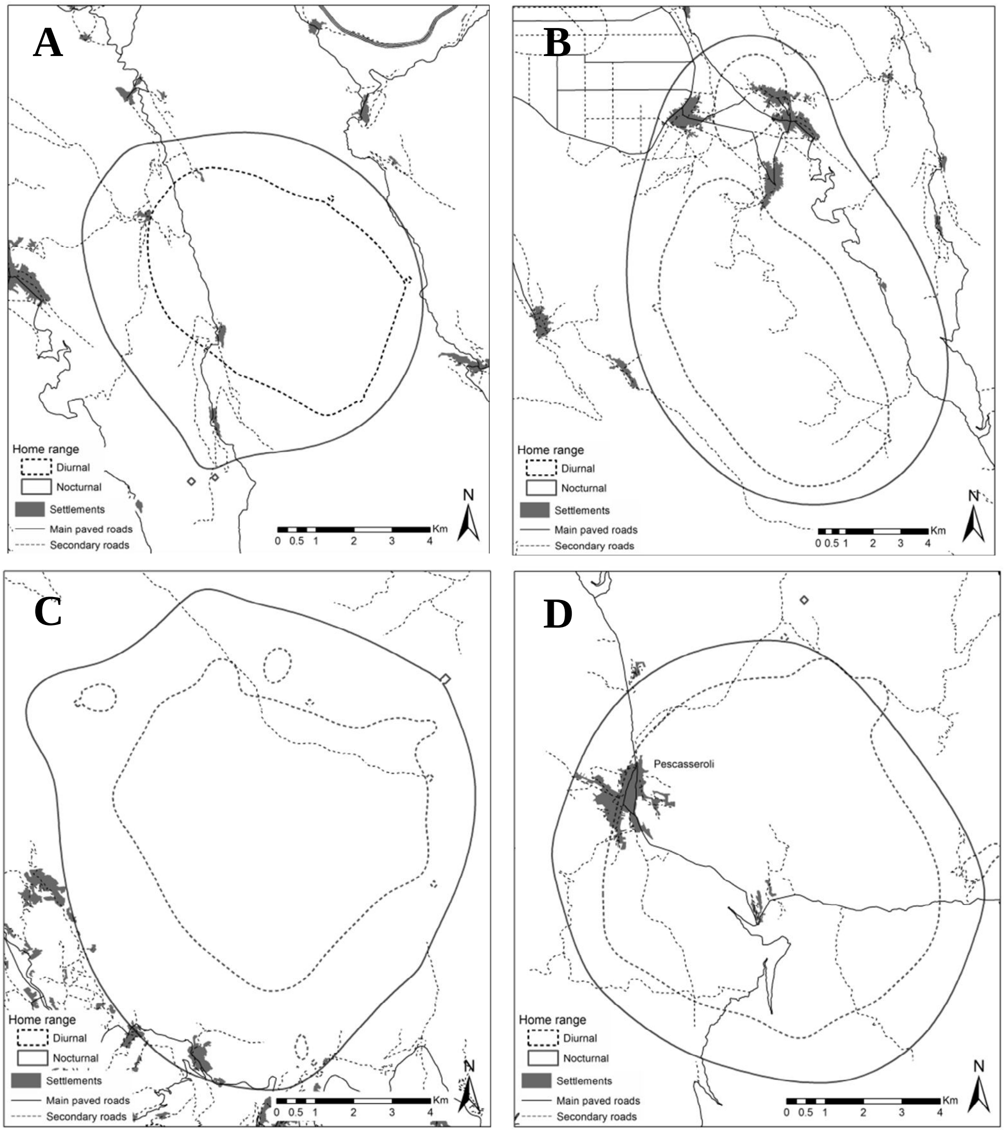
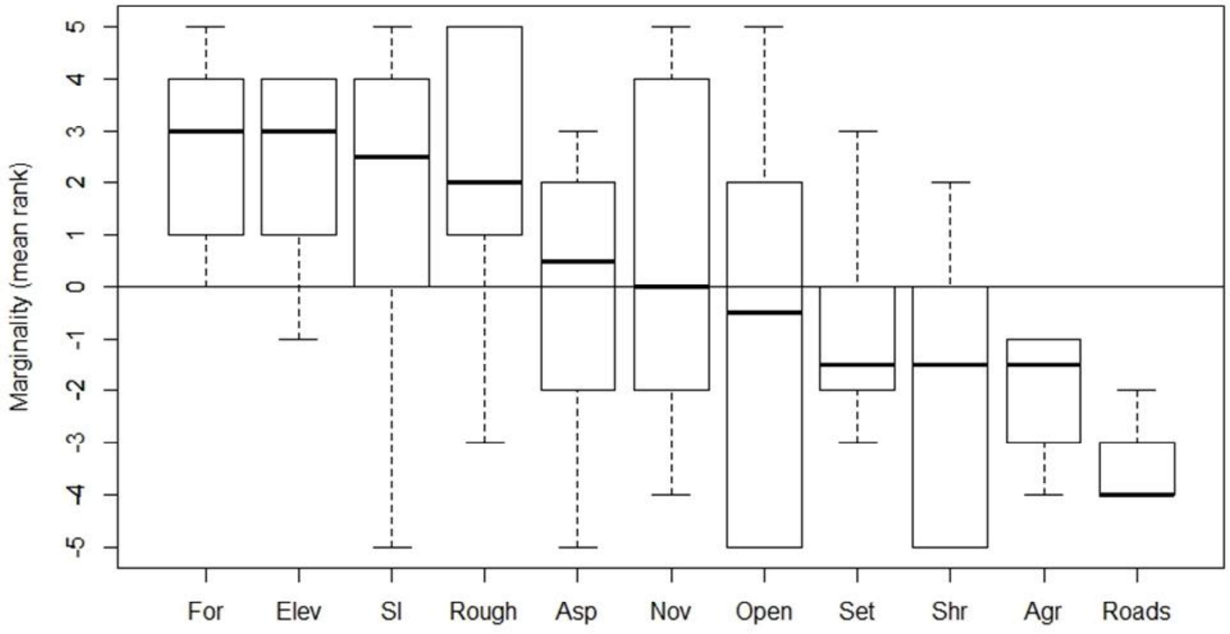


Figure 4. Boxplot of ranks of Ecological Niche Factor Analysis (ENFA) marginality values of environmental and anthropogenic covariates when comparing summer home ranges and corresponding core areas in 5 wolf packs in the central Apennines, Italy, 2008-2010 (see Supplementary Table S1 for variables description).



SUPPLEMENTARY MATERIAL

Table S1. Variables used in the Ecological Niche Factor Analysis (ENFA) to compare, on a seasonal basis, habitat composition of core areas vs home ranges of wolves in the PNALM (Central Apennines, Italy, 2008-2010).

Category [a]	**Variable**	**Label**	**Variable description**
Topographic [b]	Elevation	Elev	Altitude (m asl)
	Eastness [c]	East	Cosine transformation of aspect (degrees)
	Northness [c]	North	Sine transformation of aspect (degrees)
	Roughness	Rough	SD of elevation (m asl)
	Slope	Sl	Ground downward/upward inclination (degrees)
Environmental [d]	Agricultural fields	Agr	Cultivated land
	Forest	For	Conifer, broadleaf, and mixed forests
	Non-vegetated areas	Nov	Riparian zones, rocks
	Shrublands	Shr	Vegetation dominated by shrub species
	Open fields	Open	Pastures, sub-alpine grasslands
Anthropogenic [e]	Road density	Roads	Km/km^2 (main and secondary paved roads)
	Settlement density [f]	Set	Km^2/km^2

[a] original resolution, 20 x 20m.

[b] Digital Elevation Model provided by the Italian Military Geographic Institute

[c] Aspect was converted into the two continuous variables “northness” and “eastness” by using the sine and cosine transformations, respectively. Northness takes values close to 1 if aspect is generally northward, close to -1 if the aspect is southward, and close to 0 if the aspect is either east or west; easteness behaves similarly, except that values close to 1 represent east-facing slopes.

[d] Corine Land Cover map (European Environment Agency, 2013. Corine Land Cover 2006 seamless vector data, Version 17). We aggregated the original legend into five common land use categories.

[e] Data provided by a combination of the De Agostini, GeoNext, and TeleAtlas databases, integrated with field surveys by the National Forest Service.

[f] Including a 100m buffer

Table S2. Characteristics of seasonal home ranges and core areas of 6 wolves belonging to 5 resident packs in the Central Apennines, Italy, 2008-2010. Using Utilization Distributions (UD) estimated by the Brownian Bridge Movement Model (BBMM), core areas were delineated using a time-maximizing function based on exponential regression analysis (Vander Wal and Rodgers 2012). Seasons: summer (May-September), fall (October-December), winter (January-April).

Season	Wolf ID (pack)	Year	Time period	GPS locations	Home range (km^2)			Core area		
					BBMM 95%	2D [a]	MCP 100%	% UD	km^2	2D [a]
Summer	M22 (Villa)	2008		440	70.6	0.92	57.4	72.4	24.9	0.93
			Day	249	54.2	0.93	37.3			
			Night	191	154.7	0.93	50.4			
	F24 (Villa)	2009		643	84.7	0.92	91.4	70.2	33.1	0.94
			Day	389	65.4	0.93	70.6			
			Night	254	144.2	0.93	81.4			
	F24 (Bisegna)	2010		335	34.3	0.91	74.8	73.1	11.3	0.93
			Day	201	32.3	0.92	41.0			
			Night	134	68.4	0.90	58.1			
	M26 (Canneto)	2010		831	65.7	0.85	144.7	72.3	24.3	0.83
			Day	497	64.3	0.85	100.6			
			Night	334	90.5	0.86	128.7			
	M28 (Collelongo)	2010		879	64.5	0.87	112.2	71.1	19.4	0.87
			Day	517	54.7	0.87	79.1			
			Night	362	139.9	0.86	111.9			
Fall	F24 (Villa)	2009		450	118.7	0.92	125.3	72.4	49.9	0.95
			Day	181	128.7	0.93	100.8			
			Night	269	182.2	0.93	109.9			
	F25 (Orsara)	2009		509	71.0	0.90	96.8	70.1	27.9	0.92
			Day	197	70.6	0.91	71.4			
			Night	312	107.1	0.90	87.4			
	M26 (Canneto)	2009		413	150.4	0.87	174.9	70.1	47.6	0.85
			Day	146	147.7	0.88	142.8			
			Night	267	198.0	0.88	142.9			
Winter	F24 (Bisegna)	2010		814	49.3	0.91	82.7	71.2	17.1	0.91
			Day	362	37.7	0.92	46.8			
			Night	452	106.1	0.90	79.1			
	F25 (Orsara)	2010		832	83.6	0.90	110.7	71.1	30.2	0.90
			Day	361	88.7	0.90	88.3			
			Night	471	114.5	0.90	109.3			
	M26 (Canneto)	2010		892	108.8	0.87	237.8	71.2	37.5	0.86
			Day	393	128.5	0.87	178.1			
			Night	499	145.7	0.87	233.1			

[a] Orographically (i.e., 3D) corrected estimates of the home range are reported; to obtain the planar size of the home range, multiply BBMM home range size by the correction factor (2D).

Table S3. Top 7 Linear Mixed Models (ΔAICc $\leq$ 10, random effect= pack ID) used for model averaging aimed at assessing seasonal, circadian and anthropogenic effects on (log-transformed, 3D-corrected) home range size of 4 wolf packs in the central Apennines, Italy, 2008-2010. dn= circadian period (day vs. night); road = paved road density (km/km^2); season= summer (May-Sep), fall (Oct-Dec), winter (Jan-Apr). Marginal (R^2_m) and conditional (R^2_c) coefficients of determination are provided.

Model	AICc	ΔAICc	Weight	R^2_m	R^2_c
dn + road	18.12	0	0.55	0.79	0.99
dn + road + dn*road	18.9	0.79	0.37	0.80	0.99
dn + road + season	23.98	5.86	0.03	0.77	0.99
dn + road + season + dn*road	25.2	7.08	0.02	0.78	0.99
road	25.27	7.15	0.02	0.89	0.99
dn + season	25.93	7.81	0.01	0.66	0.98
dn + road + season + dn*season	27.11	8.99	0.01	0.75	0.99

Table S4. Values of marginality and specialization (first axis) for the habitat covariates used in the Ecological Niche Factor Analysis (ENFA) contrasting, on a seasonal basis, habitat composition within core areas with the rest of the home range in 6 GPS-tracked wolf packs in the PNALM (Central Apennines, Italy, 2008-2010). (A) Marginality values express the magnitude of the deviation of the niche relative to the available space; the higher the absolute value of the marginality, the more the niche deviates from the average conditions of the available habitat. Positive coefficients indicate preference for higher-than-mean values, while negative coefficients indicate that the species prefers values that are lower than the mean with respect to the available space. (B) Specialization values measure the narrowness of the niche around a given covariate. Agr: agricultural fields; East: eastness (degrees); Elev: elevation (meters asl); For: forest; North: northness (degrees); Nov: non-vegetated areas; Open: open fields; Roads: density of paved roads (km/km^2); Rough: roughness (meters asl); Set: density of settlements (km^2/km^2); Shr: shrublands; Sl: slope (degrees).

		(A) Marginality											
Season	Wolf ID (pack)	Agr	Shr	Set	Elev	For	Sl	Nov	Open	Rough	North	East	Roads
Summer	F24 (Bisegna)	-0.104	-0.313	-0.125	0.213	-0.233	-0.436	-0.466	0.556	-0.057	-0.023	0.010	-0.236
	F23 (Canneto)	-0.232	-0.059	-0.291	-0.087	0.583	0.489	-0.277	-0.363	0.160	0.008	-0.011	-0.191
	M26 (Canneto)	-0.279	-0.198	-0.316	0.087	0.505	0.476	-0.116	-0.281	-0.057	0.001	-0.008	-0.446
	M28 (Iorio)	-0.270	-0.573	-0.282	0.248	0.024	0.014	0.309	0.116	0.449	0.044	-0.001	-0.380
	M22 (Villa)	-0.367	-0.135	-0.449	0.328	0.356	-0.220	-0.216	-0.136	-0.493	0.020	-0.005	-0.234
	F24 (Villa)	-0.421	0.011	-0.406	0.269	0.472	0.167	-0.373	-0.249	-0.351	0.043	-0.039	-0.079
Fall	F23 (Canneto)	-0.092	-0.123	0.063	-0.058	0.409	0.621	-0.079	-0.475	0.309	-0.007	0.010	-0.299
	M26 (Canneto)	-0.079	-0.092	0.052	-0.030	0.287	0.610	0.077	-0.510	0.405	-0.013	0.002	-0.313
	F25 (Orsara)	0.520	0.383	0.059	-0.360	-0.417	-0.184	-0.058	0.232	0.338	-0.028	-0.002	0.264
	F24 (Villa)	0.073	0.424	0.350	-0.369	-0.087	-0.461	-0.238	-0.467	-0.104	0.018	0.047	0.217
Winter	F24 (Bisegna)	-0.122	-0.516	-0.250	0.076	-0.109	-0.187	-0.354	0.619	-0.227	-0.013	0.022	-0.208
	F23 (Canneto)	-0.194	0.209	-0.034	-0.095	0.333	0.420	-0.138	-0.535	0.538	0.004	0.008	-0.165
	M26 (Canneto)	-0.155	0.296	0.100	-0.138	0.239	0.353	-0.179	-0.552	0.571	-0.011	0.015	-0.116
	F25 (Orsara)	0.602	0.395	0.071	-0.298	-0.493	-0.153	0.110	0.198	0.149	-0.004	-0.008	0.220
		(B) Specialization (first axis)											
Summer	F24 (Bisegna)	-0.499	-0.368	0.046	-0.096	-0.523	0.178	-0.213	-0.491	-0.086	0.067	0.000	0.001
	F23 (Canneto)	-0.120	-0.398	-0.053	0.002	-0.634	0.036	-0.496	-0.418	-0.004	0.000	0.000	0.016
	M26 (Canneto)	0.118	0.387	-0.029	-0.005	0.618	0.005	0.450	0.498	0.032	0.000	0.000	0.044
	M28 (Iorio)	0.759	0.030	-0.621	0.001	0.153	-0.003	0.059	0.098	-0.004	0.000	0.000	-0.040
	M22 (Villa)	-0.235	-0.562	-0.058	0.001	-0.690	-0.001	-0.021	-0.385	0.000	0.000	0.000	-0.001
	F24 (Villa)	-0.529	-0.311	-0.112	0.003	-0.728	-0.010	-0.023	-0.284	0.001	0.000	0.000	-0.009
Fall	F23 (Canneto)	-0.210	-0.310	-0.015	-0.006	-0.672	-0.016	-0.509	-0.382	-0.017	0.000	0.000	-0.038
	M26 (Canneto)	0.212	0.326	0.082	0.006	0.676	-0.025	0.485	0.383	0.018	0.000	0.000	-0.048
	F25 (Orsara)	-0.095	-0.412	-0.186	-0.025	-0.738	0.326	-0.221	-0.215	-0.129	0.000	0.000	0.156
	F24 (Villa)	0.623	0.277	-0.048	0.007	0.683	-0.065	0.019	0.247	0.012	0.000	0.001	0.031
Winter	F24 (Bisegna)	-0.112	-0.100	-0.739	0.042	0.405	-0.257	0.410	-0.168	-0.006	0.000	0.000	0.048
	F23 (Canneto)	0.697	0.260	-0.025	-0.014	0.531	0.174	0.242	0.234	0.021	0.001	0.001	0.141
	M26 (Canneto)	0.218	-0.436	-0.249	-0.022	-0.499	0.325	-0.150	-0.372	0.011	-0.002	-0.002	0.429
	F25 (Orsara)	0.154	0.337	0.057	0.001	0.698	0.088	0.272	0.536	-0.063	0.000	0.000	0.005

Table S5. Home range overlap and indices of proximity between GPS-collared wolves living in adjacent packs, within the same pack (i.e., F23-M26 breeding pair), and between pack members vs floaters in the PNALM (Central Apennines, Italy, 2008-2010).

Category	Period	UDOI [a]	Proximity				
			<100 m	100–500 m	500 m–1 km	> 1km	Tot [b]
Adjacent packs							
Canneto (M26)-Orsara (F25)	Fall 2009	0.02	0	1	0	339	340
	Winter 2010	0.12	0	7	7	673	687
	Annual (2009–2010)	0.08	0	8	7	1012	1027
Same pack							
Canneto (F23-M26)	Summer 2010	1.72	255	32	8	34	329
	Fall 2009	2.51	239	36	7	56	338
	Winter 2010	1.87	565	54	24	130	773
	Annual (2009–2010)	2.08	1059	122	39	220	1440
Pack-Floater							
Villa (M22)-F21	Summer 2008	1.18	4	3	2	50	59
Villa (F24)-F21	Summer 2009	0.47	0	0	3	179	182
Canneto (M26)-F29	Summer 2010	0.28	0	1	9	442	452
Collelongo (M28)-F29	Summer 2010	0.25	0	2	3	642	647

[a] Utilization Distribution Overlap Index, with a value of 0 indicating no overlap and 1 indicating complete overlap and uniform space use; UDOI values >1 are also possible if two UDs are non-uniformly distributed and are extensively overlapped (Fieberg and Kochanny 2005).

[b] Total number of simultaneous (± 5 min) GPS locations.

Table S6. Description of extraterritorial, pre-dispersal, and dispersal movements performed by 6 GPS-collared wolves in 5 packs in the PNALM (Central Apennines, Italy, 2008-2010).

Movement type	Wolf ID (pack)	Date (from-to)	Minimum distance			Maximum Net Displacement (km)	Duration (hr)
			km	hr	days		
Extra-territorial forays	F24 (Villa)	19 Nov 2009–21 Nov 2009	22.2	24	<2	10.7	30
		22 Nov 2009–23 Nov 2009	8.5	18	<1	6.9	21
		27 Nov 2009–28 Nov 2009	12.3	15	<1	13	15
		8 Dec 2009–9 Dec 2009	11.9	10.5	<1	11.5	15
	F24 (Bisegna)	1 Jan 2010–2 Jan 2010	15.4	40.5	1	8.9	24
		7 Mar 2010–8 Mar 2010	22.1	30	<2	9.5	27
	F23 (Canneto)	16 Dec 2009–17 Dec 2009	17.9	21	<2	13.3	33
		26 Dec 2009–27 Dec 2009	13.6	24	<1	7.3	21
		4 Jan 2010–5 Jan 2010	6.9	30	<1	10.2	12
		23 Jan 2010–23 Jan 2010	8.3	18	<1	9.4	15
		4 Feb 2010–4 Feb 2010	6.6	9	<1	7.7	18
		21 Mar 2010–22 Mar 2010	10.6	12	>1	10.5	27
	M26 (Canneto)	26 Nov 2009–30 Nov 2009	18.2	15	<4	12.3	75
		16 Dec 2009–17 Dec 2009	17.9	22.5	<2	13.2	33
		27 Dec 2009–27 Dec 2009	7.3	72	<1	11.6	3
		4 Jan 2010–5 Jan 2010	7.6	30	<1	10.2	18
		25 Jan 2010–26 Jan 2010	9.2	12	<1	9.3	15
		30 Jan 2010–30 Jan 2010	11.5	15	<1	9.1	18
		1 Feb 2010–2 Feb 2010	7.5	12	<1	9.6	18
	F25 (Orsara)	5 Dec 2009–6 Dec 2009	9.4	13.5	<1	6.5	18
		28 Feb 2010–1 Mar 2010	13.7	16	<1	8.4	18
		10 Mar 2010–15 Mar 2010	31.8	15	<5	10.9	102
		3 Apr 2010–4 Apr 2010	15.5	12	<1	8.5	18
	M28 (Collelongo)	24 Sep 2010–26 Sep 2010	26.8	99	<2	17.6	36
Pre-dispersal	F24 (Villa)	12 Dec 2009–15 Dec 2009	55.2	15	<3	13.3	69
		28 Dec 2009–29 Dec 2009	37	56.5	<2	14.6	33
	M28 (Collelongo)	30 Sep 2010–6 Oct 2010	146.8	145.5	6	44.6	147
Dispersal	F24	13 Jan 2010–14 Jan 2010	50.5		<2	9.7	33
	M28 (Collelongo)	22 Oct 2010–2 Dec 2010	422.2		41	79.7	975

Figure S1. Seasonal home ranges of 5 wolf packs in the Abruzzo, Lazio and Molise National Park (central Apennines, Italy; 2008-2010). Home ranges were estimated using 95% Brownian Bridge Movement Models. Summer (May-Sep): dotted lines, fall (Oct-Dec): solid lines, winter (Jan-Apr): dashed lines.

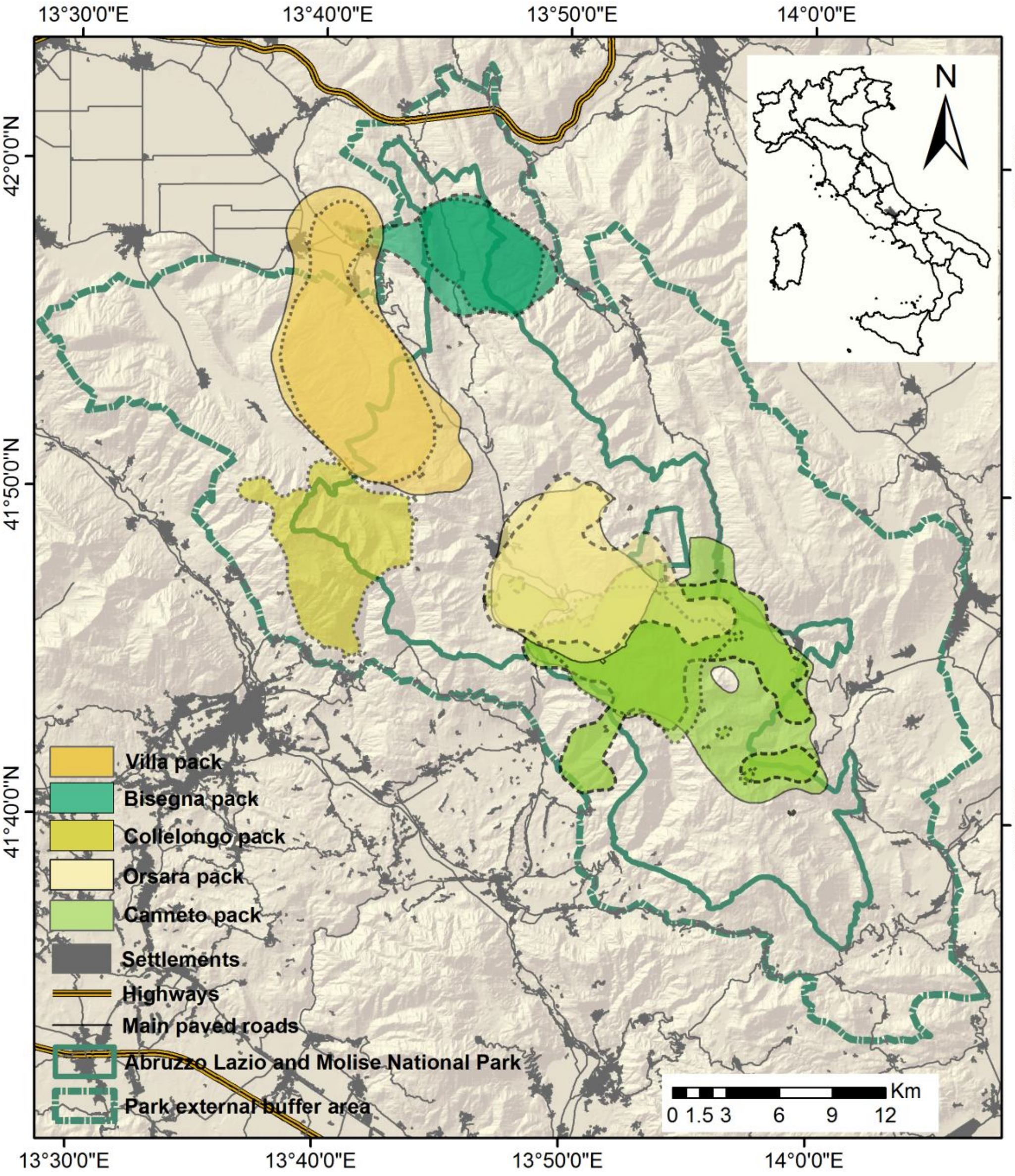


Figure S2. Scatter plot of observed vs. predicted wolf home range sizes (log-transformed). Predicted values were obtained by averaging 7 models (ΔAICc ≤10) including circadian, seasonal, and anthropogenic effects. Home range size was estimated using 95% Brownian Bridge Movement Models, with 3D-correction.

