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ASPECT OF SOME ECOLOGICAL CHARACTERISTICS OF ROE DEER (*Capreolus capreolus* L., 1758) POPULATION IN NORTH-EASTERN APENNINES, AREZZO PROVINCE, ITALY

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– Protected area "Oasi Alpe
di Catenaia"

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To Sergio

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SUMMARY

Some behavioural characteristics of roe deer (*Capreolus capreolus*) were researched. We identified the factors that contributed most to variation of daily and annual activity patterns of roe deer: light-dark cycle, seasonal variability, territorial season, and predation risk (human harassment). Secondly, during the territorial period (March-August) we measured two behavioural aspects of males simultaneously: spatial use and activity levels. Home range size did not change significantly among bimonths, but activity was the highest in the bimonth March-April, suggesting an importance of territory establishment period for roe deer bucks and supporting "low cost–low gain" mating strategy. Activity patterns during the territorial period were expected to depend more tightly on factors other than territory size, such as outside environmental and social factors. Thirdly, no correlation between genetic relatedness and spatial distance among individuals was found, indicating quite similar distribution of genetic relatedness between the sexes. This suggested that sampled population expressed no sex bias in dispersal and resulted in genetically unstructured population. In the last chapter we confirmed higher roe deer browsing pressure on Turkey oak than on chestnut in coppice areas. Also, roe deer used these areas more frequently during and after the coppicing than before, suggesting the importance of coppice for roe deer in terms of food resources, cover, and hiding places for fawns.

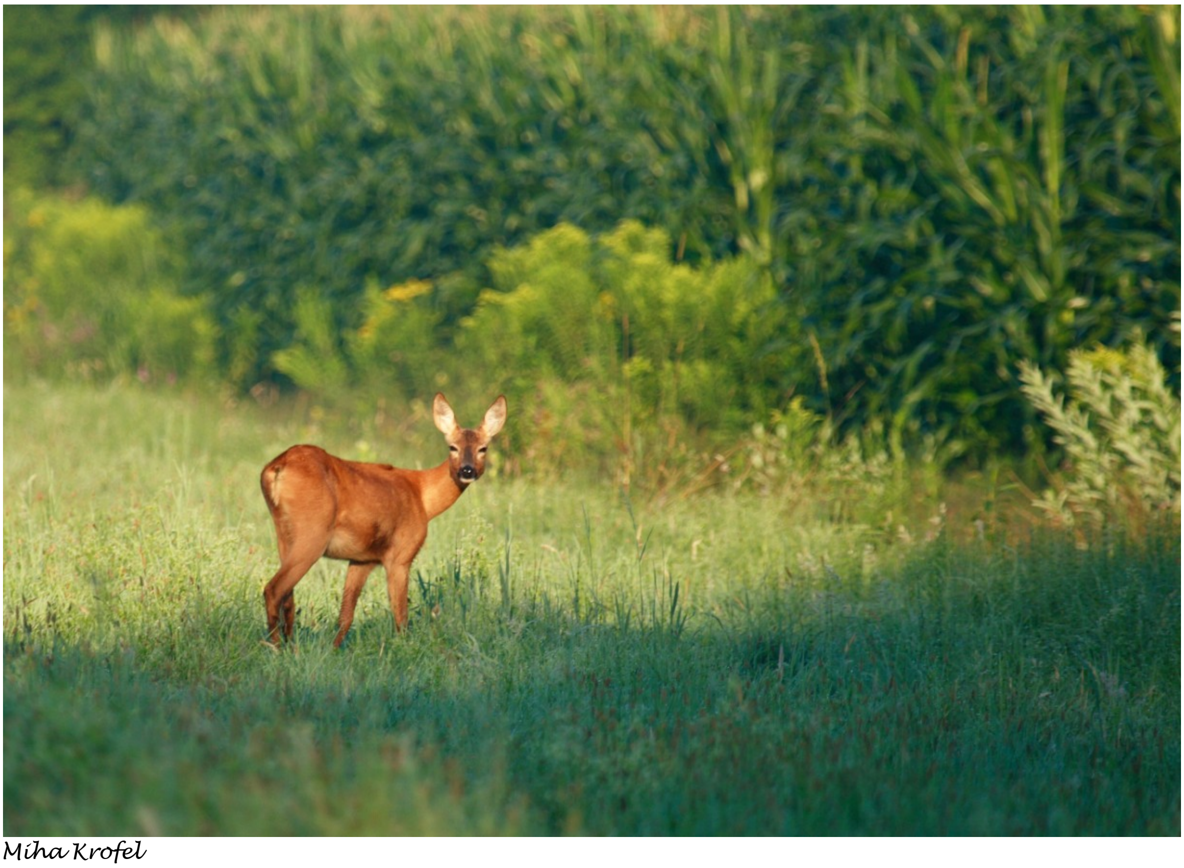
Key words: activity rhythms, behavioural ecology, browsing damage, genetic relatedness, territoriality

RIASSUNTO

In questo elaborato sono stati approfonditi alcuni aspetti dell'ecologia comportamentale del capriolo (*Capreolus capreolus*). In prima analisi, abbiamo identificato alcuni fattori che possono incidere significativamente sulla variazione dei ritmi di attività giornalieri ed annuali del capriolo; tra questi gli effetti più significativi sono relativi a ciclo luce-buio, variabilità stagionale, periodo di territorialità, rischio di predazione (disturbo antropico). Durante il periodo di territorialità (marzo-agosto) abbiamo analizzato in contemporanea due aspetti del comportamento dei maschi: uso dello spazio e ritmi di attività. Dai risultati ottenuti è emerso che le dimensioni degli *home range* non presentano differenze significative su scala bimestrale, mentre è stata rilevata una maggiore attività durante il bimestre marzo-aprile, sottolineando l'importanza di questo periodo per la scelta del territorio da parte dei maschi, e supportando l'ipotesi di utilizzo della strategia riproduttiva "*low cost-low gain*". I livelli di attività durante il periodo territoriale erano correlati al comportamento spaziale ed in particolare alle dimensioni delle aree vitali. Non è stata trovata alcuna correlazione tra parentela genetica e distanza spaziale tra gli individui, evidenziando l'assenza, su scala spaziale ridotta, sia di una struttura genetica nella popolazione sia di una differente dispersione dei sessi. Infine è stato dimostrato come i caprioli modifichino il loro comportamento spaziale in relazione alle attività dell'uomo nelle foreste. Durante i lavori di taglio dei boschi cedui queste aree sono utilizzate maggiormente in relazione all'incremento di disponibilità di risorsa trofica. I maggiori danni da brucatura da parte del capriolo avvengono nei boschi cedui di cerro.

Parole chiave: danni da brucatura, ecologia comportamentale, parentela, ritmi di attività, territorialità

Introduction



Miha Krofel

INTRODUCTION

About the species and purpose of the study

European roe deer (*Capreolus capreolus* Linnaeus, 1758) evolved in forest environment (Hewison et al., 1998), but due to its highly adaptive capability it occurs nowadays in wide variety of habitats, both natural and anthropogenic, from mild climate to the very extremes as Mediterranean shrublands and severe boreal forests. Numerous habitats that it occupies vary from early successional habitats to climax forests of all types, shrublands, moorlands, marshes, and man-influenced landscape of plantation forests, farmland mosaic, agricultural areas and even suburban gardens and parks. Occurring in a wide range of environmental conditions and expressing high behavioural plasticity with high reproductive and dispersal potentials, it became the most widely distributed and abundant large herbivore in Europe. Consequently, also one of the most hunted ones, as it is a subject of the hunting policies basically throughout its range. That's why an appropriate management of such an economical resource is urgent and a good understanding of its role in the ecosystems is required. Moreover, with geographic and demographic expansion of the roe deer also studies on inter-specific relationships in the forest and agricultural ecosystems gained on the importance. Namely, with increasing density forestry reported on roe deer damages in the silviculture and forests (e.g. Ward et al., 2004; Gill, 1992) or in the connection with the forest regeneration (e.g. Motta, 1996; Partl et al., 2002), agriculture reported on crop damages, and number of collisions between animals and vehicles increased. Besides, being roe deer prey of endangered large carnivores and likely being susceptive to competition with larger free-living herbivores or domestic herbivores, proper management of its populations became also the interest of conservation biology, population ecology, forestry.

As a consequence, roe deer has been intensively studied in the last decades. Long-term monitoring of marked populations provided detailed information on survival (Gaillard et al., 1993, 1997; Hewison & Gaillard, 1996; Pettoreli et al., 2005), population dynamics and density-dependent effects (Gaillard et al., 2003; Gaillard et al., 1998; Focardi et al., 2002; Kjellander et al., 2004a), female reproduction (Gaillard et al., 1992; Hewison et al., 2005; Kjellander et al., 2004b) and male territorial structure (e.g. Linnell & Andersen, 1998).

Parallel to that, complex behavioural adaptations throughout its range were reported for roe deer. Indeed, Andersen et al. (1998) demonstrated that much life-history variation exists. Not only that differences in behavioural traits between populations were found (e.g. from different parts of the species' range), but also great inter-individual variability within the same population was often reported (e.g. spatial behaviour: Rossi et al., 2003; Saïd et al., 2009; migration patterns: Mysterud, 1999; activity rhythms: Scheibe et al., 2009).

As reported above, the species shows a remarkable behavioural plasticity from many points of view. The purpose of this dissertation was to examine some aspects of behavioural traits in roe deer population, which inhabits a forested mountainous habitat in North-East Apennines, Italy. Specifically, we were interested in some aspects of activity rhythms, male territoriality, genetic relatedness within the population, and its browsing pressure in coppice areas.

Study area

Study area covered 120 km^2 and was located in North-Eastern Apennines in Arezzo Province, Tuscany, Central Italy (Fig. 1). Mountainous landscape was covered mainly in mixed forests. A protected area "Oasi Alpe di Catenaia" (28 km^2, 85% of forest cover), where anthropogenic influence was reduced, was included in the study area. In a non-protected part (71% of forest cover) also cultivated areas (orchards, vineyards, crop fields) and urban areas could be found (Fig. 1).

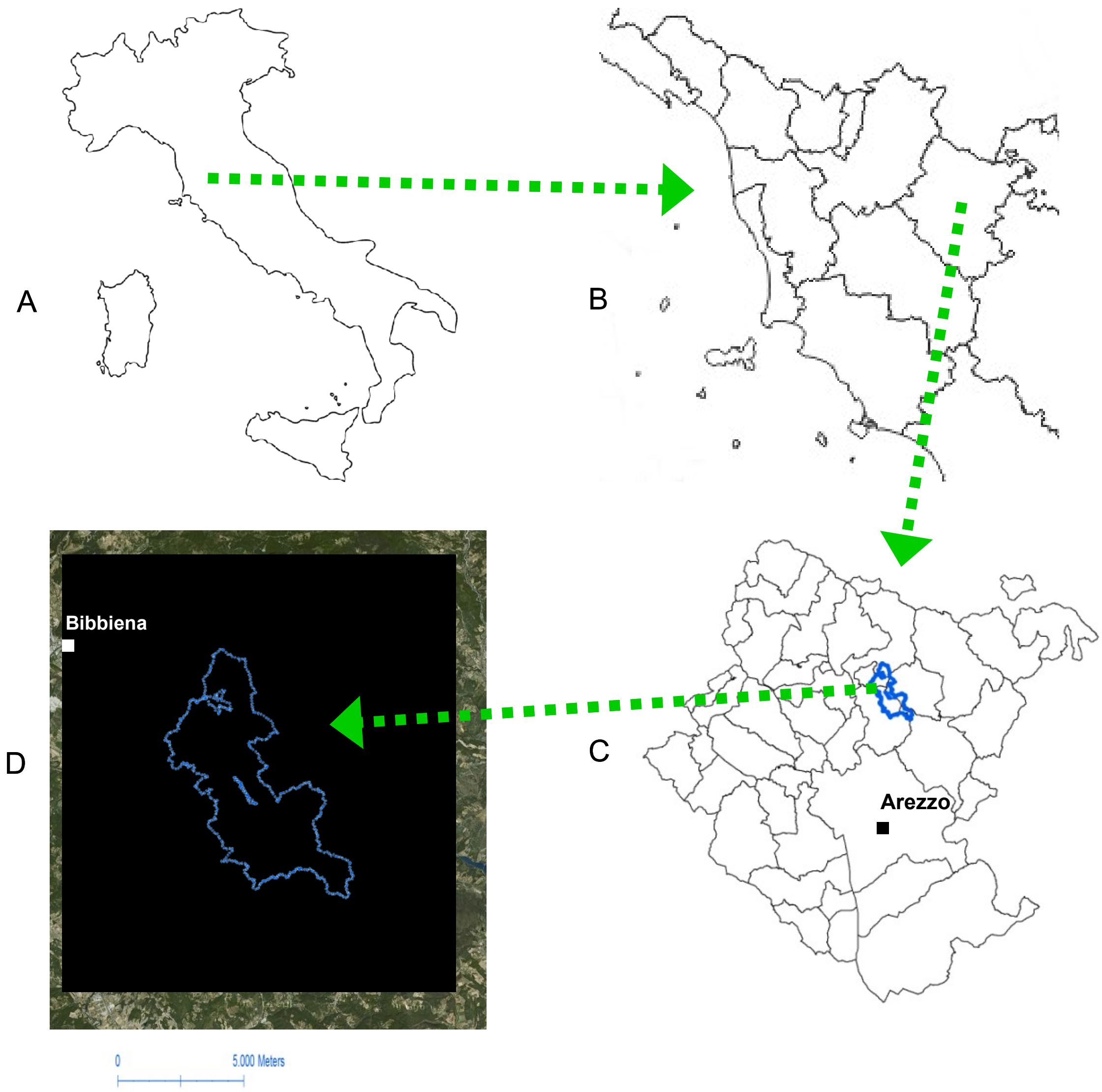


Figure 1: Location of the study area (D) with the Protected area "Oasi Alpe di Catenaia" included (outlined with blue colour), in Italy (A), Tuscany region (B), Arezzo Province (C).

Evolutionary and behavioural traits of roe deer species

SEXUAL SEGREGATION AND ACTIVITY BUDGETS

Sexual segregation, a phenomenon where males and females of a certain species use the space, habitat or forage differently outside the mating season or live in separate social groups at that time, was above all intensively studied in ungulates (Bowyer, 2004). Indeed, Mysterud (2000) brought forward a significant correlation between sexual size dimorphism and sexual segregation in ungulates: the bigger the difference in body sizes between males and females, the more likely they are to segregate and live in separate groups. Ecological segregation was partitioned in spatial, habitat, and dietary segregation by Mysterud (2000), and later Bowyer (2004) stressed that these processes are certainly not independent. Actually, sexual segregation is extremely sensitive to temporal and spatial scale, so different criteria for degree of sexual segregation should be used for each species separately (Bowyer, 2004). Bowyer and Kie (2004) proposed a niche-based assessment for defining sexual segregation in ungulates. If we consider space, habitat and diet as different niches, then overlap of two sexes of a species on one niche is likely accompanied by avoidance on another. Additionally, the status of the population and its density are likely to modify the outcome of the overlap and avoidance on certain niche combinations (Bowyer, 2004).

Sexual dimorphism in body size results in allometric differences between sexes that profoundly influence their ecology and life-history traits (Barboza & Bowyer, 2001). Allometric differences associated with increasing body size occur among ruminants because energy requirements per unit body mass scales to a power less than unity, whereas rumen volume scales directly with body mass. Consequently, larger species of ruminants, and presumably larger sex of the same species, have proportionately larger rumens than smaller ruminants, or smaller sex. Marked morphological and physiological dissimilarities between the sexes likely facilitate differential use of forage, because animals with larger rumens can digest forages of poorer quality more completely than those with smaller rumens, even though large animals require absolutely more forage (Barboza & Bowyer, 2001). **Forage selection hypothesis** thus defines causes of sexual segregation in ruminants on the basis of allometry, minimal food quality, digestive retention, and differing reproductive requirements of sexes (Barboza & Bowyer, 2001). Larger males with greater absolute metabolic requirements, feed on abundant, high-fiber forages that are degraded most efficiently by long retention in a proportionally capacious rumen (Bowyer, 2004). Small-bodied females selectively feed on low-fiber, high-quality forages to satisfy nutritional demands of gestation and lactation (Bowyer, 2004). So, females would select habitat based on food quality, while males prefer high forage biomass. Smaller females would use high quality food habitats, as they are less efficient at forage digestion that the larger males, due to a small stomach size, and quicker passage rate of food. Males on the other hand would use lower quality but higher biomass habitat than females, as they are good at digesting even low quality food.

Second, reproductive success of males and females differ. Reproductive success of males is likely influenced by size, strength, and endurance, therefore selective pressures favour behaviours that maximise growth rate and formation of energy reserves (Bowyer, 2004). Reproductive success of females is determined primarily by survival of offspring but also by engaging in activities that provide resources necessary to meet the requirements of lactation and promote survival of females (Bowyer, 2004). On such basis, **predation risk hypothesis** states that predation risk is the cause of sexual segregation, as female ruminants trade off a higher quality diet for greater security from predators during sexual segregation. Females would use relatively predator-safe habitats of often inferior food quality or quantity, as they are more vulnerable to predation than larger males. Males are predicted to use habitats with higher predation risk but abundant and high quality food to be fit competitors during the breeding season (Ruckstuhl & Neuhaus, 2002).

Ruckstuhl and Neuhaus (2002) treated social and habitat segregation as different types of sexual segregation. **Activity budget hypothesis** has thus the ability to explain the social segregation in general, without assuming the sexual differences in habitat choice (which on the other hand would be the case of first two hypotheses). The hypothesis predicts that with increasing body-size dimorphism males and females will increasingly differ in the time spent in different activities (Ruckstuhl & Neuhaus, 2002). Differences in activity budgets would make it difficult for males and females to stay in mixed-sex groups due to increased costs of synchrony to maintain group cohesion. They concluded that sexual differences in activity budgets are most likely driving sexual segregation and that sexual differences in predation risk or forage selection are additive factors. Females will compensate their lower digestive efficiency by foraging for longer than males, while males will spend more time ruminating or lying than females to digest forage, as females are less efficient at digesting forage than males. Animals with similar activity budgets will form groups, as big differences in activity budgets make synchrony of behaviour difficult and potentially costly.

However, also activity budget hypothesis was criticised by Yearsley and Pérez-Barbería (2005), when they applied the observational data to the theoretical activity model (Ruckstuhl & Kokko, 2002). With this model they could not confirm the activity budget hypothesis being the main factor driving sexual segregation. However, after improving the model by inserting additional criteria and parameters, they agreed that different activity budgets can generate sexual segregation through the mechanism of changes in population's home range. Namely, the transient spatial distribution of a population, following seasonal changes in the home range area, generated sexual segregation, if males and females differed in activity budgets.

Ruckstuhl and Neuhaus (2000; 2002) stressed that it is of crucial importance to test the different hypotheses on a wide range of ungulate species with varying degrees of sexual body size dimorphism, in order to validate the hypotheses and to understand the origin of sexual segregation. Most of the proposed hypotheses have been studied only on a single ungulate species at a time, but seeking species-specific explanations will absolutely not suffice (Main, 1998). Moreover, different studies to test the same hypotheses often led to contrary results. Since most of the research on sexual segregation has been done on sexual size-dimorphic ungulates (Ruckstuhl & Neuhaus, 2000), the need of the data on size-monomorphic ungulates is a priority. Actually, Ruckstuhl and Neuhaus (2009) stated that "the study of activity patterns in a monomorphic species could shed light on the debate on which factors cause sexual segregation in dimorphic ruminants". Ruckstuhl and Neuhaus (2002) summarised the predictions for the three hypotheses also for the case of size-monomorphic species. So, according to predation-risk hypothesis, in non-dimorphic species, females with dependent offspring are expected to use areas with lower predation risk than females without young and adult males, which are expected to form mixed-sex groups. According to forage selection hypothesis, males and females of non-dimorphic species should select the same quality food except for lactating females. So, non-lactating females and males should be found in mixed-sexed groups, while lactating females select different quality food at least during early lactation when energy demands for females are at maximum. According to activity budget hypothesis, males and females of non-dimorphic social species should be found in mixed-sex groups year round. However, due to higher energy demands lactating females are predicted to spend more time foraging than non-lactating females. From our study area Bongi et al. (2008) actually found out, that roe deer females with fawns selected different habitat type than females without fawns only in the period of giving birth and early lactation.

Monomorphic ruminants in most cases do not sexually segregate (Barboza & Bowyer, 2001), but usually live alone, in monogamous pairs, or in mixed-sex groups. Not many species of ruminants exist in which males and females are monomorphic in body size. Further on, most of them are African species (so facing quite different environmental conditions from temperate zone species, which has probably consequences also for differences in activity rhythms). For instance, territorial antelopes dik-dik (*Madoqua kirkii*) and klipspringer

(*Oreotragus oreotragus*) formed monogamous pairs, while pronghorn (*Antilocapra americana*) and African oryx (*Oryx gazella*) lived in mixed-sex groups (cited from Ruckstuhl & Neuhaus, 2002). According to activity budget hypothesis non-dimorphic ruminants should form mixed groups as digestive capabilities should be similar and hence also activity budgets (Ruckstuhl & Neuhaus, 2002). Activity synchrony helps to keep members of the group or pair in close proximity. It was suggested that a male can only be territorial and have a permanent female or females in his territory, if both sexes are of similar size and therefore have similar activity budgets (Ruckstuhl & Neuhaus, 2002). While under African conditions activity budgets of ungulates African oryx and plains zebra *Equus burchelli* (both occurring in mixed-sex groups throughout the year) have been studied already (Neuhaus & Ruckstuhl, 2002; Ruckstuhl & Neuhaus, 2009; respectively), very little is still known on year-round activity patterns of seasonally territorial species. Roe deer is one of a few size-monomorphic temperate ungulate species. Its low sexual size-dimorphism and seasonal territorialism make it a challenging species for research on activity budgets.

However, when studying activity patterns caution on deducing on proximal causes of activity levels is advised, as additional factors, e.g. environmental, ecologic, population factors can play a decisive role in masking the inner rhythms. That is, expression of the behavioural circadian rhythms (e.g. ruminating cycles) can often be modified in amplitude and phase by additional external factors (e.g. temperature, food availability, predation pressure, social interactions; Scheibe et al., 2009).

Research on activity rhythms of vertebrates and invertebrates, also plants, has been taking place for many decades already (Yerushalmi & Green, 2009; Long et al., 2007; Lincoln et al., 2006; Refinetti, 2006; Bertolucci & Foa, 1998; Boyd, 1998; Georgii, 1981). It has been oriented on one side into defining the molecular cascades of cellular mechanisms and physiological feedbacks in the control loops between outside *zeit-gebers* (*zeit-geber* is any factor that plays a role in setting or resetting an internal biological clock – Loe et al., 2007) and inside processes, and on the other side on specific behavioural responses of an animal to changes of crucial environmental factors under controlled experimental conditions. However, parallel research focused on further two aspects: on the challenge of measuring the behavioural responses of large mammals to certain environmental factors under free-living conditions (du Toit & Yetman, 2005; Loe et al., 2007; Kamler et al., 2007; Georgii & Schröder, 1983) and on developing the methodology for evaluation of deviations of observed behaviour from natural cycles due to disturbed conditions (Berger et al., 2003; Scheibe et al., 1999). Practically both issues remained constrained until the development of radio-telemetry technology. So, it has been only recently that ecological studies put into question also changes in activity patterns of free-living large herbivores and large carnivores, mostly trying to address the questions about reactions and/or adaptations of the population on the environment, which they are living in (Scheibe et al., 2009; Kaczensky et al., 2006; Wronski et al., 2006; Kowalczyk et al., 2003; Theuerkauf et al., 2003; Schmidt, 1999; Cederlund, 1989). However, despite available bibliography on natural rhythms of ungulates from more extreme environments (reindeer – Arctics: van Oort et al., 2007; Loe et al., 2007; moose, roe deer – north boreal forest: Cederlund, 1989; African ruminants: du Toit & Yetman, 2005; bushbuck – Uganda: Wronski et al., 2006; oryx – Namibia: Ruckstuhl & Neuhaus, 2009) and research on behavioural changes in semi-reserve conditions (Wallach et al., 2010; Pépin et al., 2006; Berger et al., 1999; Berger et al., 2002), data on free-living size-monomorphic ungulate from temperate forested habitats are still missing. So, our objective in this study was to define the factors that contribute mostly to variation in activity patterns of roe deer on a daily and annual temporal scale (***Chapter 1***).

In ***Chapter 1*** we thus focused our efforts in studying activity rhythms through investigation of total activity levels in a population of roe deer in forested environment of Apennine Mountains in Italy. We were interested to find out, which factors shape this species' activity patterns in this kind of environment: were the most important inner biological factors, outer environmental factors or maybe anthropogenic factors? We predicted that environmental or anthropogenic factors would be more important in shaping activity patterns of roe deer than sex. Secondly, we were interested in how the activity levels of both sexes varied throughout the year and what we could say in support to studies on African monomorphic species (oryx and zebra; see above). The question was, if seasonality was the crucial factor in shaping activity in the temperate zone and if maybe territoriality introduced some differences in the activity levels between the sexes (in the opposite to year-round stability of environmental and social factors under African conditions).

MATING SYSTEMS AND TERRITORIALITY

The term mating system is commonly defined as the general behavioural strategies adopted by individuals of both sexes in obtaining mates (Clutton-Brock, 1989). It encompasses such features as the number of mates acquired, the manner of mate acquisition, the presence and characteristics of any pair bonds, and the patterns of parental care provided by each sex (Emlen & Oring, 1977). Four different **mating systems** have been distinguished in relation to different patterns of parental care:

- **Monogamy**: a male and a female form a pair bond; favoured mainly when males and females share parental care for their young after birth (or for the eggs). It is a rare case among mammals (e.g. some bats and foxes), but very common in birds.
- **Polygyny**: a male mates with several females, while each female mates with only one male; favoured particularly when females are strongly predisposed to care for their young (for example, because of the constraints of a prolonged period of gestation and lactation). This is the case of most mammals, for which males usually provide little parental care after birth and so females take sole responsibility for the care of the young.
- **Polyandry**: a female mates with several males, while each male mates with only one female; is more infrequent and mainly appears, when the male provides most parental care (e.g. seahorses).
- **Promiscuity**: both male and female mate several times with different individuals so that there is a mixture of polygyny and polyandry; should develop when either sex may care for the eggs or young (e.g. Soay sheep *Ovis aries*).