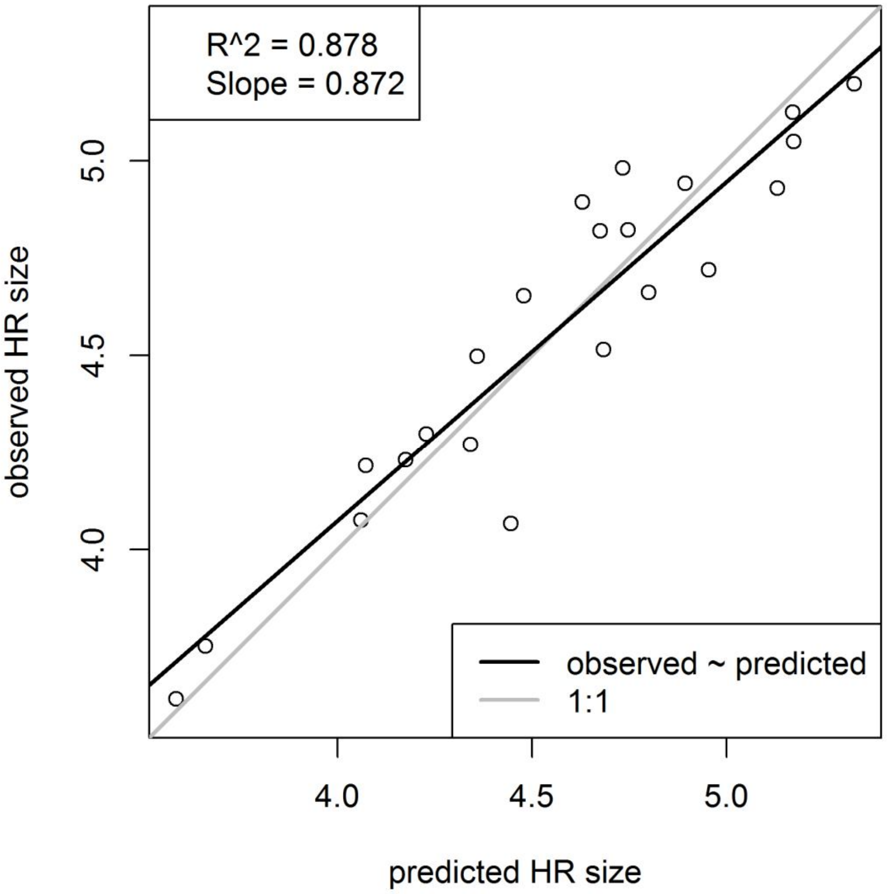


Figure S3. Summer home ranges (95% BBMM) and corresponding core areas of 4 GPS-tracked wolf packs in the Abruzzo, Lazio and Molise National Park (central Apennines, Italy; 2008-2010). Core areas were defined using a time-maximizing function (Vander Wals and Rodgers 2012).

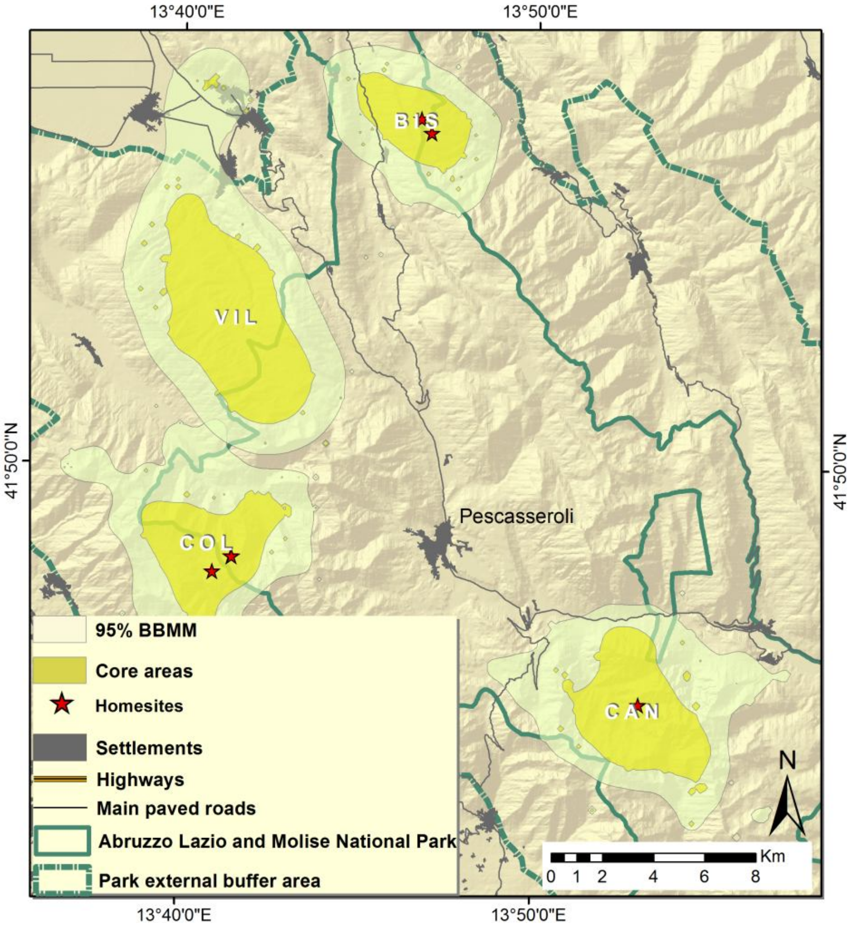


Figure S4. GPS locations collected from 4 floating wolves during the entire or part of their tracking period in the Abruzzo, Lazio and Molise National Park (central Apennines, Italy; 2008-2010).(A): wolf M22 after rejection from the Villa pack (1 Oct 2008-14 Mar 2009); (B): wolf F21 (19 May 2008-19 Aug 2009); (C): wolf F23 prior to pair-bonding (7 Dec 2008-6 Oct 2009); (D): wolf F29 (7 Jun 2010-4 Mar 2011).

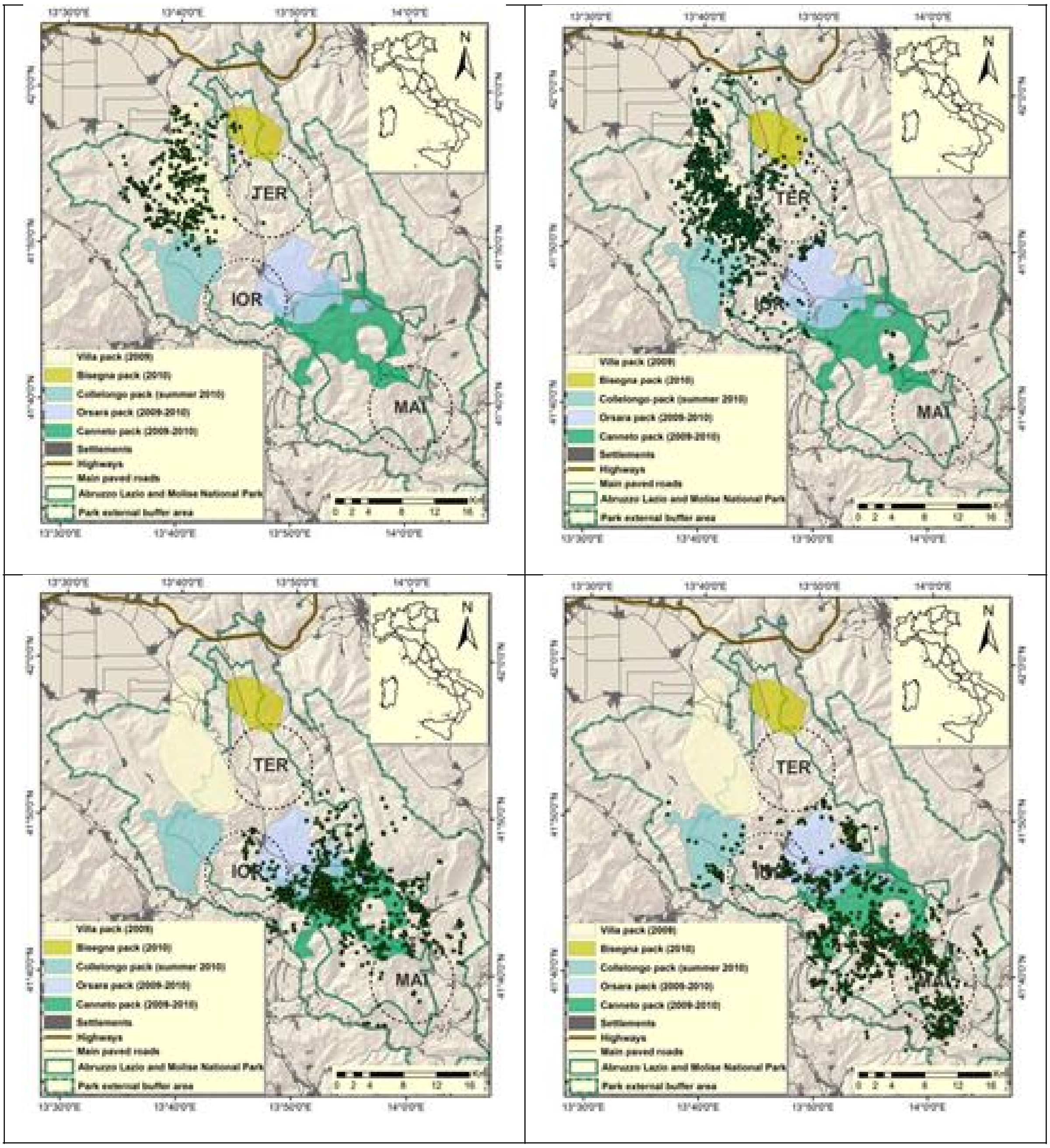


Figure S5. Distances travelled by floater wolves in the PNALM area (Central Apennines, Italy, 2008-2010) between the mean location of the first tracking day and the mean daily locations of the whole tracking period. Locally weighted scatter plot smoothing (LOESS) was applied using program R.

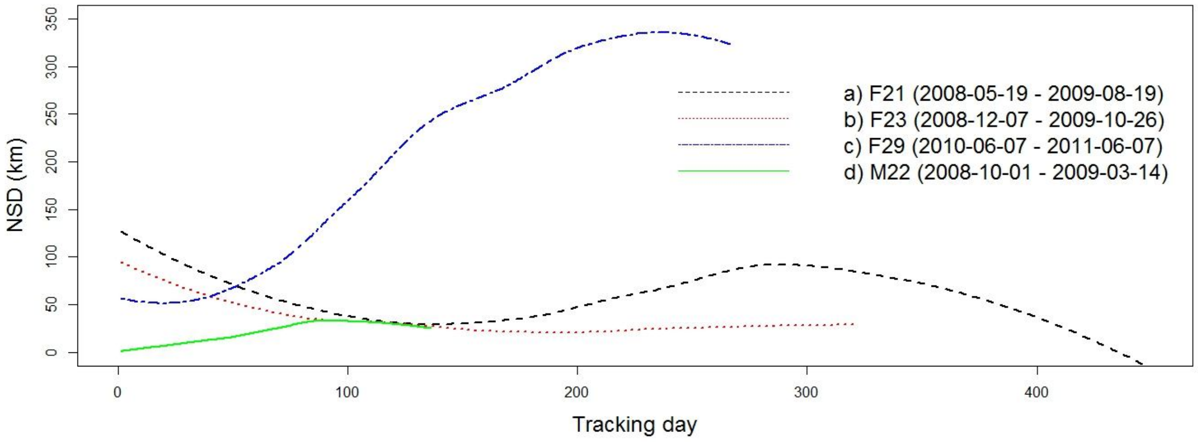
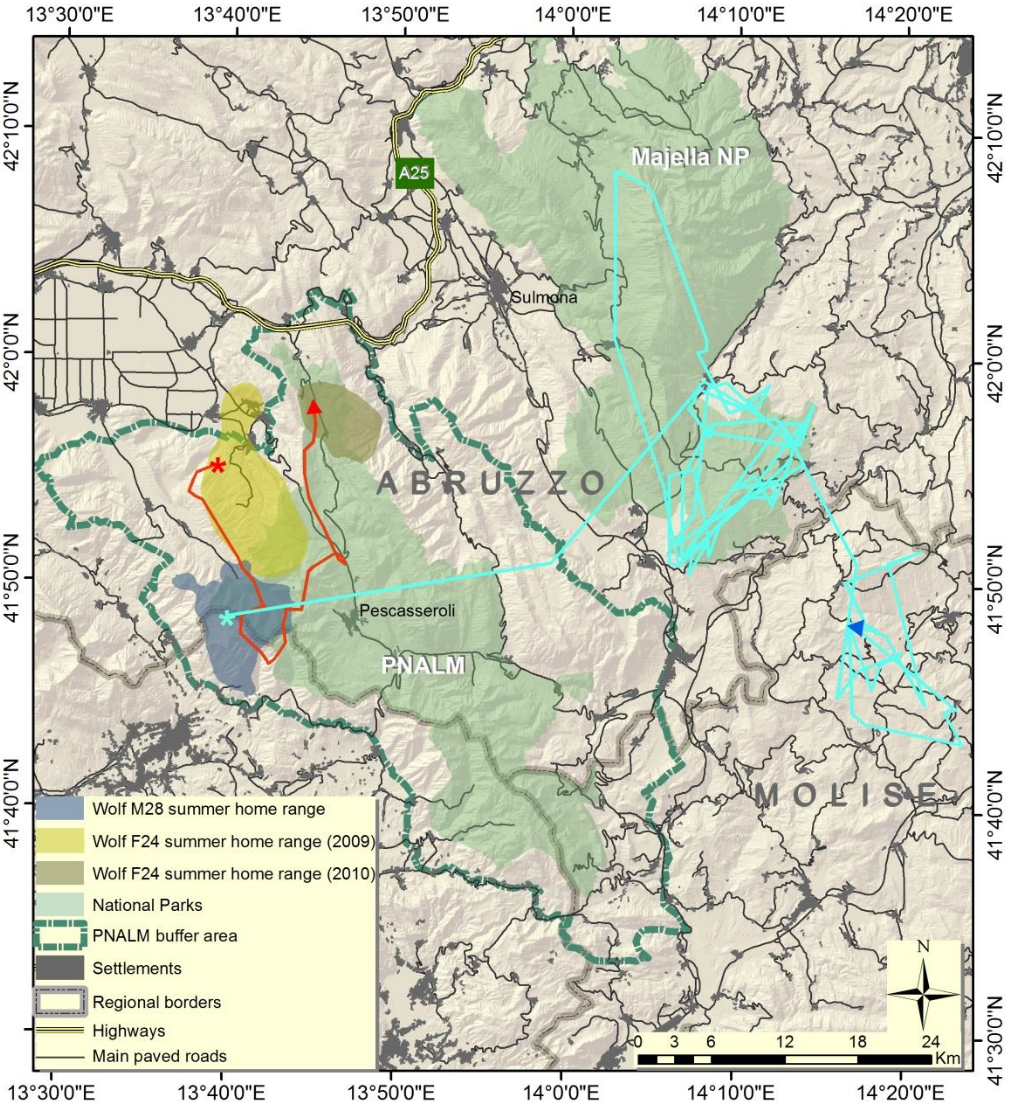


Figure S6. Dispersal trajectories of two GPS-collared wolves in the Abruzzo Lazio and Molise National Park, central Apennines, Italy, 2006-2010. Red line: wolf F24 (13-15 Jan 2010); blue line wolf M28 (22 Oct-2 Dec 2010). Asterisks denote last location in natal home range, and arrows the first location in the newly established home range (F24), or the last location recorded (M28).



CHAPTER II

MOVING AND RESTING CLOSE TO HUMANS: WOLF (*Canis lupus*) BEHAVIOURAL RESPONSES TO ROADS IN CENTRAL ITALY

INTRODUCTION

Human footprint on natural landscapes is the most pressing issue in wildlife ecology, and human-modified environments currently represent the playground for main conservation challenges (Sanderson et al. 2002). In this perspective, a first step to understand the complex process of human-wildlife coexistence would be to characterize wildlife-habitat relationships as a function of interrelating, environmental and anthropogenic factors. Road networks are well recognized to be amongst the most pervasive forms of anthropogenic disturbance (Forman and Alexander 1998), and result in a wide range of effects on wildlife populations (Forman and Alexander 1998, Coffin 2007, Fahrig and Rytwinski 2009). These span from direct mortality from vehicle collisions (Coffin 2007, Fahrig and Rytwinski 2009), to habitat loss and fragmentation (Saunders et al. 2002), and indirect alteration of individual behaviour due to increased human access to undisturbed areas (Trombulak and Frissell 2001). Globally, large-sized mammals with large home ranges are more susceptible to negative road effects, and especially to direct ones (Rytwinski and Fahrig 2012). At the same time, wide-ranging animals such as some large carnivore species proved to be able to take advantage of linear elements such as road networks in a variety of environments (e.g., *Ursus arctos*, Mace et al. 1996; *Puma concolor*, Dickson et al. 2005; *Lycaon pictus*, Abrahms et al. 2016).

Amongst large carnivores, the gray wolf (*Canis lupus*) showed to react both negatively and positively to roads along a gradient of anthropogenic disturbance across its range (e.g., Musiani et al. 1998, Ciucci et al. 2003, Whittington 2015, Zimmermann et al. 2014). On one hand, roads increase wolf mortality due to traffic accidents and facilitated access for hunters and poachers to undisturbed areas (Thiel 1985, Person and Russell 2008), and may negatively affect wolf movements (Jedrzejewski et al. 2001, Whittington et al. 2004), also reducing dispersal distances (Alexander and Waters 2000, but see also Blanco et al. 2005, Ciucci et al. 2009). On the other hand, being a highly mobile, territorial species that holds large territories, wolves may benefit from the presence of roads (Eriksen et al. 2009, Gurarie et al. 2011, Muhly et al. 2011), as they can be used to ease travel to patrol the territory (e.g., Zub et al. 2003, Barja et al. 2004) or to increase encounter rates with prey (Hebblewhite et al. 2005, Whittington et al. 2011). Several studies confirm that the perception of roads by wolves is influenced by the levels of human use, i.e., traffic volumes, associated to them and perceived as a risk of human-caused mortality (Zimmermann et al. 2014). Accordingly, wolves generally avoid highways and main roads (Thiel 1985, Kaartinen et al. 2005), but they often use low-traffic volume roads and mountain trails when human activity is low (Kunkel and Pletscher 2000, Ciucci et al. 2003, Whittington 2015). Another factor affecting wolf responses to roads is the environmental and topographic background in which roads are placed (Ciucci et al. 2003), and the strength of these response changes depending on landscape context (Beyer et al. 2013). Therefore, being habitat-mediated, wolf behavioural responses to roads should be assessed in the general framework of habitat selection modeling.

Habitat selection modeling is used to describe wildlife-habitat relationships and is considered a prerequisite for conservation planning and management (e.g., Nielsen et al. 2006, Stamps and Swaisgood 2007, Klar et al. 2008). Recently, some authors stressed the necessity to account for an animal's behavioural state (e.g., travelling, resting, foraging) when studying habitat selection (e.g., Roever et al. 2014, Abrahms et al. 2016). Pooling data among different behaviours has the potential effect to mask, overestimate or modify the strength and shape of selection patterns (Roever et al. 2014). Nevertheless, behaviour-specific habitat selection studies have received little attention (Wilson et al. 2012), likely due to the difficulty in defining animals' behavioural states from remote data (i.e., VHF and GPS telemetry locations), that lack a behavioural context (Beyer et al. 2010). In theory, spatially-explicit behavioral observations represent the most adequate tool to show how results of habitat selection studies differ when data are partitioned by animal behaviour (Wilson et al. 2012). Alternatively, different methods have been proposed to define animals' behavioural states from telemetry data, most of them consisting by using movement types as proxies of different behaviours (Van Moorter et al. 2010, Nams 2014, Gurarie et al. 2016).

Understanding space use and habitat selection patterns of wolf packs is important because residents wolves represent the reproductive portion of the population. However, wolf populations are also constituted by floating wolves that are solitary individuals seeking territories and mating opportunities, as well as old animals forced to leave their former packs through social expulsion (Fuller 1989, Fuller et al. 2003, Adams et al. 2008). Floaters are recognized to play a functional role in the population dynamics of wolves through emigration and replacement of breeding residents lost to mortality (Fuller et al. 2003, Borg et al. 2015, Cassidy et al. 2015). By moving within stable home ranges, resident wolves built a cognitive map of the environment throughout which they move, that allows them to use learned, site-specific information for navigation from place to place (Spencer 2012). On the contrary, floating wolves are generally nomadic and do not show site fidelity (Fuller et al. 2003), meaning that these individuals often move in unknown landscapes. For these reasons, a distinction should be made between resident wolves, belonging to stable packs, and floaters when assessing their habitat selection patterns.

From 2008-2010, based on 10 GPS-collared wolves in 5 packs, we assessed wolf habitat selection and behavioural responses to anthropogenic features such as roads and settlements in the central Apennines (Italy). We focused on the protected wolf population of the Abruzzo, Lazio and Molise National Park (PNALM), that represents one of the few historical strongholds of the Italian wolf population and therefore exhibits a long coexistence with human presence and activity. The PNALM features a landscape that can be considered less impacted by man-made modifications compared to other parts of Italy, and hosts a wolf population about saturation level (Boggiano et al. 2013, Molnar et al. 2015). Nevertheless, wolves in the park suffer non-trivial levels of human-caused mortality including poaching and vehicle collisions (L. Gentile, pers. comm.), and suffer higher levels of stress compared to other protected populations, possibly as a consequence of direct and indirect anthropogenic effects (Molnar et al. 2015). Moreover, although the area has long been protected, multiple human activities are present in the park and its external buffer zone (Ciucci and Boitani 2008), mainly comprising summer tourism, forestry and livestock grazing, and their intensity greatly varies throughout the year.

Our aim was to assess seasonal, circadian and habitat-mediated effects on wolf responses to anthropogenic features in the PNALM, while taking into account their behavioural state and pack affiliation. We used clusterization patterns in GPS trajectories to obtain two coarse definitions of wolf behavioural states (i.e., moving and non-moving), and we distinguished between resident and floating wolves in our habitat selection analyses. We expected wolves to 1) show stronger avoidance of roads and settlements during summer, when the intensity of human activities is higher in our study area compared to other seasons; 2) stay closer to anthropogenic features during night than daytime, as a complementary response to decreased human activity at night; 3) show stronger avoidance of roads and settlements while feeding and resting (i.e., non-moving behavioral state), when they are more susceptible to human disturbance and related human-caused mortality, and 4) show different habitat selection patterns with respect to their social category (i.e., resident pack member vs. floater), given their different life-history traits and space use behaviour.

MATERIALS AND METHODS

Study area and animals

The study was conducted in the 1,505 km^2 distribution of the core wolf population of the Abruzzo, Lazio and Molise National Park (central Apennines, Italy; Figure 1). The area is typically mountainous (elevation range: 400–2,285 m), and is characterized by a Mediterranean montane climate, with dry summers and cold, snowy winters (Piovesan et al. 2005). The most of the study area is covered by forests, followed by subalpine meadows and grasslands and intensive agricultural areas (European Environment Agency [EEA] 2006; Table S1). Forests are mostly composed by deciduous species, mainly beech (*Fagus sylvatica*), but, at lower elevations, oaks (*Quercus* spp.), maples (*Acer* spp.) and fruit trees prevail. Human population density averages 14.6 inhabitants/km^2, even though residents live aggregated in a few mountain villages and many areas are virtually void of people (Ciucci et al. 2015). Density of paved roads are 31,5 km/100 km^2 and 67.4 km/100 km^2 for main and secondary roads, respectively. Large ungulates in the study area include wild boar (*Sus scrofa*), red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*) and Apennine chamois (*Rupicapra pirenaica ornata*), whose densities, albeit formally unknown, are believed to be relatively high due to sightings and other indirect signs (R. Latini, PNALM Scientific Service, pers. comm.). Other large carnivores include Appenine brown bears (*Ursus arctos marsicanus*) and free-ranging dogs.

To capture wolves (2008-2010), modified Aldrich foot-snares (M. Collinge, Animal Damage Control, Idaho, U.S.A., pers. comm.) were set along trails and dirt roads and lured with fresh wolf scats. A mixture of ketamine hydrocloride (3.5 mg/kg) and xylazine hydrocloride (2.7 mg/kg) was administered by a blow gun (Telinject, Agua Dulce, California, U.S.A.) to immobilize captured animals, which were fitted with GPS collars (Televilt-Tellus, Followit AB, Lindesberg, Sweden; and Vectronic Pro Light-1, Vectronic Aerospace GmbH, Berlin, Germany). We configured GPS collars according to 30-day cycles, during which fix attempts were programmed every 0.5-1 hours for 10 days, and every 3 hours for the successive 20 days. We downloaded GPS locations remotely through the Global System for Mobile Communications.

Each year, we used intensive snow-tracking during winter to assess social association of GPS-collared wolves, with floaters consistently tracked alone and resident pack members frequently detected in groups of ≥ 2 wolves. We determined the minimum number of wolves in each GPS-tracked pack as the maximum number of wolves travelling together tracked throughout the winter. We also confirmed a posteriori the status of collared wolves using information derived from their GPS locations, with resident pack members sharing with other wolves the same exclusive area for ≥ 1 season, and floaters using larger areas across multiple wolf territories (Gese et al. 1988, Kamler and Gipson 2000, Gehrt et al. 2009, Hinton et al. 2016). We revealed reproduction in each instrumented pack by means of wolf howling surveys (Harrington and Mech 1983), and successively verified pup presence using remote cameras and field investigations of dens and rendezvous sites once these were vacated.

Wolf datasets

Our original dataset consisted of 12,450 wolf GPS locations collected at 3-hour intervals. Based on field tests on stationary collars the mean value of GPS error resulted of 24.4 m (±16.3 SD). We screened our dataset to detect large outliers among GPS locations following the two-step approach proposed by (Bjørneraas et al. 2010). First, we removed all fixes located farther from the median and mean location of a moving window of 20 fixes (10 fixes before and after the focal fix) than two predefined distances. The former (Δ) represents a distance that the animal could not travel within the maximum sampling interval from the median location within the moving window, and we set this distance to 100 km; the latter (μ) still represents a conservative distance from the mean location within the moving window, but it is determined from the distribution of actual distances traveled by the animals. We set μ to12 km, whose frequency within the distribution of distances travelled by wolves at 3-hr intervals was 0.04%. Second, we removed all fixes forming a spike in the movement trajectories of wolves. A spike was defined as a location for which the outgoing and incoming step length (α) exceeded 6 km, and where the cosine was less than θ=-0.97, corresponding to turning angles between 166° and 194°. Using these criteria, we identified 16 fixes (0.001%) as large outliers, distributed on 2 wolves (F24 and F29); all fixes were removed because of the Δ criterion.

After screening, we divided the original dataset according to two behavioural states of wolves: moving and non-moving. To classify a fix as a step of a moving trajectory or as a part of a non-moving cluster, we created 30 m buffers (corresponding to the upper limit of the 95% CI of the GPS error) around all GPS locations; we then dissolved the area of overlapping buffers and defined them as non-moving clusters (Zimmermann et al. 2014; Figure 2). Clusters included both consecutive locations and revisits to the same area over the entire study period. Moreover, not to include in a cluster a single fix of a moving trajectory falling within the 30 m buffer of a non-moving location, we retained within clusters only fixes for which the previous or the following GPS location was within the cluster. In this way, we classified 6,299 GPS locations as wolf cluster positions, whereas the remaining 6,151 locations were classified as moving trajectories. Unfortunately, we were not able to check in the field all clusters of GPS locations, that therefore represented an heterogeneous group of resting areas, kill sites as well as dens and rendez vous sites. Movement locations were subsamped using the *cutltraj* function in the *adehabitatLT* R package (Calenge 2006) to obtain a constant acquisition rate (i.e., 1 location/3 hrs) between consecutive locations; as a result, the final dataset for the moving behavioural state consisted of 4,243 GPS locations.

Datasets for both moving trajectories and non-moving clusters were further divided according to season and pack affiliation of wolves. We defined three seasons: summer (May-September), corresponding to reproduction, pup attendance at dens and rendezvous sites, as well as to increased human activity throughout the study area; fall (October-December), when packs are expected to reach their maximum size; winter (January-April), comprising the mating period, the peak of dispersal, and increased vulnerability of prey species due to limiting environmental conditions. Seasonal datasets were further divided according to pack association of collared wolves, that were defined as resident pack members or floaters based on field surveys (i.e., snow tracking sessions replicated during the entire study period). Therefore, we performed habitat selection analyses on five different datasets: pack members during all seasons, and floaters during fall and winter, as sample size was inadequate for floating wolves during summer (n=2).

Landscape covariates

Identical covariates were used in the analysis of selection patterns by wolves in moving trajectories and non moving locations. We investigated responses by wolves towards topographic, environmental and anthropogenic covariates. Since selection models were characterized by a small sample size (see below), we decided to limit the number of covariates to reduce as much as possible overfitting problems (Zuur et al. 2010).

Anthropogenic covariates were represented by the Euclidean distance of each wolf GPS location to the closest road (Istituto Geografico De Agostini 2001) and settlement edge (ISTAT 2011) within the study area. Roads were classified as main and secondary roads, most of which were paved. Unfortunately, we were not able to measure traffic volumes during the study period, and the classification of roads was based on the assumption that main roads connected the most populated human settlements, implying higher and more predictable human interference. On the contrary, secondary roads mainly connected smaller villages or logging areas, thus representing a lower and less regular human presence in less impacted areas of the study area. The settlements layer comprised all types of human buildings within the study area. When screening all covariates for collinearity before implementing selection models, main roads and settlements resulted highly correlated in all datasets (Pearson's r ranged from 0.60 to 0.68); for this reason, we decided to merge both layers and use them as a unique anthropogenic covariate, indicating higher human activity compared to secondary roads.

Topographic covariates comprised elevation and slope. A 20 m Digital Elevation Model (DEM) was provided by the Italian Military Geographic Institute, and was used to derive slope. Land cover data were derived from the Corine Land Cover map (European Environment Agency, 2013; Corine Land Cover 2006 seamless vector data, Version 17). We aggregated the original habitat classes into two main land use categories: 1) vegetation cover, including forest (conifer, broadleaf and mixed forests) and shrublands, whose layers were merged and used as a single covariate, and 2) open fields (pastures and sub-alpine grasslands). Agricultural fields (both intensive and extensive) were not included in the analysis, given their low presence in the study area and their spatial association with human settlements. Raster data had a pixel size of 20 m, but to take into account the potential error of GPS locations we calculated the mean habitat class value for each layer within a moving window of 30 m (corresponding to the upper limit of the 95% CI of the GPS error). All landscape covariates were standardized by computing their z-score before running further habitat selection analysis. Full models formulated for selection models comprised all covariates but elevation, which was correlated with main roads and villages (Pearson’s r range: 0.61-0.72), and open fields, due to its correlation with cover (Pearson’s r range across seasons: 0.51-0.61).

Modeling

Wolf movements

We investigated whether the locations of wolf steps (all of which were segments between locations at 3-hour intervals) in the landscape were influenced by the aforementioned covariates by means of Step Selection Functions (Fortin et al. 2015). Our analysis was based on a matched case-control design (Compton et al. 2002), that is particularly suited for assessing fine-scale habitat selection (i.e., selection along movement trajectories; 4th order selection, *sensu* Johnson 1980). We contrasted the resources at each wolf location with a set of paired ending points of random steps using conditional logistic regression (Fortin et al. 2015). For each individual, the length and turning angle of random steps were drawn from the frequency distributions of observed step length and turning angle of all other individuals (Fortin et al. 2015), but on a seasonal basis. We created 10 random steps with the same starting point per real step in the Geospatial Modelling Environment (GME) software (version 0.7.3.0; Beyer 2012).

Given the hierarchical structure of data and the expected autocorrelation within individual wolves, we used Mixed Effects Cox Models implemented in the *coxme* R package (Therneau 2015) including wolf ID as a random factor. When referring to resident pack members, we used wolf ID instead of pack ID because even if wolves belonging to the same pack generally show similar spatial behaviour (Mladenoff et al. 1995, Houle et al. 2010, Morehouse and Boyce 2013, Benson and Patterson 2015), they can show differences in finer scale, habitat use within home ranges (Benson and Patterson 2015). We checked the predictor covariates for collinearity using pairwise Pearson correlation coefficients with a correlation threshold of $r \geq 0.5$; we dropped the collinear covariates until we obtained a set of predictors whose variance inflation factor (VIF) was <3 (Zuur et al. 2010). We then defined a full model, comprising all uncorrelated covariates and the interaction term between the two anthropogenic factors and the circadian period (i.e., day or night). We assigned GPS locations (and the corresponding ending points of random steps) to daylight or night periods using the R function *solarpo*s (*maptools* R package, Lewin-Koh and Bivand 2011). To obtain robust parameter estimates, we initially performed a model selection on models with all possible combinations of covariates included in the full model for each dataset by means of Akaike Information Criterion (AIC; Burnham and Anderson 2002). We decided to retain only the top models ($\Delta AIC \leq 2$) for the following model averaging (*MuMIn* R package, Bartoń 2014), that was performed using the natural average method (Burnham and Anderson 2002, Grueber et al. 2011). Lastly, Nagelkerke Pseudo-R^2 (Nagelkerke 1991) was used to evaluate the fit to the data of all models used in the averaging procedure.

Non-movement locations

Similarly to the analysis of moving trajectories, we used conditional logistic regression to assess if and to what extent landscape covariates were selected or avoided by wolves in non-moving clusters by means of Resource Selection Functions (Manly et al. 2002). We decided to use a matched case-control design also in this case as we wanted to associate used locations of GPS clusters with a set of random points falling within a buffer with radius equal to the mean Euclidean distance travelled by wolves between consecutive clusters. Instead of positioning random points within the entire home ranges of wolves, we computed the inter-cluster distances for each individual animal using the mean x and y coordinates of GPS cluster points by season. We then used this distances to create a buffer around all clusters and, to take into account the territorial dimension of wolf spatial ecology, we finally clipped all buffers within seasonal 100% Minimum Convex Polygons (MCP 100%; White and Garrott 1990) to restrict the availability area within wolf home ranges. The number of random points to be placed within clipped buffers was proportional to their area multiplied by 10, in order to have 10 random points/km^2 of available area.

We used Mixed Effects Cox Models to implement our RSFs, and we included the cluster-ID nested within wolf pack (for resident pack members) or wolf ID (for floaters) as a random factor. We replicated the general modeling approach described for SSFs to select landscape covariates to be included in the full model and to obtain robust parameter estimates through model selection and natural averaging. Again, we were interested in assessing if wolves differently perceived roads and settlements during daylight or night periods while being in non-moving clusters. For this reason, we classified clusters as “diurnal” if at least of the 50% of their GPS locations were collected during daylight hours, and vice versa; associated random points were classified accordingly. Also in this case, we used Nagelkerke Pseudo-R^2 estimated for all models used in the averaging procedure to measure their goodness-of-fit.

RESULTS

Eight of the 10 GPS-collared wolves were permanently or temporarily members of 5 packs, including M22 who became a floater after losing his alpha status in the Villa pack, and F24 who dispersed from the Villa pack and pair-bonded with an unmarked male, establishing a new pack and reproducing in spring 2010 (Bisegna pack). Three other instrumented wolves (2 adult and 1 yearling females) were floaters when first captured; two of them maintained this status for the reminder of the study, whereas F23 pair-bonded with M26 establishing a new pack and producing pups in spring 2010 (Canneto pack). We excluded from analyses data from 1 wolf (F19) whose tracking period and acquisition rate were particularly low. The tracking period for the remaining 10 GPS-collared wolves averaged 8.5 months, ranging 1.6-19.3 months, whereas GPS success averaged 87% (range: 81–94%).

Moving trajectories

Of the 4,243 GPS locations collected throughout the entire study period that were classified as steps of wolf moving trajectories, the majority (58%) was found in forested areas, followed by pastures and alpine grasslands (18%) and shrublands (14%), with a low percentage of locations collected in agricultural fields (6%). Wolves maintained a distance of 1.7 (±0.4 SD) km from main roads and villages while travelling during the day, with lower distances detected in winter (mean =1.4 ±0.3 SD km) and higher distances detected in summer (mean =2.1 ±0.8 SD km). During the night, the distance of wolf movement locations from main roads and villages were generally lower, averaging 1.3 (±0.3 SD) km across seasons. Distances of wolf moving trajectories from secondary roads averaged 0.7 (±0.2 SD) km and 0.8 (±0.1) km during day and night periods, respectively, across all seasons.

Once models have been formulated with all possible combinations of covariates, three where used for model averaging for the fall dataset of resident pack members (range of Pseudo-R^2 =0.86-0.87), whereas the full model resulted the best one for the summer and winter datasets. When moving, resident wolves responded to the presence of main roads and villages differently across seasons and, within seasons, during daylight and night periods. During summer, wolves avoided main roads and settlements (β_{day} =0.23, P-value <0.01; β_{night} =0.18, P-value =0.01), as well as secondary roads (β_{day} =0.31, P-value <0.01; β_{night} =0.14, P-value =0.01). The opposite result was observed during winter, when wolves tended to move closer to main roads and villages (β_{day} =-0.28, P-value <0.01; β_{night} =-0.46, P-value <0.01), and the same tendency was detected during fall, but limited to night hours (β =-0.27, P-value <0.01). When considering the other environmental covariates, wolves positively selected cover during summer and winter when moving (Table 1a). Lastly, moving trajectories of wolves were characterized by flat slopes during all seasons (Table 1a).

Floaters moved closer to main roads and villages at night (mean =1.5 ±0.7 SD km) than during daylight hours (mean=1.8 ±0.6 SD km), whereas similar distances between day and night were observed for secondary roads (day: mean =0.7 ±0.1 SD km; night: mean =0.8 ±0.1 SD km, across seasons). For model averaging, six and three models were used for fall and winter datasets, respectively (range of Pseudo-R^2: fall

=0.83-0.84; winter =0.84-0.85). Floating wolves behaved similarly to resident pack members with respect to main roads and villages, as these were selected both during fall and winter, but limited to the night period (β_{fall} =-0.25, P-value =0.05; β_{winter} = -0.36, P-value <0.01). Moreover, floaters tended to move close to secondary roads in winter, both during the day and at night (β_{day} =-0.32, P-value =0.05; β_{night} = -0.27, P-value <0.01), whereas no preference or avoidance was observed for the other land cover and topographic covariates during both seasons (Table 1b).

Non-moving clusters

Of the 6,299 GPS locations collected throughout the entire study period that were classified as non-moving and other behaviours, the majority (73%) was found in forested areas, followed by shrublands (12%) and pastures and alpine grasslands (8%), with a low percentage of locations collected in agricultural fields (2%). With data collected at 3-hrs intervals, we detected an average of 3.2 ± 0.5 SD wolf clusters per day (range: 2-7 clusters per day across seasons).

Once formulated models with all possible combinations of covariates, two where used for model averaging for the fall dataset of resident pack members (range of Pseudo-R^2 =0.58-0.59), whereas the full model resulted the best one for the summer (Pseudo-R^2 = 0.62) and winter (Pseudo-R^2 = 0.61) datasets. When referring to the selection patterns adopted by resident wolves in non-moving clusters, we observed that all anthropogenic features were avoided during summer (main roads and settlements: β_{day} =0.09, P-value =0.05; β_{night} = 0.17, P-value =0.03; secondary roads: β_{day} =0.24, P-value <0.01, β_{night} =0.22, P-value =0.01), whereas only secondary roads were avoided during fall (β_{day} =0.17, P-value <0.01, β_{night} =0.13, P-value =0.03). We observed a circadian effect in the behavioural responses by wolves towards roads and settlement in winter, as they were avoided during daylight hours (main roads and settlements: β =0.01, P-value =<0.01; secondary roads: β =0.10, P-value <0.01) and selected at night (main roads and settlements: β = -0.23, P-value =<0.01; secondary roads: β = -0.18, P-value <0.01). Forested areas and shrublands represented the prevalent land cover type in wolf clusters, as they were selected during all seasons (Table 2a). Lastly, non-moving clusters of resident wolves were located in areas with lower slopes in summer, whereas steeper slopes were selected in fall and winter (Table 2a).

Only the full model was retained after model selection for both fall and winter datasets of floaters (fall: Pseudo-R^2 =0.55; winter: Pseudo-R^2 =0.52). Floating wolves behaved differently compared to resident pack members. First, main roads and villages represented again an important determinant in the probability presence of floaters in non-moving clusters. Indeed, these anthropogenic features where selected in fall and winter, both during daylight and night hours (fall: β_{day} =-0.42, P-value <0.01; β_{night} = -0.36, P-value <0.01; winter: β_{day} = -0.19, P-value =0.01; β_{night} = -0.28, P-value <0.01). Instead, no preference or avoidance was detected for this anthropogenic covariate during all seasons. If floating wolves did not show strong selection patterns for topographic and land cover covariates along moving trajectories, they selected both vegetation cover in all seasons in non-moving clusters (Table 2b). Moreover, higher slopes were selected during both fall and winter (Table 2b).

DISCUSSION

Recent studies that demonstrate large carnivore persistence in human-modified landscapes suggest that a flexible behaviour to accommodate anthropogenic disturbance through spatio-temporal responses in habitat selection patterns may be common in some of these species (e.g., Roever et al. 2010, Northrup et al. 2012, Knopff et al. 2014, Zeller et al. 2016, Basille et al. 2009, Bouyer et al. 2015, Llaneza et al. 2012, Zimmermann et al. 2014). We explored how wolves in the human-modified environment of the central Apennines adjust their patterns of habitat selection associated with their behavioral state and pack affiliation in response to anthropogenic features. Given their long coexistence history with human presence and activity, wolves in the PNALM represent a good case study to assess the flexibility of this species to live in proximity to humans.

Several studies show that wolves decrease use of roads or increase their distance from these features with increasing rate of human use (Kunkel and Pletscher 2000, Kaartinen et al. 2005, Gurarie et al. 2011, Muhly et al. 2011). Accordingly, time of day is known to influence the behavioural response of wolves to human infrastructure, likely because of diurnal activity patterns of humans. Night-bias of road use has been reported for wolves both in Europe and North America (Blanco et al. 2005, Theuerkauf et al. 2007, Hebblewhite and Merrill 2008, Zimmermann et al. 2014), as well as for other large carnivore species (e.g., Roever et al. 2010, Knopff et al. 2014, Ordiz et al. 2014). Contrarily to our expectation, we rarely found a circadian effect on wolf responses to roads and settlements, possibly due to their relatively low density in the study area, which is also accompanied by high availability of refuge areas for wolves. Instead, we found a marked seasonal effect, indicating that human interference in the PNALM is mainly related to the specific human activities carried out within the park rather than their circadian intensity, to which wolves have likely adapted their habitat selection patterns after coexisting with them for a long time.

We found that resident wolves showed a strong avoidance of main roads, settlements and secondary roads in summer, and this response was observed both along moving trajectories and in non moving locations. Wolf avoidance of anthropogenic features during summer was an expected outcome, as human activities such as tourism and livestock raising reach their maximum levels during this season. In addition, summer represents the pup-rearing period for wolves, and minimizing the probability of encountering humans has been identified as an important factor shaping wolf habitat selection during this period (e.g., Gurarie et al. 2011, Whittington 2015, Sazatornil et al. 2016). In our study population, reproduction was attested in 4 of the 5 GPS-tracked packs, and survival of both adults and pups may be severely affected if these sites are detected. As a result, such sites are often located far from sources of human disturbance (Theuerkauf et al. 2003, Capitani et al. 2006, Person and Russell 2009).

The tendency towards roads was opposite during winter, when wolves showed a preference for main roads and settlements when travelling, whereas positive selection for these anthropogenic features was limited to night hours during fall. As opposed to summer, human interference in the study area is relatively low during fall and winter, and wolves may attempt to use roads to ease travel in this season, when snow cover may represent an important factor increasing energy expenditure for locomotion (Crête and Larivière 2003). Facilitated winter movements along liner elements may serve for territory maintenance by scent-marking (Zub et al. 2003, Barja et al. 2004), and to increase hunting success (Hebblewhite and Merrill 2008, Latham et al. 2011, Whittington et al. 2011). An alternative explanation is that wolves select roads because these are concurrently selected by prey species for minerals and energy-rich food along roadside (Laurian et al. 2008, Hebblewhite and Merrill 2009). Our results are therefore in line with previous studies reporting similar preference patterns of wolves for roads and other man-made linear features in presence of low human interference (Houle et al. 2010, Gurarie et al. 2011, Soulsbury et al. 2011, Zimmermann et al. 2014).

Previous research indicates that wolves may adapt to use roads for traveling, whereas they prefer to rest further from roads and settlements (e.g., Zimmermann et al. 2014, Llaneza et al. 2016). Conversely to what expected, we detected a positive selection by resident wolves for anthropogenic features in non-moving clusters during winter, but this selection was limited to night hours. This finding may represent another characteristic of a wolf population that is well adapted to live in close proximity to humans, contrasting with other results obtained from areas where wolves recently started to recolonize human-altered landscapes (e.g., Zimmermann et al. 2014, Kojola et al. 2016), or where they are more limited by human-caused habitat modifications (Llaneza et al. 2016). The selection of anthropogenic features we reported for wolves when non moving during winter may be the consequence of the altitudinal migration of wild ungulates from high-elevation to low-elevation areas, where environmental conditions are less limiting and food availability is higher (Mysterud et al. 2011), but road and settlement densities are also higher. Similar results involving selection of areas of high human density related to higher prey availability were also reported for lynx preying on roe deer in southern Norway (Basille et al. 2009). In addition, winter selection by wild ungulates of areas of higher road densities also increases their relate of vehicle collisions (Neumann et al. 2012), thus representing an increased prey availability for wolves. Lastly, secondary roads were avoided by resident wolves in non moving clusters during fall, whereas no selection or avoidance were observed for main roads and settlements during this season. This finding may reveal that wolves are more susceptible to irregular, and therefore less predictable, traffic levels on secondary roads rather than higher, but more regular, vehicle transition on main roads when selecting non-moving clusters.

Overall, floating wolves behaved similarly to resident pack members when moving with reference to anthropogenic features. Indeed, they generally showed a positive selection for main roads, settlements and secondary roads in fall and winter. Unfortunately, the small sample size did not allow us to assess habitat selection by floaters during summer, thus we cannot confirm the general avoidance towards anthropogenic features also for wolves belonging to this category. When considering non moving clusters, floating wolves and resident pack members behaved differently, showing different responses towards anthropogenic features across seasons. Selection patterns of resident and floating wolves also differed when referring to topographic and land cover variables. On one hand, pack members showed a positive selection for vegetation cover and flat slopes along moving trajectories across seasons. On the other hand, floaters in our study population did not show significant selection or avoidance for topographic and land cover variables when traveling. Interestingly, we found different results when referring to non-moving clusters of floating wolves. Indeed, similarly to what observed for pack members, non moving locations of floaters were preferentially located in areas with high vegetation cover and characterized by steep slopes in fall and winter.

Being highly territorial (Mech and Boitani 2003), wolves tend to minimize the probability to encounter individuals belonging to adjacent packs (Mech and Harper 2002). This is particularly true for floating wolves, that move within the territorial mosaic of resident packs, and their selection patterns may be mainly influenced by the necessity to avoid pack members (Mech and Harper 2002). In addition, intraspecific aggression has been shown to regulate adult wolf survival in a density-dependent manner in Yellowstone National Park (Cubaynes et al. 2014), and wolf density in our study area is relatively high, with >5 wolves/100 km2. These observations may explain the generalist behavior of floaters when selecting habitat characteristics when moving, as well as the differences in selection for anthropogenic features in non-moving clusters compared to resident wolves. Differences in habitat selection patterns between resident and nomadic individuals have also been reported for other large carnivore species with social organization. For example, resident and transient red wolves (*Canis rufus*) and coyotes (*Canis latrans*) showed complementary patterns of selection of forest edges and roads in a human-modified, agricultural landscape of North Carolina (Hinton et al. 2015, Hinton et al. 2016).

The high plasticity that allows behavioural responses of wolves to anthropogenic features is a key factor to their persistence in human-modified landscapes, many of which have higher densities of roads and humans than in the central Apennines. The apparently well-functioning adaptation of wolves to man-made habitat features we revealed in the PNALM should not mask the fact that roads may interact with human attitudes, potentially leading to increased human-caused mortality of wolves. Indeed, despite positive attitudes towards wolves by local residents (Glikman et al. 2011), a consistence percentage of wolf mortality during the period of the study was due to poisoning or shooting (42%), and collision with vehicles (18%; L. Gentile, pers. comm.). Considering that the accessibility of wolf territories by road networks is crucial for poachers to increase their encounter rate with wolves, attention should be given to the effect of roads on wolf mortality for its management and long-term conservation in the central Apennines.

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Table 1. Coefficients, standard errors and P-values obtained by averaging the top (ΔAIC ≤ 2) logistic regression models (averaged models: ΔAIC≤ 2) for seasonal habitat selection by a) wolves belonging to resident packs and b) floaters along moving trajectories. Dashes indicate that the variable was not included in the final model.