In mammals females commonly provide most of the parental care (polygyny). The reproductive success of females is mainly limited by access to the resources necessary to breed and to meet the energy requirements of gestation and lactation (e.g., food, breeding sites), whereas the reproductive success of males is limited more by access to females. As a consequence, the distribution of females should depend primarily on resource dispersion (modified by predation pressure and the costs and benefits of associating with other individuals), while males should distribute themselves in relation to female dispersion (modified by male density) in order to gain access to as many mates as possible (Emlen & Oring, 1977). Clutton-Brock (1989) pointed out that variation in mating behaviour of male mammals depended mainly on the defensibility of females. This in turn was directly related to the density and distribution of females, female range size and female group size and stability, and indirectly to the distribution of resources. Hence, males may have two main options to gain access to females: they can either compete for females directly or they can compete indirectly, by anticipating how resources influence female dispersion and competing for resource-rich sites. Emlen and Oring (1977) and Clutton-Brock (1989) have thus proposed the following classification of **polygynous mating systems**, based on the costs and benefits of defensibility or monopolization of mates:

- In **female defence polygyny**, males gain access to females directly by following and defending one or a group of females against rivals.
    - When females live in small groups, males move over large areas searching for, associating with, and trying to mate with females in estrous, i.e. "**roving/tending**" strategy (e.g. bighorn sheep *Ovis canadensis*). This strategy should be favoured when females are spatially and temporally unpredictable (e.g. at low population densities or when food is unpredictable and females range over large areas).
    - When females live in large herds or groups, the most dominant males may attempt to defend and exclusively monopolize relatively stable groups of females, i.e. "**harem-defence**" strategy (e.g. red deer *Cervus elaphus*, feral horse *Equus caballus*).
- In **resource defence polygyny**, males gain access to females indirectly by defending a space, containing critical resources essential to females (e.g. food, birth sites) against other males, and where several females can occupy small ranges. This characterizes the "**territorial**" strategy (e.g. roe deer). This strategy is expected to be favoured when females are spatially and temporally predictable (e.g. when females occupy small ranges containing clumped and high-quality food resources).
- In **male dominance polygyny or lekking polygyny**, males aggregate into groups during the breeding season on a communal display area, where each male defends a tiny mating territory containing no resources at all and attempts to attract and court females, which visit the communal display solely for mating. This characterizes the "**lekking**" strategy (e.g. fallow deer *Dama dama*). Males in this case provide no material resources to females, only genes, and they compete directly for dominant status or position within stable assemblages. This strategy is expected to be favoured

when neither females nor their home range are economically defensible or monopolizable.

Polygyny has been associated with sexual size dimorphism; it resulted in increased sexual dimorphism among ruminants, which in turn affected sexual segregation (Bowyer, 2004). On one hand, a larger body size increases male's ability to compete successfully with other males over access to oestrous females, but on the other brings a cost in terms of higher energy requirements. Dimorphic males should therefore only associate with females when reproducing, but form male groups outside the breeding to follow optimal activity budgets (Ruckstuhl & Neuhaus, 2002). Ruminants that had a harem or tended females during the mating season were more sexually dimorphic in body size than males with monogamous mating systems or with territorial polygynous mating systems (Weckerly, 1998).

Ruckstuhl and Neuhaus (2002) suggested that male ungulates of non-dimorphic species are the only ones that can energetically allow themselves to associate with females all year round. Up to now, activity budgets were researched in those size-monomorphic ungulates, which often had a non-seasonal reproductive cycle, where females could come into oestrus all year round, like the oryx (Ruckstuhl & Neuhaus, 2009) or the zebra (Neuhaus & Ruckstuhl, 2002). In case of occurrence of bachelor groups in such non-dimorphic species, they were explained by male territorial behaviour, as the territorial and dominant males prevent other males from accessing reproductively receptive female groups. However, the roe deer, an ungulate with low sexual size-dimorphism, is territorial during half of the year and lives in mixed-sex groups in the other half of the year. Besides, females do not occur in groups, but live solitarily, likely according to an ideal free distribution (Wahlström & Kjellander, 1995; but see Vanpé et al., 2009). Also, females come into oestrous once a year only, and for a very short time (48 hours). Additionally, due to high stability of territory borders, high fidelity and long tenure (Linnell & Andersen, 1998), male roe deer do not seem to compete intensely for territories and mating in terms of attempts to enlarge their territory or occupy the resource-rich sites, which are attracted by females. Roe deer territoriality thus seems not to correspond to a conventional resource defence polygyny. Actually, a "low cost – low gain" mating tactics for roe deer males was proposed (Linnell & Andersen, 1998; Vanpé et al., 2009).

However, it is necessary to note that there is actually no discrete distinction among the three main types of polygyny, and that a realistic description of the variability of polygynous mating systems is in the sense of a continuum (a continuum from leks and territories towards harems and tending). In fact, Maher and Lott (1995) suggested that territoriality could be viewed as one point along a continuum extending from exclusivity to a system based on completely overlapping ranges and dominance. Additionally, there can be a considerable variation in behaviour within a species (behavioural plasticity), also in mating behaviour, depending on population density, habitat, climate, food resources and predation pressure (see Vanpé, 2007). A mating system shift within a population can thus result from food resource dispersion (which in turn affects female distribution), variations in age distribution, individual body size and age class, and similar. For example, roe deer yearlings and 2-year olds are physiologically already capable of inseminating females, but the territorial system of dominant, older males in majority of cases prevent such copulations. The non-territorial males are thus sometimes forced to use alternative mating tactics, such as finding unattended females through wandering on larger areas.

In ***Chapter 2*** we addressed the questions on male territoriality in roe deer. Mating systems can be understood as one of the main factors influencing the strength, form, and consequences of sexual selection (Vanpé et al., 2008). In support of what is expected from sexual selection theory, there seems to be a close relationship between party size, level of sexual dimorphism, variance in male reproductive success and the opportunity for sexual selection in ungulates (Vanpé et al., 2008). Until recently, very little was known about the opportunity for sexual selection and mating systems in ungulates with low sexual size-dimorphism or in territorial ones (but see Vanpé, 2007). Hereby our aim was to contribute to the knowledge of roe deer territoriality, with reporting for the first time on the interaction of two types of roe deer behaviour during the territorial period. Namely, we simultaneously measured spatial behaviour and activity patterns of roe deer bucks. Our objectives were to confront spatial use (in terms of home range size) and activity levels throughout the territorial period and to investigate, if changes in one behavioural aspect correspond also to changes in the other aspect of behaviour. We also hypothesised on different possible scenarios of temporal pattern of both behavioural types with the progressive territorial period, in order to describe better the temporal variation of spatial use and activity patterns. Moreover, we searched for the patterns, on basis of which we could distinguish between territorial and non-territorial males. Especially we were interested in discovering, if it was possible to distinguish both types of males on the basis of activity patterns, given that up to now such distinction was made on the basis of range use patterns only.

SOCIAL ORGANISATION OF A POPULATION FROM THE GENETICAL POINT OF VIEW

An important component of social organisation is also dispersal, which in turn affects and is affected by demography of separate populations (Linnel et al., 1998). Dispersal is a universal phenomenon that refers to the movement of an organism from its birth place to its first breeding site (natal dispersal) or from one breeding site to another (breeding dispersal; Shields, 1987). Many species of birds and mammals are faithful to their natal and breeding site, but usually one sex is more philopatric and the other one is more dispersal (Greenwood, 1980). In his review on dispersal patterns in birds and mammals, Greenwood (1980) argued that the direction of sex bias in dispersal is primarily a consequence of the type of mating system. Reproductive efforts for increased access to mates and resources and avoidance of inbreeding are important determinants of enhancing sex differences in dispersal. In polygynous mammals, intra-sexual competition is more intensive among males, which compete for females, so females, which provide all the care for the offspring, are the limiting sex (Greenwood, 1980). Females invest heavily in the offspring, so gain most from being philopatric, while males try to maximise their access to females and gain most in being dispersal. Hence males disperse more in search for available mates and male dispersal is a result of the dispersion of females. Such mate defence mating system then results in male dispersal. In the research on polygynous ground-dwelling sciurids it was even evidenced that male-biased dispersal increased with increasing level of sociality, which resulted from an increase in male dispersal rates, whereas female dispersal rates remained constant (Devillard et al., 2004). On the other hand, resource defence system was connected with male competition for resources, female choice, monogamy (so both parents helping to rear young), and female biased dispersal, which is the case in many birds (Greenwood, 1980). Female dispersal is thus a consequence of a mating system, where males partition the resources and only then females choose their mates. However, accurate and systematic data for mammals with resource defence mating system had been missing up to now, so actually there was no clear picture of dispersal patterns in these. It has been only recently, that molecular tools enabled to address this question in case of resource-defence-mating roe deer (see Coulon et al., 2006). Due to highly complex dispersal behaviour in roe deer species molecular approach can generate conclusions enabling deduction on evolutionary forces more precisely than do radio-telemetric data on limited numbers of marked animals (compare Linnell et al., 1998, and Coulon et al., 2006).

Developments in molecular biology and new molecular tools have allowed to document the reproductive characteristics, social organisation, and mating systems of many species. Before, intensive behavioural observations in the field were commonly undertaken for investigating male mating systems and social organisation, on marked and non-marked individuals. However, in case of field observations quantifying certain behaviours has several limitations, also due to taking into account small number of individuals. Then, there are limitations in research of cryptic and/or night mating behaviour and in research on species living in dense habitats. Observations also fail to evaluate the variance of behavioural type and/or reproductive success (Vanpé, 2007). On the other hand, with the development of molecular tools (especially microsatellite markers) and sophisticated analytical advances in population genetics, an alternative and direct approach is available for assessing information on reproductive success, mating systems, and social organisation by analysis on genetic relatedness (parentage) or more detailed by paternity analyses, based on the comparison of parental and offspring multilocus genotypes (see Vanpé, 2007).

Set of behaviours, not only dispersal *per se*, leads to a population structure (Comer et al., 2005). In ***Chapter 3*** we addressed the questions on genetic structure of a roe deer population on a local scale. To assess genetic structure of the population, we performed statistical correlation analyses between genetic and spatial distance measures. Actually, in the opposite of Greenwood’s hypothesis (1980), Coulon et al. (2006) evidenced that in a mosaic landscape in France dispersal in this territorial species with low level of polygyny and low level of sexual size-dimorphism was not female biased, neither on a local nor on a regional scale. On a basis of such a result, the authors suggested, that other selective forces than male mating strategy are probably responsible for the evolution of dispersal in roe deer. On the other hand, we were interested in accessing the social structure of a roe deer population that inhabited continuous, highly forested mountainous habitat of Italian Apennines, as several environmental factors might influence spatial and thus dispersal patterns in the populations (e.g. landscape structure: Coulon et al., 2004; habitat structure: Lamberti et al., 2006; Rossi et al., 2001; population density: Wahlström & Liberg, 1995). We checked for the occurrence of sex-biased dispersal on a local scale and thus of possible sexual differences in distribution of related individuals in space. We predicted that genetic distance among live-captured individuals would be negatively correlated with spatial distance among those individuals.

ROE DEER POPULATIONS IN ANTHROPOGENIC FORESTS

Roe deer populations have increased throughout the Europe since 1980 (Apollonio, 2004). As high-density populations are now widespread, managers, landowners and researchers have been faced with new challenges: monitoring increasing populations, monitoring their impact on vegetation, limiting their impact in the silviculture, protecting economical value of the forests, ensuring sufficient natural regeneration of the forests, preventing damages in agriculture, and ensuring the long-term habitat suitability and sustainability of all ecosystem elements. To achieve such objectives, numerous surveys have been undertaken and many tools have been developed, in order to evaluate the solutions for optimal management and minimising damages from both economic and biodiversity point of view (e.g. Latham, 1999; Gordon et al., 2004; Morellet et al., 2007).

Research on the interactions between ungulates and forest dynamics reported high variations in ungulate impact on vegetation in different environments. Much of the evidence highlighted, that the effects of deer damage, which most commonly included browsing, bark stripping, fraying (but also trampling, defecation and urination) depended on the density of the ungulate population in the area (Danell et al., 2003; Motta, 1996). The impacts, however, did not vary substantially only with deer density, different climate, forest composition, and seasonality, but also with different silvicultural techniques in use and with microhabitat changes on a local spatial scale (such as microclimate and vegetation communities; Bergquist et al., 2003; Jarni et al., 2004). Indeed, a high degree of variability in browsing intensity was reported also among separate sub-sampling areas, which reflected high temporal and spatial variability of biotic and abiotic factors on a local scale (Bergquist et al., 2003; Motta, 1996).

Consequently, on one side appeared the need for precise research of the browsing impacts under different environmental conditions separately, but on the other side a development of widely applicable tools for fast and low-cost long-term monitoring of ungulate-vegetation relationships became urgent, too. Indeed, in ***Chapter 4*** we combined both approaches to investigate relationship between a large herbivore and managed deciduous forest in the area with no hunting. We wanted to research the behavioural plasticity of roe deer in regards to the silvicultural technique in use and more specifically, to tree species present in the study area. Our objective was to evaluate the effects of roe deer browsing on two broadleaved tree species in coppice areas. Coppicing is quite widely applied technique of forest management in our study area (tree stems are repeatedly cut down to near ground level and in subsequent growth years many new sprouts emerge from stools, until the cycle begins again), but there is still lack of knowledge of the consequences of such technique for the roe deer behaviour in this area. On one side we were interested in the precise evaluation of the roe deer impact on vegetative tree regeneration of Turkey oak *Quercus cerris* and chestnut *Castanea sativa* through four-year survey on the re-growth of sprouts from the stools after coppicing. From the other side, we wanted to develop a little-time and -cost consuming approach for rapid representation of browsing damage in the survey area. Such tools are of crucial importance for assessing the browsing damage on larger spatial scales or for monitoring the damage in the long term, when the analytic and quantitative approach is too time consuming. Indeed, Morellet et al. (2001) stressed, that index tools should be useful and indicative, easy to use. Moreover, they are expected to be not only efficient and reliable indicators, but should involve much lower costs than traditional counts, long field observations or laboratory approaches (Morellet et al., 2001). Many methodologies have thus been developed to ease the long-term monitoring of browsing pressure, ungulate diet, and to make it applicable to wide variety of ecosystem characteristics (e.g. Morellet et al., 1999, 2001, 2003, 2007; Mysterud et al., 1999; Saïd et al., 2005).

Monitoring vegetation changes over time in response to deer browsing is in turn useful for managers to index deer abundance (Morellet et al., 2001) and consequently to keep the deer density on acceptable level from anthropogenic point of view and according to the silvicultural objectives and ecosystem characteristics (see Gordon et al., 2004) of the area under investigation. Different types of indexes of browsing pressure have thus been used in research not only to quantify the browsing pressure from the silvicultural point of view, but modifications of browsing indexes have been also developed to monitor the whole individual-population-environment relationship and for implementation in herbivores' management (e.g. Morellet et al., 2003). Moreover, monitoring can be indicative of the suitability of certain types of silvicultural techniques in use (see Bergquist et al., 2003; Jarni et al., 2004).

From this point of view, in our study we turned our attention to potential behavioural changes of roe deer, when coppicing technique had been applied. Namely, during the same period as browsing surveys we investigated also the use of coppice areas by radio-collared roe deer, in order to evaluate, if and how such areas modified spatial behaviour of roe deer. Roe deer is a selective but opportunistic feeder, which diet varies in relation to seasonal variation in plant availability (Cransac et al., 2001; Barančeková et al., 2010). Additionally, the species was proven to adapt to a wide range of environmental conditions (Hewison et al., 1998), so we expected that despite of the disturbance of forest workers the radio-collared roe deer would make use of newly available forage in form of the fallen crown foliage, which accumulated on the forest floor during the forest work.

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Chapter 1

Miha Krofel

ACTIVITY PATTERNS OF ROE DEER IN TEMPERATE FORESTED AREA

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Abstract

Rhythms of activity and rest, of feeding and drinking were proved to originate endogenously, but being susceptive to outside synchronisation. In seasonally changing environments the animals adapted to periodical changes and fluctuations of biologically significant factors in order to maximize the benefits they can derive from limited natural resources. The factors that influence activity rhythms of ungulates, monomorphic in body size, have been researched very sporadically up to now. Accordingly, activity patterns of free-living roe deer (*Capreolus capreolus*) population in forested Apennine area in Central Italy were researched in this study. A sample of 31 radio-collared roe deer was monitored by means of 24-hour long sessions of radio-tracking. According to Linear Mixed Effect model analysis activity patterns of this population resulted to be dependent on light-dark cycle, seasonal variations, and hunting period on roe deer from fixed high seats. Roe deer expressed bimodal activity pattern throughout the year, with two highest peaks of activity during dawn and dusk. Activity of the sexes differed during the territorial period of the species, from spring to summer. During the non-territorial period the activity of sexes did not differ, presumably due to absence of sexual size-dimorphism of the species. During the open hunting period roe deer showed lower activity levels than outside hunting period. Arguably this was a response to higher predation risk and a consequence of lower levels of locomotion due to the choice of closer feeding sites inside the forest cover. The prediction that roe deer would express lower levels of activity during the nights with full moon when compared to moonless nights due to elevated predation risk during the full moon, was not confirmed with our dataset. Our analysis improves otherwise very scarce knowledge of activity patterns of a size-monomorphic ungulate of the northern temperate zone.

Key words: activity rhythms, activity budget, *Capreolus capreolus*, cyclic behaviour, monomorphic ungulate, radio-telemetry, seasonality

Introduction

Circadian rhythms, e.g. 24-hour rhythm of activity and rest, of feeding and drinking etc., were proved to originate endogenously, but being susceptive to outside synchronisation (Yerushalmi & Green, 2009). For instance, one of the most effective and reliable signals in entraining (synchronising) circadian rhythms is the light-dark cycle (Scheibe et al., 2009; van Oort et al., 2007). Further on, they were proved to have an adaptive value (Yerushalmi & Green, 2009; van Oort et al., 2007): for instance, in seasonally changing environments the animals adapted to periodical changes and fluctuations of biologically significant factors in order to maximize the benefits they can derive from limited natural resources. On the other hand, the animals are faced with numerous compromises regarding optimising activity budgets (Loe et al., 2007), which play an important role in balancing individual energy reserves (Pépin et al., 2006). In temperate regions both the periodicity of influencing factors and ecological demands of animals change in respect to seasons and other factors.

Research on ungulate activity up to now revealed a strikingly similar basic daily rhythm of vast number of ungulate species. Specifically, daily pattern of activity was typically bimodal 24-hour rhythm with the highest two peaks at dawn and dusk (red deer: Georgii, 1981; Georgii & Schröder, 1983; roe deer: Cederlund, 1989, Wallach et al., 2010; white-tailed deer: Coulombe et al. 2006; Beier & McCullough, 1990; moose: Cederlund, 1989; bushbuck: Wronski et al., 2006). Such double-peaked daily rhythm is primarily influenced by light-dark cycle. In fact, the cycle of the solar day creates the temporal framework to which plants and animals have adapted through the evolution of 'biological rhythms' (van Oort et al., 2007). Such rhythms, driven by endogenous genetic 'clocks' (e.g. Yerushalmi & Green, 2009), and interplayed by exogenous *zeit-gebers* (i.e. zeit-geber is any factor that plays a role in setting or resetting an internal biological clock; Loe et al., 2007), enable organisms to anticipate and prepare for predictable changes in their environment. For instance, the signal of 24-hour light-dark cycle varies throughout the year in an entirely predicable manner at any given latitude (van Oort et al., 2007). To support this view, an absence of a crepuscular activity pattern was recently reported in Svalbard reindeer, and that was related to the absence of a zeit-geber in this particular environment (van Oort et al., 2005).

Gerkema et al. (1993) suggested, that biological rhythms are related to feeding behaviour, which especially holds true for ruminants, where digestive processes impose cyclic activity patterns with a period shorter than 24 hours. Also Berger et al. (2002) stressed that ultradian rhythms in herbivores are closely related to nutritional physiology. However, in natural conditions expression of the behavioural 24-h rhythm depends on additional external factors, which can modify phase and amplitude of the peripheral processes (i.e. masking; Scheibe et al., 2009). Factors like food availability, predation pressure, stress, climate, reproductive status, disease, competition, social status can modify, shift and also completely change (mask) the internal rhythms. Such reported changes in behaviour often suggest that factors other than maximizing energy intake play a role (Bergman et al. 2001). Moreover, high intra- and inter- individual variability of daily patterns is reported in ungulate research very often (Berger et al. 2002; Scheibe et al., 2009). The interaction between entrained internal rhythms of naturally different phases and secondary masking effects seem to be responsible for this (Scheibe et al., 2009).

Climatic factors such as temperature have been shown to affect and change activity patterns in ruminants (Belovsky, 1981; Beier & McCullough, 1990; du Toit & Yetman, 2005). Maximum daily temperatures had an effect on African oryx (*Oryx gazella*), with animals decreasing their time walking and taking less steps and bites while foraging (Ruckstuhl & Neuhaus, 2009). Animals chose their sheltering and resting places carefully, in order to experience more favourable temperature regimes and reduce heat loss (du Toit & Yetman, 2005) or not risk overheating (Maloney et al., 2005).

Moreover, activity rhythms are modified also by the presence of predators. Antipredator behaviours (vigilance, altered habitat selection, grouping, flight, etc.) reduce risk of predation, but carry costs (Creel et al., 2005). Disturbance by predators often forced ungulates to devote more time spent for vigilant behaviour (elk *Cervus elaphus*: Childress & Lung, 2003) and/or to trade preferred feeding sites for more protective cover within denser, but less-edible vegetation (elk *Cervus elaphus*: Creel et al., 2005). Such optimal balancing the benefits of risk reduction against its costs (Lima, 1998) has also consequences for activity budgets of an animal (Creel et al., 2005). Similar responses as to the predator presence show ungulates to humans during the hunting season. They trade off risk avoidance for food availability in hunted populations (Benhaiem et al., 2008). Shifts in activity were reported, as animals became more vigilant during the hunting season and chose different sites to feed, which consequently interfered with their normal rhythm of nutrition (roe deer: Benhaiem et al., 2008). If animals become more vigilant at the expense of searching for food and its processing, the intake rate might be reduced and long-term costs on fitness could be seen (Lima, 1998). Kamler et al. (2007) even argued that absence of bimodal peaks of activity of red deer in a National Park in Poland was a consequence of absence of human culling, negligible human activities, and presence of large carnivores.

As regards natural predators, their activity rhythms were often reported to adapt to those of prey (Schmidt, 1999), as they tend to adjust their temporal hunting pattern to the times when prey are most vulnerable (Theuerkauf et al., 2003). So, peaks of wolf activity also followed seasonal changes in time of sunrise and sunset (Eggermann et al., 2009; Theuerkauf et al., 2003). In East Poland, the majority of wolf prey was killed in 6 hours around sunrise and in the first four hours after sunset (Theuerkauf et al., 2003). Theuerkauf et al. (2003) argued, that the prey is expected to be most vulnerable to large carnivores' predation in dim light - dawn, dusk, and moonlight nights - as dim light provides the best hunting chances for wolves and prey are more readily available during these periods. Indeed, several studies have reported on the influence of moonlight on the nocturnal activity or the occurrence of certain behavioural patterns in ungulates (see Wronski et al., 2006). For instance, some African ungulates were more active during periods of full moon than during dark nights (Wronski et al., 2006). However, the moon has been actually recognized also as an important factor in reducing activity levels in nocturnal prey species, since at this time they are more obvious to their predators (Sabato et al., 2006). This was researched, however, mostly in case of rodents and insects in connection to rodentivores and insectivores (for the list of bibliography see: Sabato et al., 2006; Wronski et al., 2006). All the studies on rodents and insects/bats have in common the conclusion, that predators reduce their hunting activity under the full moon conditions due to lower availability of prey (see Sabato et al., 2006). In our study we were interested to find out, if roe deer change their activity patterns during moonless nights or night with full moon due to living in a wolf area.

Activity budgets of roe deer have not yet been researched in detail. The topic was usually dealt only with separate investigations on certain aspects of activity patterns of the species, but not described in a comprehensive way of total year-round activity patterns being subjected to different environmental conditions. For instance, Cederlund (1989) described activity patterns in roe deer in connection to time allocated for foraging and ruminating, Wallach et al. (2010) described feeding activity, Benhaiem et al. (2008) investigated the budget of vigilant behaviour under different risk conditions, and Batard (2010) researched the activity in the territory establishment phase.

So, in the light of previously described issues, aims of our study were to describe the year-round activity patterns of a roe deer population in a forested area with a predator presence. We measured the behavioural aspects of free-ranging roe deer population, therefore under fully natural conditions, where animals throughout the year did not suffer any additional stress caused by human interventions (e.g. enclosure keeping, habituation of observers), which is according to Scheibe et al. (1999) a necessary prerequisite for a research like this, but difficult to perform (Signer et al., 2010). We wanted to examine, which of the given sets of variables contributed most to the explanation of observed activity patterns on daily and annual time scale. More specifically, our assumptions were as follows:

1. Roe deer were expected to adjust their daily activity pattern to outside light-dark cycle, like was reported for other ungulates and also for other mammal species. If so, the pattern should also change seasonally, accordingly to annual changes in light-dark cycle. Furthermore, seasonal effect of light-dark cycle on activity would be most likely masked with seasonal temperature variations, so with thermoregulation aspect.
2. Roe deer is a sexually monomorphic species and consequently the sexes were not expected to differ in their activity patterns. However, the annual biological cycle of the species most likely dictates differences between both sexes at least during the reproductive and/or territorial period. Starting from this assumption, some differences in activity patterns between the sexes were expected also in the case of roe deer, even if just for a certain part of the year, presumably during the summer months, when the reproductive period was taking place.
3. As ungulate prey species are supposed to reduce their activity during high predation risk (Lima, 1998), and as literature findings report wolves to have the highest killing success at twilight and moonlight nights, we predicted, that roe deer in our study area would express lower activity levels during full moon nights than during new moon nights.

Material and methods

Study area

The research was conducted in a mountainous and forested habitat of Apennines in Arezzo province, Tuscany region, Central Italy. Wider study area, ca. 120 km^2 in size, was covered mainly in mixed forests, consisting of beech (*Fagus sylvatica*) and silver fir (*Abies alba*) in altitudes higher than 900 m, and of Turkey oak (*Quercus cerris*), chestnut (*Castanea sativa*), black pine (*Pinus nigra*), and Douglas-fir (*Pseudotsuga menziesii*) in altitudes lower than 900 m. A protected area “Oasi Alpe di Catenaia” (OAC) was located within the study area. It covered 28 km^2. The borders of the OAC were not fenced, thus allowing free animal trespassing at any time. The elevation of the area ranged from 330 to 1514 m a.s.l., with peaks within OAC, where snow usually fell from November till April. Two ungulate species were permanently established here at the time of the study, roe deer and wild boar (*Sus scrofa*). The natural predator species in the study area were grey wolf (*Canis lupus*) and red fox (*Vulpes vulpes*).

Hunting management differed inside and outside of the protected area OAC. Inside OAC there was a permanent banning of hunting activities, while outside hunting with hounds (target species wild boar and hares) and hunting from high seats were practiced (target specie roe deer). Open season on roe deer was from the beginning of August till middle September and from middle January till middle March every year (altogether around 14 weeks per year). Instead, from middle September till middle January hunting with hounds on wild boar and hares took place. The rest of the year (from middle March till end of July) hunting of any kind was completely forbidden.

Study animals and data collection

The activity study was carried out on free-living roe deer by means of radio-telemetry. Roe deer were captured in the vertical drop nets during the battue drives in March 2008 and March 2009. In the net captured roe deer were immediately immobilised, blindfolded, measured, fitted with the VHF radio-collars (Televilt, Sweden), and released.

VHF transmitters were equipped with activity sensors, which detected movements in any direction. Any head movement triggered a switch that changed to transmitting faster pulse rate (the pulse rate of a collar, being completely still, was 42 pulses per minute, while of a collar, that was non-stop moving, 62 pulses per minute). The number of pulse-rate changes in a specific time period may be used to index activity of an animal (Coulombe et al., 2006). Field experience with the roe deer species showed, that its pulse rates could change very dynamically and in short time frames. From this reason we listened to the signals of a certain collar until at least 10 pulses of the same rate had been counted continuously. This enabled us to discriminate between roe deer being active (at least 10 fast-rate pulses consecutively) or being inactive (at least 10 slow-rate pulses consecutively) and to avoid recording the roe deer that were in the "intermediate action" at the moment of listening. That is, we tried to avoid the errors that are in the case of variable-pulse sensors associated with head and comfort movements in resting periods or with sensors that fail to detect movements while animals are active, but keep their head still for extended periods (Coulombe et al., 2006). By doing so, we measured "total activity", where "active" behaviour meant not only activity related to different types of locomotion, but also activity related to feeding, nursing, grooming, and other social interactions.