Moving trajectories										
		a) Resident pack members								
Covariate	**Time period**	**Summer (n=7)**			**Fall (n=6)**			**Winter (n=5)**		
		β	**SE**	**P-value**	**β**	**SE**	**P-value**	**β**	**SE**	**P-value**
Slope		-0.25	0.04	< 0.01	-0.12	0.05	0.01	-0.09	0.91	0.01
Cover		0.18	0.04	< 0.01	0.07	0.04	0.09	0.20	1.22	< 0.01
Main roads and settlements	Day	0.23	1.25	< 0.01	-0.10	0.09	0.26	-0.28	0.76	< 0.01
	Night	0.18	1.20	0.01	-0.27	0.06	< 0.01	-0.46	0.63	< 0.01
Secondary roads	Day	0.31	1.37	< 0.01	0.09	0.09	0.27	0.01	1.00	0.99
	Night	0.14	1.15	0.01	-0.05	0.05	0.37	-0.03	0.97	0.55
		b) Floaters								
Covariate	**Time period**				**Fall (n=4)**			**Winter (n=4)**		
					β	**SE**	**P-value**	**β**	**SE**	**P-value**
Slope					0.10	0.08	0.20	0.11	0.06	0.07
Cover					0.13	0.08	0.09	0.09	0.06	0.14
Main roads and settlements	Day				-0.01	0.18	0.94	-0.07	0.14	0.63
	Night				-0.25	0.13	0.05	-0.36	0.10	< 0.01
Secondary roads	Day				-	-	-	-0.32	0.17	0.05
	Night				-	-	-	-0.27	0.10	< 0.01

Table 2. Coefficients, standard errors and P-values obtained by averaging the top (ΔAIC ≤2) logistic regression models for seasonal habitat selection by a) wolves belonging to resident packs and b) floaters in non-moving clusters (i.e., feeding and resting sites, as well as pup-rearing sites when referring to pack members). Dashes indicate that the variable was not included in the final model.

Non moving locations

		a) Resident pack members								
Covariate	**Time period**	**Summer (n=5)**			**Fall (n=4)**			**Winter (n=5)**		
		β	**SE**	**P-value**	**β**	**SE**	**P-value**	**β**	**SE**	**P-value**
Slope		-0.17	0.03	< 0.01	0.10	0.04	0.01	0.29	0.02	< 0.01
Cover		0.51	0.04	< 0.01	0.38	0.04	< 0.01	0.57	0.03	< 0.01
Main roads and settlements	Day	0.09	0.05	0.05	-0.05	0.05	0.33	0.01	0.04	< 0.01
	Night	0.17	0.08	0.03	-0.11	0.07	0.14	-0.23	0.04	< 0.01
Secondary roads	Day	0.24	0.05	< 0.01	0.17	0.04	< 0.01	0.10	0.03	< 0.01
	Night	0.22	1.25	0.01	0.13	0.06	0.03	-0.18	0.04	< 0.01

		b) Floaters					
Covariate	**Time period**	**Fall (n=4)**			**Winter (n=4)**		
		β	**SE**	**P-value**	**β**	**SE**	**P-value**
Slope		0.83	0.05	< 0.01	0.46	0.04	< 0.01
Cover		0.18	0.06	< 0.01	0.45	0.06	< 0.01
Main roads and settlements	Day	-0.42	0.10	< 0.01	-0.19	0.07	0.01
	Night	-0.36	0.08	< 0.01	-0.28	0.07	< 0.01
Secondary roads	Day	0.04	0.08	0.60	0.09	0.06	0.11
	Night	-0.07	0.07	0.33	-0.12	0.07	0.10

Figure 1. Location of the study area in the Abruzzo, Lazio and Molise National Park (Central Appenines, Italy; 2008-2010) and spatial distribution of moving trajectories and non-moving locations of 4 resident wolf packs.

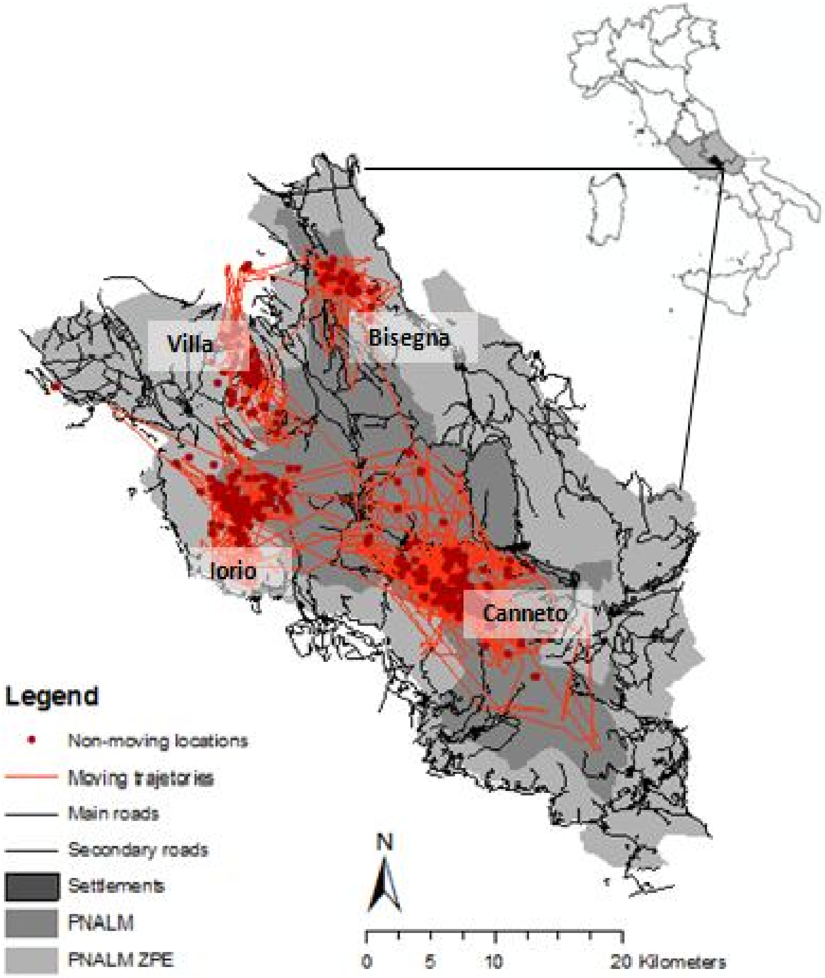
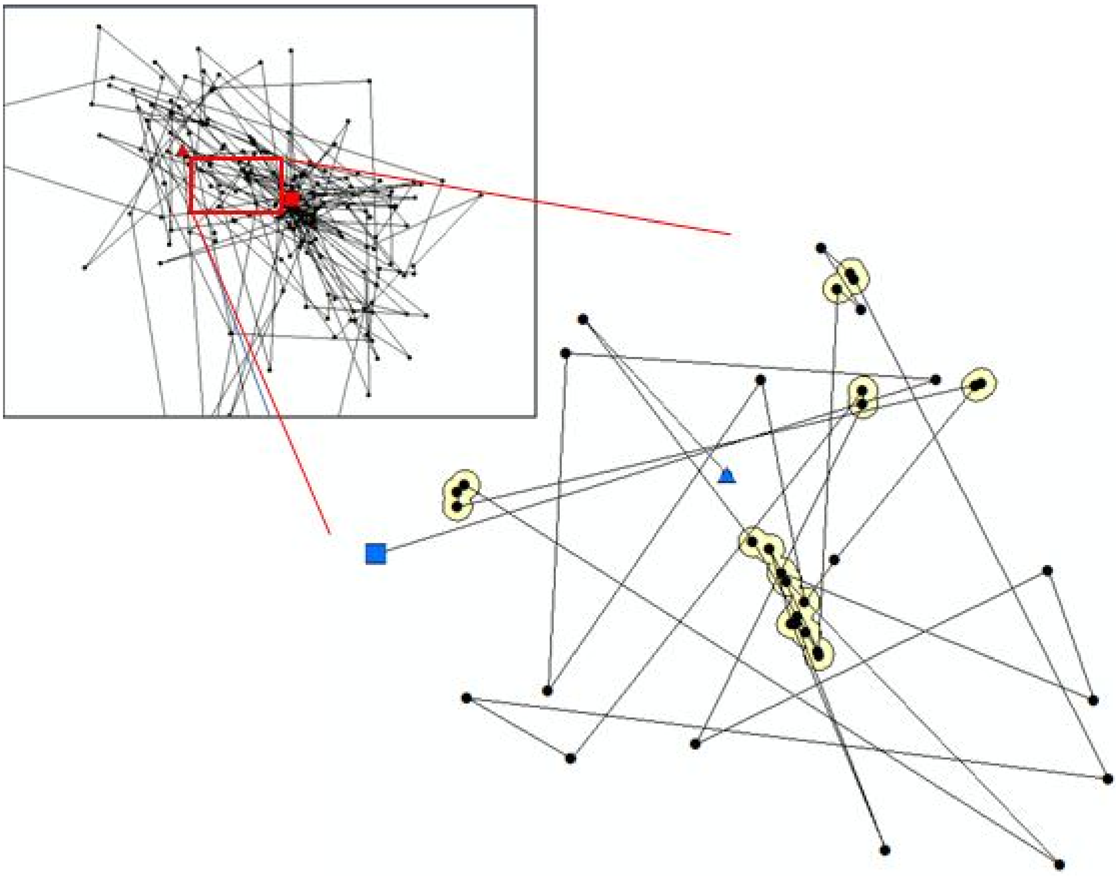


Figure 2. GPS locations collected from one wolf in the Abruzzo, Lazio and Molise National Park (Bisegna pack, summer 2010; inset), and movement analysis of GPS-positioning data showing how positions were separated into single positions >30 m from the nearest position, and cluster positions. Triangles and squares represent the first and last locations of GPS trajectories, respectively.



SUPPLEMENTARY MATERIAL

Table S1. Topographic, land cover and anthropogenic covariates measured in the study area, within home ranges of resident wolf packs and areas used by floating wolves in the Abruzzo, Lazio and Molise National Park (2008-2010). Estimates referring to resident packs and floaters are annual means, obtained by averaging values obtained from summer, fall and winter home ranges and areas used by wolves.

Spatial feature	**Area** (Km^2)	**Elevation** (m a.s.l.)	**Slope** (degree)	**Forest** %	**Shrubland** %	**Agricultral fields** %	**Open fields** %	**Main roads** (km/100 km^2)	**Secondary roads** (km/100 km^2)	**Settlements** (km2/100 km^2)
Study area	*1.505*	*1338.3*	*20.5*	*53.6*	*18.4*	*5.6*	*22.6*	*31.5*	*67.4*	*0.8*
Bisegna pack	94.3	1270.2	19.2	35.3	40.4	9.8	18.1	38.2	75.5	2.9
Canneto pack	221.5	1477.1	23.1	59.3	15.2	2.1	19.3	29.4	50.2	0.5
Iorio pack	96.5	1488.2	24.9	67.7	24.3	0.1	13.5	14.7	31.8	0.1
Orsara pack	116.4	1446.7	20.2	64.3	14.7	2.8	15.7	30.1	60.3	1.8
Villa pack	117.6	1264.8	15.9	53.8	25.6	1.2	19.1	21.4	65.7	2.8
F21 area	684.3	1394.7	20.4	47.9	35.4	4.7	12.3	22.9	55.8	1.1
F23 area	573.4	1492.4	20.3	49.1	34.4	4.8	10.9	32.4	59.1	0.9
F29 area	685.1	1305.7	21.2	57.8	25.6	7.1	9.6	38.9	54.3	1.2
M22 area	233.4	1160.54	15.6	50.2	22.3	15.8	15.4	30.4	71.5	1.8

CHAPTER III

WHAT TO EAT, AND WHERE? WOLF (*Canis lupus*) DIET AND SELECTION OF FEEDING SITES IN THE HUMAN-MODIFIED ENVIRONMENT OF CENTRAL APENNINES, ITALY

INTRODUCTION

Food habits of wolves have represented one of the most studied aspects of wolf biology, providing a quite extensive description of prey consumed by wolves and their selection patterns (Okarma et al. 1995, Peterson and Ciucci 2003). Compared to the more pristine environments of North America, where wolves consume large wild ungulates (i.e., moose, elk, caribou, mule and white-tailed deer), wolves in Europe mostly consume large and medium-sized wild ungulates, supplemented by livestock or other anthropogenic food sources, especially where wild prey had been extirpated or severely reduced (Meriggi and Lovari 1996, Newsome et al. 2016). More recently, following recovery of wild ungulates across Europe (e.g., moose and red deer in Scandinavia and central Europe; wild boar and roe deer in southern Europe), they act as important prey for many European wolf populations (Zlatanova et al. 2014). However, wolves may still forage on anthropogenic foods whenever they are available and easily accessible (Vos 2000, Capitani et al. 2004, Gazzola et al. 2005, Migli et al. 2005, Torres et al. 2015). Especially in human-altered landscapes, dietary responses of wolves to the availability of anthropogenic food sources strongly affect their ecology and conservation, as well as the adequacy of conflict mitigation management; therefore, a detailed knowledge of prey consumption by wolves in human-altered environments is essential (Newsome et al. 2016)

Italy hosts one of the most important wolf source population at the European scale (Boitani 2003). On the verge of extinction in the late 1960s (Zimen and Boitani 1975), wolves in Italy dramatically recovered both in numbers and range by means of natural recolonization (Ciucci and Boitani 2010). The restoration of wild ungulate communities throughout the Apennine and the Alpine ranges since the late 1970s was among the main factors facilitating this range expansion, according to which wolves in Italy shifted from a diet mostly composed of garbage, offal remains, and livestock (MacDonald et al. 1980, Boitani 1982) to one comprising one or two wild ungulate species, selected within 2- or multi-prey systems (Capitani et al. 2004, Mattioli et al. 2004, Meriggi et al. 2011). Based on these studies, wild boar and roe deer are the two most common prey species of wolves, supplemented by other locally abundant wild ungulates (e.g., red deer, fallow deer). Nevertheless, wolves may consume domestic ungulates to a significant extent also in these multi-prey systems (Capitani et al. 2004, Gazzola et al. 2005, Imbert et al. 2016). This findings lead to conclude that a dietary response towards wild ungulates is not sufficient per se to prevent economic and social conflict over wolf conservation. A multitude of food-habits studies have been conducted in the human-modified environments of Italy, including the Alps (Gazzola et al. 2005, Marucco et al. 2008) and the northern Apennines (Ciucci et al. 1996, Capitani et al. 2004, Mattioli et al. 2004, Milanesi et al. 2012, Meriggi et al. 2015, Imbert et al. 2016). However, little knowledge is available on the food-habits of wolves in the central Apennines, where a diversified community of wild ungulates is accompanied by widespread livestock raising. In this context, few published study investigated to what extent domestic and wild prey are integrated into the wolf diet (Patalano and Lovari 1993, Pezzo et al. 2003).

Wolf diets are generally assessed by scat analysis, in which the researcher identifies hairs in scat to quantify prey consumed by a carnivore (Reynolds and Aebischer 1991, Klare et al. 2011). Scat analysis is widely used because the approach is noninvasive, relatively quick to apply, and large samples can be collected (Litvaitis 2000). Scat analysis is also a particularly useful supplement to radiotracking, direct observation, and snow-tracking in studies of diet selection. On the other hand, scat sampling represent a critical phase in scat analysis, since random sampling of scats in the field is rarely feasible, and potential biases may occur if non-independent groups of scats are collected (Huggard 1993a). In particular, social species such as wolves can produce highly correlated clusters of scats, especially if these are collected at den and kill sites (Marucco et al. 2008). Because foraging ecology in wolves is influenced by a high degree of social cohesion within packs (Packard 2003), the pack is the level at which factors such as prey composition (Jedrzejewski et al. 2000), and spatio-temporal variation in prey density and distribution (Okarma et al. 1995, Metz et al. 2012) concur to shape prey consumption by wolves. This is of fundamental importance when designing dietary studies based on scat analysis, as collecting scats irrespectively of the spatial arrangement of the wolf population may lead to incorrect conclusions and sampling bias (Steenweg et al. 2015).

More recently, field surveys of clusters of global positioning system (GPS) telemetry locations have been proposed as a method to identify kill sites and describe carnivores’ diet (Sand et al. 2005, Zimmermann et al. 2007, Webb et al. 2008, Knopff et al. 2009). The majority of GPS cluster studies on wolf kill sites have been undertaken in winter, when wolf tracks are easier to follow thanks to snow cover, and prey remains are therefore easier to find (Sand et al. 2005, Webb et al. 2008). Assessing wolf diets during the summer is more challenging, because small prey, such as roe deer fawns and red deer calves, are rapidly consumed (Peterson and Ciucci 2003), and the lack of snow makes tracking wolves more difficult. Moreover, GPS-cluster methods can be biased toward large-size prey and might not accurately reflect total wolf diet composition (Sand et al. 2005).

In this study, we used both scat analysis and GPS cluster checking to investigate the feeding ecology of wolves in the Abruzzo, Lazio and Molise National Park (PNALM; central Apennines, Italy). Here, wolves have always coexisted with humans, to the extent that PNALM was one of the most important historical strongholds for wolves in the Italian peninsula (Zimen and Boitani 1975). The wild ungulate community in the PNALM is amongst the richest in Italy, but wolf-livestock conflict still represents an issue (Cozza et al. 1996). Verified livestock losses due to predation are compensated by park administration, but this seems not to be sufficient to prevent illegal killing of wolves by poisoning or shooting (Ciucci and Boitani 2010). However, since a general positive attitude of local people towards wolf have been reported (Glikman et al.

2012), such actions don’t seem to be supported by the majority of residents, and poaching is likely under the responsibility of a very few individuals. Unfortunately, no quantitative data are available on abundance and distribution of both wild and domestic ungulates in the PNALM. Nevertheless, we deemed important to 1) provide information on the feeding ecology of wolves in the human-modified landscape of central Apennines, and 2) assess the extent of livestock consumption by wolves to inform conflict mitigation management. To this aim, we first applied log-linear modeling to dietary data obtained from scats analysis to investigate wolf diet composition in the PNALM (2005-2008), as well as its inter-pack and seasonal. Given that scat analysis only reveals what the wolves ate and not necessarily what they killed, we integrated our analysis with data obtained from GPS cluster checking (2009-2010), by which we could also determine if a feeding site was associated to a kill or scavenging event, thus providing more information on wolf use of wild and domestic ungulates. Lastly, we investigated wolf use of carcasses and selection patterns of kill and scavenging sites.

MATERIALS AND METHODS

Study area

This study was carried out in the Abruzzo, Lazio and Molise National Park (PNALM) and its external buffer zone in the Central Apennines, Italy (1,505 km^2). The area is typically mountainous, with elevations ranging from 400 to 2,285 m, and is characterized by a Mediterranean montane climate. Vegetation types are dominated at higher altitudes by pure stands of beech (*Fagus sylvatica*) forests and, at lower altitudes, by mixed forests where other species (*Quercus* spp., *Acer* spp.) predominate. Valley bottoms are covered mostly by abandoned fields, pastures and seasonally cultivated fields near human settlements. Above 1,800 m, the beech forests give way to alpine grasslands that have been degraded by centuries of grazing. Wild ungulates include wild boar (*Sus scrofa*), red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*) and Apennine chamois (*Rupicapra pirenaica ornata*), whose densities, albeit formally unknown, are relatively high based on sightings and pellet group counts (R. Latini, PNALM Scientific Service, pers. comm.). European hares (*Lepus europaeus*) are also quite common. Livestock represents one of the main economic activities for local people, and husbandry methods routinely adopted in the PNALM vary considerably (Fico et al. 1993). Cattle (*Bos taurus*) and horses (*Equus ferus caballus*) are generally left on semi-free pastures in the mountainous areas from April to October. Foals are born mainly in April-May, while the calving season is concentrated in May-June. Although permanent supervision of herds is mandatory, this rule is often broken for economic reasons. Sheep (*Ovis aries*) and goats (*Capra hircus*) are kept on pasture from June to October and are generally guarded by shepherds and guarding dogs. At night, the animals are kept in roped-off enclosures. The lambing season lasts all year long, with peaks in January-February and September-October. Lambs are usually slaughtered within three to four months and, therefore, only adults are present in flocks in summer.

Diet analysis

Data collection

From 2005 to 2008 we periodically collected wolf scats year-round along systematic transects within wolf territories as delineated by a combination of different monitoring techniques, i.e., show-tracking and wolf-howling, combined with non-invasive genetic sampling (Boggiano et al. 2013). Only non-weathered and collectible scats (*sensu* Floyd et al. 1978) were included in the analysis. Time since deposition was estimated according to the last sampling effort, wolf activity in the area, scat appearance, exposure and weather conditions. In collecting scats, we attempted to distribute sampling efforts equally among 4 of the 7 packs comprising the core of the PNALM wolf population (Iorio, Mainarde, Orsara and Villa packs). This choice was made primarily for reasons related to the possibility of collecting a sufficient number of scats/pack to obtain an accurate diet estimate, as well as to take into account the spatial variation of prey availability among packs. Each collected scat was assigned to a given pack if it was within the pack home range, or if it was collected during intensive monitoring (i.e., snow-tracking or wolf-howling surveys) of a given pack. Scats collected on the field can be autocorrelated (i.e., they can be produced following the same kill or meal; Huggard 1993b), with the risk of generating pseudoreplicates (Marucco et al. 2008). To reduce autocorrelation, collection sessions of wolf scats we programmed according to a rotational time schedule within each pack to maximize the probability of collecting scats produced by different feeding events. We also pooled scats deposited at the same carcass or marking point and sharing the same content and estimated deposition time, and averaged scats collected along the same snow-tracking trajectory that shared the same prey species.

Laboratory procedures and quantification methods

Scats were collected in nylon bags, labelled and frozen (-30°C) prior to analysis. Afterwards, they were autoclaved for operator safety and processed according to standard laboratory procedures (Reynolds and Aebischer1991), adapted to wolf scats as described elsewhere (Ciucci et al. 1996, Ciucci et al. 2004). The point-frame method (Chamrad and Box 1964) was employed for the identification and quantification of scat content. Mammal hairs in each scat were recognized by microscopic examination of the cuticular pattern and the medulla in comparison to hairs from mammals collected in the study area. Wild and domestic ungulate species were further classified as juvelines (≤ 4-5 months) and adults (> 4-5 months) on the basis of microscopic hair features detectable from birth to the first autumn molt (Ciucci, P., Boitani, L., Pelliccioni, E. R., Rocco, M., and Guy 1996). Accuracy of observers (n=3) in identifying mammal hairs was evaluated through a pre-analysis blind test on a sample of 120 hairs of mammal species collected from local populations. Observers were evaluated both at the species and age levels, with an average reported accuracy of 98.1 % (±0.9) and 98.1 % (±0.8) for species and age class identification, respectively.

We used the percentage of occurrence based on the number of equivalents (*sensu* Floyd et al. 1978) for the quantification of scat content. Equivalents represent the sum of the volumetric proportions of each food component in the single scat (Floyd et al. 1978) and thus return the number of scats expected if each of these was composed solely by that particular food category. For log-linear analysis (see below), frequency based on the number of equivalents was considered more appropriate than the number of scats or occurrences because equivalents reduce autocorrelation of the data and weight occurrences based on whole scat proportions. Non-food items (i.e., those of no nutritive value believed to be ingested either intentionally or involuntarily; Reynolds and Aebisher 1991) were excluded from analyses. Moreover, to focus our log-linear analysis on the most important prey types, we excluded those food items with average seasonal occurrence per pack < 3%.

Modelling

We used log-linear models to assess the multivariate effects of spatial (wolf pack) and temporal (season) variables on diet composition. Log-linear models are one of the specialized cases of Generalized Linear Models (GLMs) for Poisson-distributed data and represent a valuable tool to analyze multi-way contingency tables that involve three or more categorical variables (Agresti 2003). In log-linear analyses the conditional relationship between variables is analyzed by taking the natural logarithm of the cell frequencies within a contingency table, and parameter estimates may be expressed as odds (main effects) and odds ratios (interaction coefficients; Knoke and Burke - Jeansonne 2012). For the scope of the study, seasons were broadly defined as summer (April–September) and winter (October–March), mostly to account for differences in space use patterns by wolves and livestock availability. Scats were allocated to summer or winter season according to their estimated deposition time. Due to small sample size, we pooled data across age classes of ungulate species and years; therefore, we were not able to assess the effect inter-annual variability on wolf diet composition.

We applied log-linear modelling based on a 3-dimensional table (i.e., pack, season and prey category). We formulated the saturation model (i.e., the one with no degrees of freedom, whose estimated frequencies correspond exactly to the observed data):

$$\mathrm{Ln}(Fijk) = \mu + \lambda_i A + \lambda_j B + \lambda_k C + \lambda_{ij} AB + \lambda_{ik} AC + \lambda_{jk} BC + \lambda_{ijk} ABC$$

where Ln(Fij) is the log of the expected cell frequency of the cases for cell ijk in the contingency table (i.e., the number of equivalents for each prey category); μ is the overall mean of the natural log of the expected frequencies; λ terms each represent “effects” which the variables have on the cell frequencies; A, B and C are the variables prey composition, season and pack; i, j and k refer to the categories within the variables. Cattle and horses, summer and Iorio pack were used as reference levels for parameter estimation. To find the best model that best fitted the data we performed a model selection on models with all possible combinations of covariates included in the saturation model by means of Akaike Information Criterion with small sample size correction (AICc; Burnham and Anderson 2002). We decided to retain only the top models (ΔAICc ≤ 2) for the following model averaging (*MuMIn* R package, Bartoń 2014), that was performed using the natural average method (Burnham and Anderson 2002).We provided Pseudo-R^2 estimate to evaluate the goodness-of-fit of the final model to our data (Nagelkerke 1991).

GPS cluster checking

Data collection

Between 2009 and 2010, 7 wolves (3 males and 4 females, all adults) were fitted with GPS collars (Televilt-Tellus, Followit AB, Lindesberg, Sweden; and Vectronic Pro Light-1, Vectronic Aerospace GmbH, Berlin, Germany). To identify feeding sites (i.e., predation and scavenging events), GPS collars were programmed with an intensive schedule designed to obtain a location every hour (wolf F21 and M22) or 30 minutes (all other wolves) for 10 days each winter month (i.e., December-April). On the remaining days, collars were programmed to obtain locations every 3 hours. We focused our study on the intensive schedule only. Every morning during the intensive schedule periods, all wolf locations during the previous 24 hours were plotted in a metric grid system (1:100,000) using ArcGIS (version 10.2.2) to identify clusters of locations compatible with kill or scavenging events. The criteria to identify a cluster were two or more successive locations with a maximum distance between them ≤ 200 m, based on the assumption that wolves spend >1 hour on medium-large prey (Sand et al. 2005). This procedure was repeated with new data accumulating during the study period, and all new clusters were intensively searched in the field (using their geometric centre to easily reach them) for possible prey remains within a 100-m radius around all single cluster locations. Once we found prey remains, surveyed clusters with new positions were not visited, due to the fact that they likely represented different visits to the same feeding event, rather than the use of a new feeding site (Webb et al. 2011). If we detected signs of a kill while approaching a cluster, we left it undisturbed until 1 week after wolf presence (Sand et al. 2005). Moreover, we did not visit GPS clusters until at least 2 days after the last GPS positioning of wolves to minimize disturbance. In addition, we randomly selected and visited 20% of all single positions in the field (Sand et al. 2005).

We classified surveyed clusters into feeding clusters (with prey remains), resting clusters (with signs of bed sites in the snow), track clusters (with signs of travelling only), and unknown clusters (with no signs of wolves). Feeding clusters were further classified as kill or scavenging events. Kill sites were characterized by the presence of dispersed blood, signs of a struggle, bite marks, or subcutaneous hematoma in prey carcasses (Treves and Karanth 2003). Conversely, scavenging sites were identified when prey remains did not show any sign of predation. Feeding clusters and single positions were searched for body parts and other remains that could help determine the sex, age, condition of prey and its time of death. Species of ungulate carcasses found were identified from hair and skeletal remains whereas sex was determined by visual inspection of reproductive organs, or by presence of antler pedicles. We acknowledge that smaller prey items might have gone undetected with our collection method we used (i.e., either the prey was completely consumed or the remains were carried away from the feeding site).

Description of use of feeding sites

We provided the following characteristics of kill and scavenging sites: 1) first prey consumption time, defined as the time difference between the first and last location of GPS clusters used to find carcasses; 2) temporal window of carcass use by wolves; to this aim, we created 100 m buffers (i.e., the same distance used to searching for prey remains at GPS clusters) around carcasses; we then selected all locations falling within them and calculated the time difference between the last and first location; this analysis was based on the assumption that locations within 100 m from the carcass represented an actual use of it by the wolf; 3) number of wolf visits within the aforementioned temporal window (visits defined as both series of sequential locations and single locations); and 4) proportion of locations of carcass use collected during four circadian periods, i.e., dawn, day, dusk and night. Lastly, we created 30 m buffers around all locations comprised in the temporal window of use of each carcass (30 m corresponded to the upper 95% CI of GPS error); we then dissolved the area of overlapping buffers and defined them as "satellite" clusters. We calculated the number of satellite clusters associated to each feeding site, and their distance to the corresponding carcass.

Selection of kill and scavenging sites

To describe selection patterns of kill and scavenging sites, we considered topographic, land cover and anthropogenic variables. Topographic variables comprised elevation and slope; a 20 m Digital Elevation Model (DEM) was provided by the Italian Military Geographic Institute, and was used to derive slope estimates in ArcGIS (version 10.2.2). Land cover data were derived from the Corine Land Cover map (European Environment Agency, 2013; Corine Land Cover 2006 seamless vector data, Version 17), from which we aggregated the original habitat classes into four main land use categories: 1) forest (conifer, broadleaf and mixed forests), 2) agricultural fields (both intensive and extensive), 3) shrublands and 4) open fields (pastures and sub-alpine grasslands). Original raster data had a pixel size of 20 m, but we calculated the average density of land use categories within a moving window of 100 m, i.e., the same distance used to find carcasses. Anthropogenic covariates were represented by the Euclidean distance (km) of each wolf kill/scavenging site to the closest road (Istituto Geografico De Agostini 2001) and settlement edge (ISTAT 2011). Roads were classified as main (connecting the most populated human settlements) and secondary (connecting smaller villages or logging areas), most of which were paved. The settlements layer comprised all types of human buildings within the study area. All landscape covariates were standardized by computing their z-scores before running selection models.

To assess environmental characteristics of wolf kill and scavenging sites, we fitted logistic regression models (Hosmer and Lemeshow 2000), in which the response variable took values 1 (when the site was used) or 0 (when the site was available but unused; Boyce and McDonald 1999). The response variable was modeled for dependence on predictor variables in the context of the Generalized Linear Mixed Models (GLMMs) framework to accommodate autocorrelation and variation in sampling intensity, by adding wolf ID as random effect. Since this effect was negligible (see "Results" and Table S4), we removed it from the model, thus resuming to a Generalized Linear Model (GLM) framework to perform the analyses. In an initial phase we conducted exploratory analyses within predation and scavenging subsets. We checked the predictor variables for collinearity using pairwise Pearson correlation coefficients with a correlation threshold of $r \geq 0.5$; we dropped the collinear covariates until we obtained a set of predictors whose variance inflation factor (VIF) was <3 (Zuur et al. 2010). We derived a full additive model for each subset, combining all variables that were selected on the basis of the exploratory analysis. We first performed a model selection on models with all possible combinations of covariates included in the full model for each subset by means of Akaike Information Criterion with small sample size correction (AICc; Burnham and Anderson 2002). We then decided to retain only the top models (ΔAICc ≤ 2) for the model averaging approach. We used a natural average method, so that parameter estimates from models that provided little information about the variation of the response variable were given little weight. On the averaged model, we lastly performed goodness-of-fit tests using receiving operating characteristics (ROC; Boyce et al. 2002). All the analyses were performed in R (*MuMIn* package: Bartoń 2014; *lme4* package: Bates et al. 2015; *ROCR* package: Sing et al. 2005).

RESULTS

Diet analysis

Scat sample collected and diet description

Between 2005 and 2008, we collected a total of 1,408 scats. Of these, 80.3% (n=1,131) were collected within the territories of the 4 sample packs, with an average (± SD) number of scats per pack of 283 (± 24). Only these scats with certain assignment to a specific pack were used for further analysis, and 91.0% (n=1,029) of them were considered independent, i.e., representative of different meals and used for log-linear modeling. The remaining 19.7% (n=277) of wolf scats were collected in the rest of the park territory; specifically, this percentage of scats was constituted for 94.6% (n=262) of scats collected within the territories of the other 3 packs permanently present in the area, and for 5.4% (n=15) of scats whose assignment to a specific pack was uncertain.

We found a total of 19 food categories and 6 non-food categories (Supplementary material; Table S1). Food items represented the 61.6% (n=1,562) of total occurrences (n=2,535), and mainly comprised mammals (99.7% of occurrences; n=1,557), followed by birds (feathers and bones, 0.26% of occurrences; n =4) and fruits (Prunus spp., 0.1% of occurrences; n=1). Non-food items were mainly represented by plants (Fagus spp. and grasses, 91.0%; n=973), soil (6.1%; n=59) and rocks (1.5%; n=15). Other categories, such as insects and other materials, constituted the remaining 1.5% (n=15) of total occurrences of non-food items. Mammal species identification was not always possible, since 0.83% (n=13) of total occurrences of this class was indicated as undetermined. Moreover, for the 2.1% (n=27) of scats it was not possible to quantify the volume of the prey species due to low food content; these scats were not considered for further analysis. Mammal species were present as a single item in 81.3% (n=1,041) of scats, as two food items in 16.2% (n=207), and three or more items in the remaining 2.5% (n=33).

Based on equivalents, the 5 most frequent prey categories in the wolf diet in the PNALM were cattle and horses (29.7% ±13.3 SD), wild boar (22.7% ±9.1 SD), roe deer (17.2% ±8.7 SD), sheep and goats (16.0% ±2.7 SD) and red deer (10.5% ±6.5 SD). These main prey species were identified in wolf scats from all monitored packs (Figure 1). Amongst wild ungulates, chamois was also present in the diet of all wolf packs, although in a very low percentage (i.e., 1.5%). When referring to age classes, wild boar piglets represented an important component of wolf diet, comprising 47% (±17 SD) of this prey category. Similarly, roe deer fawns, red deer and cattle calves represented 27% (±12.1 SD), 24% (±8.5 SD) and 27% (±11.6 SD) of total number of equivalents of these prey categories. On the other hand, foals were used by wolves to a lower extent, and represented 10% (±0.9 SD) of consumed sheep and goats. Overall, juveniles were consumed more during summer compared to winter (Welch Two Sample t-test: $t = 4.09$, $df = 35$, P-value < 0.01; Figure 2).

Log-linear models

From a total of 1,131 scats, 1,080 equivalents were included in log-linear modelling, referring to the 4 sample packs within the study area and encompassing 7 seasons and 4 years (from 2005 to 2008; in 2005, scats were collected in all packs during winter only). According to model selection, the model that best fitted the data comprised all variables included in the saturation model, with the exception of the third order interaction between pack, prey category and season (Table S2). Overall, the odds that wild boar, roe deer, sheep and goats and red deer were consumed by wolves were 12%, 15%, 28%, and 38% lower than cattle and horses, respectively. We detected a difference in wolf diet among packs. In particular, wild boar was more consumed in the Mainarde, Orasara and Villa packs compared to cattle and horses in the Iorio pack. In addition, roe deer and red deer were more consumed in the Orsara and Villa packs compared to cattle and horses in the Iorio pack (Table 1). Similarly, sheep and goats were more consumed in the Mainarde and Villa packs compared to cattle and horses in the Iorio pack (Table 1). Lastly, we detected a seasonal variation for the consumption of two prey categories, i.e., wild boar and roe deer. The former was more consumed during winter compared to cattle and horses during summer, whereas the opposite result was observed for roe deer (Table 1). The final model showed a good fit to our data, as indicated by Pseudo-R^2 estimate =0.94.

GPS cluster checking and feeding site analysis

Cluster characteristics

We obtained 1,538 hourly locations from 2 wolves (one male and one female, both floaters) and 7,022 half-hour locations from 5 wolves (3 females and 2 male), of which 3 belonged to 3 resident packs, whereas the remaining 2 were floaters. Out of the totality of GPS locations collected during the intensive sampling, 26.3% were classified into 635 clusters. Of these, 315 (49.6%) were resting sites and 204 (32.1%) were track clusters that occurred as a result of wolves traveling along the same routes several times during the study period. At 66 clusters (10.4%), we did not find sings of wolves. We found prey remains at 50 (7.8%) of total clusters, including 1 chamois, 5 roe deer, 14 red deer, 5 wild boar, 6 cows, 2 goats, 2 hares, 4 horses and 11 sheep. Moreover, 20 carcasses were found at single locations, and included 1 roe deer, 3 red deer, 2 wild boar, 1 cow, 1 dog, 4 horses, 1 pig, 6 sheep and poultry (*Gallus gallus domesticus*). Out of 70 carcasses, 24 were identified as predation (or probable predation) events, whereas 40 were identified as scavenging sites. For the remaining 6 carcasses it was not possible to determine the cause of death.

The predation subset mainly comprised wild ungulates (81.8%), whereas the opposite trend was observed for the scavenging subset, in which 70% of carcasses were of domestic ungulates. First consumption time for predation events averaged 2.33 (±2.85 SD) hours, whereas the mean temporal window of carcass use was 20.3 (±26.2 SD) hours. Wolves almost always revisited predation sites (87.5%), with an average number of visits of 2.0 (±1.6; range: 0-4), and the most of their locations of carcass use were distributed between daylight (37.7% ±17.7 SD) and night (47.2% ±23.7 SD) periods. When referring to scavenging sites, wolves spent an average of 2.4 (±2.8 SD) hours at carcasses during their first visit. In this case, the temporal window of carcass use averaged 23.5 (±36.5 SD) hours, and the maximum number of visits was 7 (mean =2.0 ±1.6 SD). Extreme cases (n=5) were represented by wolves showing a longer temporal window of carcass use (up to 8.9 days), during which they used extended portions of their ranges and visited more feeding sites before using the carcass again. Locations of carcass use were mainly distributed between daylight (36.9% ±14.8 SD) and night (49.7% ±19.3 SD) periods.

Number of satellite clusters used by wolves did not differ between the predation and scavenging subsets (Welch Two Sample t-test: t =1.75, df = 62.2, P-value = 0.09). In 38% of total feeding sites, wolves did not stopped in satellite clusters during the temporal window of carcass use. This cases referred to carcasses visited only once by wolves, as well as to feeding sites that were left by wolves for short periods, during which animals travelled for an average of 498.4 m (±581.9 SD) before coming back to the carcass. In 10% of cases, wolves used one satellite cluster between the first and second visit to the carcass, whose distance from the feeding site ranged 144.2-806.4 m (mean =410.6 ±299.4 SD); wolves used this satellite clusters for an average of 3.6 hours (±1.9 SD). In all other cases, excluding those in which the temporal window of carcass use was >2 days, wolves used a mean number of 1.3 satellite clusters (range: 2-6), whose distances from carcasses averaged 1048.3 m (±842.2 SD).

Selection of kill and scavenging sites

Full models for both predation and scavenging subsets comprised all variables but elevation, open fields and distance to settlements, based on correlation analysis (Table S3). In both cases, adding individual wolf identity as a random factor in the full model did not substantially improve the proportion of variance explained (Table S4). When referring to the predation subset, 6 models were used for the averaging approach (Table S5a). The averaged model by means of multi-model inference (Table 2a) indicated that wolf kill sites were preferentially located in forested areas (β= 0.65, P-value =0.05) and shrublands (β= 0.82, P-value <0.01), with flat slopes (β= -0.52, P-value =0.03). The integral of ROC (Figure 3a) was equal to 0.81, which indicates a statistically significant difference between the fitted model and the null one. Also for the scavenging subset 6 models were used for the averaging approach (Table S5b). In this case, the averaged model (Table 2b) showed that scavenging events were generally located in shrublands (β= 0.39, P-value =0.01) and close to main roads (β= -0.64, P-value =0.01). Also in this case, the integral of ROC =0.75 (Figure 3b) provided a good model fit to the data.

DISCUSSION

We investigated the feeding ecology of wolves in the human-modified environment of central Apennines, Italy. In the continuum of anthropogenic disturbance that can be observed across the European wolf range, the central Apennines represent a middle ground between the more pristine environments that can be found in northern Europe and the agricultural landscapes inhabited by wolves in southern Europe. Our study area supports a diverse community of wild ungulates, and concurrently features widespread livestock grazing, which represents one of the main economic activities for local people. These are the conditions in which wolf-livestock conflict represents a challenge for wolf conservation, and accurate knowledge of wolf feeding habits is needed to drive conflict mitigation strategies.

In line with generalities inferred at the biogeographical scale (Zlatanova et al. 2014, Newsome et al. 2016), our study concurs in depicting the diet of wolves in southern Europe as a composition of both wild and domestic prey, the latter being increasingly consumed in human-modified landscapes featuring intense livestock production (Vos 2000, Migli et al. 2005, Torres et al. 2015). When focusing on the Italian wolf population, previous research showed an important use of livestock in the southern-central Apennines, whereas in the northern Apennines wild ungulate occurrence increases (Ciucci et al. 1996, Meriggi and Lovari 1996, Capitani et al. 2004, Mattioli et al. 2004) and in the western Alps the use of livestock is negligible and wolf diet is mainly characterized by wild prey (Capitani et al. 2004, Gazzola et al. 2005). Our results fit this general trend, as wolf prey consumption in the PNALM reflected a multi-prey system dominated by cattle and horses, abundantly supplemented by wild boar. Roe deer, red deer and sheep and goats represented secondary prey species, whereas chamois were only occasionally consumed by wolves in the PNALM. Scarcity of wild ungulates is believed to enhance wolf predation on domestic livestock (Meriggi and Lovarit 2016). However, this is unlikely in our study area, where densities of wild ungulates, albeit formally unknown, are relatively high based on sightings and pellet group counts (R. Latini, PNALM Scientific Service, pers. comm.).

The reason underling primary use by wolves of cattle and horses in the PNALM is better represented by the fact that livestock is locally abundant and that methods of livestock husbandry may favor wolf targeting on domestic ungulates. For example, the preference for cattle may depend on the herder’s habit of leaving herds grazing unguarded all year round. Similarly, the practice of hobbling unguarded adult horses in high-elevation pastures to prevent long-range movements makes this ungulates more vulnerable to wolf predation (Patalano and Lovari 1993). Overall, wolves could prefer domestic ungulates instead of wild ones because of their highly clumped distribution in known pastures (reduction of search time and greater opportunity of prey choice), their low capability of detecting and avoiding predators due to domestication (greater probability of successfully attacking the prey), and their low efficiency of escape behavior (reduction of attack failures). However, as described by the data obtained by GPS cluster checking, all carcasses of cattle and horses were associated to scavenging events, rather than to wolf kills. Although this finding derives from a small sample, it indicates that wolf use of cattle and horses in the PNALM may be mostly due to the presence of carcasses of animals dead from other causes (e.g., disease, rough terrain, starvation). These are frequently available to wolves as they are generally abandoned on the pastures due to the inaccessibility of the sites and the high costs associated with their removal. However, it is important to consider that GPS cluster data were available for winter months only; thus, it is not possible to infer conclusions for the summer season. Differently from cattle and horses, sheep and goats are traditionally guarded by shepherds accompanied by effective livestock guarding dogs, and are generally protected into predator-proof shelters during the night. We accordingly documented that sheep and goats were less consumed by wolves compared to cattle and horses in the PNALM. However, sheep and goats carcasses found during winter were represented by both scavenging and predation events. Therefore, even if cattle and horses were more used by wolves compared to sheep and goats, these may represent the first target species to consider when planning prevention strategies to reduce livestock loss.

When referring to wild ungulates, wild boar was the most used prey species by wolves in the PNALM. Accumulated evidence from different ecological settings and multi-prey systems along the Italian Apennines clearly indicate that the wild boar is the primary prey of wolves (Mori et al. 2017), as also revealed in other south European wolf populations (e.g., Migli et al. 2005, Zlatanova et al. 2014, but see Barja 2009). Wild boars are highly gregarious, and their large and conspicuous groups, often clustered in resource-rich patches, are easily detectable by wolves and likely correspond to high encounter rates and reduced searching time (Mattioli et al. 1995). Compared to larger wild ungulates, the size of wild boars in Mediterranean countries may correspond to a particularly profitable prey/wolf mass ratio, increasing capture success and reducing handling time (Mattioli et al. 2011, Imbert et al. 2016). Productivity in the wild boar is the highest among other wild ungulates (Massei et al. 2015), allowing for a large proportion of particularly vulnerable age classes to wolves (i.e., piglets, juveniles, and subadults; (Mattioli et al. 2004, Imbert et al. 2016). In addition, postnatal body growth occurs more slowly in wild boar with respect to roe deer (Gaillard et al. 1992), the second most popular prey species for the wolf in Italy. All the above factors concur to explain why wild boars often constitute the bulk of the diet in many different wolf-prey systems in southern Europe.

The wolf is considered an opportunist species (Peterson and Ciucci 2003). A trophic opportunist consumes the food which occurs most abundantly, changing its diet depending on food availability. Unfortunately, we could not contrast prey consumption patterns by wolves against relative abundance, structure, distribution, and accessibility of both wild and domestic ungulates, as this information was not available in the study area. Nevertheless, we found that the relative proportions of cattle and horses vs wild boar consumption by wolves in the PNALM varied spatially, i.e., among the four studied packs, possibly reflecting variability in prey abundance and accessibility across space and time. In particular, the Iorio pack was the one that mainly relied on cattle and horses, which represented almost the half of the diet in term of occurrences (44.4% ±15.7 SD). All other prey categories showed an average percentage of occurrence lower than 15%. Similarly, cattle and horses were the most consumed prey category in the Mainarde pack (37.2% ±22.5 SD), although wild boar also represented an important food item (29.0% ±4.9 SD). On the opposite side, wild boar and roe deer were the main prey species in the diet of the Villa pack (29.8% ±6.0 SD and 26.5% ±16.2 SD, respectively). Lastly, the Orsara pack showed the highest use of wild ungulates, with wild boar, roe deer and red deer representing with similar proportions 64% of wolf diet. Overall, our results reinforce the observation that wolves are highly adaptable when referring to prey selection, as we revealed a variation in wolf diet at a small spatial scale, i.e., between adjacent packs, likely reflecting differences in prey availability within single territories as well as learning and cultural tradition of single packs (Sand et al. 2016).

We also found a seasonal difference in wolf diet. The two main prey category, i.e., cattle and horses and wild boar, resulted to be more used during summer and winter, respectively. This result is in line with the notion that the primary period of concern regarding livestock loss is summer and early fall, when livestock generally graze freely on high elevation pastures, often in high densities, with little to no monitoring (Barja 2009). In addition, grazing season timing coincides with the wolf pup-rearing season. The nutritional demands of wolves are considerable during this period, due to the need to satisfy growing pups. This potentially heightens the wolf use of livestock throughout the grazing season. All these factors become even more advantageous during summer, when calves are largely vulnerable to predation (Mech and Peterson 2003)

Share of juveniles in wolf diet varied between seasons and among prey categories. We found that wild boar piglets constituted a relevant share of wild boar remains in wolf scats (47.4% ±17.2 SD). A substantial consumption of wild boar piglets and juveniles has been repeatedly reported in the Italian Apennines (Mattioli et al. 1995, Ciucci et al. 1996, Gazzola et al. 2005) and elsewhere in southern Europe (Jedrzejewski et al. 2000, Nores et al. 2008), and it has been related to their greater vulnerability compared to wild boar adults (Mattioli et al. 2011). Consumption of wild boar piglets, however, was mainly limited during summer, maybe reflecting their higher availability during this season. Cattle calves, red deer calves and roe deer fawns represented about one fifth of occurrences of these prey species in wolf diet. Calves have a quite predictable distribution, require minimal handling, and the risks of injury upon attack are negligible; thus, as predicted by the optimal foraging theory (Stephens and Krebs 1986) they are expected to maximize energy gain over handling time. In the PNALM calves are largely available to wolves since the very first weeks of life as, in free-ranging cattle herds, births are delivered without any control; accordingly, their consumption resulted higher during summer compared to winter. On the contrary, sheep and goats were mainly consumed as adults, as foals represented only 10.3% ±0.9 SD of this prey category in wolf diet.

Thanks to data obtained from GPS cluster checking, we were able to provide a description of wolf use of carcasses, as well as their selection patterns of kill and scavenging sites. Wolves in the PNALM showed a clear tendency to revisit carcasses, for both kill and scavenging events, and often stopped in areas in the proximity of carcasses between revisits. Wolf kill sites during winter in the PNALM were characterized by flat slopes, and were preferentially located in forested areas and shrublands. Overall, the environmental attributes of the kill sites identified in this study are consistent with the natural history of wolf hunting behaviour. The wolf is a coursing predator that may chase prey over extended distances (Peterson and Ciucci 2003). Ungulates are killed primarily in flat, snow-covered areas where flight ability may have been inhibited, particularly where deeper snow associated with such topographic characteristic could hinder ungulate locomotion, and therefore offer an advantage to the predator (Husseman et al. 2003). Although distance to main roads of kill sites resulted not significant in the averaged model, its predictor weight was relatively high; therefore, it is plausible to assume that, with a bigger sample size, we would have found a selection for main roads in wolf kill sites during winter. This would have been in line with previous research, as roads provide convenient travel corridors that likely increase prey encounter rates (Kunkel and Pletscher 2000), while open habitats associated to them likely facilitate prey detection (Creel et al. 2005). Concurrently, the high forest and shrubland cover we recorded at kills sites of wolves may reflects the fact that their chases simply ended in areas where vegetation was denser.

Wolves are facultative scavengers (Peterson and Ciucci 2003), and their use of carrion is therefore assumed to vary according to its availability in space and time. Winter represents the limiting season for wild ungulates in temperate ecosystems (Telfer and Kelsall 1984), meaning that their carcasses are generally more available for wolves during this season. Accordingly, out of 70 carcasses we found during the study period, more than the half (n = 40) were associated to scavenging events. To be noticed, wild ungulates carcasses in the PNALM do not represent the only source of carrion for wolves during winter, as cattle and horses are often left grazing in pastures year-round. Indeed, carcasses of wild ungulates and cattle and horses were present in similar percentages (30% and 35%, respectively) in wolf scavenging sites, whereas sheep and goats represented the most of the remaining part of scavenging events (30%). Similarly to what observed for kill sites, scavenging sites of wolves in the PNALM were preferentially located in shrublands. The selection for this environmental feature may be related to inter-specific interactions with other scavengers species present in the study area. For example, the PNALM features a high presence of free-ranging dogs, whose competition with wolves has been reported to heighten wolf stress levels in this area (Molnar et al. 2015). Due to their lower fear of humans, free-ranging dogs tend to scavenge in open habitats, which are more associated with human presence (e.g., villages and hunting glades). Thus, the fact that wolves preferred to use carcasses in areas with more ground cover may be the result of a spatial segregation with free-ranging dogs mediated by habitat type, as previously reported for other scavenger species (Lemon 1991, Green et al. 1997). In addition, carcasses in open areas are more easily detected by all avian scavengers, that may lower consumption time of carcasses by wolves and thus, reduce their overall food intake (Vucetich et al. 2004). Another environmental feature that characterized wolf scavenging sites was the proximity to main roads.