During each monitoring session active or inactive status was attributed to each radio-collared roe deer every 15 minutes, with the sessions lasting for 24 hours. The animals were monitored from one or two high peaks in the study area, using receivers "Wildlife Materials" and directional hand-held antenna. The intensive data collection period started in September 2008 and lasted till March 2010. During this time 41 sessions of 24-hour duration each, were undertaken. On average, 17 animals were monitored per session. Data of 31 animals were included in the final analysis, 17 females and 14 males.

Maxim Dallas semiconductor i-button temperature sensor was used for measuring the air temperature. The sensor was placed 1,5 meter above the ground in white wooden ventilated box without lower surface, which protected the sensor from the direct weather influences. The sensor was programmed to record the temperature every 15 minutes.

Then, 24 hours of each session were grouped into 4 day periods. Dawn and dusk periods were defined as 3-hour lasting periods around sunrise and sunset, respectively. The exact times of sunrise and sunset for each session were assessed through sunrise/sunset calculator (www.hia-iha.nrc-cnrc.gc.ca/sunrise_adv_e.html), providing the standard times of solar rise/set for the Chitignano location: 11°52′ E, 43°39′ N. An hour and half before and after this time were considered as dawn/dusk periods for our analysis. The two periods in-between were night and daylight, whose duration was changing throughout the year in respect to the sun-light cycle.

Moreover, hunting period was a binomial predictor variable, describing whether hunt on roe deer was opened at the time of certain activity session or not. Hunting with hounds took place in autumn only. In order to avoid colinearity with season, we did not consider hunting with hounds as additional variable for the model. Moon was a predictor variable defining the sessions that were undertaken at the day of full or new moon or maximum up to three days before or after. To all other days in the lunar cycle the value "other" was attributed.

Data analysis

Level of activity was expressed as percentage of active records out of all (four) records each hour. In order to detect the effects of abiotic and biotic factors on roe deer daily activity pattern, we analyzed activity percentage data using Linear Mixed Effect (LME) models with restricted maximum likelihood method. For the purpose of fitting an LME model the hourly activity data of each individual were averaged into four periods of the day and this percentage was arcsin square root transformed (dependent variable) in order to satisfy the normality condition. Roe deer identity, repeated through successive sessions and day periods, was considered as a random factor, since animals were monitored for periods of varying length, to avoid pseudo-replication of data (Machlis et al., 1985). Fixed factors with the following levels were considered in the LME: sex (males, females), season (spring: March-May; summer: June-August; autumn: September-November; winter: December-February), day period (night, dawn, day, dusk), age class ("1", "2-5", "≥6" years old), hunting period on roe deer opened (yes, no), moon (full, new, other). Sensor derived air temperature, also averaged into four day periods, was considered as a covariate. The Pearson correlation coefficient was run beforehand to build a correlation matrix among the predictor variables. Categorical variable "Precipitation" that measured, if there was raining or snowing during the session and for how long, was found to be correlated with the "Temperature" variable, so it was omitted from the final analysis. 2-way interactions between variables were also considered (see Table 1 for the list of these).

18 model structures were constructed, trying to shape the best description of influential terms on roe deer activity patterns (Table 1). The model with the lowest value of Akaike Information Criterion (Burnham & Anderson, 2002) was selected as the best one, and for that the parameter estimates were calculated. LME pairwise comparisons without adjustment for

multiple comparisons (LSD method) were performed to test the differences in the activity levels due to fixed factors.

Additionally, as we wanted to test the potential influence of full and new moon on roe deer activity, a new database with night data only was considered. It included for each session and for each animal the average of hourly activity, starting from ca. one hour and half after sunset and finishing ca. one hour and half before the sunrise (see text above for the explanation of day periods duration). So, depended variable was mean percentage of activity during the night, arcsin square root transformed. Another 18 model structures were constructed, trying to shape the best description of influential terms on roe deer night activity patterns (Table 2). Then we used the same statistical approach of fitting the LME models as described above.

All analyses were performed using the SPSS 13.0 program (SPSS inc., 1989-2004). Significance was set at $p \leq 0.05$ in all tests.

Results

Roe deer expressed a 24-hour bimodal activity pattern in all seasons (Fig. 1). The peaks of daily periodic cycles were most pronounced at dusk and dawn, and this pattern persisted throughout the year. The distance between these two highest peaks was increasing in the first half of the year, and decreasing again in the second half of the year, reflecting increasing and decreasing length of the day (Fig. 1).

After fitting 18 LME models, the best one included the following fixed factors: sex, season, day period, and hunting period on roe deer (Tab. 1). Season, day period, and hunting period significantly affected roe deer activity patterns, as well as three out of four 2-way interaction terms: sex*season, sex*day period, and season*day period (Tab. 2).

More into detail, seasonal differences contributed to significant variation in roe deer activity. Activity in autumn (mean ± SE: 34,39 % ± 0,57) was significantly lower than activity in all other seasons (LME pairwise comparisons: spring, summer, and winter, $p < 0,001$ in all cases). Activity in overall did not differ significantly among spring, summer, and winter.

Then, significant influence of interaction term sex*season highlighted the seasonal differences in activity between the two sexes ($p < 0,001$). Males expressed significantly higher activity level in spring than females (males: 42,35 % ± 1,05; females: 35,32 % ± 0,82). Also in summer males (42,19 % ± 0,92) were still noticeable more active than females (36,65 % ± 0,74). On the other hand, sexes did not differ in their activity levels either in autumn or in winter.

Also day periods significantly affected variation in roe deer activity patterns. Activity during the dusk (47,17 % ± 0,68) was the highest among all day periods (significantly higher than activity during the dawn, day, or night; LME pairwise comparisons: $p < 0,001$ in all cases). Secondly, activity during the dawn (39,17 % ± 0,71) was significantly higher than activity during the day and night ($p < 0,001$ in both cases). Finally, activity during the daylight (32,76 % ± 0,43) was significantly higher than activity during the night (30,84 % ± 0,48) ($p = 0,017$), which made the night as the part of the day with the lowest activity levels for the roe deer.

Additionally, there was also significant variation in roe deer activity among day periods in different seasons (Fig. 2). Dusk activity was the day period with the highest activity in all four seasons (spring: 48,30 % ± 1,39; summer: 47,92 % ± 1,31; autumn: 44,36 % ± 1,27; winter: 48,27 % ± 1,47). There was only one exception, that arrived up to the levels of dusk activity, and that was dawn in winter (47,65 % ± 1,59). It was the only one not being lower than dusk activity levels and was significantly higher than dawn activity during spring (36,50 % ± 1,43), during summer (38,21 % ± 1,29), and during autumn (35,48 % ± 1,21). Night activity level was the highest during the summer (34,28 % ± 1,01) and the lowest during the winter (25,46 % ± 0,69), with the difference being significant. On the other hand, daylight activity was significantly lower during autumn (26,78 % ± 0,81) than in other three seasons. In winter, the daylight activity was significantly higher than night activity.

Then, activity of roe deer was significantly higher when hunting on roe deer was closed (38,30 % ± 0,38) than when it was opened (35,70 % ± 0,57; LME pairwise comparisons: p < 0,001).

Night activity data

The Linear Mixed Effect model, that best described the variation in activity of 31 radio-collared roe deer during the night time, did not include the moon influence (Tab. 3). The variables, which contributed significantly to explanation of activity variation during the night, were sex, hunting period, and temperature (Tabs. 3 and 4). Males (33,69 % ± 0,76) were significantly more active during the night than females (28,97 % ± 0,59; LME pairwise comparisons: p = 0,021). Then, during the closed hunting season (32,39 % ± 0,59) the night activity was higher than during the open season (27,41 % ± 0,75; LME pairwise comparisons: p < 0,001). Differently from the previous model, roe deer activity during the night depended also on variation of the outside temperature. Namely, the temperature had a positive effect on the night activity levels (B = 0,050 ± 0,007; p < 0,001).

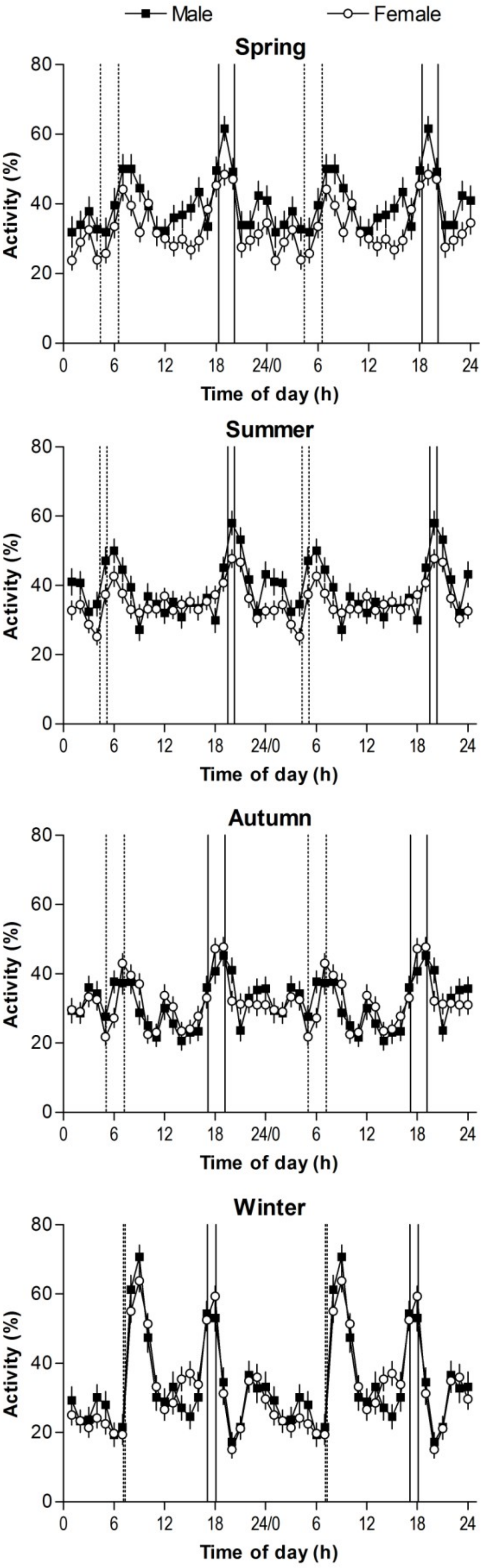

Figure 1: Daily activity pattern of 31 radio-collared roe deer, varying in four seasons (in mean % activity ± SE), in North-East Apennines, Central Italy. Dotted vertical lines represent the dawn interval for each season and solid vertical lines the interval of dusk. The intervals include the sum of the twilight periods in each season. Roe deer showed a 24-hour bimodal activity cycles with two most pronounced peaks at dawn and dusk in each season. Spring: March, April, May; Summer: June, July, August; Autumn: September, October, November; Winter: December, January, February.

Table 1: Model selection on activity data of 31 radio-collared roe deer from Alpe di Catenaia, North-East Apennines, Central Italy. Linear mixed models were fitted to the data, each one with different set of terms included (X). The parameter estimate was run for the model with the lowest value of Akaike Information Criterion (AIC) (model number 1, on the top in bold). For details on the predictor variables see text under Material and Methods. ΔAIC = difference in the AIC value between a given model and the one with the lowest AIC.

Nr. model	***Sex***	***Season***	***Day period***	***Age class***	***Temperature***	***Hunt selection***	***Moon***	***sex*season***	***sex*day period***	***sex*age class***	***sex*temperature***	***sex*hunt selection***	***season*day period***	***season*age class***	***season*temperature***	***season*moon***	***day period*age class***	***day period*temperature***	***day period*moon***	***age class*hunt selection***	***hunt selection*moon***	**AIC**	**Δ AIC**
1	**X**	**X**	**X**			**X**		**X**	**X**			**X**	**X**									**-2253,42**	**0**
2		X	X		X	X							X		X			X				-2250,56	2,86
3	X	X	X		X	X		X	X		X	X	X		X			X				-2234,24	19,18
4	X	X	X					X	X				X									-2233,46	19,97
5	X	X	X		X			X	X		X		X		X			X				-2225,06	28,36
6		X	X			X																-2199,80	53,62
7	X	X	X			X																-2196,31	57,12
8	X	X	X	X		X		X	X	X		X	X	X			X			X		-2188,11	65,32
9		X	X		X																	-2173,95	79,47
10	X	X	X	X	X			X	X	X	X		X	X	X		X	X				-2171,33	82,09
11	X	X	X	X	X	X		X	X	X	X	X	X	X	X		X	X		X		-2170,58	82,85
12	X	X	X	X	X	X	X															-2169,20	84,22
13	X	X	X		X	X	X	X	X		X	X	X		X	X		X	X		X	-2161,61	91,81
14		X	X		X		X															-2159,67	93,75
15	X	X	X	X	X	X	X	X	X		X	X	X		X	X		X	X	X	X	-2136,80	116,62
16	X	X	X	X	X		X	X	X				X	X	X	X	X	X	X			-2114,10	139,33
17	X	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	-2111,01	142,42
18	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	-2092,26	161,17

Table 2: Results of the best LME model, explaining variation in activity patterns of 31 radio-collared roe deer, Alpe di Catenaia, North-East Apennines, Central Italy.

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	29,164	2461,145	**< 0,001**
Season	3	900,001	19,765	**< 0,001**
DayPeriod	3	1952,250	188,574	**< 0,001**
HuntSelection	1	936,729	33,337	**< 0,001**
Sex	1	29,164	1,782	0,192
Season * DayPeriod	9	1976,668	13,561	**< 0,001**
Season * Sex	3	901,500	7,286	**< 0,001**
DayPeriod * Sex	3	1951,425	5,557	**0,001**
HuntSelection * Sex	1	939,637	0,196	0,658

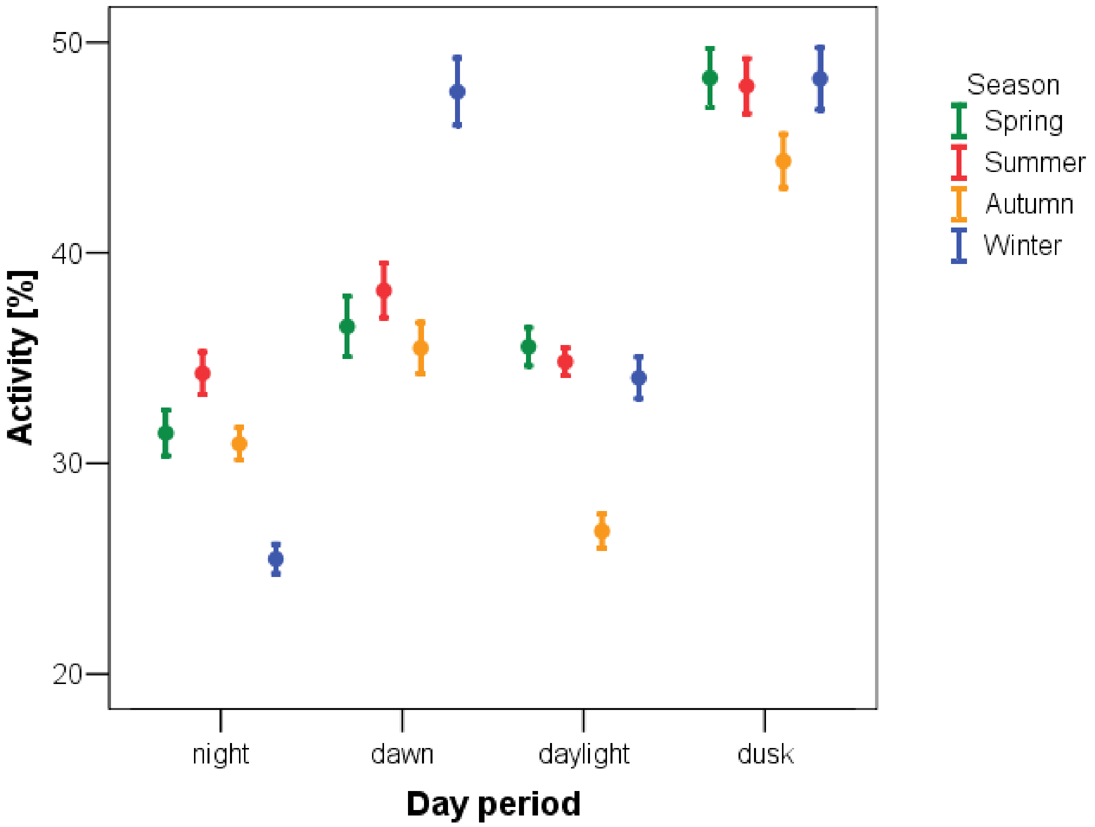

Figure 2: Average activity levels (in %) of 31 radio-collared roe deer during different day periods (night, dawn, daylight, and dusk) among the seasons (spring, summer, autumn, and winter) in Alpe di Catenaia, North-East Apennines, Central Italy.

Table 3: Model selection on night activity data of 31 radio-collared roe deer from Alpe di Catenaia, North-East Apennines, Central Italy. Linear mixed models were fitted to the data, each one with different set of terms included (X). The parameter estimate was run for the model with the lowest value of Akaike Information Criterion (AIC) (model number 1, on the top in bold). For details on the predictor variables and choice on night data see text under Material and Methods. ΔAIC = difference in the AIC value between a given model and the one with the lowest AIC.

Nr. model	***Sex***	***Season***	***Age class***	***Temperature***	***Hunt selection***	***Moon***	***sex*season***	***sex*age class***	***sex*temperature***	***sex*hunt selection***	***sex*moon***	***season*age class***	***season*temperature***	***season*hunt selection***	***season*moon***	***age class*hunt selection***	***age class*moon***	***temperature*moon***	***hunt selection*moon***	**AIC**	**Δ AIC**
1	**X**			**X**	**X**															**-903,24**	**0**
2	X	X		X	X															-884,14	19,10
3	X	X			X															-874,57	28,67
4	X	X	X	X	X	X														-865,44	37,80
5		X		X		X														-864,38	38,86
6		X		X	X								X	X						-860,23	43,01
7	X	X			X		X			X				X						-855,67	47,57
8	X	X					X													-850,21	53,03
9	X	X		X			X		X				X							-846,03	57,21
10	X	X		X	X		X		X	X			X	X						-839,18	64,06
11	X	X	X	X			X	X	X			X	X							-807,57	95,67
12	X	X	X		X		X	X		X		X		X		X				-807,35	95,89
13	X	X	X	X	X		X	X	X	X		X	X	X		X				-791,02	112,22
14	X	X		X	X	X	X		X	X	X		X	X	X			X	X	-786,15	117,09
15	X	X	X	X	X	X	X		X	X	X		X	X	X			X	X	-775,47	127,77
16	X	X	X	X		X	X	X	X		X	X	X		X		X	X		-744,76	158,48
17	X	X	X	X	X	X	X					X	X	X	X	X	X	X	X	-742,34	160,90
18	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	-720,14	183,10

Table 4: Results of the best LME model, explaining variation in activity patterns of 31 radio-collared roe deer during the night only, Alpe di Catenaia, North-East Apennines, Central Italy.

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	95,002	843,245	**< 0,001**
Sex	1	26,786	6,028	**0,021**
HuntSelection	1	318,552	18,832	**< 0,001**
Temperature	1	280,652	52,751	**< 0,001**

Discussion

Our results showed a significant influence of light-dark cycle and seasons on activity patterns of roe deer in temperate forest (*confirmed Prediction 1*). Roe deer showed a polyphasic daily pattern with two main peaks corresponding to varying sunset and sunrise times during the year. A circadian pattern of roe deer included ultradian components, which were likely related to feeding behaviour. Actually, species specific pattern of feed uptake and digestion strongly determines all other activities (Scheibe et al. 1999). Also, due to small body size and consequently less convenient stomach volume/body size relationship (see Mysterud, 1998) the roe deer has less time to ruminate and so needs to forage more frequently than larger cervids (Cederlund, 1989). So, the roe deer, due to its selective feeding strategy and consequently relatively short bouts of rumination, is recognised as a highly active animal (Cederlund, 1989). Ultradian peaks thus likely reflected the rumination process in roe deer.

Following, with the variation of the seasons the daily activity patterns were altered in level and also frequency structure. Firstly, the distance between the two highest daily peaks of activity depended on the annual solar cycle, so on the day length. More precisely, the distance of the dawn peak from the peak of dusk was enlarging through the first half of the year, together with the daylight prolongation, and was shortening in the second half of the year, when the ratio day/night was becoming smaller. Secondly, the lowest activity was recorded during the autumn, immediately after the rutting period of roe deer. Presumably, activity levels dropped after the rutting period so drastically, as the territorial period of the species ended and territorial behaviour of males disappeared (see Johansson & Liberg, 1996). Arguably, in autumn majority of the activity of roe deer corresponded to feeding cycles only and less activity to social interactions.

Dusk was the day period with the highest roe deer activity in all seasons, and it did not differ among the seasons. On the opposite, the other of the two highest peaks, dawn activity, was absolutely the highest in winter. Correspondingly, the night activity in winter was the lowest among all seasons. We expected this result being the consequence of thermoregulation effect during the coldest season of the year. Due to limited movements, animals conserved energy during the winter nights, which are the coldest part of the year. Deer were most likely active less frequently and for shorter time, as even the change of bed sites costs energy. However, the regular feeding bouts that force ruminants to be active in certain intervals were sufficient reason that roe deer started feeding immediately at dawn in winter, after being quite inactive for the night. Also red deer in Poland in winter expressed the lowest activity levels just before sunrise (the coldest part of the day; Kamler et al., 2007). To support this view, modelling of nocturnal activity data only revealed a significant influence of night temperatures on roe deer night activity. The lower the night temperatures the lower was activity level and vice-versa. So, shift of activity into the period of the dawn and daylight during the winter could indeed be adaptation to colder temperatures. Actually, Kilpatric (2003) already provided some evidence for role of thermoregulatory costs in animals' foraging decisions. If the ambient temperatures were below an animal's lower critical value, foraging in sunlight would lower thermoregulatory costs and lead to longer foraging time. So in our case we can speculate that the feeding bout during the dawn in winter lasted for a long time. In case of reindeer a positive effect of temperature on activity levels was reported during the winter also, which meant that reindeer temporarily reduced feeding time when exposed to extremely low temperatures and precipitation in winter (Loe et al., 2007). Moreover, in our study area night activity was highest during the summer, when outside temperatures were more convenient than during other parts of the summer day. With such shift of, most likely, longer and more frequent feeding bouts during the summer night (in addition to elevated levels of social interactions during this season), the peak at dawn in the summer was much less pronounced. In fact, 24-h periodicity was in our study extremely expressed in winter season, and less in other seasons. Similarly was reported for red deer, where the two peaks of activity during dawn and dusk were more pronounced in winter than in summer (Georgii & Schröder, 1983). Anyway, for the roe deer of northern latitudes this was not the case, as Cederlund (1989) reported less evident 24-h periodicity of roe deer in winter than in other seasons.

Activity budget hypothesis proposes that the main force, driving social sexual segregation, is the difference in activity budgets between males and females (Ruckstuhl & Neuhaus, 2002). As smaller females have proportionally smaller rumen than larger males (Bowyer, 2004), they need to forage more often than males, while males will spend more time ruminating or lying than females to digest forage (Ruckstuhl & Neuhaus, 2002). Differences in activity budgets would make it difficult for males and females to stay in mixed-sex groups due to increased costs of synchrony to maintain group cohesion (Ruckstuhl & Neuhaus, 2002). However, Yearsley & Pérez-Barbería (2005) provided evidence that activity budgets alone are insufficient to explain the high degree of sexual segregation observed in the field, though highlighted the trend that seasonal changes in the home range areas can generate sexual segregation, if males and females differ in activity budgets. It has been argued, that the study of activity patterns in monomorphic species would have been much necessary to help uncovering some doubts about the postulated hypotheses on the evolutionary aspect of sexual segregation (Ruckstuhl & Neuhaus, 2009), knowing that monomorphic ruminants do not sexually segregate (Bowyer, 2004) and that sexual dimorphism in body size is a key factor in the evolution of sexual segregation (Mysterud, 2000). Roe deer is one of few species that are monomorphic in body size (Ruckstuhl & Neuhaus, 2009): sexual difference in body mass is below 5% (Yearsley & Pérez-Barbería, 2005). Indeed, our study confirmed a general non-significant influence of sex on variation of roe deer activity in the course of the year; nevertheless, sex was a highly influential term on variation of activity in connection with seasons. Activity between the two sexes differed only during the territorial period of males, i.e. during spring and summer months – during autumn and winter average activity between males and females was not different. We confirmed that the onset of the territorial period could mask a general pattern of activity, where sexes do not differ in their activity patterns to a substantial degree (*confirmed Prediction 2*). Indeed, as roe deer do not lower the forage intake during the rut, additional activity levels to those of feeding activity cycles, could be expected in case of males in spring and summer due to their territorial behaviour (marking, patrolling, defending, searching). Similarly, in the study of sexually size-monomorphic ruminant African oryx, Ruckstuhl and Neuhaus (2009) discovered that male and female oryx did not differ in their activity budgets. Also the study on plains zebra (*Equus burchelli*) showed the same results: similar-sized stallions and mares did not differ in activity budgets (Neuhaus & Ruckstuhl, 2002). However, the seasonal differences between the sexes were not confirmed in case of these two African monomorphic species (oryx: Ruckstuhl & Neuhaus, 2009; zebra: Neuhaus & Ruckstuhl, 2002), where the year-round breeding season might obscure potential seasonal differences in optimal activity budgets (Neuhaus & Ruckstuhl, 2002).

Concluding from our results, different activity rates between males and females in spring and summer were most likely a consequence of male sexual and territorial annual cycle: in spring the period of territory establishment and defence begins, which demands higher daily activity due to territorial behaviour (Johansson & Liberg, 1996). Correspondingly, autumn is the period, when territorial spatial structure is collapsing, males lose their antlers, and winter mixed-groups are starting to appear. At this time sexual differences in activity were not noted anymore. Our results demonstrate that among many factors, affecting social sexual segregation, also seasonal territoriality can modify or condition social segregation by sexes in a non-dimorphic species, as well. Hereby was presented, that sexes differed on the basis of their activity budgets during the period of territoriality, but not during the time of mixed-sex groups. Nevertheless, a more precise study on activity rhythms of both sexes during the territorial period is needed, in order to clarify on the amplitude, direction, and causes or adaptation of such difference.