This result may be the consequence of the altitudinal migration of wild ungulates from high-elevation to low-elevation areas, where environmental conditions are less limiting and food availability is higher during winter (Mysterud et al. 2011). Main road density is generally higher along valley bottoms, that are selected in winter by wild ungulates. Higher densities of prey likely means higher availability of carcasses, which may explain wolf selection of main roads for scavenging during winter. Winter selection by wild ungulates of areas featuring higher road densities also increases their relate of vehicle collisions (Neumann et al. 2012), thus representing an increasing availability of vehicle-killed prey for wolves. In addition, we cannot dismiss the possibility that scavenging by wolves could be related to butchery remains or illegal carcass dumps close to human settlements.

In line with previous research in human-altered environments, we reported a case of a wolf population preying on both wild and domestic ungulates. However, cattle and horses appeared a relevant food item to support the studied wolf packs. In addition, wolves appeared to recurrently rely on livestock carcasses, and this mechanism may also alter wolf ecology and behaviour (Newsome et al. 2015) and interfere with ecological processes (Wilmers et al. 2003). Minimizing livestock accessibility should then be achieved to mitigate social conflicts over wolf conservation in the PNALM.

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Table 1. Model coefficients, standard errors and P-values of the variables included in the log-linear model of wolf diet in the Abruzzo, Lazio and Molise National Park, central Apennines, Italy (2005-2008); stars represented interaction terms; "Iorio pack", "cattle/horses" and "summer" are used as reference levels for wolf pack, prey composition and season variables, respectively.

Variable	**Estimate**	**Standar error**	**P-value**
(Intercept)	3.25	0.11	<0.01
Mainarde	-1.10	0.23	<0.01
Orsara	-0.72	0.20	<0.01
Villa	-0.77	0.23	<0.01
Red deer	-0.96	0.25	<0.01
Roe deer	-1.87	0.37	<0.01
Sheep/goats	-1.27	0.24	<0.01
Wild boar	-2.05	0.34	<0.01
Winter	-0.73	0.18	<0.01
Mainarde*Red deer	0.63	0.61	0.31
Orsara*Red deer	1.67	0.44	<0.01
Villa*Red deer	1.64	0.48	<0.01
Mainarde*Roe deer	0.74	0.42	0.07
Orsara*Roe deer	1.10	0.33	<0.01
Villa*Roe deer	1.09	0.36	<0.01
Mainared*Sheep/goats	1.19	0.37	<0.01
Orsara*Sheep/goat	0.34	0.39	0.39
Villa*Sheep/goat	0.86	0.38	0.02
Mainarde*Wild boar	1.84	0.47	<0.01
Orsara*Wild boar	1.55	0.43	<0.01
Villa*Wildboar	1.61	0.44	<0.01
Mainarde*Winter	1.16	0.31	<0.01
Orsara*Winter	0.42	0.29	0.15
Villa*Winter	0.44	0.32	0.16
Red deer*Winter	-0.09	0.55	0.86
Roe deer*Winter	-1.01	0.48	0.03
Sheep/goats*Winter	-0.08	0.39	0.84
Wild boar*Winter	0.91	0.46	0.05

Table 2. Model averaged coefficients, standard errors and P-values of the variables included in the 6 models retained by means of AICc model selection for a) kill sites and b) scavenging sites of wolves in the Abruzzo, Lazio and Molise National Park, central Apennines, Italy (2009-2010); the predictor's weight of each term is provided.

Variable	Averaged estimate	Std. Error	P-value	Pred. Weight
a) Kill sites				
Forest	0.65	0.32	0.05	1
Shrublands	0.82	0.21	< 0.01	1
Slope	-0.52	0.24	0.03	1
Distance to main roads	-0.47	0.26	0.07	0.77
Distance to secondary roads	-0.35	0.28	0.22	0.46
Agricultural fields	-0.41	0.51	0.42	0.29
b) Scavenging sites				
Shrublands	0.38	0.12	< 0.01	1
Distance to main roads	-0.62	0.25	0.01	1
Distance to secondary roads	-0.41	0.25	0.10	0.82
Slope	-0.32	0.19	0.09	0.69
Agricultural fields	0.13	0.14	0.35	0.26
Forest	0.10	0.23	0.67	0.11

Figure 1. Consumption of main prey categories by wolves in Abruzzo, Lazio and Molise National Park (central Apennines, Italy). Based on scat analysis (2005-2008), percent occurrence based on number of equivalents is shown; values are mean percentages across sampling units (i.e., pack/season/year).

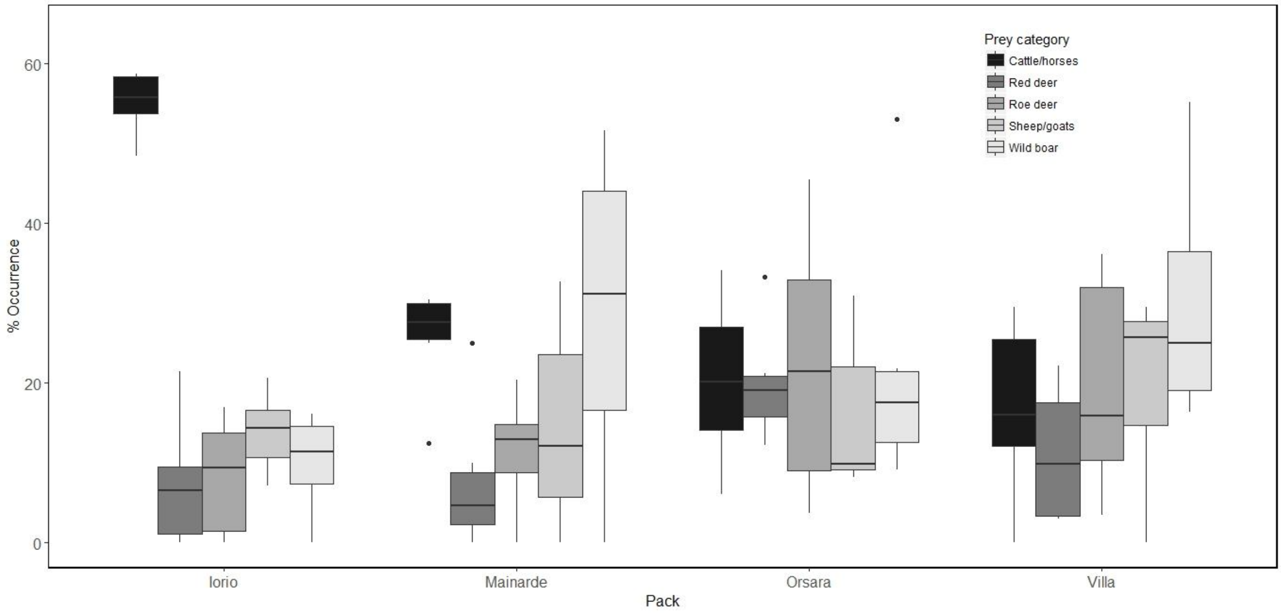


Figure 2. Seasonal share of juveniles in the wolf diet in the Abruzzo, Lazio and Molise National Park, central Apennines, Italy; percent occurrence based on number of equivalents is shown; values are mean percentages across pack and sampling years (2005-2018).

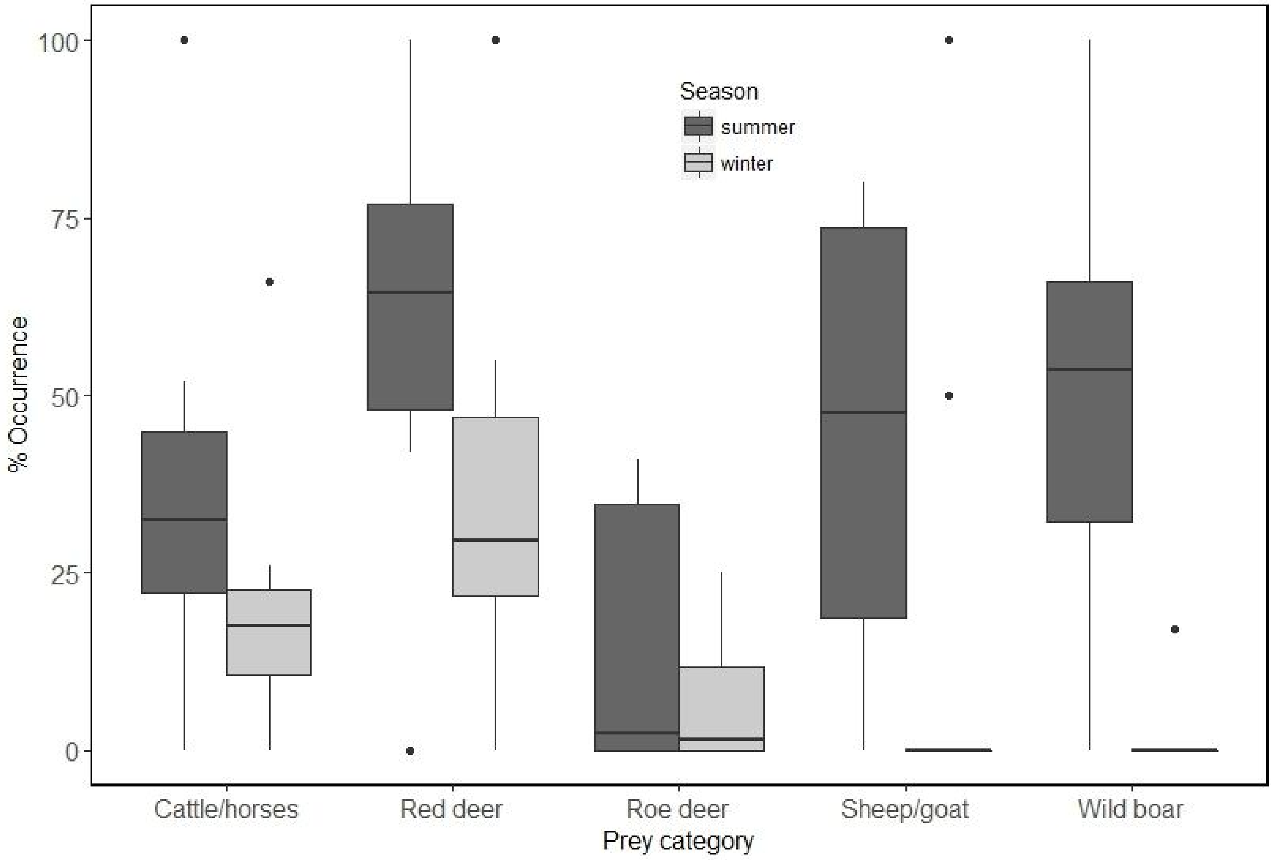
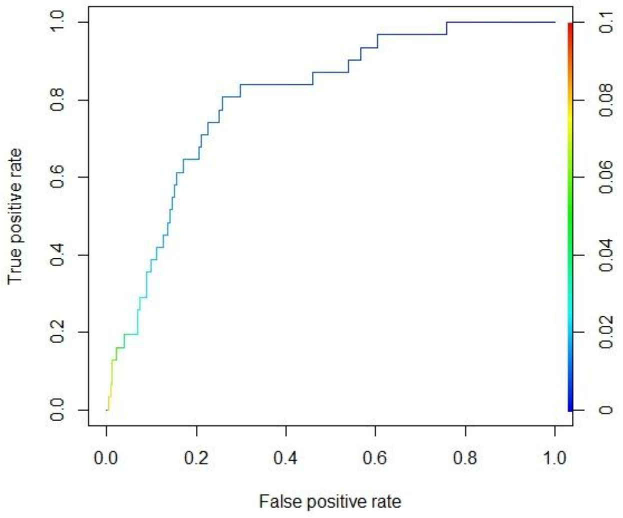
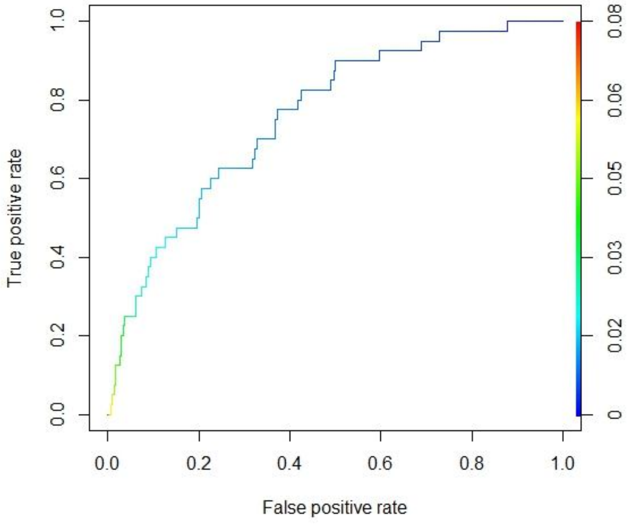


Figure 3. The receiver operating characteristics (ROC) curve from the averaged models' fit for a) kill sites and b) scavenging sites selection by wolves in the Abruzzo, Lazio and Molise National Park, central Apennines, Italy (2009-2010). ROC curves compare sensitivity (false negative rate) versus specificity (true positive rate) across a range of values (cut offs) for the ability to predict a dichotomous outcome. The more the specificity increases, the more sensitivity decreases (i.e. false positive rate increases). The more the curve displays an asymptotic shape, the bigger is the area under the ROC curve, and the better is the fit of the model.

a)



b)



SUPPLEMENTARY MATERIAL

Table S1. Food and non-food categories found in wolf scats (n =1,408) in the Abruzzo, Lazio and Molise National Park, central Apennines, Italy (2005-2008). Scats were collected year-round within the territories of 4 sample packs.

Food categories			
Mammals	Wild ungulates	Chamois	*Rubicapra pyrenaica ornata*
		Red deer	*Cervus elaphus*
		Roe deer	*Capreolus capreolus*
		Wild boar	*Sus scrofa*
	Domestic ungulates	Cattle	*Bos tauros*
		Goat	*Capra hircus*
		Horses	*Equus caballus*
		Pig	*Sus scrofa domesticus*
		Sheep	*Ovis aries*
	Canids	Dog	*Canis lupus familiaris*
		Red fox	*Vulpes vulpes*
		Wolf	*Canis lupus lupus*
	Lagomorphs	Hare	*Lepus europeus*
		Rabbit	*Oryctolagus cuniculus*
	Others	Dormouse	*Glis glis*
		Micromammal	Unknown
		Mole	*Talpa europaea*
Birds			Unknown
Fruit		Blackthorn	*Prunus* spp.
Non-food categories			
Plant material	Grasses		*Brachipodium* spp.
	Broadleaf		*Fagus silvatica*
Soil			
Rocks			
Insects			Unknown
Other (wood, paper, plastic, fabric)			

Table S2. Model selection table for log-linear modeling of diet analysis of wolves in the Abruzzo, Lazio and Molise National Park, central Apennines, Italy (2005-2008). Stars indicate interaction terms.

Pack	**Prey**	**Season**	**Pack*Prey**	**Pack*Season**	**Prey*Season**	**Pack*Prey*Season**	**df**	**logLik**	**AICc**	**ΔAICc**	**weight**
+	+	+	+	+	+		28	-474.322	1021.2	0	0.97
+	+	+	+	+	+	+	40	-455.103	1028.3	7.13	0.03
+	+	+	+		+		25	-489.798	1042.5	21.25	0
+	+	+		+	+		16	-519.47	1075.9	54.67	0
+	+	+	+	+			24	-509.505	1078.8	57.56	0
+	+	+	+				21	-528.285	1107.4	86.16	0
+	+	+			+		13	-540.439	1110.1	88.89	0
+	+		+				20	-532.806	1113.5	92.32	0
	+	+			+		10	-546.261	1114.4	93.2	0
+	+	+		+			12	-563.28	1153.3	132.08	0
+	+	+					9	-583.924	1187.4	166.17	0
+	+						8	-588.312	1193.8	172.63	0
	+	+					6	-590.976	1194.7	173.44	0
	+						5	-595.542	1201.6	180.36	0
+		+		+			8	-610.992	1239.2	217.99	0
+		+					5	-631.704	1273.9	252.69	0
		+					2	-638.322	1280.7	259.53	0
+							4	-636.503	1281.3	260.12	0
							1	-643.394	1288.8	267.6	0

Table S3. Correlation matrix for the set of variables chosen to investigate selection by wolf of a) kill sites and b) scavenging sites in the Abruzzo, Lazio and Molise National Park (PNALM; central Apennines, Italy); 2009-2010. Agr = agricultural fields, Elev = elevation, For =forest, Open =open fields, Shrub =shrublands, Main_r = distance (km) to main roads, Sec_r = distance (km) to secondary roads, Sett = distance (km) to settlements. Relevant correlations are indicated by a star.

a)

	Agr	**Elev**	**For**	**Open**	**Shrub**	**Sl**	**Main_r**	**Sec_r**	**Sett**
Agr	1.00	-0.35	-0.28	-0.05	-0.06	-0.33	-0.18	0.02	-0.28
Elev	-0.35	1.00	0.10	0.06	-0.21	0.32	0.60	0.29	0.66
For	-0.28	0.10	1.00	-0.63	-0.40	0.33	0.12	-0.19	0.27
Open	-0.05	0.06	-0.63*	1.00	-0.18	-0.26	-0.03	0.19	-0.10
Shrub	-0.06	-0.21	-0.40	-0.18	1.00	-0.08	-0.15	-0.06	-0.20
Sl	-0.33	0.32	0.33	-0.26	-0.08	1.00	0.21	0.23	0.24
Main_r	-0.18	0.60*	0.12	-0.03	-0.15	0.21	1.00	0.28	0.72
Sec_r	0.02	0.29	-0.19	0.19	-0.06	0.23	0.28	1.00	0.19
Sett	-0.28	0.66*	0.27	-0.10	-0.20	0.24	0.72*	0.19	1.00

b)

	Agr	**Elev**	**For**	**Open**	**Shrub**	**Sl**	**Main_r**	**Sec_r**	**Sett**
Agr	1.00	-0.41	-0.30	-0.08	-0.07	-0.38	-0.17	0.13	-0.27
Elev	-0.41	1.00	0.11	0.06	-0.20	0.31	0.57	0.20	0.66
For	-0.30	0.11	1.00	-0.60	-0.39	0.35	0.14	-0.22	0.25
Open	-0.08	0.06	-0.60*	1.00	-0.17	-0.20	-0.05	0.18	-0.09
Shrub	-0.07	-0.20	-0.39	-0.17	1.00	-0.12	-0.18	-0.10	-0.21
Sl	-0.38	0.31	0.35	-0.20	-0.12	1.00	0.25	0.13	0.22
Main_r	-0.17	0.57*	0.14	-0.05	-0.18	0.25	1.00	0.23	0.73
Sec_r	0.13	0.20	-0.22	0.18	-0.10	0.13	0.23	1.00	0.16
Sett	-0.27	0.66*	0.25	-0.09	-0.21	0.22	0.73*	0.16	1.00

Table S4. Comparison between a full model including a random effect of the individual wolf and the same model without the random component; models refer to a) kill sites and b) scavenging sites selection by of wolves in the Abruzzo, Lazio and Molise National Park (PNALM; central Apennines, Italy); 2009-2010. The percentage of variation explained by the random effect of the individual (PVR) is presented. Agr = agricultural fields, Elev = elevation, For =forest, Open =open fields, Shrub =shrublands, Main_r = distance (km) to main roads, Sec_r = distance (km) to secondary roads, Sett = distance (km) to settlements.

Model	**Res. dev.**	**% variation random**
a) Predation		
Model random: Logit(P)~ Agr + For + Shrub + Sl + Main_r + Sec_r + (1 Wolf_ID)	216.0	PVR=3.16e-13
Modelfix: Logit(P)~ Agr + For + Shrub + Sl + Main_r + Sec_r	216.2	
b) Scavenging		
Model random: Logit(P)~ Agr + For + Shrub + Sl + Main_r + Sec_r + (1 Wolf_ID)	411.5	PVR=3.16e-13
Modelfix: Logit(P)~ Agr + For + Shrub + Sl + Main_r + Sec_r	412.8	

Table S5. List of models retained by AICc model selection (ΔAIC<2) for a) kill sites and b) scavenging sites selection by wolves in the Abruzzo, Lazio and Molise National Park (PNALM; central Apennines, Italy); 2009-2010. Agr = agricultural fields, Elev = elevation, For =forest, Open =open fields, Shrub =shrublands, Main_r = distance (km) to main roads, Sec_r = distance (km) to secondary roads, Sett = distance (km) to settlements.

Candidate models (ΔAICc ≤ 2)	**df**	**logLik**	**AICc**	**ΔAICc**	**weight**
a) Kill sites					
Used_Avail~ For + Shrub + Sl + Main_r	5	-206.21	422.40	0	0.28
Used_Avail ~ For + Shrub + Sl	4	-207.64	423.30	0.86	0.18
Used_Avail~ Agr + For + Shrub + Sl + Main_r	4	-207.65	423.30	0.88	0.18
Used_Avail~ For + Shrub + Main_r	5	-206.93	423.90	1.46	0.13
Used_Avail~ For + Shrub + Sl + Main_r	6	-206.01	424.00	1.61	0.12
Used_Avail~ For + Shrub + Sl + Main_r + Sec_r	6	-206.11	424.20	1.82	0.11
b) Scavenging sites					
Used_Avail~ Shrub + Sl + Main_r + Sec_r	5	-108.43	226.90	0	0.28
Used_Avail~ Shrub + Main_s + Sec_r	4	-109.64	227.30	0.41	0.23
Used_Avail~ Sl + Main_r + Sec_r	6	-108.11	228.30	1.38	0.14
Used_Avail~ Agr + Shrub + Main_r + Sec_r	4	-110.30	228.60	1.73	0.12
Used_Avail~ Agr + Shrub + Sl + Main_r + Sec_r	5	-109.31	228.60	1.75	0.12
Used_Avail~ Agr + For + Shrub + Sl + Main_r + Sec_r	6	-108.33	228.70	1.81	0.11

CHAPTER IV

CIRCADIAN ACTIVITY OF WOLVES (*Canis lupus*) IN THE HUMAN-MODIFIED ENVIRONMENT OF CENTRAL APENNINES, ITALY

INTRODUCTION

The circadian rhythm of animal activity is a persistent feature of many species (Cloudsley-Thompson 1961). The times that animals are active during the day are influenced by a number of physiological and environmental factors, such as the quality of their vision in bright and weak light (Kavanau and Ramos 1975, Nielsen 1983), seasonal and circadian variation in environmental characteristics (Mech 1970, Beltran and Delibes 1994, Avenant and Nel 1998, Manfredi et al. 2011), intra-specific avoidance (Geffen and MacDonald 1993, Schwartz et al. 2010), intra-guild interactions (Hayward and Slotow 2009, Lucherini et al. 2009), availability and activity of prey species (Harmsen et al. 2011, Podolski et al. 2013) and predation pressure (Lima and Dill 1990), including human-caused mortality (Baker et al. 2007, Murray and St Clair 2015, Ordiz et al. 2016). Furthermore, individual characteristics such as sex, reproductive condition and social status may contribute to variation in circadian activity patterns (Zalewski 2001, Kolbe and Squires 2007, Tsunoda et al. 2009). Modern Global Positioning System (GPS) technology allows researchers to obtain detailed, quantitative field data for activity phasing of wild ranging animals, but their interpretation still remains a challenging task as it depends on knowledge of the integrated influences of the above factors.

Large carnivores can synchronize circadian activity patterns to those of their prey if this increases hunting efficiency (Curio 1976). This relationship has been shown in various carnivore species such as black-backed jackals (*Canis mesomelas*, Ferguson et al. 1988), jaguars (*Panthera onca*) and pumas (*Puma concolor*, Harmsen et al. 2011). At the same time, prey may decrease predation risk by reducing activity when predators are active (Lima 2002), or by shifting their habitat use in response to predator presence (Caro 2005, Laundré et al. 2010). However, large carnivores living in human-dominated landscapes have been shown to behave as both predators and prey (Ordiz et al. 2011), as a consistent proportion of their adult mortality is often human-induced (Woodroffe and Ginsberg 1998). Accordingly, large carnivores exhibit behavioral plasticity in circadian activity patterns to avoid anthropogenic interference ranging from tourism and recreational activity (Olson et al. 1998, George and Crooks 2006) to direct exploitation by hunting (Kitchen et al. 2000). For example, mountain lions became more nocturnal when human activity increased (Van Dyke et al. 1986) and coyotes (*Canis latrans*) resumed diurnality after human persecution ceased (Kitchen et al. 2000). The ability of large carnivores to adapt their spatio-temporal behaviour to varying levels of human pressure is considered a fundamental trait for their persistence in human-dominated environments.

The wolf (*Canis lupus*) is a pack-living, highly mobile species that have to range over wide areas to meet its essential life needs, and is therefore more susceptible to come into direct contact with humans (Packer et al. 2013). At the same time, wolves are adaptable animals (Mech and Boitani 2003), and in the last decades they proved to be the most successful amongst large carnivores in persisting in human-dominated landscapes throughout Europe (Chapron et al. 2014). Here, centuries of persecution have likely favored wolves that avoid humans (Linnell et al. 2002), both spatially or temporally (Theuerkauf et al. 2003b). Overall, activity patterns of wolves differ among study areas depending on the relative importance of factors affecting their time budgets, such as sun periodicity (Theuerkauf et al. 2009), prey activity (Theuerkauf et al. 2003a) and human interference (Vila et al. 1995, Ciucci et al. 1997, Kusak et al. 2005). Wolves were found to be active in the day and at night in areas with low human presence (i.e., Canada: Kolenosky and Johnston 1967; Alaska: Fancy and Ballard 1995), to have bimodal activity patterns where human interference is intermediate (e.g., Poland: Theuerkauf et al. 2003a), and to show nocturnal behaviour (e.g., Spain: Vilà et al. 1995; Italy: Ciucci et al. 1997; Croatia: Kusak et al. 2005). Nocturnal activity has generally been interpreted as the behavioural response adopted by wolves to avoid anthropogenic disturbance in the human-dominated landscape of Southern Europe.