In our study area the main predator of roe deer is grey wolf. Wolves in SE and E Poland were reported to be crepuscular, being the most active at dusk and dawn (Theuerkauf et al., 2003; Eggermann et al., 2009). As Theuerkauf et al. (2003) suggested that prey is expected to be most vulnerable to wolf predation in dim light (dawn, dusk, and moonlight nights), we were wondering, if night activity levels of roe deer in our study area depended on the moon phase. Speculatively, if there existed an influence of full and new moon on roe deer activity, it could be expressed through shifts in activity patterns during the darkest part of the day, the night. Consecutively, we tested for the differences in night-time activity related to the minimum (new moon) and maximum (full moon) of the phase of the lunar month. Even if there are reports on the influence of moonlight on the nocturnal activity of ruminants (Wronski et al., 2006), we could not confirm any variation in roe deer activity due to the full or new moon (*Prediction 3 not confirmed*). Similarly, also study on bushbuck (*Tragelaphus scriptus*) detected no difference in nocturnal activity rates between night with intense moonlight and night with little or no moonlight (Wronski et al., 2006). They proposed that a reason for that was a strong circadian rhythm with little or no plasticity in that species. However, we would not consider this reason applicable in the case of forest-dwelling roe deer in our study area, as we have previously confirmed quite wide frame of plasticity in its activity patterns in the course of the year. Then, in the study on radio-collared maned wolves (*Chrysocyon brachyurus*) it was discovered that they travelled significantly less on the nights of full moon when compared to the new moon (Sábato et al., 2006). Arguably, maned wolves reduced their nocturnal activity during the nights of the full moon as a consequence of reduced activity of prey (rodents) during the full moon and also due to prey's habitat selection shift during the nights with full moon into covered habitats (Sábato et al., 2006). Presumably in this way maned wolves were economising energy either due to reduced prey availability either due to increased kill efficiency (Sábato et al., 2006). Actually, wolves in East Poland killed 1,8 times more than expected at dawn, dusk, and during moonlight nights, whereas they killed much less than expected during the day (Theuerkauf et al., 2003).

To sum up, it seemed that in our study area the moon did not have a noticeable effect on roe deer activity patterns. Most likely other factors played a more significant role in shaping roe deer activity, and thus different moon phases in such highly forested area like ours did not primarily influence the variation in activity. Obviously, for confirmation of our results and evaluation of lunar importance in temperate forests additional studies based on the night activity all over the year with more precise weather data and also on the activity of predator – wolf, are needed.

Hunted ungulate populations were often reported to change their behaviour as a response to time of open hunting season (e.g. Benhaiem et al., 2008). Actually, roe deer in our case resulted significantly more active outside stalking season than during stalking season on roe deer. Hunters mostly built their high seats on the edge of forest or in clear-cuts or beside open meadows, all of which were perceived like places of high probability for roe deer to forage. Arguably, lowered activity of roe deer during the hunting season was a consequence of trading better feeding sites for safer forest cover. Different responses to perceived higher risk may have important consequences for roe deer energy budgets, if reducing time available for foraging and interfering with selection of forage-rich sites (Benhaiem et al., 2008). Additionally, in our study site hunting with hounds took place in autumn, when the activity of roe deer was the lowest. In the future research, authors will take into account variation in roe deer activity for days with and without hunting in the same season. In this way it will be possible to discriminate effect of seasonality from effect of hunting harassment on roe deer activity rhythms.

However, the risk of predation during the hunting season was not perceived differently by male and female roe deer, which is the opposite finding as for the size-dimorphic elk by Creel et al. (2005). Also the study on Sardinian mouflon revealed that males and non-reproductive females did not differ in their flight response to predation risk, arguably due to low sexual dimorphism of the mouflon (Ciuti et al., 2008). Therefore, one could argue, that predation risk perception by both sexes is connected with the degree of body size-dimorphism of the species. Additionally, another group, namely females with fawns, should be separated from females without fawns for the further research on the activity patterns of roe deer. Namely, being size-monomorphic species, roe deer females with different reproductive status could differ more than the difference in activity between males and females in general is. To support the idea, a study of antipredator behaviour of females with fawns in our study area already revealed different habitat selection by females with fawns and females without fawn, but just during the summer season, when fawns were adopting their hiding strategy (Bongi et al., 2008). Arguably, such difference in spatial use could be reflected also in different activity patterns, as females with fawns express higher overall energy demands due to gestation and lactation. Especially as differences in time budget between lactating and non-lactating female mountain goats *Oreamnos americanus* were already proven (Hamel & Côté, 2008).

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Chapter 2

Miha Krofel

TERRITORIAL BEHAVIOUR OF MALE ROE DEER IN FORESTED HABITAT AS REVEALED FROM TELEMETRY STUDY OF SPATIAL BEHAVIOUR AND ACTIVITY PATTERNS

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Abstract

Territories of male roe deer, which are established during spring and summer months, are likely a resource holding territories in sense of access to females. Territoriality in male roe deer is so an important trait, which conditions both yearly breeding success and spatial structure of the population. During this period territorial behaviours (marking, patrolling and defending territory, aggression) occur, which have influence on the activity patterns of males. The prevailing feeding-resting-feeding rhythm is in this period accompanied by elevated activity due to social interactions. Till now the actual territoriality was described indirectly on the basis of site-specific dominance and patterns of range use. In this study, on the contrary, our aim was to describe the territorial behaviour of 13 radio-collared roe deer males not only on basis of their spatial use, but also in relation with their activity patterns. We investigated, if patterns of spatial behaviour coincided with the activity patterns, modelling bimonthly home range size data and activity data. Among the radio-collared bucks we found spatial indices for describing both, territorial and non-territorial males. We confirmed that a 3-year old buck can be still non-territorial or defend his first territory. Then, yearlings expressed both, dispersal behaviour and a stationary sub-ordinate behaviour. Home range size was proven not to vary in different bimonths, so likely the territories corresponded to the whole home range of a respective male. On the other hand, activity patterns decreased with the proceeding territorial period, indicating the importance of the territory establishment phase for this species. The finding is in accordance with proposed "low risk – low gain" strategy in male roe deer, where males invest relatively little during one mating season, but are dependent on being able to defend a territory over several seasons, thus the low annual investment in mating trades for a multi-year tenure. However, with our dataset we could not confirm a simple connection between activity patterns and home range sizes, the reason being likely an influence of other social, environmental, and population factors that modified this relationship.

Key words: activity rhythms, *Capreolus capreolus*, home range, monomorphic ungulate, seasonality, territoriality

Introduction

Mating systems were suggested to be connected with sex biased juvenile and breeding dispersal, inter-sexual competition for mates, and degree of investment in resources (Greenwood, 1980). In ungulates polygyny is strongly prevalent, but many male mating systems to obtain mates are present (e.g. roving, harem defence, lekking, and territoriality; Owen-Smith, 1977; Clutton-Brock, 1989; Isvaran & Clutton-Brock, 2007). Studies on different groups of mammals have shown that with some exceptions level of sexual dimorphism is commonly linked to the level of polygyny (Alexander et al., 1979). However, sexual size-monomorphic species have received much less attention in the research of the variations in mating system or sexual selection than sexually highly-dimorphic species (Lott, 1991). Also, the territoriality phenomenon in ungulates has still not been well understood, maybe due to location-dependent dominance and the potential role of both phenotypic traits and territory characteristics, as these represent difficulties in expressing predictions on sexual selection (Owen-Smith, 1977). Indeed, the causes, evolution, and function of territoriality in mammals had been well recognised issues in scientific literature in the last decades (Vanpé, 2009b; Liberg et al., 1998; Greenwood, 1980). After diverging opinions, territoriality in male roe deer (*Capreolus capreolus* L.) had nevertheless been recognized as a mating tactics (resource defence polygyny mating system) and territories being resource-holding territories (Wahlström, 1994; Liberg et al., 1998). Territory size, location, and habitat quality can be considered as sexually selected traits in ungulate males (see Andersson, 1994), as these territory characteristics may determine the number of potential mates that a territorial male can monopolise within its area of dominance, and males usually compete strongly to establish and defend the best territories in term of access to females and resources (Vanpé, 2007). The estimate of male reproductive success showed that this is not entirely the case in roe deer males, as they seemed to gain no obvious benefit from defending a better-quality territory in sense of enhanced access to females (Vanpé, 2009b): male’s access to females was conditioned by territory size, but not by its habitat quality, which is not fully in line with the conventional resource defence polygyny strategy. Rather than direct access to females, a territory can enable a male to court and mate without interference due to site-specific dominance (Hoem et al., 2007) – a spatially localised dominance system. Linnell and Andersen (1998) actually proposed a “low risk–low gain” territorial strategy in roe deer with emphasis on high survival and long tenure, where little change of territory borders occurs among the years and males express extremely high site fidelity to their first territory (tenure for consecutive years). Further support for this strategy was provided also by Hoem et al. (2007) with the finding that even in direct fights between two territorial males no territory loss occurred.

Male territoriality in roe deer is a seasonal phenomenon, where territories are established in spring and maintained until the rutting period is over. The onset of the rutting period vary through the geographical range of roe deer distribution with the latitude and longitude, even with the altitude of a singe area, or from year to year (Danilkin & Hewison, 1996). For European populations the territorial period is most often reported to last from March till August (Liberg et al., 1998; Johansson et al., 1995; Johansson & Liberg, 1996), with July and August being the period of reproduction.

Little direct evidence for roe deer territoriality has been published so far, but those studies that were dealing with this issue, commonly used the criteria like “defended, marked area”, “site-specific dominance”, “exclusivity”, “non-tolerance”, “patterns of range use” for distinguishing between territorial and non-territorial animals (Liberg et al., 1998). However, no one has tried to combine such spatial criteria with the evaluation of activity patterns before, with the exception of Batard (2010), who on the other hand investigated spatial behaviour and activity levels of bucks during the period of territory establishment only. Most authors regarded the whole home range during the territorial season as a territory (e.g. Batard, 2010; Johansson & Liberg, 1996), and so did we in present study. Territories can thus support the holder during the territorial period, and non-territorial males, females and their fawns (Liberg et al., 1998). In any case, measures of home range size are the most commonly used animal space use estimators in ecological research (Börger et al., 2006). In several studies an extremely high inter-individual variation in home range sizes of roe deer was reported, also with regards to time scale considered (annual, seasonal, monthly; e.g. Börger et al., 2006; Rossi et al., 2003). On the other hand, in case of every singular individual, a very high stability of roe deer male territories seems to be their general characteristic (Linnell & Andersen, 1998).

Intra-specific variations in mating strategies in ungulates have been already reported. Similarly, also in the case of roe deer not all males are territorial, so there could be recognised both territorial and non-territorial males during spring and summer. In most cases the direct connection is age of males, as empirical evidence shows, that yearlings never become fully territorial, and as first territories are actually established at three or four years of age, depending on the population density (Liberg et al., 1998). Majority of yearlings spent their summer on a territorial area, often overlapping several territories (Danilkin & Hewison, 1996), and occupying larger home ranges, possibly as a consequence of being frequently harassed. They are generally expelled from a territory of an adult and thus show great spatial instability. Then, "subadult" males (2 years of age) were reported as both, sometimes being able to defend their own territories (so being treated as "adults") and sometimes being non-territorial and behaving differently from both yearlings and males of 3 or more years of age (see Liberg et al., 1998). In support of this, genetic evidence revealed that yearly breeding success was still lower for the 2-year olds than for the prime-aged males (Vanpé et al., 2009a). In fact, two different spacing tactics in subadults were recognised, the so called "satellites" and "peripherals". The difference is in the proximity of their range with the centre of territory of an adult male and in the frequency of getting in the conflict situations with the territorial male (see Liberg et al., 1998). Furthermore, aggression of dominant males is sometimes the proximate cause of dispersal in ungulates (Mysterud et al., 2002), also in the case of roe deer (Wahlström, 1994). Wahlström (1994) evidenced, that early matured yearlings, the ones with larger antlers, were harassed stronger by the adult territorial males than peers with smaller antlers (late developers), and therefore were more likely to disperse early. Moreover, males can also lose their territories due to old age, hence restricting their movements to a small area, or becoming nomadic (Liberg et al., 1998; Vanpé et al., 2009b).

Roe deer are active both during the day and night, with their activity peaking at twilight (Pagon et al., in prep., *Chapter 1*; Danilkin & Hewison, 1996; Cederlund, 1989; Wallach et al., 2010). Such bimodal 24-hour rhythm with the highest two peaks at dawn and dusk has been proved for other ungulates, as well (red deer: Georgii & Schröder, 1983; white-tailed deer: Coulombe et al. 2006; moose: Cederlund, 1989; bushbuck: Wronski et al., 2006). Typically, in a course of the day ruminants alternate between feeding and resting behaviours (Cederlund, 1989), so expressing bouts of activity and inactivity. Also Gerkema et al. (1993) suggested, that biological rhythms are often related to feeding behaviour, which especially holds true for herbivore animals, where digestive processes impose cyclic activity patterns with a period shorter than 24 hours. Berger et al. (2002) actually stressed that such ultradian rhythms in herbivores are closely related to nutritional physiology.

Anyway, activity levels vary with the variation of the seasons, also in terms of differences between both sexes (Pagon et al., in prep., *Chapter 1*). The most pronounced seasonal difference in activity patterns was found in spring and summer (corresponding to the territorial period), when males expressed elevated activity levels compared to females; this was not the case in autumn and winter, where no difference in activity between sexes was found.

Arguably, during the territorial and/or reproductive period additional territorial, reproductive behaviours occur in roe deer males, and this expectedly has consequences in elevated levels of total daily activity. For instance, territories are defended through aggression (fights, chases) and marking (rubbing the antlers and forehead against woody or stiff herb stems - i.e. fraying, and scraping the ground with front hooves), which in turn influence the activity patterns of bucks. All male age classes are performing marking behaviour, but it occurs with highest frequency in adult males and with the lowest in yearlings (Johansson & Liberg, 1996). The same authors reported also average densities of scrapes and rubs up to at least 100 per ha of each type. Finally, also barking was reported to be linked to the expression of territoriality among bucks, as enabled to locate and identify them, and to be related to dominance (Reby et al., 1999).

Activity rhythms with connection to spatial movements were described up to now only in dimorphic ungulates with high degree of sexual segregation (e.g. red deer: Georgii & Schröder, 1983; bighorn sheep: Ruckstuhl, 1998). For roe deer, however, data on activity levels in direct connection to territory holding are very scarce (but see Batard, 2010). Activity patterns in roe deer were usually described only in sense of feeding, foraging cycles (Cederlund, 1989; Wallach et al., 2010), but not in connection to territoriality. Our objective was to describe the territorial behaviour of male roe deer on the basis of comparison of their spatial use and activity patterns. As territory size was proven to be one of the most important traits of the roe deer territory (as breeding success depended on that; Vanpé et al., 2009b), we wondered if changes in spatial behaviour are connected to changes in activity levels. We wanted to describe the space use of males in the territorial period, to assess the territorial space structure of radio-collared males, and to describe activity patterns of these males.

Our *first prediction* was, that in our study territories covered the entire home ranges, so home range sizes would neither increase neither decrease for separate individuals once the territory has been established. For example, we did not expect even the reproductive territories to be significantly smaller than territories in previous bimonths, as Vanpé et al. (2009b) demonstrated that breeding success of a male depended on the size of its reproductive territory.

Then we tested the variation of activity through the bimonths. The highest activity levels could be recorded during the reproductive period, when mating takes place, or during the territory establishment phase, when males start occupying their territories. However, as territory marking by males occurred throughout the territorial period and actually peaked in July (Johansson & Liberg, 1996), and as males faced constant intrusions by other (especially non-territorial) males in vicinity throughout the territorial period (Hoem et al., 2007), we predicted, that the activity levels will be the same (high) throughout the territorial period (*Prediction 2*).

Further on, we investigated, if different spatial behaviour corresponds to certain activity levels, so if changes in one type of behaviour condition changes in the other behaviour. As Vanpé et al. (2009b) reported, that territory size was linked to the breeding success, we predicted, that certain spatial profiles of males could be connected to distinctive activity levels among spatial clusters (*Prediction 3*).

The results from ***Chapter 1*** (Pagon et al., in prep.) suggest extremely important synchronisation of roe deer activity rhythms with the light-dark cycle. Thus, we expected males to express a bimodal activity pattern with the highest activity peaks at dusk and dawn also during the territorial period, nonetheless that during this period some modification of activity patterns could be expected due to increased social interactions (*Prediction 4*).

Material and methods

Study area

The research was conducted in a mountainous and forested habitat of Apennines in Arezzo province, Tuscany region, Central Italy (11°54′ E, 43°39′ N; UTM). Study area, ca. 120 km^2 in size, was covered mainly in mixed forests, consisting of beech (*Fagus sylvatica*) and silver fir (*Abies alba*) in altitudes higher than 900 m, and of Turkey oak (*Quercus cerris*), chestnut (*Castanea sativa*), black pine (*Pinus nigra*), and Douglas-fir (*Pseudotsuga menziesii*) in altitudes lower than 900 m. A protected area "Oasi Alpe di Catenaia" (OAC), covering 28 km^2, was located within the study area. The elevation of the area ranged from 330 to 1514 m a.s.l., with peaks within OAC, where snow usually fell from November till April.

Habitat composition and forest management practices differed inside and outside OAC (see Grignolio et al., in press for more details). Outside OAC, deciduous coppice forests (mainly oak, *Quercus spp,* and chestnut) were prevalent (58% of the study area outside OAC) with a harvest frequency of 20 years. These forests were characterised by a high density of young trees, and by rich undergrowth vegetation. On the other hand, high deciduous forests (43%; mainly beech) and conifer forests (13%), characterised by scarce undergrowth vegetation, prevailed inside OAC, where harvest frequency was around 50-70 years. Shrubs occurred inside OAC in 14% of the surface and outside OAC in 9%. Outside OAC also cultivated areas (orchards, vineyards, crop fields, etc.) and urban areas could be found.

Inside OAC two ungulate species were permanently present at the time of the study, roe deer and wild boar (*Sus scrofa*). The natural predator species in the study area were grey wolf (*Canis lupus*) and red fox (*Vulpes vulpes*). Inside OAC there was a permanent banning of hunting activities, while outside hunting with hounds and selective hunting from high seats was practiced.

Data collection

Roe deer were captured in battue drives using vertical drop nets. They were blindfolded, measured, fitted with VHF Televilt (Sweden) radio-collars with activity sensors, and released. Radio-telemetric techniques were used later for tracking them. 13 males were monitored in the territorial period 2009, from March till August. Individual males were attributed an age class each, on a basis of teeth wear age estimation at the capture site. 6 males were six or more years old, 5 males were from 2 to 5 years old, and 2 males were yearlings – one year old.

Firstly, for the purpose of this study we used discontinuous radio-tracking with triangulation method, using receivers "Wildlife Materials" and hand-held Yagi antennas. The location of each collared animal was defined on the basis of three bearings, usually from the distance of 100 to 500 meters. We distributed the locations uniformly during the 24 hours and separated the consecutive locations by an interval of at least 12 hours, in order to avoid temporal and spatial autocorrelation (White & Garrott, 1990).

Secondly, activity sensors in VHF transmitters detected movements in any direction. Any head movement triggered a switch that changed to transmitting faster pulse rate. The number of pulse-rate changes in a specific time period may be used to index activity of an animal (Coulombe et al., 2006). Field experience with the roe deer species showed, that its pulse rates could change very dynamically and in short time frames. From this reason we listened to the signals of a certain collar until at least 10 pulses of the same rate had been counted continuously. This enabled us to discriminate between roe deer being active (at least 10 fast-rate pulses consecutively) or being inactive (at least 10 slow-rate pulses consecutively) and to avoid recording the roe deer that were in the "intermediate action" at the moment of listening. More specifically, we tried to avoid the errors that are in the case of variable-pulse sensors associated with head and comfort movements in resting periods or with sensors that fail to detect movements while animals are active, but keep their head still for extended periods (Coulombe et al., 2006). With such technique we recorded the total activity of the individuals, thus not only activity related to locomotion, but also activity related to other behaviours, such as feeding, grooming, and other social interactions.

During each monitoring session active or inactive status was attributed to each radio-collared roe deer every 15 minutes, with the sessions lasting for 24 hours each. For more details on the methodology of activity data assessment see Pagon et al., in prep. (*Chapter 1*). 15 sessions of activity monitoring were performed in the study period 2009. On average, 8 males were monitored per session.

For the purpose of activity data analysis 24 hours of each session were grouped into 4 day periods. Dawn and dusk periods were defined as 3-hour lasting periods around sunrise and sunset, respectively. The exact times of sunrise and sunset for each session were assessed through sunrise/sunset calculator (www.hia-iha.nrc-cnrc.gc.ca/sunrise_adv_e.html), providing the standard times of solar rise/set for the Chitignano location: 11°52′ E, 43°39′ N. An hour and half before and after this time were considered as dawn/dusk periods for our analysis. The two periods in-between were night and daylight, whose duration was changing throughout the year in respect to the sun-light cycle.

Data analysis

Three bimonthly home ranges of each animal, using Ranges VI software (Kenward et al., 2003) were calculated (March and April, May and June, July and August). The latter home range was the so called reproductive home range. Home ranges were estimated with the 90% Kernel method (Börger et al., 2006), on the basis of 10 to 16 fixes per home range, on average 14. For the purpose of cluster analysis also 50% Kernel home range sizes were calculated. Altogether, 34 bimonthly home ranges were assessed. Overlap between pairs of neighbouring bimonthly territories was also assessed with Ranges VI software. Overlap between home ranges of two males was calculated as overlap of smaller home range over the larger home range. Percentage of vegetation types in each home range was estimated using Arc Map 9.2 software (ESRI Inc., Redlands, USA).

Then, to asses the variation in bimonthly home range size of 13 roe deer males a Linear Mixed Effects (LME) model was fitted to the data of 34 bimonthly home ranges. The dependent variable, 90% Kernel home range size in hectares, was natural log transformed in order to satisfy the normality condition. Roe deer identity, repeated through bimonths, was considered as a random factor, to avoid pseudo-replication of data (Machlis et al., 1985). Two fixed factors with the following levels were considered in the LME: age class (“1”, “2-5”, and “≥6” years old) and bimonths. Percentage of broadleaved forest and percentage of shrubs included in each home range were considered as covariates. Percentage of coniferous forest within the home ranges appeared to be correlated (Spearman correlation: $p<0,05$) with the amount of broadleaved forest, so this variable was omitted from the analysis. Initially, all main effects and 2-way interactions were included in the LME. Subsequently we began with model simplification, removing the least significant terms in a stepwise fashion, starting with the highest-order interactions, and keeping the marginally significant interaction terms that enabled us to produce the appropriate final model.

Following, to be able to connect the spatial patterns of radio-collared males with their activity levels throughout the territorial period, cluster analysis was undertaken. The aim was to group males with similar spatial behaviour. As only for 8 males the complete activity dataset for the whole territorial period was available, we included only these males in the cluster analysis and later in the activity data analysis. Quantitative variables that described the spatial profile of each male and contributed to the evaluation of the most appropriate cluster structure were: home range size 90% Kernel [ha], home range size 50% Kernel [ha], overlap of the preceding bimonthly home range over the successive ones for the same male [%], distance between the centres of the successive bimonthly home ranges for the same male [m], and percentage of different vegetation types included in each home range (three most important ones: deciduous forest, coniferous forest, and shrubs) [%]. Each of these factors consisted of three variables, one for each bimonth. The variables were standardized before the clustering algorithm took place.

To obtain a stable result more techniques were applied. Several outcomes were considered to impose successive techniques and to verify the stability of the final result. The algorithms for data classifications (search of the clusters) were hierarchic agglomerative technique and then k-means method. For the first one the measure of the diversity level between the pairs of data was a matrix of Euclidean distances, which was calculated by following techniques: single linkage, complete linkage, and average linkage. The results were visualised with dendrograms. Separate subsets of home range data were created in order to find the best combination of spatial variables and verify the previous classifications on larger datasets. On the basis of division level among the groups and stability of a certain pattern one technique and one combination of spatial variables were chosen.

Moreover, in order to detect among others also the effects of clusters of different spatial behaviour on roe deer activity patterns, we fitted the LME model also to the data on activity patterns of 8 roe deer males (as only these were attributed a certain cluster based on spatial behaviour). Level of activity was expressed as percentage of active records out of all (four) records each hour. For the purpose of fitting an LME model the hourly activity data of each individual were averaged into four periods of the day and this percentage was arcsin square root transformed (dependent variable) in order to satisfy the normality condition. Roe deer identity, repeated through successive sessions and day periods, was considered as a random factor. Fixed factors with the following levels were considered in the model:

bimonths (March-April, May-June, July-August), day period (night, dawn, daylight, dusk) and cluster category (3 clusters for 8 individuals). After fitting 3 main effects and their interaction terms in the first model, model simplification took place in the same manner as described above. LME pairwise comparisons without adjustment for multiple comparisons (LSD method) were performed to test the differences in the activity levels due to fixed factors.

LME modelling was performed with SPSS 13.0 software (SPSS inc., 1989-2004) and cluster analysis was done in R 2.11.1 software (R Development Core Team, 2009).

Results

The 90% Kernel home range sizes were calculated for 13 radio-collared roe deer males for three bimonths in the territorial period 2009. The largest home range sizes were measured in March-April. After that, in May-June the maximum size of home ranges decreased and average size stabilised. In March-April the average home range size (mean ha ± SE) of roe deer males was 109,7 ± 31,9 ha (n=12, range from 10,8 to 341,8 ha), in May-June 47,1 ± 18,4 ha (n=11, range from 9,2 to 221,0 ha), and in July-August it measured 47,9 ± 14,5 ha (n=11, range from 12,6 to 164,9 ha).

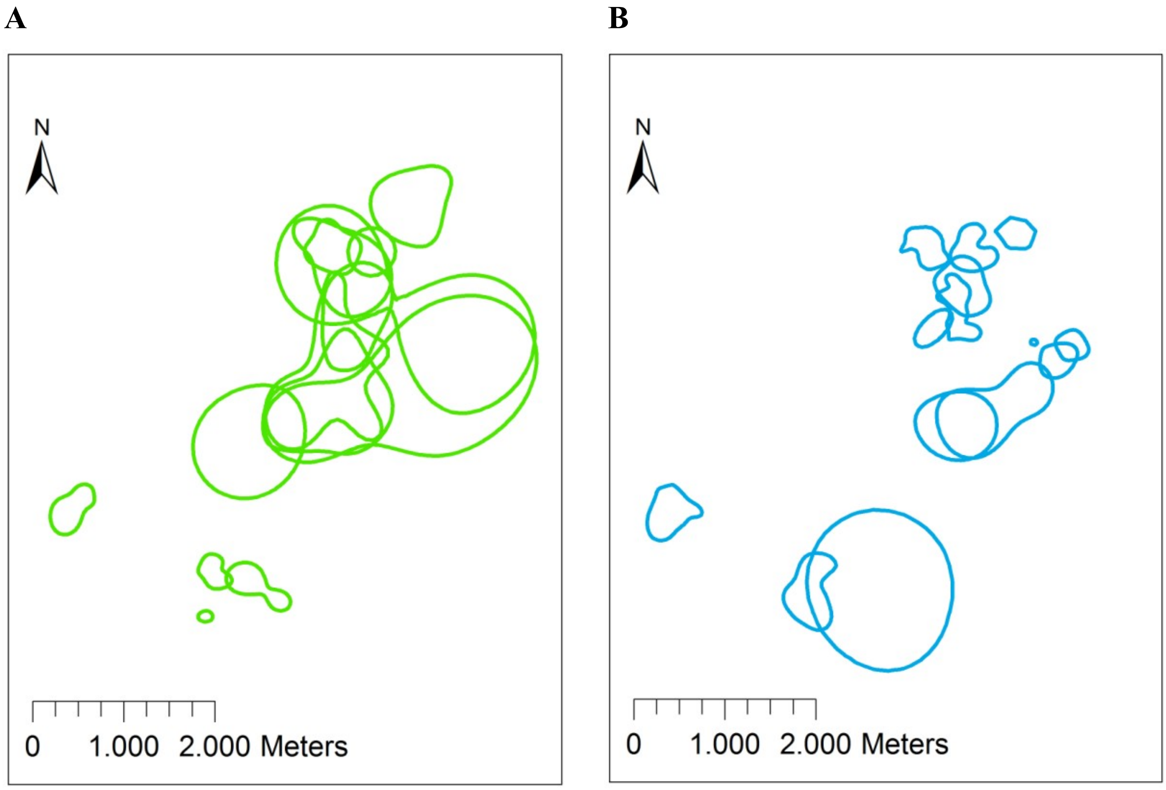
After fitting an LME model to the home range size data (Tab. 1), a significant effect of amount of shrubs on the home range size variation has been revealed ($F_{1;7,2}$=5,810; p=0,046). Additionally, two interaction terms, shrubs*age class ($F_{1;8,0}$=5,836; p=0,042) and shrubs*bimonths ($F_{2;7,1}$=8,362; p=0,014) had a significant influence on home range size variation. Factors that were marginally influencing were also age class and amount of broadleaved forest within a home range, and their interaction: broadleaved forest*age class. However, the model did not detect any differences in home range size variation in the course of the bimonths ($F_{2;9,9}$=0,585; p=0,576; Tab. 1).

Table 1: Results of the LME model, explaining variation in bimonthly home range size (90% Kernel) of 13 radio-collared roe deer males from March till August 2009, Alpe di Catenaia, North-East Apennines, Central Italy.

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	9,893	25,069	**0,001**
Age class	2	12,859	3,206	0,074
Bimonths	2	9,885	0,585	0,576
Broadleaved forest	1	10,144	4,393	0,062
Shrubs	1	7,175	5,810	**0,046**
Age class * Bimonths	4	10,403	2,083	0,155
Broadleaved forest (Age class)	2	12,960	2,861	0,094
Shrubs (Age class)	1	8,039	5,836	**0,042**
Shrubs (Bimonths)	2	7,072	8,362	**0,014**

More into details, amount of shrubs in the home range had a positive effect on the home range size (B=0,542 ± 0,335), so more shrubs there were included in the home range, the larger the home range was. Home range size of roe deer males varied differently also in regards to percentage of shrubs in different bimonths. In the first bimonth (March-April) both large (over 300 ha) and intermediate (between 100 and 300 ha) home ranges included the highest percentages of shrubs (up to 15%). However, in the last bimonth (July-August) even the largest home ranges included low percentage of shrubs (up to 3%), and during May-June, almost none of the home ranges actually included shrubs. Moreover, broadleaved forest had a marginally significant effect in shaping home range size of roe deer males. The higher was the percentage of broadleaved forest in the home range, the smaller was the size of the home range. However, home range size variation did not depend on the temporal aspect of progressive territorial period alone (non-significant influence of variable "bimonths"), neither did on the interaction term age class*bimonths. So, males of different age classes did not change their home range size differently through the bimonths of the territorial period. Both, prime-aged males (age class "2-5" years) and older males (age class "≥6" years old) had the largest home range in March-April and the smallest home range in May-June, whereas during the bimonth July-August the home range size was of intermediate value.

Actual spatial distribution of 13 radio-collared roe deer males is shown on Fig. 1. Actually, majority of them occupied neighbouring areas. 9 adult males, which were monitored throughout the territorial period, expressed a high degree of site fidelity, as all reproductive home ranges could be found in the same area as their March-April home ranges. The average overlap (% mean ± SE) of the preceding bimonthly home ranges over the successive ones for the same male was 69,7 % ± 4,9 (n=27 overlaps, 9 males; range from 8,0 to 100 %). For older males the average overlap for the respective male was 69,6 % ± 5,6 (n=18 overlaps, 6 males; range from 23,0 to 100 %) and the same for the prime-aged males: 69,8 % ± 10,0 (n=9 overlaps, 3 males; range from 8,0 to 100 %). There was only one excursion of an older male out of his usual area noted, as it left its home range area immediately after its recapture in the nets in late March 2009. It stayed 2,4 kilometres away from its successive home range centre, as he returned to his zone after one month, in the last week of April. The temporary shift of part of a home range was believed to be only a reaction due to the recapture stress, as the male was well known from the previous years of radio-tracking. Basically, the first part of its March-April home range overlapped more than 95 % over the successive May-June home range.



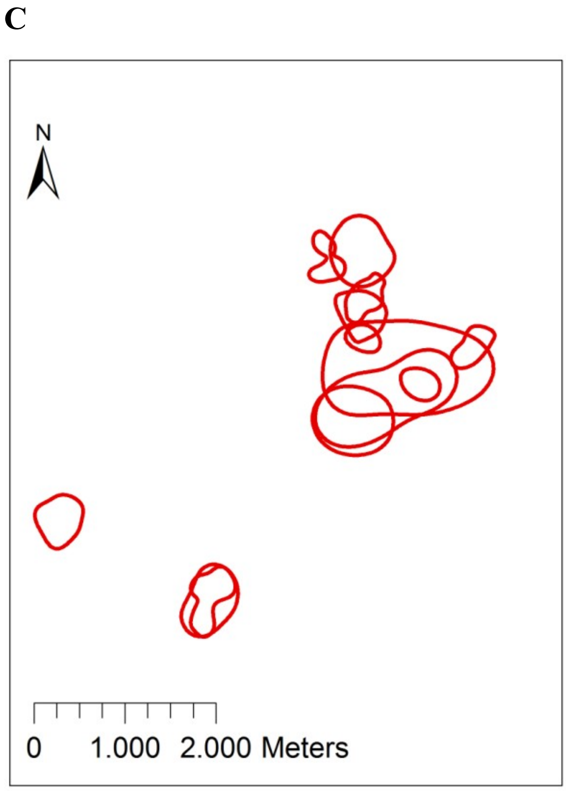


Figure 1: Spatial distribution of 34 bimonthly 90% Kernel home ranges of 13 radio-collared roe deer males, monitored during the territorial period March-August 2009 in forested area Alpe di Catenaia, North-East Apennines, Central Italy. **A**: Bimonthly home ranges of March-April, n=12; **B**: May-June, n=11; **C**: July-August, n=11.

The two yearlings expressed quite different spatial behaviour from one another. One of them expressed extremely stationary spatial behaviour, as it occupied the same and quite small area throughout the territorial period (size of bimonthly HR from 10,8 ha to 31,7 ha). Interestingly, it spent all the territorial period in the reproductive area of another territorial radio-collared prime-aged male. On the other hand, the second yearling abandoned his March-April home range and moved in the second week of May to another area 4,4 kilometres away. Its home range size increased from March-April to May-June from 59,0 to 221,0 ha. In fact, its May-June home range represented the largest home range of this bimonth, being three times larger than the second largest May-June home range.

Decreasing overlap among the neighbours with the progressing territorial period well illustrated male roe deer territoriality (see also Fig. 1). During three bimonths we registered 22 events of neighbouring and overlapping bimonthly home ranges of 8 adult males. For March-April home ranges the average overlap between the neighbours was 18,2 % ± 3,7 (range from 0 to 71,4 %), average overlap of May-June home ranges dropped to 5,7 % ± 2,8 (range from 0 to 48,7 %), and average overlap of July-August home ranges stabilised at 8,8 % ± 3,6 (range from 0 to 56,3) (Fig. 2).

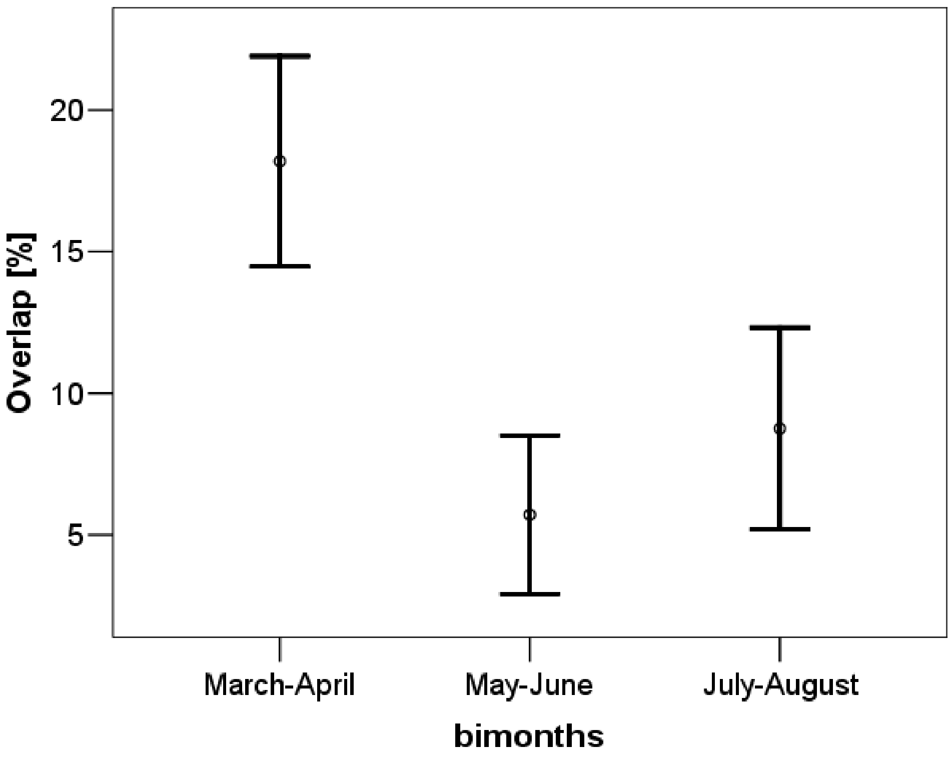


Figure 2: Average overlap (± SE) between pairs of neighbouring bimonthly home ranges (March-April, May-June, July-August) of 8 adult roe deer (≥ 2 years old) during the territorial period 2009 in Alpe di Catenaia, North-East Apennines, Central Italy. Overlap between home ranges of two males was always considered as overlap of smaller home range over the larger home range.

After assessing cluster analyses to different sets of spatial variables it turned out, that two combination sets of measurements for allocating 8 individuals to groups on the basis of their spatial behaviour were the best ones and gave the same outcome. The more parsimonious one was chosen as our final result and it included the combination of six variables, based only on the bimonthly sizes of home ranges. So, three bimonthly 90% Kernel home range sizes and three bimonthly 50% Kernel home range sizes were enough to produce three distinctive and consistent groups of males on the basis of "complete linkage" hierarchic clustering (Fig. 3) and k-means method with k=3, as well. The clustering produced two groups of two males each and one group of four males.

In the first group there were two older males from age class "≥6" years (tagged "440" and "705"; see Fig. 3). For one of them the more precise age estimation was available: it was more than 10 years old. The average home range size for these two males (± SE) was 130,26 ± 3,58 ha in March-April, then 65,30 ± 12,11 ha in May-June, and 88,75 ± 13,94 ha in July-August.

The second group of two males consisted of one prime-aged male of 3 years of age (tagged "745") and one old male of 9 years of age (tagged "500"). Their average home range size in March-April was 220,47 ± 87,22 ha, in May-June 13,43 ± 4,26 ha, and in July-August 27,38 ± 13,73 ha. Unlike the first group these two males hold much smaller home ranges during May-June and July-August. In the remaining group there were one yearling, two older males of ≥6 years old (one of them was 7 years old), and one young male of 3 years of age. This group was characterised by very stable home range holding, as home range sizes were quite small during all three bimonths (March-April: 30,48 ± 13,16 ha; May-June: 24,61 ± 2,85; July-August: 20,08 ± 2,51 ha).

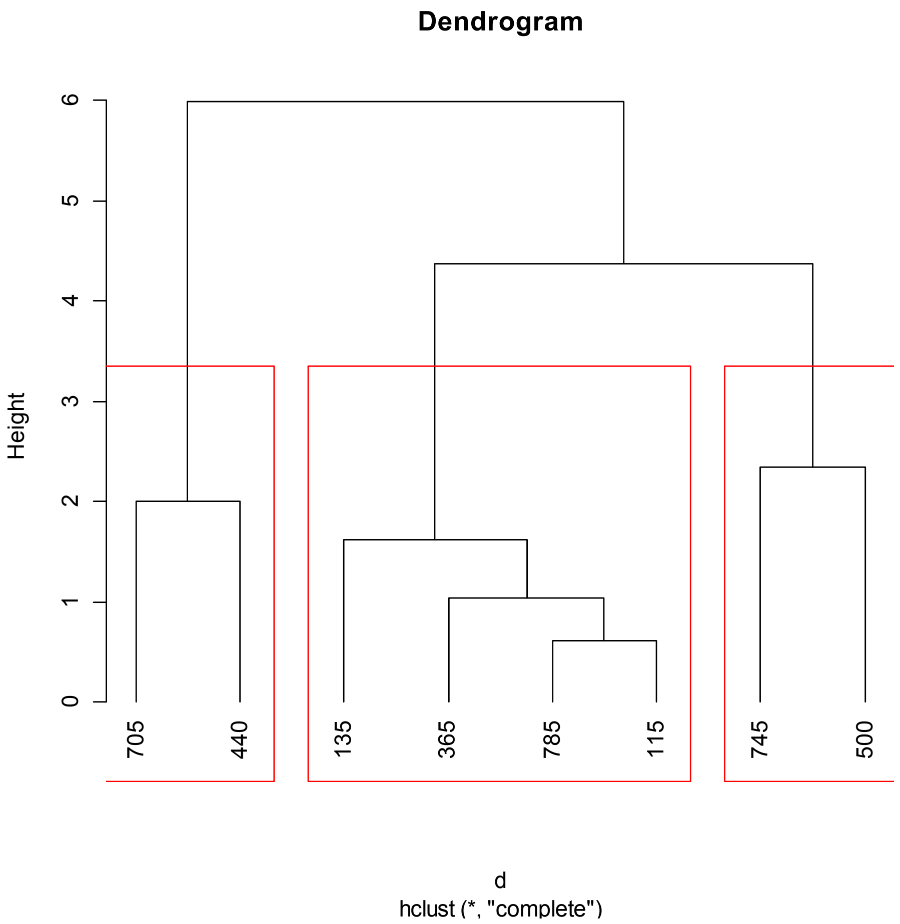


Figure 3: Dendrogram as the outcome of cluster analysis on six spatial variables (see the text for more details) of 8 roe deer males with the "complete linkage" hierarchical clustering. Dataset of roe deer males from March till August 2009, Alpe di Catenaia, North-East Apennines, Central Italy.

Then, LME modelling of activity data revealed a significant effect of bimonths, day period, cluster, and of interaction term day period*cluster on male roe deer activity variation during the territorial period (Tab. 2). However, the interaction term bimonths*cluster did not affect the variation in activity patterns, so no variations in activity among different clusters of spatial use in different bimonths were expected.

Table 2: Results of the LME model, explaining variation in activity patterns of 8 radio-collared roe deer males from March till August 2009, Alpe di Catenaia, North-East Apennines, Central Italy.

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	206,226	11894,600	**< 0,001**
Bimonths	2	119,876	5,626	**0,005**
Day period	3	147,938	40,349	**< 0,001**
Cluster	2	204,986	12,584	**< 0,001**
Bimonths * Cluster	4	117,906	1,128	0,347
Day period * Cluster	6	147,744	3,650	**0,002**

To specify, males were significantly more active during the dusk (52,96 % ± 1,57) than during other day periods (LME pairwise comparisons: p<0,001). Activity during the dawn (43,01 % ± 1,54) was also significantly higher than activity during the night (38,78 % ± 1,21) and daylight (37,00 % ± 0,84; p < 0,001 in both cases). Activity levels during the night were also higher than during the daytime (p = 0,009).

Then, influencing factor on activity levels was also spatial behaviour of males, as the cluster of one young (3 years) and one old (9 years) male (“745, 500”) showed significantly higher activity levels (47,78 % ± 1,69) than the cluster of two older males “440, 705” (40,50 % ± 1,44) or than the third cluster with the rest of the males (42,97 % ± 0,99) (LME pairwise comparisons: p < 0,001). The latter two clusters did not statistically differ in their activity levels (p = 0,73).

Thirdly, also the variable bimonths was shaping variation of males’ activity patterns. Activity levels were the highest in the first bimonth (March-April: 44,00 % ± 1,50) and the lowest in the last bimonth (July-August: 42,11 % ± 1,06), with the difference being significant (LME pairwise comparisons: p = 0,001). The average activity of May-June was on the intermediate level (43,52 % ± 1,25) and not different with neither of the other bimonths (p = 0,088 and p = 0,112, respectively). It turned out, that on the monthly scale the highest levels of roe deer activity took place in April and later decreased in May and June, until the activity levels stabilised and remained on comparable level from June till August.

Moreover, males with different spatial use differed in their daily activity patterns (significant effect of the interaction term day period*cluster). Namely, cluster “745, 500” expressed significantly higher activity levels during the night than other two clusters. Cluster “440, 705” was significantly less active at dawn than other two clusters. During the daylight and dusk the clusters did not statistically differ in their activity levels.

Discussion

During the territorial period we measured two behavioural patterns of roe deer males simultaneously: spatial use and activity levels. Our analysis suggested that there existed no simple relationship between age class of roe deer males, their home range size, and activity patterns.

Both small and large home range sizes could be found in both adult age classes, prime-aged males ("2-5" years) and older males ("≥6" years). Spatial clusters were actually not connected with age classes at all, as apart from the cluster with two old males (most likely non-territorial – see explanation below) the other two clusters included males of different age classes each. In fact, bimonths and variation of behaviour of different age classes within the bimonths had no effect on the home range size variation during the territorial period. Variation in home range sizes was thus not connected to temporal aspect of progressive territorial period and that could be the indirect proof that attributing the whole home range as the actual territory is likely correct (*Prediction 1 confirmed*). Only if there was an obvious decrease in the home range size with time, it might be suggested, that the actual territory was only the breeding site and not the whole home range (Batard, 2010). However, as home range sizes did not change in time significantly, territories might actually cover the entire home ranges. Nevertheless, home ranges in March-April were in average larger than successive home ranges. We suggest this being an indication, that territories were not yet established from the beginning of March on, but likely the establishment phase took place later in that bimonth, for instance in April. Also Rossi et al. (2003) reported that during the winter home ranges of males were larger than in spring, which was connected to their territory establishment phase.

Interestingly, the quantity of shrubs appeared to be an important factor to influence the home range size of roe deer males. Actually, Saïd and Servanty (2005) proved that home range size decreased with increasing amount of (forest) edge. If we speculate that males in our study, which stayed mostly in forested area, had less amount of edge in the home range than males with the shrubby area included, we would expect the latter to actually decrease their range sizes. However, the opposite happened. One has to bear in mind that we measured no trophic quality of the shrubby areas on our study site neither the quantity of edge that they create with certain distribution. However, food resources can shape spatial use to a high degree (Saïd et al., 2009). For a highly productive environment in France Saïd et al. (2009) proved that variation in home range size of female roe deer depended on the spatial variations in habitat quality. Females adjusted the range size to include more patches of habitat (so increased the range size), if they occurred in low quality areas. Actually the direct connection was the amount of food resources, so that all females occupied an area with similar quantity of dry biomass. Being roe deer an income breeder (for females – Andersen et al., 2000) one could expect that males have similar trophic needs. If in our less productive environment males with the shrubby area in the home range didn't satisfy their needs for quantity of available biomass, they might consequently enlarge their home range size.

In contrast to spatial behaviour, activity patterns were found to decrease with the proceeding bimonths (*Prediction 2 not confirmed*). A study of Johansson and Liberg (1996) confirmed that marking behaviour of male roe deer was performed only during the territorial season, from March till August, and was completely absent from September till February. Additionally, Pagon et al., in prep. (*Chapter 1*) provided evidence from the same study area, that activity levels of males were higher than those of females only in spring and summer, i.e. during the territorial period. So, most likely the elevated levels of activity were due to the territorial behaviour of males. The highest activity of males in our study was registered in April. Presumably, the decrease in activity after June on was a consequence of some changes in the context of territorial behaviour, as it is highly unlikely that such changes would occur in the usual feeding-resting rhythm of ungulate ruminants. Namely, roe deer, being an income breeder, does not rely on fat reserves to reproduce or to raise fawns (for females – Andersen et al., 2000), so males would need to regularly restore their energy reserves, as well. Moreover, as the fraying and scraping activities in roe deer were reported not to decrease till August and actually peaked in July (Johansson & Liberg, 1996), we speculate, that such high activity levels, registered in our study in the first bimonth, also still in May, were actually a consequence of higher locomotor activity at that time, and not just of a beginning of marking behaviour. Batard (2010) proposed that such observation likely corresponded to the period of territory establishment and we would agree on this issue as a most probable explanation. In this case, males would concentrate the highest energy expenditure in certain periods only to avoid continuous high-activity states due to the territorial behaviour. This supports the theory of "low cost – low gain" tactic for roe deer by Linnell and Andersen (1998), where males invest relatively little during one mating season, but are dependent on being able to defend a territory over several seasons. In fact, due to the high stability of territory net in the landscape it was suggested that territory boundaries are fixed and even outlast the tenancy of any single individual (Hewison et al., 1998). High fidelity to territorial sites is connected to little costs, as site familiarity increases foraging efficiency, improves predator detection, reduce costs of aggression and combating with neighbours, but the low annual investment in mating trades for such long (multi-year) tenure (Linnell & Andersen, 1998). Indeed, in our study the activity of males during the reproductive period was lower than activity during the territory establishment phase several months before, which is a new information, but supporting the existing theory.

From spatial point of view, all adult males were already in March-April more or less in the area that they would had occupied during the rut. Beside the short distance between the centres of successive home ranges also high overlap among the three home ranges of the same male indicated high site fidelity of adult males throughout the territorial period. None of the monitored adult males notably increased its territory during the successive bimonths, so there was no indication of possible shifts in home range and consequently absence of territory holding. Among the three clusters that we obtained, the most numerous one was characterized by small home range sizes, that did not exceed 70 ha in the first bimonth (March-April) and were never larger than 30 ha in the following two. However, it included all three age classes, namely, one yearling, one 3-year old, and two older males, with the latter having the highest activity level. Despite the difference in activity levels among the age classes, we believe, that all of these males were territorial (except the yearling, being subordinate on the territory of another prime-aged male; see below). Secondly, the cluster consisting of a 3-year old and a 9-year old male was believed to include the territorial males, as well. Their spatial pattern was characterized by a very large home range in the first bimonth (more than 140 ha, even 300 ha for the 3-year old) and a very small home range in the next two bimonths (less than 40 ha). The reason for the older male to have larger home range in March-April was most likely the temporary shift from his usual area due to recapture stress. Later he returned and remained in a well-defined area. The 3-year old also changed his area during first bimonth, but remained in a very restricted area during the following two bimonths (10 ha). It also expressed incredibly high activity levels, actually the highest of all monitored males. The most likely explanation was that this male was territorial, only being late in his territory establishment, probably due to being this his first territory. To support this idea, its activity levels did not decrease after occupying smaller area, but they remained on a very high level. So, most likely it established its first territory and due to not knowing the neighbours and the area, it was forced to remain highly active due to more interactions (Batard, 2010). In support for this, Johansson and Liberg (1996) reported, that first-year-territory holders expressed higher intensity of marking than the neighbours from the previous years, which already knew each-other.

On the other hand, the cluster with two old males, one being around 13 years old already, was characterised with big home range sizes throughout the territorial period (more than 70 ha even the reproductive home range). We believe that these two males failed to defend their own territories, especially because they wandered largely over the reproductive territories of other radio-collared males. In fact, Liberg et al. (1998) reported that old males often become nomadic and do not hold territories anymore, in connection with their failure to maintain large antlers (Vanpé et al., 2009a). Moreover, there was a third 3-year old male in our study (not included into cluster analysis due to lack of complete activity dataset), which obviously wasn't able to defend a territory, too, and expressed similar spatial behaviour like these two nomadic old males. Its home range during July-August was up to 8-times larger than the reproductive territory of other 4 prime-aged males. Probably it had to move on larger area to avoid the territory holders, as it was actually using the area of other radio-collared territorial males. It was reported from several populations (e.g. Batard, 2010) that 2 years old males ("subadults") were mostly not able to defend their territories at that age, and that first-territory holders were usually 3- or 4-years olds. We confirmed that in our study area not all 3-years olds are able to hold a territory yet. Taking into consideration a study of Vanpé et al. (2009a) a high variation in yearly breeding success of 3-year olds might be expected in this population, as well.

Concluding, we strongly suggest, that future analysis of male roe deer territoriality are oriented into more intensive data collection on spatial behaviour and activity patterns (having more and shorter temporal periods of spatial use and activity levels to compare) and these are combined with field observations (frequency and intensity of marking behaviour, aggressive interactions, dominance – subordinate behaviour, mating acts) and/or genetic analysis to obtain spatial distribution of paternities. Following the same individuals over several years in this case would also give an insight on variations in the territorial behaviour.

Despite that spatial behaviour was found to have the effect on activity patterns, the statistical connection was found just for one cluster and more thorough look discovered that mainly due to elevated activity levels of one 3 year old buck. We believe that *Prediciton 3* could be confirmed just partly. For example, older males from one spatial cluster had the same activity level as an old male in the other spatial cluster. Also, a male of 3 years of age from the cluster of large home range during March-April expressed extremely higher overall activity levels than a 3-year old from a cluster with quite small range sizes during the entire period of territoriality. And vice-versa, males with similar spatial use in the largest cluster (4 males) differed in activity levels. To support the idea, different clusters of spatial behaviour did not differ in their activity patterns in different bimonths. So the tendency in the activity patterns through bimonths was apparently not different among the clusters of spatial behaviour. Speculatively, there might exist more variation in the activity patterns inside the clusters than among them as territorial period progressed in time.

We suggest that spatial clusters actually reflected the territorial status of the males rather than being connected directly to activity patterns or age classes. This confirms great inter-specific variability of roe deer species, like was reported already in several other studies (e.g. Scheibe et al., 2009), but also suggests that it would be necessary to increase the sample size in order to obtain more stable and reliable spatial clusters. Moreover, also Mysterud (1999) concluded that males with large home ranges were able to patrol their territories as frequently as those with small ranges. Inter-individual variability in activity during the territorial period could be then connected with several other factors, which were not considered in our study. Marking among other could likely depend on spatial characteristics of the territory (Johansson et al., 1995), age and "type" of the neighbours (yearlings, non-territorials with "sneaky" strategies, first-year neighbours, already known neighbours), and with the variation in marking we would expect also variations in activity levels among males. Then, activity patterns were proved by Pagon et al., in prep. (*Chapter 1*) to be strongly influenced by outside environmental conditions.

To support the idea, the influence of day periods on variation of activity patterns of bucks in this study was remarkable. Males showed a distinctive bimodal daily pattern of activity with the highest activity levels at dusk and dawn (*Prediction 4 confirmed*). Indeed, the light-dark cycle has been recognised as the strongest and the most reliable entraining signal in nature and being so, it is among the most effective ones in entraining (synchronising) circadian rhythms (Scheibe et al., 2009; van Oort et al., 2007). In fact, such synchronisation of activity peaks with dusk and dawn have been widely observed in many other ungulate species (red deer: Georgii & Schröder, 1983; white-tailed deer: Coulombe et al. 2006; moose: Cederlund, 1989). Such circadian rhythm, conditioned by light-dark variations, was already proven to exist throughout the year in this studied roe deer population (Pagon et al., in prep., *Chapter 1*).