In a previous study conducted in the central Apennines, Italy, Ciucci et al. (1997) found that wolves' activity was concentrated during night hours, although evidence provided by this study was based on data recorded from one VHF-collared pack. At the time when the study was conducted, wild prey communities were virtually absent in the area, and wolves were forced to mainly rely on anthropogenic food sources such as rubbish tips in proximity of human settlements. The access to additional food sources in human-dominated environments has been shown to influence animal behaviour, habitat selection and movement patterns (e.g., Ordiz et al. 2013, Flack et al. 2016). For instance, the availability of a human-related food source caused a behavioral dichotomy in a brown bear population in north-eastern Turkey, with individuals engaging in seasonal migrations depending on the relative availability of dumps within their home ranges (Cozzi et al. 2016). In central Italy, wolves visited more than one rubbish tip during extensive nocturnal movements, likely shifting their activity patterns towards nocturnal behaviour to maximize food intake while avoiding direct contacts with humans (Ciucci et al. 1997). Nowadays, the ecological context of central Apennines has changed, as wild ungulates became widely available for wolves following roe deer (*Capreouls capreolus*) and red deer (*Cervus elaphus*) reintroductions, and wild boar (*Sus scrofa*) introduction in the 70s (Apollonio and Lovari 2001).

The aim of our study was to assess wolf circadian activity in the renewed ecological conditions of central Apennines by using GPS data collected from 10 wolves in 5 packs in the Abruzzo, Lazio and Molise National Park (PNALM). Activity patterns of wolves feeding on wild ungulates might be similar to those of their preferred prey species if this increases predation success (Curio 1976). However, human presence might still represent one of the main determinants of wolf activity patterns, possibly causing nocturnal behaviour as previously describe by Ciucci et al. (1997). At the same time, environmental characteristics such seasonality in temperate regions have been shown to influence wolf activity patterns (Theuerkauf 2009). Besides environmental characteristics, seasonal changes in the PNALM comprise yearly variation in human activity, that is mainly concentrated during summer due to recreational activities and livestock. Lastly, summer months also represent the critical pup-rearing period for wolves, during which wolf movements are constrained by the need to return regularly to dens and rendezvous sites (Mech and Boitani 2003). Taking into account the aforementioned factors potentially affecting wolf activity patterns in the PNALM, the objectives of our study were 1) to describe wolf circadian activity using GPS locations collected at high temporal resolution; 2) to assess the effect of human presence on wolf activity patterns by using road and settlement densities as proxies of it; specifically, we expected wolf activity to be more concentrated during night in presence of higher road and settlement densities; 3) to assess the effect of ecological correlates such as season and social status (i.e., breeding pairs vs. non-breeding wolves, as well as resident pack members vs. floaters) on wolf circadian activity.

MATERIALS AND METHODS

Study area and animals

The study area (1,505 km^2) is centered in the Abruzzo, Lazio and Molise National Park (PNALM) and its external buffer zone in the Central Apennines, Italy (Figure 1). This represents one of the historical strongholds of the Italian wolf population, where wolves have been surviving close to humans since historic times. The area is typically mountainous, with elevations ranging from 400 to 2,285 m. The climate is continental; during winter months, snow cover is normally shallow and discontinuous in the valley bottoms, but may significantly increase at higher elevations. Vegetation types are dominated by pure stands of beech (*Fagus sylvatica*) forests at higher altitudes and by mixed forests at lower altitudes, where other species such as *Quercus* spp., *Fraxinus* spp. and *Acer* spp. predominate. Above 1800 m beech forests give way to alpine prairies and grasslands that have been degraded by centuries of grazing. Valley bottoms are covered mostly by abandoned fields, pastures and seasonally cultivated fields. Human population density averages 14.6 inhabitants/km^2, even though residents live aggregated in a few mountain villages (Ciucci et al. 2015), whereas paved road density averages 39.2 km/km^2. Wild ungulate community is amongst the richest in the Appennines, including wild boar (*Sus scrofa*), red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*) and Apennine chamois (Rupicapra pirenaica ornata). Both wolves and brown bears (*Ursus arctos marsicanus*) are present in the PNALM.

Between December 2008 and October 2010, 10 wolves (5 adult males, 4 adult females and 1 yearling female) were live-captured using dragged, modified Aldrich foot-snares (M. Collinge, Animal Damage Control, Idaho, U.S.A., pers. comm.). Wolves were equipped with GPS collars (Televilt-Tellus, Followit AB, Lindesberg, Sweden; and Vectronic Pro Light-1, Vectronic Aerospace GmbH, Berlin, Germany), and locations were remotely downloaded through the Global System for Mobile Communications (GSM). During winter months (January-April), collars were scheduled according to 30-day cycles, during which fix attempts were programmed every 0.5-1 hours (high rate) for 10 days, and every 3 hours (low rate) for the successive 20 days; during the rest of the year, collars' schedules only included the low acquisition rate. We used repeated snow-tracking sessions to assess social status (i.e., resident pack member vs. floater) of GPS-collared wolves during winter. It is more difficult to accurately ascertain wolf social status and association within packs during summer (Messier 1985). Therefore, in these months, we assessed the association of instrumented wolves, as well as reproduction in each pack, by means of wolf-howling surveys (Harrington and Mech 1982).

Wolf data and patterns of activity

We focused our analysis of wolf activity on the intensive schedules of GPS collars, i.e., with locations collected at half-hour intervals, due to its higher temporal resolution and average acquisition rate (see Results section). This dataset was available for five of the 10 collared wolves and focused on winter months only (January-April). Wolf circadian activity was described by calculating the Euclidean distance travelled by animals between consecutive locations during the 24 hrs, as movement rates have been shown to be a good proxy for wild-ranging animals' activity. To this aim, we divided the 24-hour day into 4 time periods, i.e., dawn, daylight, dusk and night. Dawn and dusk periods (2 hours each) were defined on a seasonal basis and averaged across years, as one hour before and after local sunrise and sunset times, respectively (Ciucci et al. 1997); length of daylight and night periods varied accordingly. We then calculated the mean distances travelled by wolves within the half-hour intervals of GPS locations. We also used the half-hour dataset to modeled wolf circadian activity with respect to two anthropogenic factors (see below), using paved road (Istituto Geografico De Agostini 2001) and settlement (ISTAT 2011) densities (km/km^2 and (km^2/km^2, respectively) as proxies of human interference. To calculate road and settlement densities when missing locations were present, we interpolated wolf trajectories using the *adehabitatLT* R package (Calenge 2006). We used ArcGIS (version 10.2.2) for all spatial analyses.

To assess seasonal and social status effects on wolf activity, we used the entire GPS dataset, that was available year-round and for all collared wolves. This dataset consisted of GPS locations collected at 3 hours intervals, and therefore included all data obtained during periods of low acquisition rate as well as systematically sub-sampled data obtained during periods of intensive acquisition rate. We defined three seasons: summer (May-September), corresponding to reproduction and increased availability of livestock and higher human activity throughout the study area; fall (October-December), when packs are expected to travel together at their maximum size, and conditions are less limiting for ungulate species; winter (January-April), comprising the mating period, the peak of dispersal, and conditions (e.g., snow cover) that make prey species more vulnerable to predation. Previous research indicate that in gregarious carnivores whose societies are structured by a dominance hierarchy, social status and reproductive condition may affect circadian activity patterns (e.g., Kolowski et al. 2007, Tsunoda et al. 2009). Accordingly, we classified wolves as resident pack members, breeding pair members (i.e., wolves engaging in pup attendance at dens and rendezvous sites) and floaters (i.e., non-territorial wolves that move more or less temporarily over the mosaic of territorial packs; Fuller et al. 2003).

Modeling

To assess the effect of anthropogenic features on wolf activity, we used a Bayesian approach for model formulation with estimation using Markov chain Monte Carlo (MCMC) methods. We analyzed the data with a Bayesian approach for two reasons; first, the models were quite complex, including both random effects (wolf effects) and autoregressive terms (temporal effects), and second, the large number of missing values (calculating 30-min distance traveled by wolves was not possible when GPS locations were missing) are handled by data augmentation, i.e., treating missing values as unknowns to be predicted. We used a linear model to assess how the response variable (i.e., the square root of distances travelled by wolves between consecutive locations; we transformed the data using the square root to make them normally distributed) was affected by the covariates time interval (48 levels), wolf ID (5 levels), and paved road and settlement densities (continuous covariates). We used two different models, in which we added the anthropogenic effects separately, based on their correlation coefficient (Pearson's r =0.72). The model formulation was the following:

$$\boldsymbol{y = \lambda_j + \alpha_k + \beta_j x_{density} + \epsilon}$$

where the parameter λj is the effect of daily half-hour interval j (j = 1, 2, . . . , 48), αk is the random effect of individual wolf k (k = 1, 2, . . . 5), assumed to be distributed as N(0, σ^2_{wolf}), $x_{density}$ is the road and settlement density, with time of day dependent regression coefficient β_j, and ϵ is the noise term accounting for unexplained variation in the travelled distances and assumed distributed as N(0, σ^2_j), i.e., normal distributed with time of day dependent variance. The same modeling approach was used to assess how wolf activity was affected by the covariates time interval (8 levels), wolf ID (10 levels), season (3 levels) and wolf social status (3 levels). Overall model formulation used for the 3 hrs dataset was the following:

$$\boldsymbol{y = \lambda_j + \alpha_k + \eta_{jseason} + \eta_{jsocial} + \epsilon}$$

where the parameter λ_j is the effect of daily 3-hour interval j (j = 1, 2, . . . , 8), α_k is the random effect of individual wolf k (k = 1, 2, . . . 10) assumed to be distributed as N(0, σ^2_{wolf}), $\eta_{jseason}$ is the time-dependent effect of season, and $\eta_{jsocial}$ is the time-dependent effect of social status (i.e., resident pack member vs floater). For all models, we included temporal dependence between measurements by assuming that the effect of a given time interval depended on the previous one. Specifically, we assumed:

$$\boldsymbol{\lambda_j = \nu \cdot \lambda_{j-1} + \epsilon_j}$$

$$\boldsymbol{\lambda_1 \sim N(0, 1000)}$$

$$\boldsymbol{\epsilon_j \sim N(0, \tau^2)}$$

where v is the autoregressive coefficient, and the variance parameter τ^2 controls the level of smoothing of the time effect. A large value of τ^2 induces low degree smoothing, whereas a small value gives heavy smoothing. In the models fit we chose to set $\tau^2 = 1000$ (low degree smoothing), due to the large amount of data, which in itself induces smooth time–effect estimates. For the categorical variables season and social status, we set fall and pack member as reference levels, and uninformative normal priors N(0, 10.000) were assumed for the remaining levels, as well as for the regression coefficients of continuous covariates. Similarly, for the regression coefficients of continuous covariates (i.e., road and settlement densities), uninformative normal distributions were assumed *a priori*. For all variance components in the models, we assumed a priori that the inverse variance (the precision) was gamma distributed with *Ga* (0.0001, 0.0001), i.e., a distribution with expectation 1 but with a large variance, which is commonly used for precisions.

We estimated the unknown model parameters by Bayesian posterior means using MCMC methods in OpenBUGS (Lunn et al. 2000). Due to the large number of observations, convergence was relatively fast and assessed by visual inspection of trace plots. Convergence was obtained within a burn-in of 5,000 iterations, but we ran a further set of 15,000 iterations with a thinning of 10, yielding 1,500 samples for each parameter as basis for posterior estimation. The estimated posterior distributions for the model parameters provided point estimates (mean) and credible intervals (lower 2.5% and upper 97.5% percentiles of the estimated distribution). We considered effects to be statistically significant if the credible intervals of the corresponding parameters did not contain the zero value (Kruschke 2011).

RESULTS

Eight of the 10 GPS-collared wolves we captured between 2008 and 2010 were permanently or temporarily members of 5 packs, including one male who became a floater after losing his alpha status and one female who established a new pack after a period of floating; the remaining two wolves were floaters during their whole tracking periods. Moreover, 3 of the 8 wolves who belonged to resident packs comprised a breeding pair and one reproductive female.

We used a total of 7,027 locations from 5 wolves for the half-hour dataset, whose average acquisition rate was 90.9%. Mean position (3D) dilution of precision (PDOP) of GPS locations was 4.5 (±2.8), and 95% of locations were 3-dimensional fixes, which generally have < 20 m error (Di Orio et al. 2003). The descriptive analysis of distances travelled by wolves during the 24-hours revealed that wolf circadian activity patterns were characterized by an overall higher activity during night hours (mean distance travelled = 368.7 m ±56.8 SD), but we also observed two peaks of activity around dawn (mean distance travelled = 420.7 m ±39.9 SD) and dusk (mean distance travelled = 409.6 m ±62.5 SD); overall, wolf activity was lower during daylight hours (mean distance travelled = 301.6 m ±49.1 SD, Figure 2). The results obtained from the models formulated using the half-hour dataset showed no substantial differences in wolves' circadian activity with respect to both road and settlement density (Figure 3a and 3b; Table S1 and S2, respectively).

The 3 hours dataset comprised 11,297 locations, whose average acquisition rate was 87.0%. Again, PDOP values (= 4.7 ±3.2) and percentage of 3-dimensional fixes (90.5%) confirmed the high quality of GPS locations used in the Bayesian modeling. The results obtained from the models formulated using the 3 hours dataset showed that wolf activity changes seasonally, with wolves being generally less active during daylight hours in summer compared to fall (Figure 4a, Table S3). Moreover, we found that social status affected wolf activity patterns, as both breeding wolves and floaters were found to be more active during the day, and less active around dusk compared to pack members (Figure 4b, Table S4).

DISCUSSION

We investigated wolf circadian activity patterns with respect to different ecological and anthropogenic correlates in the human-modified environment of central Italy. Interestingly, wolves seemed to respond to changes in the ecological context of central Apennines consisting in the renewed presence of wild prey. Indeed, wolves switched their circadian activity from a substantially nocturnal behaviour to bimodal activity patterns, with activity peaks around dawn and dusk. Amongst wild ungulates, wild boar, roe deer and red deer are the main prey species for wolves in the PNALM (Chapter IV). Whereas wild boar are mainly nocturnal (Boitani et al. 1994, Podgórski et al. 2013), roe deer (Cederlund 1989) and red deer (Kamler et al. 2007) usually show bimodal activity patterns peaking around dawn and dusk. Therefore, temporal selection of crepuscular phases for hunting is likely the reason of wolf activity patterns we observed in central Apennines. Moreover, hunting strategies have evolved to maximize kill rates, so the killing success of wolves might be higher if they hunt at times when they have maximum performance. Since the vision of wolves is best adapted to crepuscular light (Kavanau and Ramos 1975, Roper and Ryon 1977), they probably find it easier to locate and kill wild prey around dawn and dusk. Good vision is particularly important when attacking prey, not only to ensure that a kill is made but also to prevent fatal injuries to the predator (Asa and Mech1995). From an evolutionary perspective, it should be disadvantageous for wolves to adjust their circadian activity patterns and become nocturnal to avoid humans unless the risk of being killed is high. Indeed, by reducing their daytime and crepuscular activity, wolves foraging in the wild may fail to effectively exploit the available food resources. Unfortunately, due to lack of accurate data on prey activity and distribution in our study area we could not assess the direct effect of this fundamental factor on wolf temporal behaviour.

On the other hand, nocturnal activity to avoid humans might be an advantageous option for wolves that do not rely on hunting in crepuscular light and mainly feed on anthropogenic food sources such as garbage and livestock carrion. Garbage is a good resource for wolves because it is always available regardless of season or environmental conditions, it is predictable in both space and time, it is highly clumped (e.g. in residential areas), and it is always replenished after use (Beckmann and Berger 2003). However, feeding on dumps close to human buildings increases the risk to come into direct contact with humans. Accordingly, nocturnal behaviour of wolves has been interpreted as a behavioral response complementary to human activity in Southern Europe (e.g., Vila et al. 1995, Ciucci et al. 1997), allowing wolves to exploit anthropogenic food sources while minimizing risks of human-caused mortality. In these conditions, however, wolves completely lose their ecological role (e.g., Crooks and Soulé 124 1999, Ripple et al. 2014), with unknown consequences for the dynamics of the biotic community in which they live. Even if we observed wolf activity patterns that are ascribable to animals preying on wild ungulates (Theuerkauf et al. 2003a), we will not be able to assess if and to what extent wolves are restoring their role of apex predators in the PNALM until accurate data on wolf foraging behaviour and prey abundance and distribution will be available for further analyses.

Wolves living in human-dominated landscapes can adopt two different strategies to minimize contacts with humans: spatial or temporal avoidance. Spatial avoidance has been shown by previous studies throughout the current wolf range (e.g., Theuerkauf et al. 2003b, Kaartinen e al. 2005, Chavez and Gese 2006, Karlsson et al. 2007, Kojola et al. 2016), although it might be correlated with wolves selecting habitats that are less accessible by humans (e.g., dense forested areas, mountains). Ciucci et al. (1997) found that wolves in the central Apennines prefer to establish their home ranges in areas of low human presence. Indeed, even if human activity centres (e.g., villages, roads, agricultural areas) where present within wolf home ranges, these were generally located toward their outer part, where wolf presence was minimal and essentially nocturnal. The lack of both road and settlement density effects on wolf activity patterns we observed in the PNALM may be related to their spatial avoidance towards anthropogenic features. Indeed, if territories feature habitat characteristics that allow wolves to spatially avoid humans, it may be that wolves do not have to alter their circadian activity to avoid anthropogenic disturbance. In the PNALM, wolves select core areas whose environmental and topographic features ensured proper concealment from humans and where human accessibility is particularly limited (Chapter II). As a consequence, even if living in a human-modified landscape, wolves in this part of Italy may benefit of the presence of enough undisturbed areas and may not have the necessity to temporally avoid humans. Nevertheless, it should be remembered that these results are limited to winter season, when human activity in our study area is much limited compared to summer. Therefore, more data covering the entire year should be collected to assess the effect of seasonal variation in anthropogenic disturbance on wolf circadian activity.

When referring to ecological factors, we observed a seasonal affect on wolf activity, with wolves being less active during summer days. This result may be related to three different causes, or the combination between them. First, higher presence of human activities in the PNALM during summer months may lead wolves to reduce their activity during daylight hours. Indeed, mountain tourism and livestock grazing at high-elevation pastures are widespread in the park, and areas that are generally void of people during fall and winter are characterized by higher human presence in summer. Second, the reason for a lower activity of wolves during summer may be related to denning (e.g., Eggermann et al. 2009), as wolf movements are restricted around dens and rendez vous sites during the pup-rearing season (Harrington and Mech 1982, Ballard et al. 1991, Schmidt et al. 2008, Tsunoda et al. 2008). Lastly, daytime heat during summer may represented a limiting factor for diurnal movements of wolves in summer (e.g., Theuerkauf 2009). We also found that wolves social status affected their activity patterns, as both breeding wolves and floaters were found to be more active during daylight hours, and less active after dusk compared to pack members. During the period of reproduction, wolves (especially breeding females) spend much of their time at denning and rendez vous sites (Ballard et al. 1991, Harrington and Mech 1982). Because other wolves from the pack usually assure the feeding of breeding females during this time (Mech et al. 1999), females do not have to maintain an activity pattern based on hunting. If other wolves cannot supply the breeding female with enough food, her activity pattern should be shaped both by hunting and by the need to attend pups. In Spain, such a situation caused breeding females to leave the den mainly in the day, whereas they were mainly active at night in other periods of the year (Vila et al. 1995). Vila et al. (1995) assumed that in Spain, where packs are usually small, it would be the safest for females to leave the den for hunting during the day when sunlight would help keep the unattended pups warm and potential predators are less active. This is likely to be the case also for our study area, where breeding wolves pairs represented the only adult members of their newly established packs, and were brown bears may represent a danger for wolf pups. On the other hand, territoriality may be the mechanism explaining the increased diurnality of floaters. Territoriality in wolves serves to space packs to avoid excessive amounts of conflict and mortality (Mech 1973, Packard and Mech 1980, Mech and Harper 2002). Among wolf packs, territories are maintained both through nonaggressive (e.g., howling and scent marking) and aggressive behavior. Aggressive interactions may sometimes lead to the death of individual wolves, and these mortalities have been used as a measure of intra-specific strife within wolf populations (Mech and Boitani 2003). Temporal avoidance of pack members may represent a strategy adopted by floaters to minimize the risk to encounter cospecifics, while maximizing their freedom of movement over the mosaic of territorial packs.

Temporal avoidance of human activity may allow large carnivores to maintain their distribution range, and even expand it, in human-dominated landscapes (e.g., Basille et al. 2009, Kindberg et al. 2011). This plasticity in large carnivore behavior may have both positive and negative effects in terms of distribution, fitness, and performance of their ecological function as apex predators (Ordiz et al. 2014). Although limited by both sample size and resolution, our results are the first to provide a GPS-based description of circadian activity by multiple packs in a source wolf population in Italy. The main result of this study show that wolves in the central Apennines modified their activity patterns in response to changes in the biotic community of this area. If in the 80s wolves lived in a human-modified environment with few or no possibility to feed on wild ungulates, nowadays they still live in close proximity to humans, but can integrate their space use and circadian activity according to their hunting behaviour. Along with results from studies on a variety of large carnivore species (Kitchen et al. 2000, Boydston et al. 2003, Hebblewhite and Merrill 2008, Ordiz et al. 2016), our findings suggests that changes in circadian activity patterns of large carnivores is useful as a proxy of anthropogenic influences on natural ecosystems (Seryodkin et al. 2013).

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Figure 1. Study area in the Abruzzo, Lazio and Molise National Park, central Apennines (Italy), as defined by the locations of 10 GPS-collared wolves, with locations collected at 3-hr intervals and used to assess seasonal and social effects on circadian activity patterns (small figure), and 5 GPS-collared wolves, with locations collected at half-hour intervals and used to assess road and settlement density effects on circadian activity patterns (large figure).

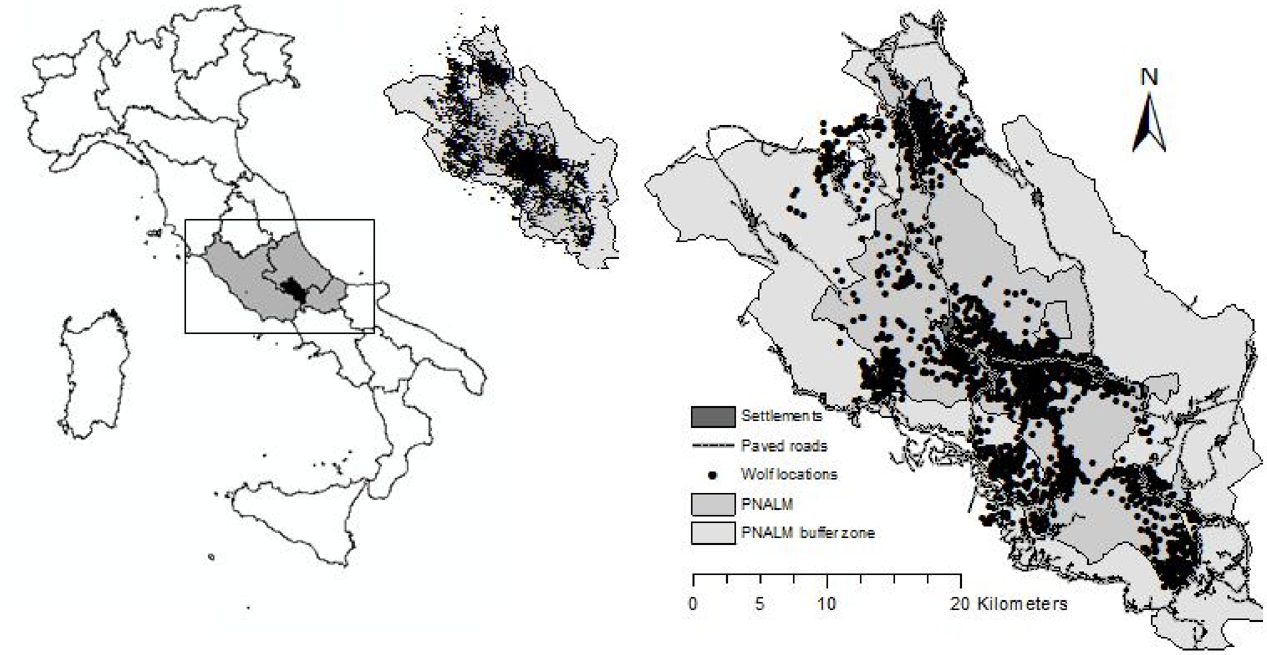


Figure 2. Trend of mean distances ± SD (error lines) travelled by wolves (n =5 pack members in 4 packs) between consecutive GPS locations during the 24-hours in winter (January-April; 2008-2010) in the PNALM, Central Apennines, Italy. Dawn and dusk periods are delimited by dashed lines; dawn and dusk were defined on a seasonal basis and averaged across years, as one hour before and after local sunrise and sunset times, respectively.