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Chapter 3

Miha Krofel

GENETIC RELATEDNESS IN A FOREST ROE DEER POPULATION

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Abstract

The genetic structure of a population is determined by the dispersal, demography, and mating system of a species and is profoundly linked to its social behaviour and population dynamics. In this study we investigated genetic relatedness in a sample of radio-collared roe deer from a population inhabiting forested area in the Italian Apennines. We calculated the Queller and Goodnight relatedness coefficient from genotypes obtained by nine microsatellite loci. Our results suggested that there was no correlation between genetic and spatial distance neither in case of females nor in males. The two sexes showed a similar distribution of genetic relatedness, which contrasted with the hypothesis of sex-biased dispersal in the population. On the contrary, gene flow in the population seems to be high. Both limited geographical scale in this study and the high forest cover could have favoured the observed pattern.

Key words: *Capreolus capreolus*, dispersal, genetic structure, microsatellites, social organization, spatial behaviour, territoriality

Introduction

The genetic structure of a population is determined by the dispersal, demography, and mating system of a species and is profoundly linked to its social behaviour and population dynamics (Shields, 1987). Mating systems were proposed to be the cause of sex bias in dispersal, through the mechanisms of increased access to mates or resources and the avoidance of inbreeding.

Greenwood (1980) described the patterns of sex-biased dispersal among birds and mammals in connection to their mating strategies. In a mate-defence strategy, males compete directly for females, so their dispersion is determined by female distribution, and thus males are expected to disperse more than females (in search of available mates and to avoid inbreeding). The strategy results in female philopatry and in the evolution of matrilineal social organisation (Greenwood, 1980). Being this strategy typical of the majority of mammals, this prediction was widely supported, also by mathematical models (Perrin & Mazalov, 2000; Perrin & Goudet, 2001). For instance, a model for population expansion, termed “rose-petal hypothesis” that considered structure, distribution, and dispersal in the population, was applied to the white-tailed deer (*Oducoileus virginianus*) by Porter et al. (1991). The hypothesis proposed that female white-tailed deer expand as a series of overlapping home ranges, as female offspring establish home ranges, which overlap that of their mother, have low dispersal rates, and are highly philopatric. Such spatial structure of home ranges was referred to as “petals of rose” (Porter et al., 1991). Therefore, this hypothesis predicts a negative correlation between genetic relatedness and spatial distance among white-tailed deer females (Comer et al., 2005). However, Porter et al. (1991) proposed a contrasting model for white-tailed deer behaviour, a “gas diffusion” model, in which individuals disperse from the areas with highest population density to areas with lower density until equilibrium is reached. Such case then leads to little genetic structure and weak relationship between genetic and spatial distances or its total absence (Comer et al., 2005). Finally, while Porter et al. (1991) confirmed “rose-petal hypothesis” in their study population of white-tailed deer, Comer et al. (2005) suggested, that such genetic structure can be modified by other factors, in their case by hunting regimes.

On the other hand, for resource-defence mating strategy Greenwood (1980) predicted that males would be the philopatric sex, as they benefit from local knowledge of the site for acquiring and defending resources, on basis of which females then choose the mates. Thus, males would result being competitive for resources, and females would be the dispersing sex due to female choice of male resources and inbreeding avoidance. However, most of the ungulate species that exhibit resource defence polygyny live in Africa, so up to now there has been a lack of evidence to confirm the predictions for a resource-defence strategy in ungulates living in temperate environments. It was only recently, that Greenwood's predictions were tested on roe deer (*Capreolus capreolus*), a medium-sized cervid with resource-defence polygyny mating system (Liberg et al., 1998; Coulon et al., 2006), but the outcome did not support the prediction on female-biased dispersal (Coulon et al., 2006).

However, social systems may differ also from population to population, as a response to different ecological factors and constraints, and management practices (Lott, 1991). Actually, Moore and Ali (1984) suggested that dispersal could be influenced by competition and Wahlström and Liberg (1995) proposed a model for density dependent sex-specific dispersal in roe deer. At low densities dispersal should be male biased, as females remain philopatric, because costs of dispersal likely exceed benefits, and males are forced to leave due to aggression of adults males. Namely, due to abundant food resources at low densities yearlings become well-developed, and as such are perceived as competitors to adult males and are thus expelled from the area (Wahlström, 1994). At intermediate densities dispersal should be high for both sexes, as now also females disperse due to limitations in food resources. On the opposite, at high densities the model predicts a low dispersal for both sexes, as competition for food is too intense for yearlings to reach the critical weight threshold to cope with the increased energy expenditure of the dispersal (Wahlström & Liberg, 1995).

Behavioural differences affect the social structures in different ungulate species (e.g. white-tailed deer: Comer et al., 2005; wild boar *Sus scrofa*: Iacolina et al., 2009). Social system of a roe deer makes it a unique European cervid from several points of view. For instance, the territorial period lasts for half a year (from March till August; see *Chapter 2*), which means the territories are defended several months before the reproductive period that lasts from mid-July to mid-August. Territorial behaviour of males during the territorial period is strongly linked to hormonal cycles (secretion of testosterone) and antler cycle (the antlers are ossified during the territorial period; Sempere et al., 1992). They defend the territory against intruders with aggression, intolerance, marking and displaying behaviour. However, the dominance is site-specific (Liberg et al., 1998; see *Chapter 2*). Females, on the other hand, are non-territorial. During summer they live solitarily or with their fawns in overlapping home ranges. During the non-territorial period the social organisation of a roe deer population differs, as formation of small matriarchal groups begins in autumn. These, so called family units are a basic social unit during the late autumn and winter, consisting of few adults, their offspring, and some yearlings (Hewison et al., 1998). Family units are often accompanied by one or two bucks (Hewison et al., 1998). In general, both sexes seem to show high fidelity to the home range site either throughout the year either seasonally only (Cargnelutti et al., 2002), and both sexes disperse, as yearlings or as two-year-olds (Linnell et al., 1998). Secondly, recently it has been proven that territoriality of a roe deer does not conform entirely to the features of conventional resource defence polygyny (Vanpé et al., 2009). Vanpé et al. (2009) thus confirmed the hypothesis of Linnell & Andersen (1998) that male territoriality rather conforms to an “always stay” and “low risk – low gain” mating strategy, which differ from territoriality in some other ungulate species, especially African ones. Thirdly and most importantly for our argument, from a study in a mosaic landscape in France it was recently proven, that dispersal was not female biased, neither on a local nor on a regional scale (Coulon et al., 2006). Thus, studying relatedness in a roe deer species can contribute to better knowledge of its genetic and social structure in different environmental conditions.

The purpose of our study was to associate the knowledge of spatial behaviour of radio-collared roe deer to the findings on their genetic structure, in order to get an insight in the social organisation in a forest roe deer population on a local scale. Actually, the species is adapted to a wide variety of habitats, which can imply among others also a wide variation in the social structure and spatial behaviour among roe deer populations (Hewison et al., 1998; see Hewison et al., 2001; Cargnelutti et al., 2002). We might observe different relations between spatial distance and relatedness, as previously reported in Coulon et al. (2006), due to certain outside factors, for instance different environmental conditions, landscape structure (Coulon et al., 2004), habitat structure (Lamberti et al., 2006; Rossi et al., 2001), population structure, and population density (Wahlström & Liberg, 1995).

In this study we combined pairwise spatial distances, obtained from telemetric monitoring of radio-collared individuals, and pairwise relatedness among the same individuals, calculated on the basis of microsatellite genotyping. We wanted to test the occurrence of sex-biased dispersal on a local scale in a roe deer population, occupying a continuous forested habitat in Central Italy. Given that roe deer species exhibits quite different degree of sociality during the territorial and during the non-territorial period, we were interested in examining the socio-spatial structure of the population among different seasons, as well. Thus we predicted that genetic relatedness would be negatively correlated with spatial distance for one or both sexes. Genetic relatedness would be thus higher among females than among males in case of male-biased dispersal and vice-versa in case of female-biased dispersal.

Materials and methods

Study area

The study was carried out in Alpe di Catenaia, a mountainous and forested area situated in the Arezzo Province, Tuscany, Central Italy, with an extension of 120 km^2. A protected area “Oasi Alpe di Catenaia” (OAC), covering 28 km^2, was located within the study area (Fig. 1). The elevation of the area ranged from 330 to 1514 m a.s.l., with medium altitudes from 900-1000 m, and with peaks within OAC, where snow usually fell from November till April. Study area was covered mainly in mixed forests (85% in OAC, 71% outside the OAC), consisting of beech (*Fagus sylvatica*) in altitudes higher than 900 m, and of Turkey oak (*Quercus cerris*), chestnut (*Castanea sativa*), black pine (*Pinus nigra*), silver fir (*Abies alba*) and Douglas-fir (*Pseudotsuga menziesii*) in altitudes lower than 900 m. Deciduous coppice forest, characterized by a high density of young trees and by rich undergrowth vegetation, was prevalent outside the OAC. On the opposite, high deciduous forest and conifer forest with scarce undergrowth vegetation prevailed inside OAC. Shrubs covered 14% of the OAC and 9% of the area outside OAC. Outside OAC also cultivated areas (orchards, vineyards, crop fields) and urban areas could be found.

Ungulates, permanently present in the study area at the time of the study, were roe deer *Capreolus capreolus* and wild boar *Sus scrofa*. The natural predator species were grey wolf *Canis lupus* (Mattioli et al., 2004) and red fox *Vulpes vulpes*. Roe deer was ubiquitous in the study area and its density was assessed every year with battue drive census, resulting in an average density of 31,0 ± 1,9 deer/km^2 for last ten years. Inside the OAC there was a permanent banning of hunting activities, while outside hunting with hounds and hunting from high seats were practiced. During the open season for roe deer, which usually lasted around 14 weeks per year, this species was subjected to hunting with rifles from high seats only.

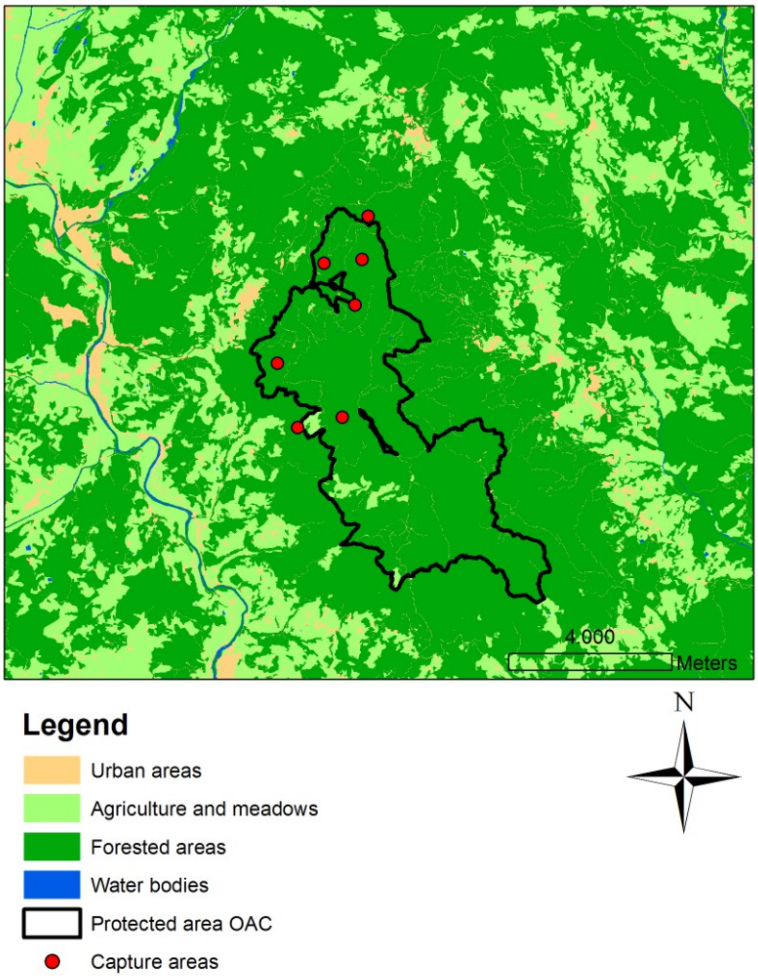


Figure 1: Study area with the protected area "Oasi Alpe di Catenaia" (OAC) in North-East Apennines, Central Italy. The protected area is outlined with black line and forest cover is indicated with dark green colour. Battue areas, where roe deer were captured and fitted with radio-collars, are indicated with red dots.

Spatial data collection and analysis

Roe deer were captured in battue drives using vertical drop nets from 2002 to 2009. Battues were located within or in close proximity to the protected area (Fig. 1). Animals were hand-caught, blindfolded, measured, and a sample of hairs was taken for genetic analysis. They were fitted with VHF Televilt (Sweden) radio-collars and then released. Radio-telemetric techniques were used for monitoring them. For the purpose of this study we used discontinuous radio-tracking with triangulation method, using hand-held receivers "Wildlife

Materials" and directional hand-held Yagi antennas. The location of each radio-collared animal was defined on the basis of three bearings, usually from the distance of 100 to 500 meters. The localisation of the individuals took place 8 to 12 times per month from 2002 till 2010. We distributed the locations uniformly during the 24 hours and separated the consecutive locations by an interval of at least 12 hours, in order to avoid temporal and spatial autocorrelation (White & Garrott, 1990).

Seasonal home ranges of each animal, using Ranges VI software (Kenward et al., 2003), were estimated with the 90% Kernel method (Börger et al., 2006) on the basis of more than 20 fixes per season (24 fixes on average). Seasonal home ranges of 66 individual animals were assessed. Seasons were defined as follows: spring (March, April, May), summer (June, July, August), autumn (September, October, November), and winter (December, January, February). Euclidean distances between the Kernel centres of seasonal home ranges were calculated for each pair of radio-collared roe deer for a given season and a given year.

Genetic analyses

Hair samples for DNA isolation were obtained from 66 roe deer, which had been captured between March 2002 and March 2009 and equipped with radio-collars (see above). DNA was extracted from hairs using Instagene Matrix (Bio-Rad, Hercules, California, USA) and then stored at -20°C. Samples were genotyped with 9 polymorphic microsatellites: Roe1, Roe6, Roe8, Roe9, NVRTH16, NVRTH24, ILSTS011, OarFCB304 and RT (Tab. 1). Each PCR reaction was performed in a 10 µl reaction volume containing 3 µl of DNA, 2 pmol of each primer, 100 µM of each dNTPs, 2.5-3 mM $MgCl_2$, 1 PCR buffer and 0.5 U of Taq DNA polymerase (Euroclone, Siziano, Italy). The PCR conditions consisted of a first cycle of denaturation at 95°C for 3 minutes, 35 cycles at 95°C for 30 seconds, annealing temperature (50-65°C, see Tab. 1) for 30 or 45 seconds, 72°C for 30 seconds, and a final extension at 72°C for 10 minutes. Subsequently, PCR products were checked by electrophoresis on a 2% agarose gel. Allele sizing was performed by capillary electrophoresis on ABI PRISM 3100 automatic sequencer (Applied Biosystems).

Table 1: Microsatellite features and PCR conditions for nine polymorphic loci genotyped for 66 roe deer from Alpe di Catenaia, Italy, captured in drop nets from 2002 to 2009.

Locus	Primer Sequence	Temp. Annealing	Dye	Nr of alleles	Size Range
Roe1	F-AAATTTGGCTCTGCAATCGG R-ACACAAAAGCCACCCAATAC	55-50	6-FAM	4	120-132
Roe8	F-5'-AAGCCGCGCTTGAAGGAG R-5'-ATCAAGCTCCCCTCTTCG	55-50	HEX	8	67-85
Roe9	F-5'-TTGGCGTCATTCCAACAGAG R-5'-TCACAGCAGAATGTCATCTG	55-50	6-FAM	7	161-177
NVHRT16	F-ATTCTAAGCCCAAATAATCTT R-TCTAAGGGGTCTGTGTCTT	60-50	HEX	6	156-170
ILSTS011	F-GCTTGCTACATGGAAAGTGC R-CTAAAATGCAGAGCCCTACC	60-50	6-FAM	6	265-275
Roe6	F-5'-GTTCCTAGCCCAGTGCTC R-5'-TGCAGACCTGGCAGAC	60-50	NED	11	79-109
RT1	F-5'-TGCCTTCTTTCATCCAACAA R-5'-CATCTTCCCATCCTCTTTAC	65-56	FAM	9	223-235
OarFCB304	F-CCCTAGGAGCTTTCAATAAAGAATCGG R-CGCTGCTGTCAACTGGGTCAGGG	65-60	FAM	9	160-174
NVHRT24	F-TGTGGACTATAGGGAGC R-GTGTACAAAAAGTGATTGAGT	55	NED	8	127-145

As concerns genetic data analysis, we used Genetix (Belkhir et al., 2001) to assess the level of genetic variability in the sampled population, through the estimation of observed heterozygosity (H_O) and expected heterozygosity (H_E), the number of alleles for each locus and their frequency in the sample. Moreover, F_{IS} was also calculated for the overall population and its significance tested with permutations.

MicroChecker (Van Oosterhout et al., 2004) was used to evaluate the possible presence of genotyping errors, dropout, or null alleles on the 9 loci, while deviations from the Hardy-Weinberg equilibrium (HWE) and from the linkage equilibrium were evaluated by Genepop 4 (Raymond & Rousset, 1995). The significance of the tests was corrected for multiple comparisons with the Bonferroni sequential procedure (Rice, 1989).

The matrix of pairwise relatedness among sampled individuals was calculated with GenAlEx 6 software (Peakall & Smouse, 2005). Relatedness was estimated using the r_{xy} statistic as coefficient of relatedness (Queller & Goodnight, 1989) that ranges from -1 to 1. Values close to zero indicate low relatedness, i.e. alleles are randomly extracted from an outbred population, whereas relatedness values much higher than zero are expected for pairs that are more related than random pairs in the population. We compared the r_{xy} distributions obtained for female (n=34) and male (n=32) roe deer in the sample, using Student t-test to test the difference.

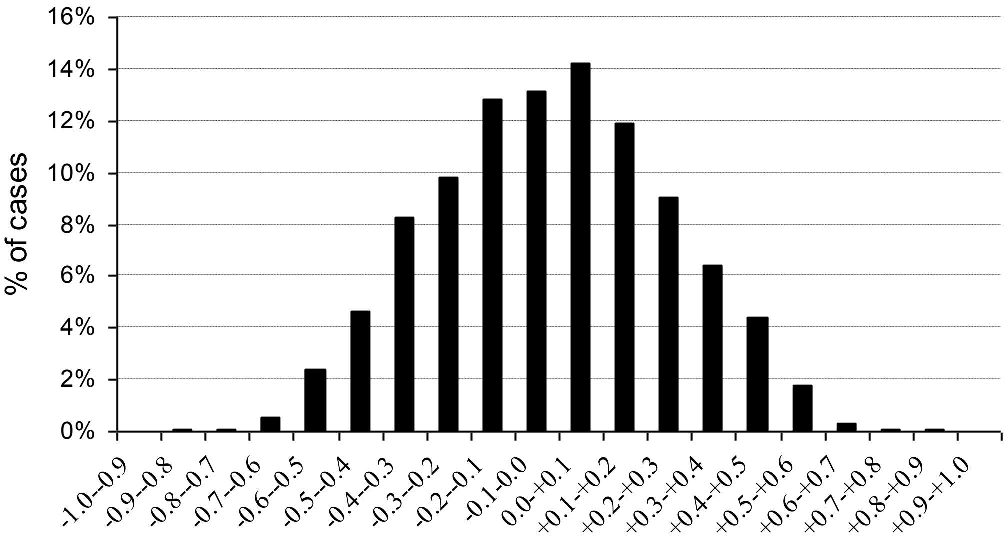
In order to test the hypothesis of a negative correlation between genetic relatedness and geographical distance, we performed a Mantel test (Smouse et al., 1986) with GenAlEx 6. The test compares the correlation between the observed spatial and genetic matrices to correlations obtained by 999 random permutations of the matrix columns and rows (Smouse et al., 1986). Mantel tests were performed on a seasonal basis. Thus for every season a genetic (r_{xy}) matrix and a matrix of Euclidean distances between seasonal home range centroids were computed, both jointly and separately for the two sexes. Only seasons when at least three animals had been monitored, were considered in the analysis.

Results

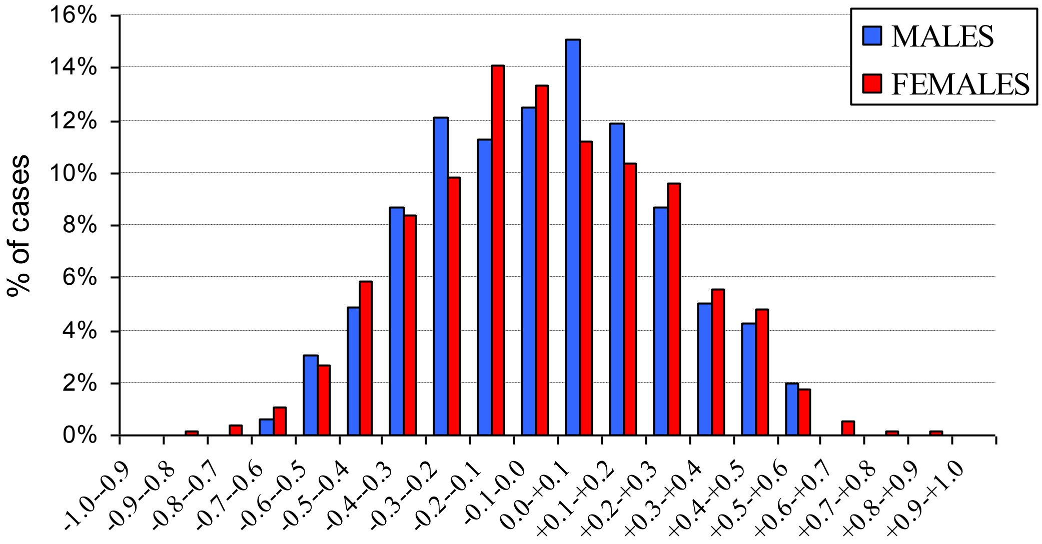
A total of 51 different alleles (3-12 alleles per locus, k=5,7) were found in the population. Average observed heterozygosity (H_O=0,588) and expected heterozygosity (H_E=0,570) were similar. Proportion of missing data was 3,7 %. No evidence of genotyping errors, dropout, or null alleles was found on 9 loci by MicroChecker. The overall F_{IS} in the population was -0,0317 (not significant when tested against permutations in Genetix) and it showed no significant deviation from HWE across loci (χ^2=18,2; d.f.=18; P=0,4398). A single locus showed a significant excess of heterozygotes (Roe08; P=0,0043), while no deficit was found on any microsatellite. Similarly, linkage disequilibrium was not found for any pair of microsatellites, when the significance of the test was corrected for multiple comparisons by the Bonferroni sequential procedure (36 tests, minimum P-value=0,0248). Therefore, the assumption of independence was verified for the microsatellite set in use.

The average relatedness coefficient in the population was very close to zero (-0,015 ± 0,266). The overall distribution of r_{xy} values (Fig. 2a) fitted a normal distribution (Kolmogorov-Smirnov normality test: P=0,200). Distributions for females and males (Fig. 2b) did not differ (two-sample t-test: t<0,001; d.f.=1055; P=1,000; both means equal to -0,0314). Moreover, the proportion of pairs with r_{xy}>0,2 was only slightly higher in females than in males (23 % vs. 20 %).

No significant correlation between relatedness and spatial distance was found in the population (P values >0,05 for all seasons and both sexes). As expected, most correlation coefficients (R) were negative (Fig. 3), but none of them was significant neither in the overall dataset nor in the datasets of two sexes separated, for all seasons. However, higher seasonal variation of R was observed in males than in females (Fig. 3).



a)



b)

Figure 2: Distribution of r_{xy} coefficient of relatedness (Queller & Goodnight, 1989) in 66 roe deer from Alpe di Catenaia, Italy, captured from 2002 to 2009. a) Overall distribution, b) separated distributions for males and females.

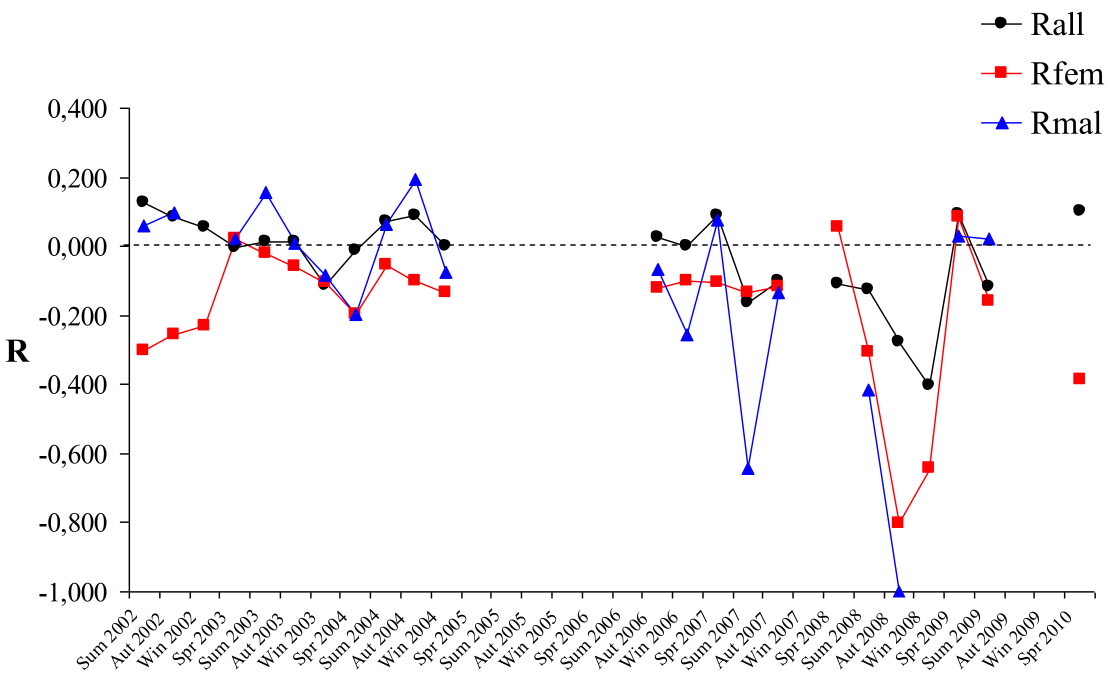


Figure 3: Seasonal variation of Mantel test correlation (R) between genetic relatedness and Euclidean distance among centres of seasonal home ranges of 66 roe deer from Alpe di Catenaia, Italy, monitored from 2002 to 2010. Gaps represent the periods when the number of monitored animals or the number of fixes was below the selected threshold (at least three individuals/season and at least 20 fixes/individual/season).

Discussion

Our results demonstrated the occurrence of a similar pattern of relatedness between male and female roe deer within a forest population in the Italian Apennines. The outcomes did not support the prediction, that closely related roe deer have home ranges in vicinity or overlapping, as no correlation between relatedness and spatial distance among home ranges was found, neither for females nor for males. Consequently, through genetic and spatial distance measures we did not confirm any difference in dispersal patterns between the two sexes.

In this context, our results did not support the hypotheses of sex-biased dispersal. Firstly, there was no support for the hypothesis on female-biased dispersal in resource defence mating system, where females should disperse due to mate choice and to avoid inbreeding (Greenwood, 1980). The results were thus in accordance with the outcomes of Coulon et al. (2006), who proved the absence of a female-biased roe deer dispersal in a mosaic landscape in France. Secondly, also there was no support provided for the concept of "rose petal" genetic structure, which predicted high degree of female philopatry and thus high negative correlation between genetic and spatial distance for females (Porter et al., 1991).

Such result can be a consequence of several influencing factors. Firstly, our study was carried out on a spatially quite limited, local scale, where the data of live-captured roe deer only were included in the analysis. Captures took place within an area of less than 30 km^2. We can thus not exclude the possibility that such local scale of genetic measures was not sufficient to reflect the dispersal patterns in the whole population or to detect the same patterns as an analysis on a wider, regional scale would. However, also a research on roe deer relatedness that was applied on a regional scale (2200 km^2-area), but accounted for local and regional scale separately, proved an absence of a female-biased dispersal on both spatial scales (Coulon et al., 2006). In fact, their results even suggested that the opposite might be true, so a possibility of a slight male-biased dispersal pattern in roe deer was indicated. Moreover, a study on an international scale stressed both, that there was evidence for female philopatry resulting in mitochondrial genetic differentiation and population structures, and that dispersal differences between both sexes are small (Nies et al., 2005). Namely, in this study the authors compared the genetic structure among the populations on the basis of mitochondrial and nuclear DNA. They found out that due to the absence of correlation between the mitochondrial and nuclear distances different patterns of differentiation between the mitochondrial and nuclear genome must had been taking place among the sampled populations. Only mitochondrial distances were significantly correlated with geographic distances (Nies et al., 2005).

Secondly, the variety of dispersal patterns (temporally and spatially, within and between the sexes) actually observed within the roe deer populations was reported to correspond to a variety of environmental conditions and population characteristics (see Linnell et al., 1998). Based on radio-telemetry techniques only minor differences in dispersal rates between sexes have been found, but wide diversity of dispersal rate and patterns across the species' range had been reported (Linnell et al., 1998). For instance, the above mentioned genetic research by Nies et al. (2005) on roe deer populations in Central Europe found philopatry of females and dispersal of males in one population, the opposite situation in another, and found no differences in the remaining seven. Then, Coulon et al. (2004) stressed that for investigating correlations between genetic relatedness and spatial distances, landscape features should be taken into account, as the degree of habitat fragmentation can affect individual dispersal and, consequently, the genetic structure of the whole population. In fact, roe deer population in our study site inhabited continuous forest, so comparisons of the outcomes with other populations, inhabiting a mosaic landscapes, should be made with caution, as dispersal patterns are expected to differ. Additionally, presence of a continuous habitat might favour gene flow in the population and thus explain the lack of genetic structure observed in the study population.

Finally, the model by Wahlström and Liberg (1995) suggested, that dispersal in roe deer depends on population density. According to their model, there should be no sex difference in dispersal rates at intermediate to high densities, which corresponds to our outcomes of this study. The model of Wahlström and Liberg (1995) predicts high dispersal rates for both sexes at intermediate densities and low dispersal for both sexes at high densities. Applying these predictions, we would expect a significant negative correlation between genetic relatedness and spatial distance, if both sexes were philopatric (in case of high density). As we did not obtain such significant correlation, we have no evidence that any of the sexes is philopatric in our study area. However, caution is advised before making such conclusion, as we might have sampled too small proportion of the population to detect the correlation between genetic and spatial measures. Anyway, it seems more likely that both sexes disperse at a sufficient rate to shuffle the genes in the population, and consequently limit the genetic structure of the population.

To sum up, additional analyses on larger spatial scale should be undertaken in this study area, in order to confirm the observed absence of genetic structure on a local scale in the studied Apennine population, and secondly, to account for additional factors that could influence the dispersal patterns, like population density and landscape features.

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Chapter 4

Miha Krofel

ROE DEER (*Capreolus capreolus* L.) BROWSING EFFECTS AND USE OF CHESTNUT AND TURKEY OAK COPPICE AREAS

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Abstract

- Roe deer (*Capreolus capreolus*) browsing pressure on vegetative regeneration of Turkey oak (*Quercus cerris*) and chestnut (*Castanea sativa*) and roe deer use of coppiced areas were investigated.
- In the Apennines, Central Italy, six experimental areas were chosen, where fenced (ungulate access excluded, protected P) and non-fenced (ungulate influence present, non-protected NP) plots were established after coppicing. From 2002 to 2005, each plot was surveyed twice a year, and number, biomass, collar diameter, and total height of the sprouts were measured.
- Roe deer had a different effect on the re-growth of Turkey oak and chestnut sprouts. After four years, chestnut did not show any browsing-related damage, while in Turkey oak biomass and height of the sprouts in fenced plots significantly differed from those in non-fenced plots.
- The results agreed with an experimental browsing index. The outcome is relevant because to assess the effects on a larger scale, a swift and reliable field tool is necessary since analytic and quantitative approaches cannot be applied.
- Monitoring of 62 adult radio-collared roe deer confirmed an increase in the use of coppiced areas. In particular, the utilization index showed a higher use of these areas during and after the coppicing.

Key words: *Castanea sativa*, coppice, deer, forest damages, *Quercus cerris*

Introduction

Over the last few decades, the relationships between wild ungulates and forest ecosystems have been investigated in depth by means of different methods that have focused on the complexity of interactions between forest fauna and forest vegetation (Jorritsma et al., 1999; Reimoser et al., 1999; Sipe and Bazzaz, 2001; Partl et al., 2002; Horsley et al., 2003). This has been a particularly relevant issue since the 1960's in Italy, where, like in other European countries, dramatic social changes in rural as well as mountainous areas have resulted in the progressive abandonment of crop lands, fallows, and hayfields and, consequently, into a marked forest expansion (Bätzing et al., 1996; Höchtl et al., 2005). Over the last two decades, the total surface of forested areas in Italy increased from 8,6 to 10,5 million hectares (INFC 2007). At the same time, clear-cut and coppice methods on large areas were criticised. Therefore, silvicultural systems and practices, shaped according to natural processes and functions within the forest ecosystem and oriented towards a sustainable forest management, became more and more common. In addition, since the 1980's the total surface of protected forested areas in Italy noticeably increased, also on account of the establishment of a considerable number of national as well as regional parks. These processes resulted in the decline of overall wood exploitation and the increase of structural complexity of the stands. Consequently, the productivity and structural complexity of forest ecosystems in Italy consistently improved in a relatively short time.

Among the consequences was creation of favourable conditions for the expansion and reintroductions of many animal species. This was also the case of wild ungulates, which were often regarded as rare species until the 1970's and which population densities increased significantly afterwards (Apollonio, 2004). Recent estimates showed that since 1980 roe deer (*Capreolus capreolus*) and fallow deer (*Dama dama*) populations in Italy increased by 300%, while red deer (*Cervus elaphus*) population increased by 600% (Apollonio, 2004). As a consequence, damage to agricultural crops or forest stands became more and more frequent and the need for forest adjustment and hunting management plans dramatically increased, also on account of the changes in the status and structure of herbivore populations.

This phenomenon was not limited to Italy. Latham (1999) pointed out several cases of high ungulate density in other European regions, where such density was actually found to be much higher than expected under natural or non-human-influenced conditions. Accordingly, the forest damage caused by ungulate browsing was more and more frequently recorded and forest owners began to complain about the negative effects of ungulates on forest vegetation. Therefore, high deer density was argued to be a determinant of a decrease in the long-term habitat suitability (Tremblay et al., 2005) and to indirectly threaten the sustainability of deer population size because of the increasing lack of resources (Caughley, 1970; Forsyth and Caley, 2006). On the other hand, silvicultural practices were found to be unlikely to restore natural regeneration as long as the presence of deer populations at high density were reported (Beguin et al., 2009).

The browsing effects vary to a substantial degree according to deer density (Gill, 1992; Reimoser, 1986), environmental factors such as the climate, and natural resource management such as the silvicultural techniques, which are meant to design the spatial and temporal distribution of resources (Hannan and Whelan, 1989; Motta, 1995; Putman, 1996; Morellet and Guibert, 1999; Mysterud and Ostbye, 1999; Reimoser and Ellenberg, 1999; Morellet et al., 2007). In fact, a high degree of variability in browsing intensity was reported also among separate sub-sampling areas, which reflects high temporal (seasonality) and spatial (microclimate, vegetation communities etc.) variability of biotic and abiotic factors on a local scale (Motta, 1995; Bergquist et al., 2003; Jarni et al., 2004). Therefore, it is advisable that the relationship between browsing pressure and damage is defined for each habitat and management practice, to be able to predict variations in browsing effects due to changes in the demographic characteristics of ungulate populations and/or changes in management practices (Motta, 1995).

Browsing pressure is often dependent also on the characteristics of ungulate populations and interspecific interactions in forest ecosystems (Morellet et al., 2007). The suitability of certain types of European habitats for ungulates and the relationships between ungulates and forests were already investigated in the context of previous research (Ratcliffe, 1992; Kay, 1993; Tabor, 1993; Putman, 1994; Sipe and Bazzaz, 2001; Partl et al., 2002; Horsley et al., 2003). Some studies found out that deer browsing had a strong effect on forest restoration, reducing plant size and changing biomass distribution (Partl et al., 2002; Drexhage and Colin, 2003) and regeneration under different management regimes (Beguin et al., 2009). To estimate such browsing damage is a very complex issue (Canham et al., 1993; Reimoser et al., 1999), which was more frequently explored in relation to conifer species (Welch et al., 1991; Gill, 1992; Mysterud and Østbye, 2004; Heuze et al., 2005), and poorly investigated in relation to broadleaved species and vegetative regeneration in coppice woods (Kay, 1993; Putman, 1994). In addition, the use of clear-cut areas by deer is an important factor for both forest and game management and yet poorly documented.

The aim of this study was to assess the effects of roe deer population on vegetative regeneration of two broadleaved tree species in coppiced areas, in order to gain a better understanding of the interactions between coppice forests and roe deer spatial behaviour in Apennine forest ecosystem. In particular, we analysed the effect of roe deer browsing on vegetative regeneration of Turkey oak (*Quercus cerris*) and chestnut (*Castanea sativa*), two of the most important broadleaved species in Italy, for their environmental as well as economic value, in connection to coppice exploitation. After the coppicing, the effect was surveyed for the period of four years, in order to analyse development of the degree of browsing through time and to compare the regeneration capacity of the two broadleaved species. The effects of browsing were assessed both by means of direct measurements on sprouts' growth and indirect tools represented by rapid estimates of the percentage of damaged sprouts. In fact, the reliability of the indirect method was a key issue in order to assess the effects of deer browsing on a large scale, since analytic and quantitative approach cannot be applied.

In addition, we investigated roe deer use of coppice areas by monitoring a sample of radio-collared roe deer by means of radio-tracking techniques. The aim was to improve the knowledge on roe deer spatial behaviour in the Apennines, which is still poorly understood (Lamberti et al., 2001; Rossi et al., 2001; Rossi et al., 2003; Lamberti et al., 2004), and to evaluate its use of coppiced areas. So, we performed an analysis on roe deer movements in relation to different periods of forest coppicing, uncovering thus how roe deer was influenced by forest works, and consequently by variations in the type and availability of food resources.

Material and methods

Study site

In the Apennines, coniferous and deciduous forest stands are present, with the latter being prevalent. In particular, pure and mixed deciduous forests of beech (*Fagus sylvatica*), chestnut, Turkey oak and downy oak (*Quercus pubescens*) are present in high percentage (INFC 2007). As regards forest management, the coppice system is mostly adopted in private forests, which amount to about two thirds of the total forested area of the Apennines. Coppice areas are mainly concentrated in the lower mountain vegetation belt, where oaks and chestnut are the most common tree species. On the contrary, the high forest system prevails in public areas, which are concentrated in the upper mountain belt and dominated by beech forests. These environments are very important to ungulates (Jedrzejewska and Jedrzejewsky, 1998) and represent most suitable habitats to roe deer and wild boar (*Sus scrofa*), whose population densities are the highest among ungulate species in Italy (Apollonio, 2004).

The study was carried out in a mountainous area in the province of Arezzo (Tuscany, Italy, 43°48’N, 11°49’E). The site was a protected area “Oasi Alpe di Catenaia” (OAC). Altitude in the area ranged from 330 to 1514 m a.s.l. The climate was temperate, with hot and dry summers, and cold and rainy winters. The mean annual rainfall was 1224 mm and the mean annual temperature was 9,5°C. Eighty-four percent of OAC area was covered by forests, mainly mixed deciduous woods, where the coppice management system was still in use. The main tree species were chestnut, Turkey oak, and beech. Forest stands were managed by the Regional Forest Service (“Comunità Montana del Casentino”) according to a forest management plan. The maintenance of the coppice system for obtaining both fire wood and timber wood was allowed in prescribed areas only, mainly concentrated at lower altitudes, where Turkey oak and chestnut prevailed. The ungulate community consisted of roe deer and wild boar. Density of roe deer population was estimated every year, from April to June, with drive census (Tab. I). Hunting was strictly forbidden inside OAC, but was practiced outside. The natural predators of roe deer were grey wolf (*Canis lupus*) and red fox (*Vulpes vulpes*).

Table I: Population densities of roe deer in the study area from 2002 to 2005.

Year	2002	2003	2004	2005	Mean ± SE
Summer density [Nr. individuals/km^2]	35,3	32,8	34,9	28,0	32,8 ± 1,7

Effects of roe deer browsing on sprouts' growth

In February 2002 six experimental areas, each of the size of one hectare, were established as the representatives of coppice stands. The six stands were at the end of rotation period, when dominated by Turkey oak or chestnut. A preliminary dendrometric survey was first carried out, in order to determine their representative condition and characteristics before the treatment. The number of shoots and basal area of chestnut in the experimental plots ranged from 1123 to 3939 ha^{-1} and from 21,7 to 32,3 m^2 ha^{-1}, respectively, while the respective values ranged from 2181 to 2410 ha^{-1} and from 29,0 to 30,0 m^2 ha^{-1} for Turkey oak plots. Subsequently, in March 2002 all six experimental stands were coppiced, with the release of 50-60 standards per hectare, in accordance with the procedure traditionally used in Italy. In each experimental area two permanent plots of about 200 m^2 of size were established, one of which was fenced (protected P), while the other was left accessible to roe deer browsing (non protected NP). In each plot, all stools were permanently numbered and surveyed. In order to accurately and quantitatively analyse the effect of roe deer on sprouts and stool development, we recorded number of sprouts, collar diameter (d), total height (h) of sprouts, and number of recently browsed sprouts for each stool. The procedure was carried out at the end of 2002 and then at the beginning and at the end of each growing season, from 2003 to 2005.

Moreover, in order to obtain rapid estimates of browsing damage, each stool was classified according to the percentage of browsed sprouts, as shown in Table 2. In this way we established an easy and little time-consuming approach to assess and analyse the dynamics of deer browsing with reference to different tree species (Turkey oak and chestnut). In particular, we compared the percentage of stools in each damage class and calculated a browsing index (BI) as the average damage rank recorded in each sampling plot. In addition, the correlation between BI and growth losses in non-fenced plots was determined by Pearson's correlation coefficient (*r*).

Specific allometric equations were elaborated in order to assess the effect of deer browsing on the aboveground biomass of stools and sprouts more precisely. Representative samples of 100 chestnut and Turkey oak undamaged sprouts were collected outside and yet close to the experimental plots. Collar diameter and total height of each sprout were subsequently measured in the laboratory, so that the average parameters for all the stools were obtained. Later, the sprouts were dried in an air stove at 85 ± 2°C until constant weight was reached. The data obtained were used to elaborate specific allometric relations for predicting woody biomass (dependent variable), based on two independent variables (d^2h), according to a simple linear model $y = a + bx$. This enabled us to estimate the biomass of each sprout and, consequently, of each stool. The effect of deer browsing was assessed by comparing the data from fenced *vs* non-fenced stools by means of one-way ANOVA (Statistica, Statsoft Inc.).

Table II: Classification and ranking of browsing damage in regards to the percentage of browsed sprouts on each stool.

Damage class	Percentage of damaged sprouts	Damage rank
No damage	0	0
Slight damage	< 30%	1
Medium damage	30-60%	2
Heavy damage	> 60%	3

Radio-telemetry and roe deer use of habitat

From March 2002 to March 2005 we captured 62 adult roe deer (26 males and 36 females), using vertical drop nets. All animals were equipped with Televilt VHF radio-collars and subsequently located by means of discontinuous radio-tracking, using “Televilt RX-8910 HE” and “TRX 1000-S Wildlife Materials” receivers and four-element hand-held Yagi antennas. Each month 12 or more locations were obtained by triangulation for each animal. We distributed telemetry locations uniformly over the day and separated consecutive fixes by an interval of at least 12 hours to avoid autocorrelation (Van Winkle, 1975). Accuracy of fixes was determined in the field, using test transmitters placed in various habitats (Harris et al., 1990). Accuracy testing resulted with an error polygon of 1 hectare. The average error polygon was smaller than the average size of separate vegetation communities, which was relatively large in our study area (mean ± SE: 216,3 ± 92,7 ha), so it was possible to determine roe deer habitat use in this area. For the purpose of this study we considered only the fixes that were within coppiced areas and grouped them into three periods: before, during, and after the coppicing. Radio-tracked roe deer were using two Turkey oak coppiced areas, indicated with abbreviations “C17-1” and “D5-1”. All data necessary for the habitat use analysis were obtained and modified with the programme ArcView GIS 3.2.

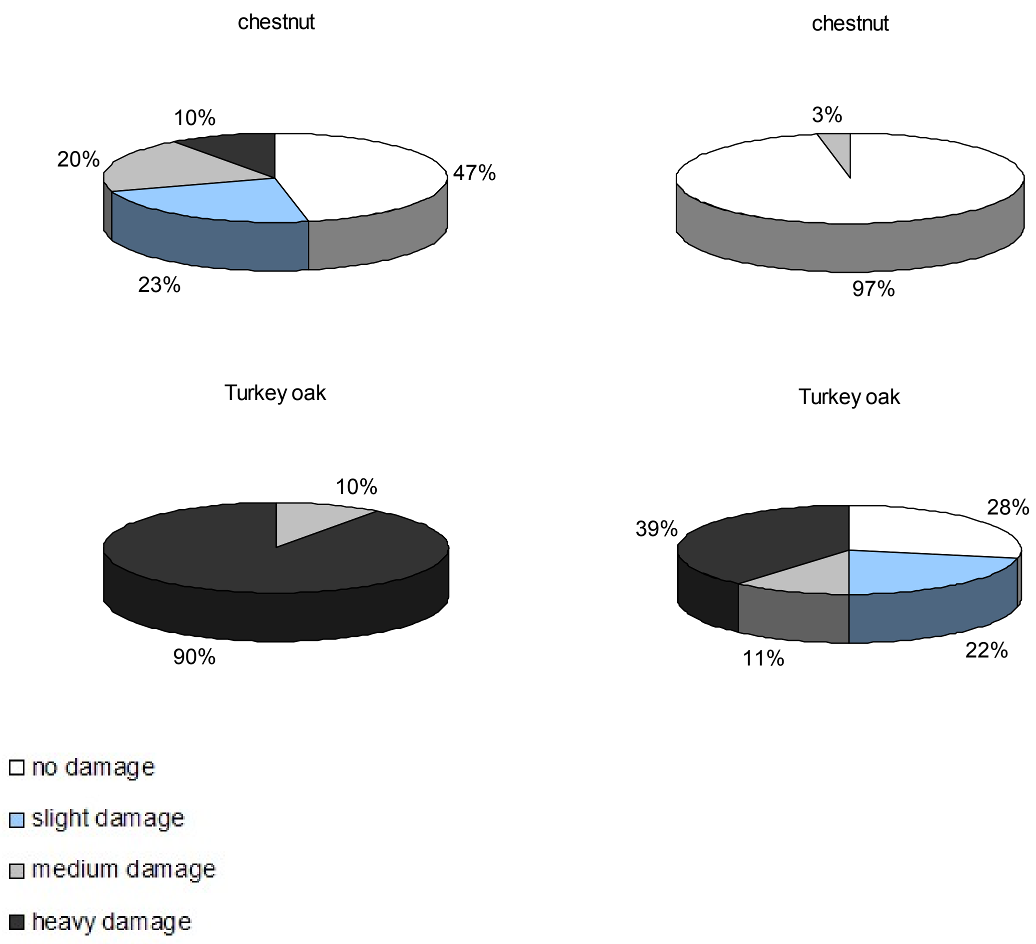
We calculated the utilization index of coppiced areas, where differences in roe deer use of each area were assessed using the formula below. For each period (before, during, and after the coppicing) we calculated the average number of fixes per deer in each area, and divided the outcome by the duration of the period (number of months). Duration of the periods was assessed like follows: “before” lasted from the beginning of deer monitoring to the beginning of the coppicing; “during” lasted as long as the coppicing; “after” lasted from the end of the coppicing to the end of monitoring. We used a chi-square test for statistical analyses: the observed value was assumed to represent the utilization index for each period, while the expected value was assumed to represent the utilization index, as assessed throughout the whole study period.

$$\frac{\sum_{i=1}^{n} \text{fix}_{i} \Big/ \sum_{i=1}^{n} \text{roe deer}_{i}}{\sum_{x,j,z=1}^{n} \text{month}_{x,j,z}}$$

Results

Effects of roe deer browsing on sprouts growth

Effect by roe deer on vegetative regeneration of chestnut and Turkey oak stools in non-fenced plots differed during the year immediately after coppicing, as well as in the years after that. In fact, one year after coppicing 30% of chestnut stools in non-fenced plots were quite damaged, while in Turkey oak every single stool was severely browsed (Fig. 1a). Four years after the coppicing, 50% of Turkey oak stools still suffered severely from roe deer browsing, while chestnut plots showed almost no damage (Fig. 1b).



(a) **one year** **(b)** **four years**

Figure 1a and b: Effect of roe deer browsing on chestnut and Turkey oak stools one year (a) and four years (b) after coppicing.

Different level of browsing damage on chestnut and Turkey oak stools was clearly indicated by the browsing index BI (Fig. 2). In non-fenced plots, Turkey oak stools had markedly higher (roughly three-fold higher) average BI than chestnut stools. In both species, the BI showed a decreasing trend through the years following the coppicing. However, while in chestnut plots the BI approached zero, it still measured over 0,5 four years after coppicing in Turkey oak plots.

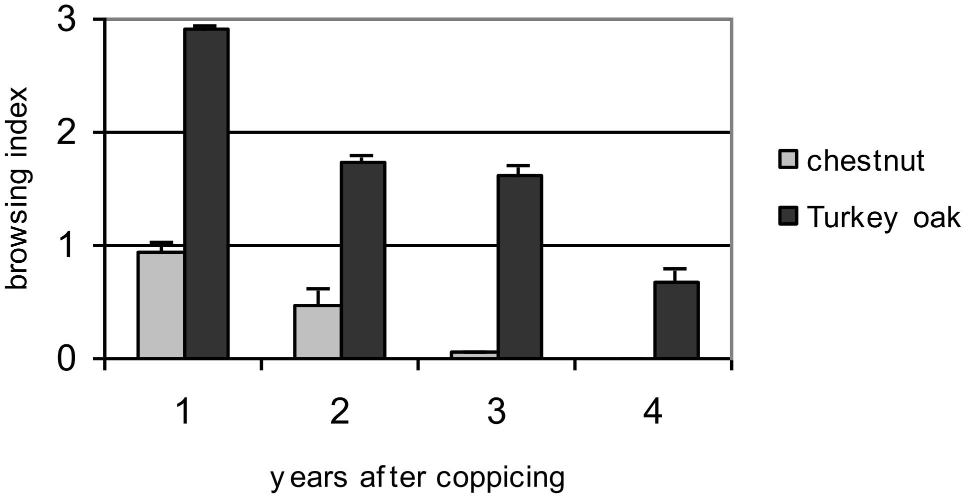


Figure 2: Comparison of the effect of roe deer on chestnut and Turkey oak sprouts as revealed by browsing index: mean (±sd) browsing index through four years after coppicing.

Different effects of deer browsing could also be observed in relation to the height of sprouts. Roe deer browsing did not have a significant effect on the height of sprouts in chestnut already one year after coppicing (Fig. 3). In contrast, in Turkey oak plots the height of the sprouts in fenced plots was found to statistically differ from that observed in non-fenced plots (one-way ANOVA: F = 105,85; p<0,001) (Tab. III; Fig. 3). In Turkey oak plots, the height of the sprouts from fenced and non-fenced plots still differed even four years after the coppicing (one-way ANOVA: F = 13,87; p<0,001) (Tab. III; Fig. 3).

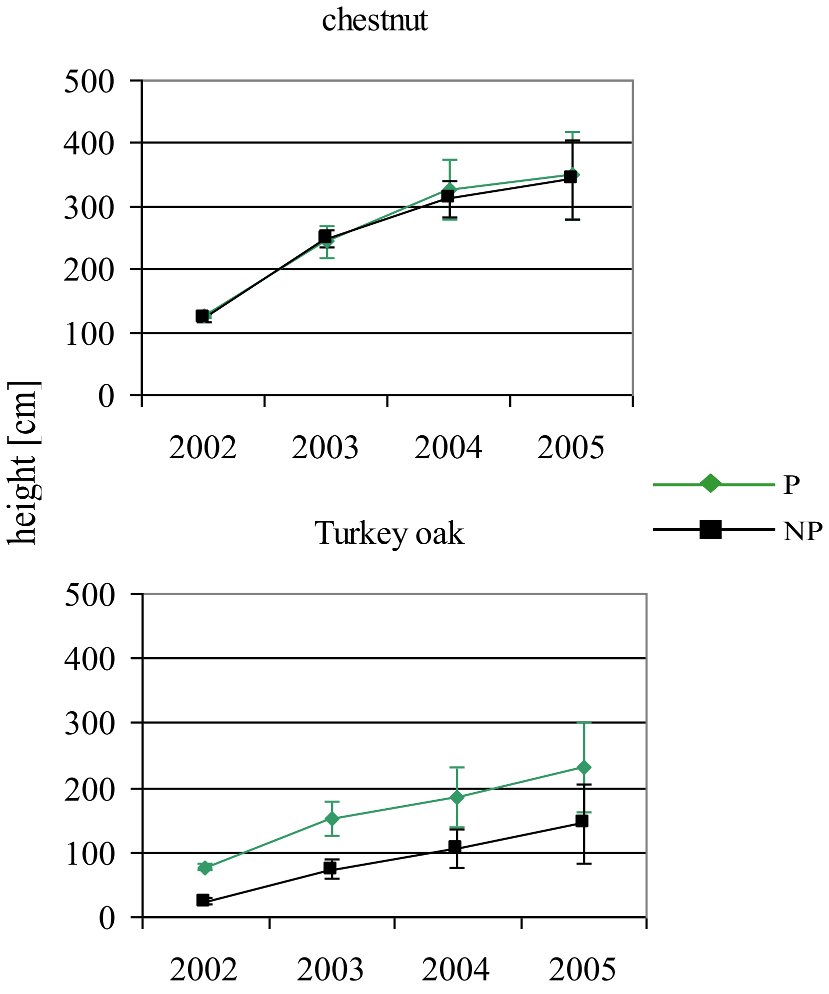


Figure 3: Mean (± sd) height of chestnut and Turkey oak sprouts, protected (P) and non protected (NP) from roe deer browsing, from 2002 till 2005.