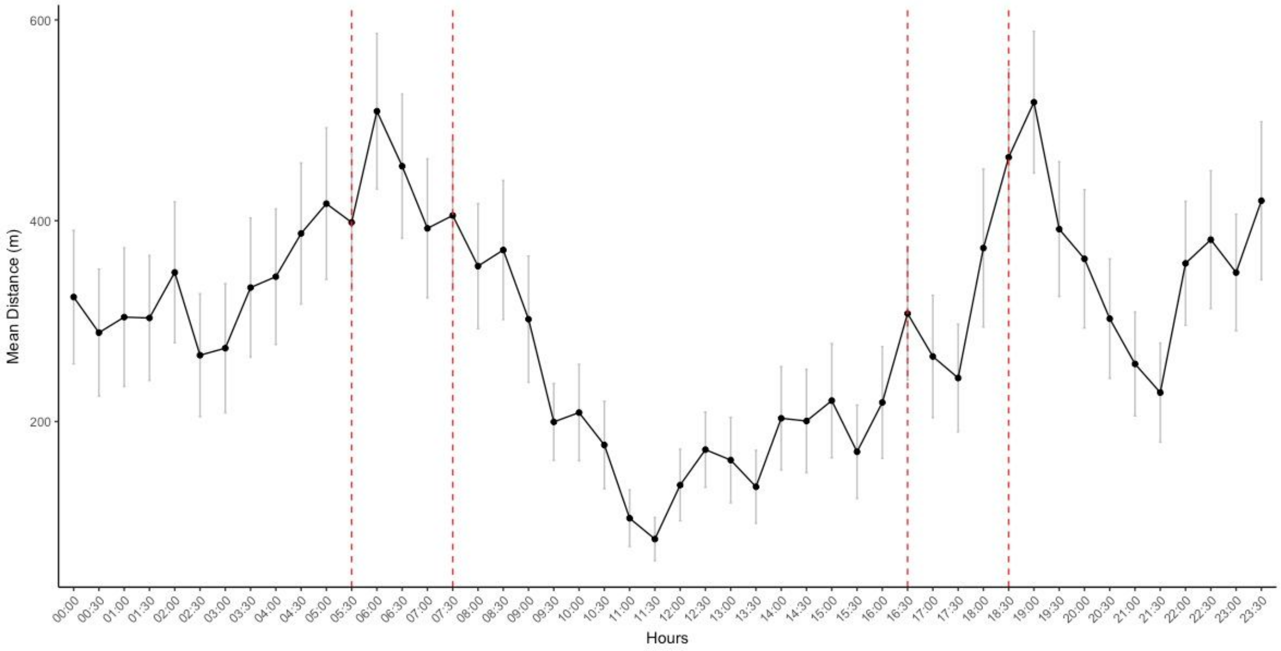
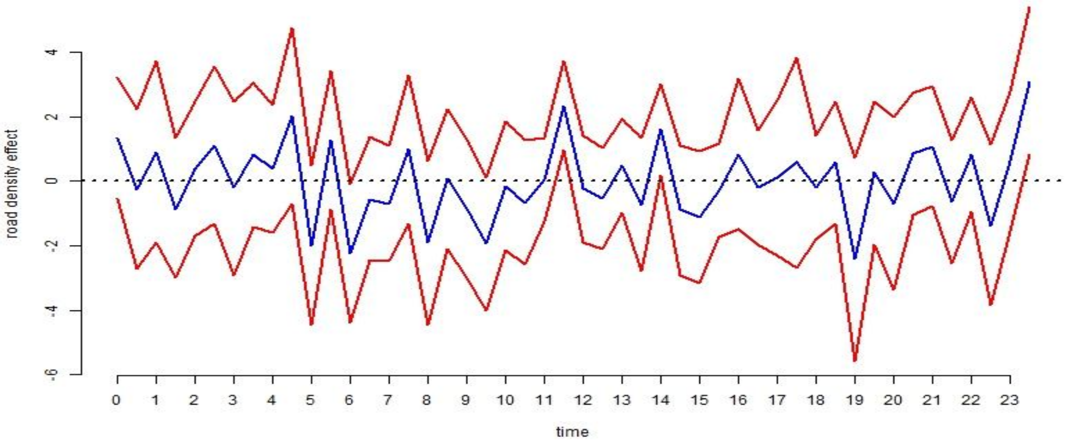


Figure 3. Posterior mean estimate (blu line) and the 95% credible intervals (red lines) for the time-dependent linear effects of a) road density and b) settlement density on expected wolf movement in the PNALM, 2008-2010. Both densities had no significant effect on expected wolf movement, because the zero-line is well inside the credible intervals for almost all time points.

a)



b)

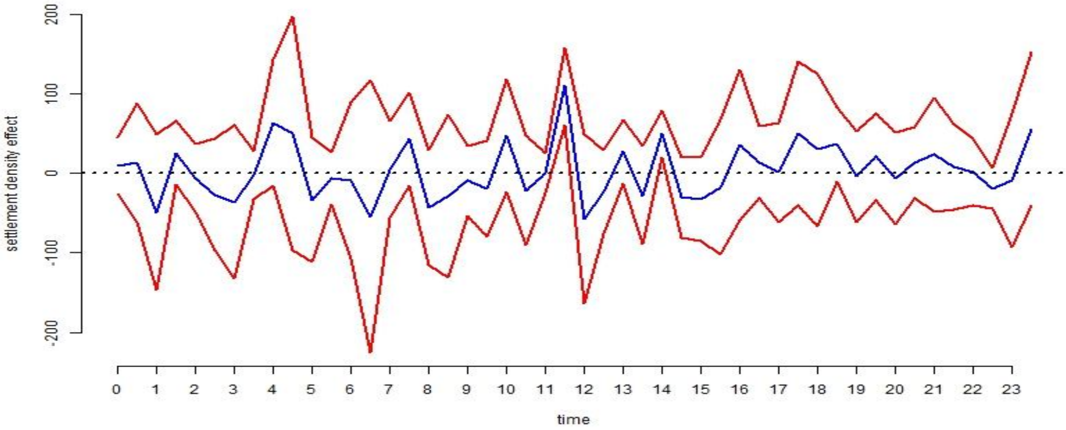
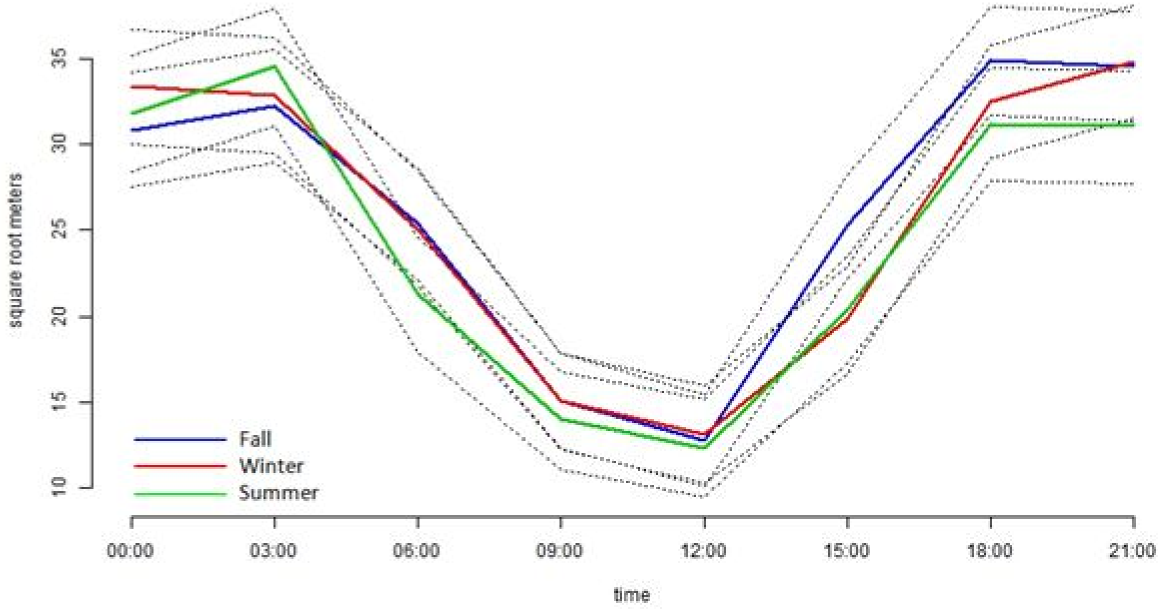
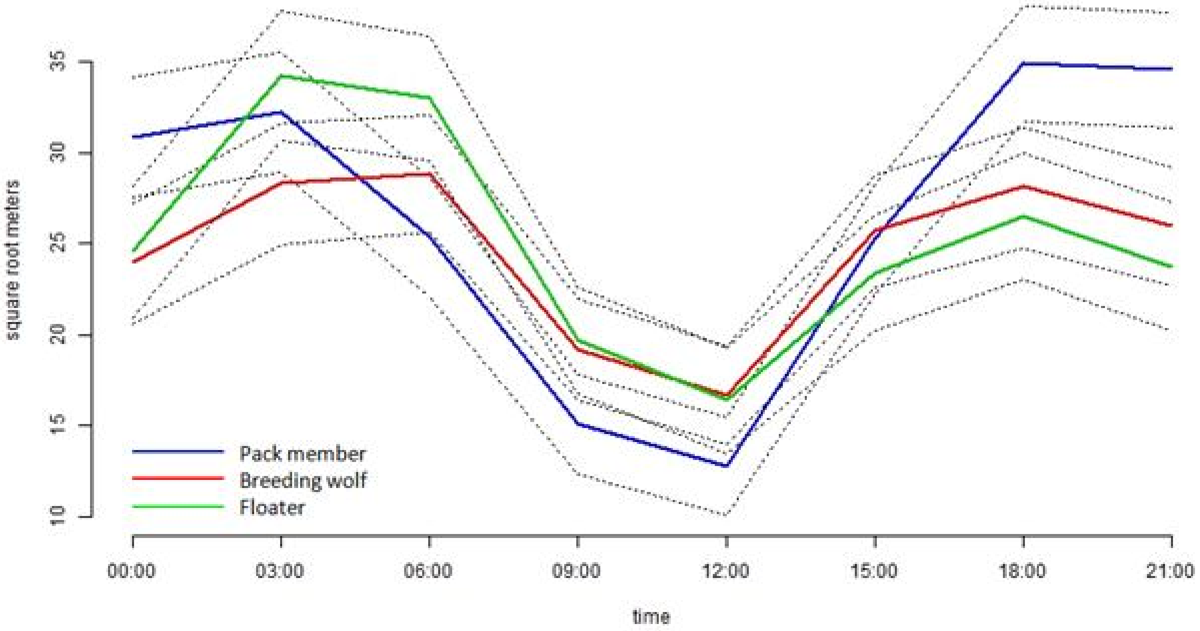


Figure 4. Activity patterns of 10 wolves a) during different seasons and b) with different social status in the PNAM, Central Apennines, Italy (2008-2010); the mean of the time effects (solid lines) and the 95% credible intervals (dashed lines) are shown.

a)



b)



SUPPLEMENTARY MATERIAL

Table S1. Posterior mean estimate, median estimated and corresponding 95% credible intervals for the time-dependent linear effect of paved road density (km/km2) on expected wolf movement in the PNALM, 2008-2010; bold lines indicate a significant road density effects on expected wolf movements.

		Beta coefficient for road density effect				
Time point	**Time (hh:mm)**	**MEAN**	**SD**	**25% CI**	**MEDIAN**	**95% CI**
1	00:00	1.35	0.96	-0.53	1.34	3.21
2	00:30	-0.26	1.25	-2.70	-0.26	2.22
3	01:00	0.89	1.43	-1.90	0.87	3.72
4	01:30	-0.86	1.10	-3.00	-0.87	1.33
5	02:00	0.38	1.06	-1.69	0.38	2.48
6	02:30	1.11	1.24	-1.32	1.12	3.56
7	03:00	-0.21	1.36	-2.91	-0.20	2.48
8	03:30	0.84	1.13	-1.42	0.85	3.04
9	04:00	0.39	1.01	-1.58	0.39	2.37
10	04:30	2.02	1.39	-0.71	2.01	4.76
11	05:00	-2.01	1.27	-4.46	-2.02	0.49
12	05:30	1.28	1.09	-0.87	1.27	3.44
13	06:00	-2.24	1.09	-4.38	-2.24	-0.10
14	06:30	-0.57	0.97	-2.45	-0.57	1.38
15	07:00	-0.69	0.91	-2.48	-0.71	1.11
16	07:30	1.01	1.18	-1.33	1.02	3.29
17	08:00	-1.88	1.31	-4.44	-1.87	0.64
18	08:30	0.07	1.11	-2.08	0.07	2.24
19	09:00	-0.83	1.09	-3.00	-0.83	1.31
20	09:30	-1.93	1.04	-3.99	-1.93	0.11
21	10:00	-0.14	1.01	-2.15	-0.14	1.86
22	10:30	-0.67	0.98	-2.59	-0.66	1.26
23	11:00	0.05	0.66	-1.23	0.04	1.34
24	**11:30**	**2.35**	**0.70**	**0.96**	**2.36**	**3.73**
25	12:00	-0.23	0.84	-1.90	-0.23	1.42
26	12:30	-0.54	0.79	-2.09	-0.54	1.03
27	13:00	0.49	0.73	-0.97	0.49	1.93
28	13:30	-0.72	1.05	-2.78	-0.73	1.36
29	**14:00**	**1.60**	**0.72**	**0.18**	**1.60**	**3.01**
30	14:30	-0.88	1.03	-2.91	-0.87	1.12
31	15:00	-1.10	1.05	-3.14	-1.09	0.93
32	15:30	-0.29	0.74	-1.73	-0.29	1.17
33	16:00	0.82	1.19	-1.50	0.82	3.17
34	16:30	-0.18	0.90	-1.95	-0.18	1.60
35	17:00	0.10	1.22	-2.31	0.11	2.50
36	17:30	0.58	1.65	-2.67	0.60	3.82

37	18:00	-0.19	0.82	-1.80	-0.19	1.41
38	18:30	0.58	0.97	-1.33	0.59	2.47
39	19:00	-2.42	1.60	-5.58	-2.43	0.74
40	19:30	0.27	1.13	-1.96	0.26	2.48
41	20:00	-0.69	1.37	-3.35	-0.69	1.98
42	20:30	0.86	0.97	-1.04	0.87	2.75
43	21:00	1.08	0.96	-0.78	1.07	2.94
44	21:30	-0.62	0.97	-2.53	-0.62	1.26
45	22:00	0.82	0.92	-0.96	0.81	2.62
46	22:30	-1.37	1.28	-3.83	-1.38	1.15
47	23:00	0.63	1.11	-1.55	0.65	2.77
48	23:30	3.09	1.17	0.82	3.10	5.39

Table S2. Posterior mean estimate, median estimated and corresponding 95% credible intervals for the time-dependent linear effect of settlement density (km2/km2) on expected wolf movement in the PNALM, 2008-2010; bold lines indicate a significant road density effects on expected wolf movements.

		Beta coefficient for settlement density effect				
Time point	**Time (hh:mm)**	**MEAN**	**SD**	**25% CI**	**MEDIAN**	**95% CI**
1	00:00	9.079	18.32	-26.59	8.785	44.76
2	00:30	12.82	38.13	-61.58	12.78	88.43
3	01:00	-49.81	50.06	-147	-50.64	49.01
4	01:30	25.71	20.36	-13.88	25.62	66.28
5	02:00	-5.825	21.44	-48.04	-5.989	36.6
6	02:30	-27.41	35.93	-97.33	-27.22	43.81
7	03:00	-36.15	49.2	-132.8	-36.16	61.03
8	03:30	-2.437	15.15	-33.05	-2.438	27.18
9	04:00	63.58	40.51	-16.14	63.85	143.6
10	04:30	50.02	74.05	-96.48	49.84	196.4
11	05:00	-33.97	40.03	-111.6	-34.14	45.13
12	05:30	-6.443	16.53	-38.97	-6.515	26.44
13	06:00	-8.763	49.81	-107.3	-8.776	88.95
14	06:30	-55.22	87.4	-225.7	-55.57	116.9
15	07:00	3.878	31.05	-56.86	3.669	65.65
16	07:30	44.09	29.73	-15.41	44.28	101.2
17	08:00	-43.1	37.19	-115.6	-42.85	28.41
18	08:30	-28.11	52.79	-130.7	-27.7	73.56
19	09:00	-9.076	22.56	-53.22	-9.089	34.85
20	09:30	-18.94	30.66	-79.7	-18.81	40.78
21	10:00	47.17	36.61	-23.83	47.13	118.6
22	10:30	-21.86	35.14	-90.99	-21.47	47.1
23	11:00	0.1992	12.55	-24.5	0.1357	24.85
24	**11:30**	**110**	**24.45**	**60.76**	**110.2**	**158.1**
25	12:00	-57.38	54.03	-163.7	-57.32	48.86
26	12:30	-23.6	26.27	-75.39	-23.64	28.87
27	13:00	27.17	20.34	-13.12	27.16	67.22
28	13:30	-28.03	31.07	-89.68	-28.13	33.72
29	**14:00**	**49.77**	**15.19**	**20.27**	**49.8**	**79.3**
30	14:30	-30.48	25.78	-81.55	-30.39	19.87
31	15:00	-33.02	26.95	-85.52	-32.94	19.24
32	15:30	-17.98	43.56	-102	-18.08	67.62
33	16:00	35.43	48.19	-58.65	35.45	130.5
34	16:30	13.91	23.06	-31.64	14.13	59.18
35	17:00	1.065	31.54	-61.49	1.127	62.79
36	17:30	50.38	46.14	-40.82	50.81	140.7
37	18:00	29.87	48.75	-66.25	29.92	125.4
38	18:30	36.49	23.9	-10.39	36.58	83.03
39	19:00	-3.995	28.96	-61.33	-4.17	53.15

40	19:30	20.51	27.81	-34.46	20.29	74.94
41	20:00	-6.216	29.22	-63.86	-6.097	51.3
42	20:30	13.76	22.56	-30.7	13.8	57.83
43	21:00	23.4	36.83	-48.41	23.46	94.9
44	21:30	8.297	27.38	-46.16	8.314	61.87
45	22:00	1.465	21.67	-40.52	1.337	43.9
46	22:30	-19.21	13.32	-44.84	-19.24	7.097
47	23:00	-9.163	42.94	-93.54	-8.45	74.73
48	23:30	55.87	49.08	-39.91	56.24	152.3

Table S3. Posterior mean estimate, median estimated and corresponding 95% credible intervals for the time-dependent linear effect of season on expected wolf movement in the PNALM, 2008-2010; fall = reference level; bold lines indicate a significant road density effects on expected wolf movements.

			Beta coefficient of season effect				
Season	**Time point**	**Time (hh:mm)**	**MEAN**	**SD**	**25% CI**	**MEDIAN**	**95% CI**
Winter	1	00:00	2.53	1.55	-0.56	2.55	5.59
	2	03:00	0.59	1.57	-2.49	0.59	3.69
	3	06:00	-0.29	1.48	-3.21	-0.29	2.60
	4	09:00	0.06	1.19	-2.25	0.07	2.42
	5	12:00	0.45	1.18	-1.87	0.45	2.79
	6	**15:00**	**-5.46**	**1.39**	**-8.16**	**-5.45**	**-2.77**
	7	18:00	-2.34	1.51	-5.36	-2.34	0.58
	8	21:00	0.27	1.52	-2.71	0.29	3.24
Summer	1	00:00	0.99	1.68	-2.31	0.99	4.28
	2	03:00	2.25	1.75	-1.18	2.25	5.65
	3	**06:00**	**-4.10**	**1.63**	**-7.32**	**-4.11**	**-0.87**
	4	09:00	-1.02	1.30	-3.61	-1.01	1.51
	5	12:00	-0.35	1.30	-2.90	-0.35	2.18
	6	**15:00**	**-4.92**	**1.51**	**-7.88**	**-4.91**	**-2.00**
	7	**18:00**	**-3.74**	**1.62**	**-6.97**	**-3.73**	**-0.60**
	8	**21:00**	**-3.53**	**1.64**	**-6.69**	**-3.55**	**-0.33**

Table S4. Posterior mean estimate, median estimated and corresponding 95% credible intervals for the time-dependent linear effect of wolf social status on expected wolf movement in the PNALM, 2008-2010; non-breeding pack members = reference level; bold lines indicate a significant road density effects on expected wolf movements.

			Beta coefficient of social status effect				
Social status	**Time point**	**Time (hh:mm)**	**MEAN**	**SD**	**25% CI**	**MEDIAN**	**95% CI**
Breeding wolf	**1**	**00:00**	**-6.88**	**1.68**	**-10.17**	**-6.88**	**-3.56**
	2	**03:00**	**-3.92**	**1.68**	**-7.23**	**-3.91**	**-0.66**
	3	**06:00**	**3.51**	**1.62**	**0.33**	**3.52**	**6.70**
	4	**09:00**	**4.10**	**1.35**	**1.47**	**4.10**	**6.76**
	5	**12:00**	**3.93**	**1.37**	**1.30**	**3.94**	**6.61**
	6	15:00	0.41	1.55	-2.71	0.41	3.46
	7	**18:00**	**-6.85**	**1.66**	**-10.06**	**-6.85**	**-3.59**
	8	**21:00**	**-8.63**	**1.63**	**-11.88**	**-8.62**	**-5.48**
Floater	**1**	**00:00**	**-6.30**	**2.01**	**-10.23**	**-6.29**	**-2.39**
	2	03:00	1.96	2.00	-1.97	1.97	5.80
	3	**06:00**	**7.59**	**1.93**	**3.81**	**7.59**	**11.35**
	4	**09:00**	**4.59**	**1.63**	**1.35**	**4.60**	**7.76**
	5	**12:00**	**3.61**	**1.64**	**0.36**	**3.63**	**6.79**
	6	15:00	-1.99	1.82	-5.61	-1.97	1.50
	7	**18:00**	**-8.45**	**1.93**	**-12.19**	**-8.43**	**-4.69**
	8	**21:00**	**-10.90**	**1.93**	**-14.71**	**-10.88**	**-7.15**

CONCLUSIONS

The aim of my PhD research was to assess how wolves adapt their spatio-temporal niche in the human-modified environment of central Apennines, Italy. By focusing on a wolf population characterized by a long coexistence history with humans, my research gives insights on wolf adaptations to human interference, whose knowledge is fundamental to improve wolf conservation strategies in human-dominated landscapes.

The spatial niche of wolves in the PNALM was influenced by human presence at different scales. First, I revealed a response by wolves to humans at the home range scale, including larger home ranges detected during night and in areas of higher road density. Thus, human influence on wolf spatial niche manifested itself in two ways. First, larger nocturnal home ranges indicate that wolves in the PNALM display spatial avoidance of higher levels of human activity during daylight hours by restricting their daytime movements. Second, habitat fragmentation caused by roads likely force wolves to increase home range size to reach a minimum threshold of undisturbed area to meet their ecological needs, such as the presence of refuge areas for resting and attending pups. In addition, wolf spatial responses to human presence were habitat-mediated, as core areas were preferentially established at higher elevation and in the more forested portions of the home range. Overall, these landscape characteristics reflect safe places, where human accessibility, and related risk of human-caused mortality, are low.

When focusing on habitat selection patterns within home ranges, I found that wolf responses to anthropogenic features mainly varied according to a seasonal effect. In particular, I found that wolves strongly avoided roads during summer, when human disturbance is relatively high in the PNALM due to tourism and livestock raising at high-elevation pastures. On the other hand, wolves were found to select roads during winter, when human activity is low and snow cover may represent an important factor increasing energy expenditure for locomotion; in these conditions, wolves are well known to use roads for ease of travel. In line with previous research, I observed that the spatial segregation adopted by wolves towards anthropogenic features enables them not only to coexist with humans, but also to take advantage of man-made habitat modifications in presence of low human interference.

Human influence on the PNALM wolf population also extended to its feeding ecology. Indeed, I found that, despite a high availability of wild ungulates, wolves consistently consume livestock in this area. This observation is linked to the husbandry methods currently used in the PNALM, that often involve free-ranging herds with little to none supervision. These conditions not only heighten the conflicts with farmers and the subsequent risk of retaliatory killing of wolves due to livestock losses, but may also alter wolf ecology and behaviour and interfere with ecological processes. Minimizing livestock accessibility should then be achieved not only to mitigate social conflicts over wolf conservation, but also to restore wolf ecological role as apex predator and, consequently, the integrity of biotic community dynamics.

In the central Apennines, wolves have faced improved ecological conditions during the last decades, characterized by reintroduction (roe deer and red deer) or introduction (wild boar) efforts of their natural prey species that took place in the 1970s. Before these ecological changes occurred, wolves in this area have proved to be able to survive in absence of wild ungulates, mainly relying on anthropogenic food sources such as garbage and livestock. In these conditions, wolf activity patterns were characterized by a nocturnal behaviour, that was interpreted as a complementary response adopted by wolves to exploit the available food sources, i.e., rubbish tips in proximity to human settlements, while minimizing encounters with humans. Nowadays, wolves can rely on wild ungulates, amongst which wild boar and roe deer resulted the preferred prey species in the PNALM. My analysis of wolf temporal niche reflected these renewed ecological conditions, as wolves showed bimodal activity patterns, with activity peaks around dawn and dusk, that likely reflect wolf selection of crepuscular light for hunting wild ungulates. Therefore, wolf temporal niche in the PNALM currently seems to be shaped by the interaction between human presence and hunting strategies, rather than being mainly determined by the temporal avoidance of human activities.

Overall, my PhD research show that the spatial arrangement, habitat use, activity, and movements of wolves in the human-modified landscape of central Apennines are highly integrated to make the best functional compromise between the necessity to achieve all fundamental biological requirements such as prey and cover, and the need to avoid any direct form of disturbance by humans. Nevertheless, potential limits of my research are represented by the small number of GPS-collared wolves, as well as by the lack of accurate data on prey abundance and distribution in our study area. Moreover, similar studies are needed from less productive and more human-altered ecosystems to fully appreciate the spatio-temporal flexibility of wolves in highly populated countries such as Italy.