Differences in the sprout diameter due to browsing were also assessed. One year after the coppicing there was no significant difference between the diameters of the sprouts from fenced and non-fenced plots in chestnut as well as Turkey oak (Fig. 4). On the contrary, in Turkey oak plots the mean diameter of the sprouts was significantly smaller in non-fenced plots than in fenced (Tab. III; Fig. 4) since the second year after coppicing on.

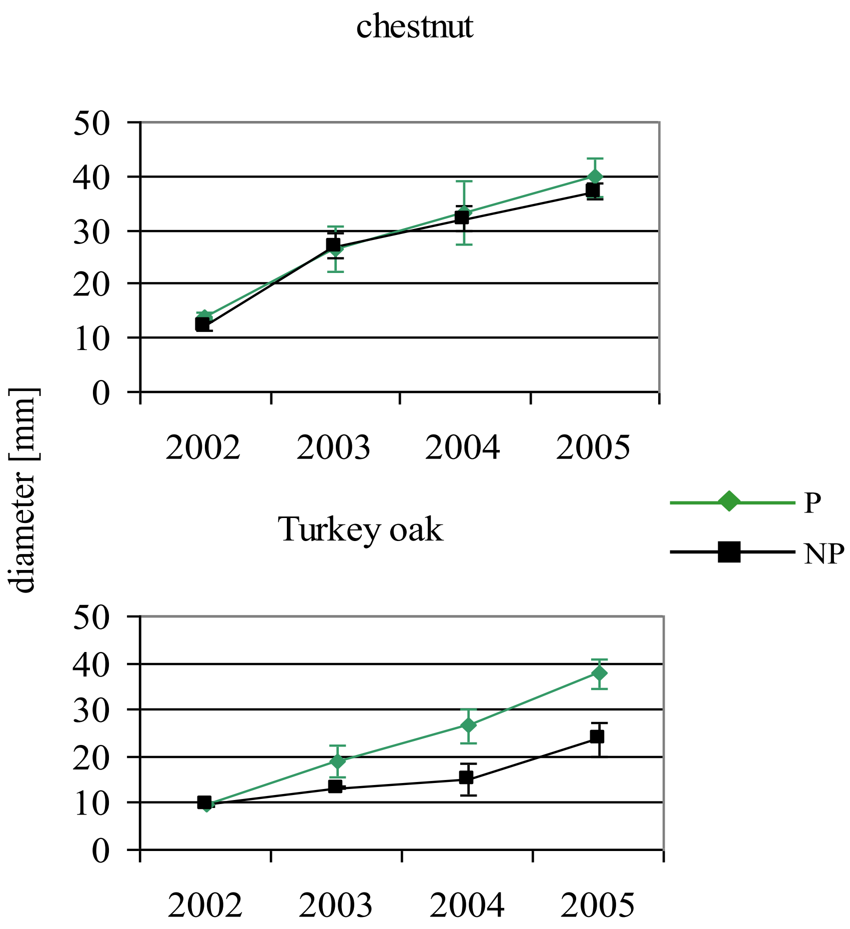


Figure 4: Mean (± sd) collar diameter of chestnut and Turkey oak sprouts, protected (P) and non protected (NP) from roe deer browsing, from 2002 till 2005.

Number, height, and diameter of sprouts affected the average stool biomass. In chestnut plots no difference in stool biomass was found between fenced and non-fenced plots, while in Turkey oak plots the average stool biomass in fenced plots was higher than in non-fenced plots (Tab III; Fig. 5).

Pearson's correlation coefficient between BI and growth losses, expressed as difference in percent between sprouts height and stools biomass from fenced and non-fenced plots, for chestnut as well as Turkey oak, was calculated. We confirmed a significant correlation between BI and losses in height of sprouts (r=0,859; p=0,006), and between BI and losses in biomass of stools (r=0,659; p=0,075).

Table III: Analysis of variance of height and diameter of sprouts and biomass of stools in Turkey oak coppice in fenced and non-fenced plots for first and fourth year after the coppicing.

		sprout height [cm]					
		mean	**n. obs.**	**SD**	**GDL**	**F**	**P**
2002	**Fenced**	77,80	20	21,50	1/38	105,85	0,000
	Non-fenced	24,50	20	8,60			
2005	**Fenced**	228,70	19	73,80	1/33	13,87	0,0007
	Non-fenced	145,00	16	55,80			

		sprout diameter [cm]					
		mean	**n. obs.**	**SD**	**GDL**	**F**	**P**
2002	**Fenced**	1,00	20	0,20	1/38	0,0002	0,988
	Non-fenced	1,00	20	0,40			
2005	**Fenced**	3,76	19	0,99	1/33	18,68	0,0001
	Non-fenced	2,35	16	0,93			

		stool biomass [g]					
		mean	**n. obs.**	**SD**	**GDL**	**F**	**P**
2002	**Fenced**	23,30	20	13,10	1/38	17,16	0,0001
	Non-fenced	9,80	20	6,40			
2005	**Fenced**	824,28	19	558,66	1/33	14,53	0,0005
	Non-fenced	260,50	16	207,96			

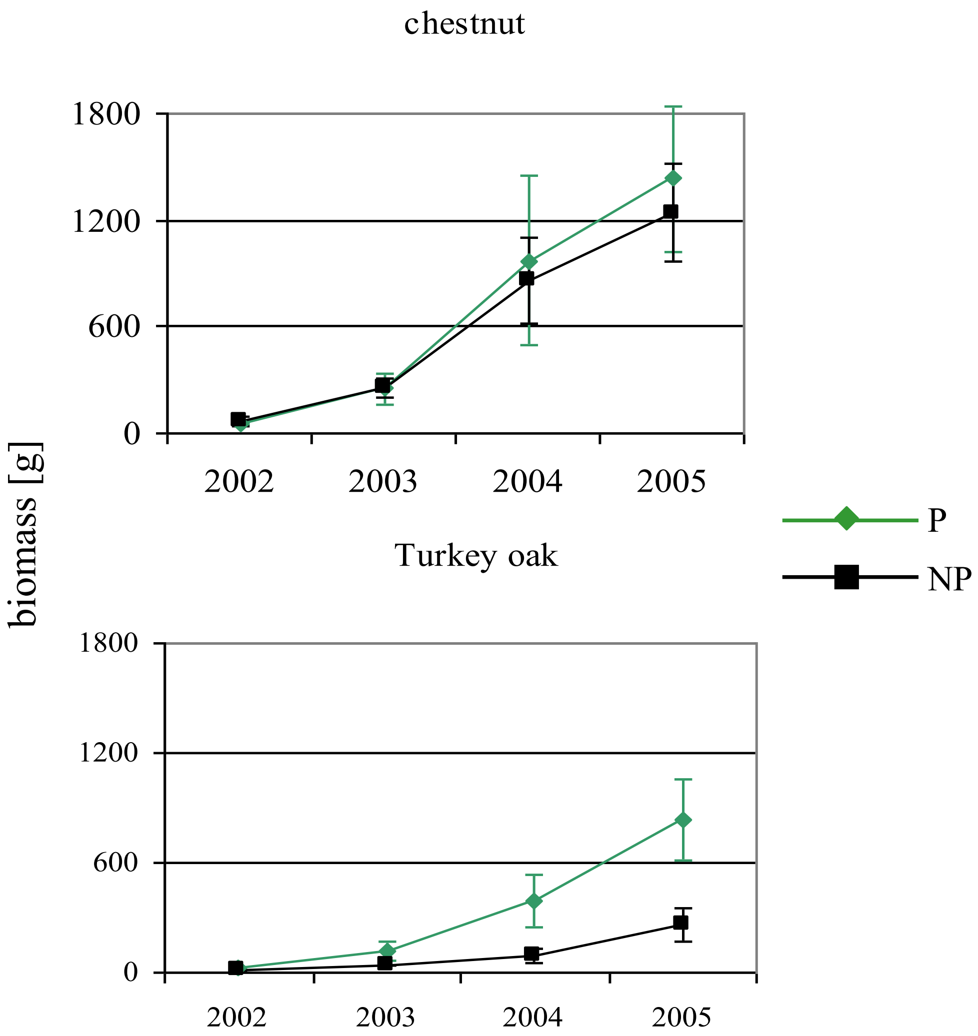


Figure 5: Mean (± sd) biomass of chestnut and Turkey oak stools, protected (P) and non protected (NP) from roe deer browsing, from 2002 till 2005.

Roe deer use of coppice areas

The use of coppiced areas by radio-collared roe deer appeared to be more frequent during and after the coppicing. In details, the utilization index for two Turkey oak stands varied significantly according to the period: during and after the coppicing, roe deer spent more time in the coppice areas than before (χ^2=12,91; df=2; p<0,01; and χ^2=6,56; df=2; p<0,05 for the areas "C17-1" and "D5-1", respectively; Fig. 6). The stands were used significantly more frequently during the harvest.

Therefore, we took into account also the distribution of fixes in different periods of the day (i.e. night, dawn, daylight, dusk), which showed a more intense use during the night for both Turkey oak stands during the coppicing. In details, in "D5-1" area, night fixes represented 23,4% of the total fixes before the harvest, 27,3% during the harvest, and 0% after the harvest. In "C17-1" area, night fixes represented 34,8% of the total fixes before the harvest, 100% during the harvest, and 0% after the harvest.

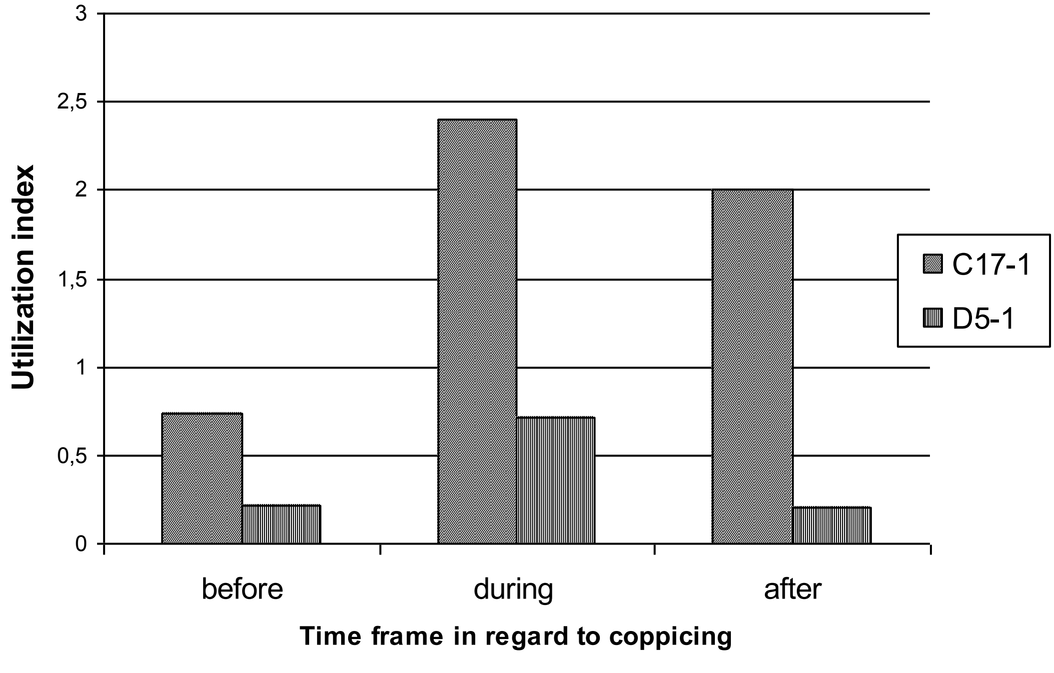


Figure 6: Utilization index for the use of two Turkey oak coppice areas (tagged "C17-1" and "D5-1") by radio-collared roe deer in three different periods with respect to wood harvesting (before, during and after coppicing) from 2002 to 2005.

Discussion

Our study revealed different levels of roe deer browsing pressure on Turkey oak and chestnut. In particular, damages were more severe in Turkey oak than in chestnut stands, consequently a high degree of selection of feeding sites was evident.

Some studies pointed out that deer might use woods to a different degree according to their structure and composition. Tree species like balsam fir (*Abies balsamea*) and most deciduous species were palatable to deer (Potvin et al., 2003; Casabon and Pothier, 2007; Tremblay et al., 2007). In our study differential use of deciduous coppice areas by roe deer was noted, as its effect on the sprouts growth was completely different in Turkey oak than in chestnut stands. Specifically, only low levels of damage were observed in chestnut coppice, where no significant reduction in growth was recorded due to roe deer browsing. Deer browsing was shown to affect the individual tree survival only to a small extent, especially after its first 5 years of growth (Black et al., 1979). However, it can still represent an economic problem, as considerable damage in tree recruitment was reported due to deer browsing. For instance, yew (*Taxus baccata*) showed retarded recruitment under heavy roe deer browsing pressure (Mysterud and Østbye, 2004); silver fir (*Abies alba*) suffered considerable damage from red deer (*Cervus elaphus*) browsing (Motta, 1995) and balsam fir from white-tailed deer (*Odocoileus virginianus*) browsing (Beguin et al., 2009). In our study, the height of sprouts in Turkey oak coppice differed significantly between fenced and non-fenced plots. Still, as the browsing damage was recorded to decline in non-fenced plots through the years, decrease in stool biomass could be connected to browsing on lateral Turkey oak sprouts. In fact, in case of the sprouts that succeeded to grow above the browsing height, browsing appeared on the lateral sprouts.

In the first year after coppicing browsing index was noticeably lower in chestnut than in Turkey oak stands. Faster growth of chestnut sprouts (height increase of one meter per year roughly) prevented roe deer browsing on chestnut already since the second year after the coppicing. Moreover, lower level of browsing damage in chestnut stands was most likely connected to roe deer preference for Turkey oak over chestnut. The outcomes were consistent with the findings of previous studies on *Quercus* spp., which were most susceptible to browsing (Gill, 1992; Kuiters and Slim, 2002). Additionally, roe deer was reported to feed highly selectively (Bergman et al., 2005; Moser et al., 2008; Jarni et al., 2004). Given that the selection of browsing sites can be explained by differences in the food quality (Jarni et al., 2004), different taste of Turkey oak and chestnut sprouts could accordingly likely contribute to the observed browsing pattern. Speculatively, different concentration of tannins in chestnut and Turkey oak may encourage selective feeding. For instance, Bergvall et al. (2006) reported that deer preferred plants with low tannin concentrations. Also, a negative relationship was found between the height and palatability of 1-year-old seedlings (Laitinen et al., 2002). In fact, as pointed out by Ward and Young (2002), severe browsing on sprouts may induce high carbohydrate demands as a growth response. This would consequently limit the production of tannins and increase the palatability of sprouts to deer. On the contrary, plants that are browsed to a lower degree, like chestnut in our study, may produce more tannins so their palatability to deer even decreases. Moreover, shoots within browsing height were more vulnerable than shoots above the browsing height (Rooke et al., 2004). Thus, all these factors likely contributed to differential pattern of browsing between Turkey oak and chestnut stands. However, Fickel et al. (1998) pointed out that roe deer unexpectedly showed a high tannin-binding capability, probably due to proline containing enzymes mixed to parotid salivary proteins, which enabled them to hydrolyse and condense tannins. Nevertheless, in case of choice - like in our study - they most likely choose plants with lower concentration of tannins.

The results of detailed analysis of diameter, height, and biomass dynamics, and those obtained from an experimental browsing index agreed to a high degree, which supports the reliability of the latter approach. The outcome is relevant, as such fast field tool arguably represents a useful management tool (Espleta et al., 2006). Namely, in order to assess the effects of deer browsing on vegetative regeneration on larger scale, a swift and reliable field tools are necessary since analytic and quantitative approaches cannot be applied.

The radio-tracking data on collared roe deer individuals were consistent with the vegetation survey findings for Turkey oak stands. The number of radio-localisations inside coppice areas increased above all during as well as after the wood harvesting. During the harvest high quantities of biomass, e.g. crown foliage, immediately became available for roe deer consumption. Roe deer approached these sites at night, when foresters were gone, and foraged on the foliage. The utilisation index for these sites was higher in the period of harvesting and then in the following three years than in the period before harvesting. Our study thus provided evidence that roe deer used the areas, where forest works were undertaken. However, animals approached these sites during the night after the foresters were gone, and thus avoided the direct anthropogenic disturbance during the day.

Another important feature of our study sites was vegetation density. This is connected to the visibility and consequently, to the possibility for animals to use such stands as hiding places. In our study site the recruitment of sprouts was especially high in the first and second year after the coppicing. That is why the middle layer of vegetation was so dense, which consequently represented an optimal environment for hiding fawns, as well as safe resting places for adults (Mysterud and Ims, 1999). Indeed, dense coppice stands were selected by roe deer females with fawns as part of their anti-predator strategy (Bongi et al., 2008). On one hand, our results provided evidence for the importance of Turkey oak coppice as a feeding place and as a shelter for roe deer in our study area. On the other hand, our results confirmed a non-negligible effect of roe deer on vegetative regeneration of Turkey oak. These outcomes highlighted a possible role of roe deer in influencing competition between Turkey oak and chestnut in the first years after coppicing. Similarly, a study from Netherlands showed that oak (*Quercus robur* and *Quercus petraea*) was out-competed by beech in the forest canopy due to ungulate browsing pressure (Kuiters and Slim, 2002).

When taking into consideration the current roe deer density in the study area and its browsing pressure, one could argue that an increase in roe deer density could influence the Turkey oak regeneration to an inacceptable degree from the economic point of view. Several studies found deer density to be positively correlated with occurrence and intensity of browsing damage (Welch et al., 1991; Gill et al., 1996; Putman, 1996; Beguin et al., 2009). However, not only deer density but also other ecological factors were crucial for the un/successful regeneration process of forest vegetation (Kramer et al., 2006; Heuze et al., 2005). Well, in our study area roe deer density has recently been quite stable and its management plans are designed to maintain their current density. Thus, browsing pressure cannot be expected to decrease under such circumstances, also because natural predators – wolves do not seem in the position to regulate the present roe deer population.

To sum up, this study highlighted the roe deer preference for coppiced areas from several reasons. Forest management plans, based on the coppice system, thus need to account for the creation of areas where roe deer gather in higher numbers to forage, but also due to finding safe places to rest and hide. Periodic wood harvesting provides large herbivores with resources in terms of available crown foliage, higher number of sprouts, and suitable hiding as well as resting places. One may argue that forest management plans that maintain coppice woods have a positive effect on roe deer population, as they provide more favourable habitat for roe deer compared to high-forest management. However, from an economical point of view, one should also notice that roe deer held back the vegetative regeneration process in

Turkey oak coppice, thus modifying forest structure development and reducing the economic value of these stands.

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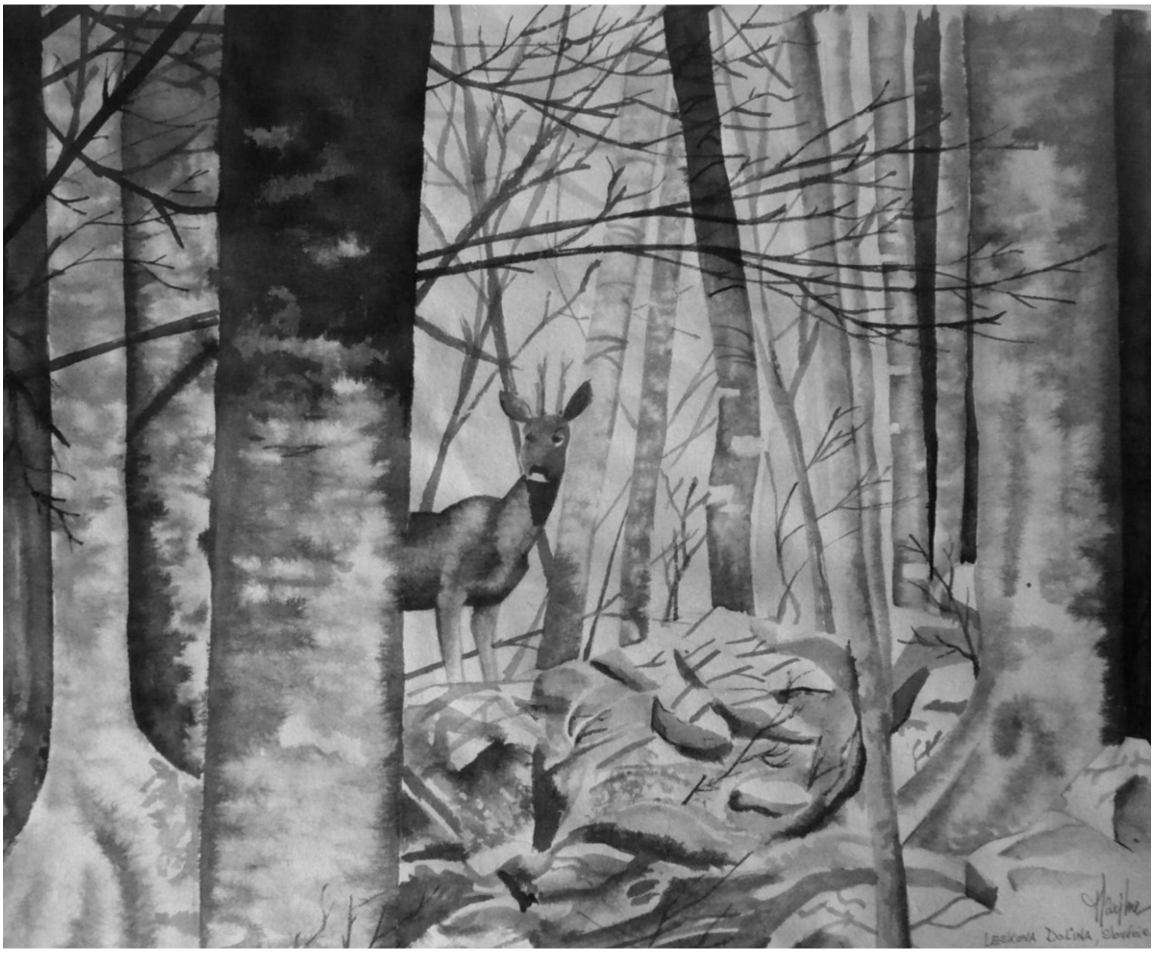
Discussion



Maxime Pastore

DISCUSSION

In this dissertation we researched some aspects of behavioural ecology of roe deer population inhabiting forested area of North-Eastern Apennines in Central Italy.

In ***Chapter 1*** we investigated activity patterns of roe deer. Knowledge of activity rhythms of a population is important for both, basic and applied biology. Namely, research on activity budgets of a species helps to understand better energy demands of the species, its relationship with the environment, and helps to predict its responses to potential changes in the environment. Moreover, through comparing activity rhythms among populations we can obtain indices on appropriate management of protected areas and their role in the landscape, on sustainable management of the environment, and can contribute to the guidelines for re/introductions and hunting bag structures.

Our results suggested that a territorial species with low sexual size-dimorphism from temperate zone adjusted its activity patterns to:

i) light-dark cycle
ii) seasonal variability of environmental conditions,
iii) territoriality (mating system),
iv) predation risk, here measured in terms of human hunting pressure.

According to Berger et al. (2003) rhythms of physiological functions and related behaviours are essential components of the ecological relations between an organism and its environment. We confirmed that outside environmental factors, anthropogenic factors, and social/mating system of a monomorphic species, all contributed to shaping activity patterns and thus were influencing and modifying also the inner rhythms. Our results supported the findings of other studies in that activity patterns reflected the actual environmental conditions, including variation of factors among the seasons (Pipia et al., 2008).

The synchronisation of activity with the light-dark cycle, the strongest and the most reliable entraining signal in nature, was apparent. The light-dark cycle was indeed reported to be among the most effective signals in entraining (synchronising) circadian rhythms (Scheibe et al., 2009; van Oort et al., 2007). Roe deer in our study expressed daily bimodal activity pattern, where the peaks of activity occurred during dawn and dusk, with peak at the dusk being significantly higher. Moreover, bimodal pattern of roe deer activity followed seasonal changes in duration of daylight and night. Hence annual activity rhythm, as well, was influenced by seasonally varying light-dark cycle. Additionally, daily variation in both phase and amplitude of activity was also dependent on variation of the seasons.

Difference in activity patters between the sexes was apparent during the spring and summer months, which coincided with the territorial period of the species. The differences were most likely a consequence of male sexual and territorial annual cycle: in spring the period of territory establishment and defence begins, which demands higher total daily activity due to territorial behaviour (Johansson & Liberg, 1996). The sexual cycle of male roe deer appeared to be initiated by an endogenous rhythm in winter and was then maintained by hormonal changes resulting from increasing photoperiod in spring (Sempere et al., 1992). In spring the concentration of plasma testosterone starts to increase in roe bucks (Sempere et al., 1992), influencing the initiation of sperm production in their reproductive organs, shedding of velvet, and expression of territorial behaviour. On the other hand, testosterone level in the blood of roe bucks, that had reached its maximum during the peak of rutting at the beginning of August, after the rut deceases quickly, which causes gradual end of sperm cells production, decrease in size and mass of testicles, and other physiological changes. Correspondingly, autumn is the period, when territorial spatial structure is collapsing, males lose their antlers, and winter mixed-groups starts to appear. Actually, the lowest activity was recorded during the autumn, immediately after the rutting period of roe deer. Presumably, activity levels dropped after the rutting period so drastically, as the territorial period ended and majority of the activity corresponded to feeding cycles and less activity to social interactions (Johansson & Liberg, 1996).

The activity budget hypothesis proposes that sexually size-dimorphic males and females segregate into different groups due to incompatibility of activity budgets and movement rates, independent of possible sexual differences in forage selection (Conradt, 1998; Ruckstuhl, 1998, 1999). Sexual differences in activity budgets would be expected to increase with increasing sexual size dimorphism up to a level where synchrony of behaviour is too costly and sexual or size segregation occurs (Ruckstuhl & Neuhaus, 2002). Thus, males and females of non-dimorphic social species should be found in mixed-sex groups year round (Ruckstuhl & Neuhaus, 2002). This prediction was confirmed with the studies on two African size-monomorphic ungulates. In case of ruminant African oryx (*Oryx gazella*), Ruckstuhl and Neuhaus (2009) discovered that male and female oryx did not differ in their activity budgets. Moreover, the study on plains zebra (*Equus burchelli*) showed the same results: similar-sized stallions and mares did not differ in activity budgets (Neuhaus & Ruckstuhl, 2002). The authors concluded that the year-round possibility of conceiving likely forced the animals to synchronise their time budgets, which might be a major reason for the lack of difference in body size between males and females. However, our data on roe deer from temperate zone showed, that territoriality modified the otherwise lack of difference in the activity levels between the sexes. Indeed, based on the literature findings also Ruckstuhl and Neuhaus (2000) summed up that factors such as clumped food distribution, differential predator pressure, or territoriality in males may override effects of body-size differences.

To sum up, predictions of activity budget hypothesis for sexual segregation in dimorphic species were hereby confirmed also with the dataset on activity levels of monomorphic species, roe deer. Roe deer males and females showed the same activity patterns while staying in mixed-sexed groups (autumn, winter), but differed in their activity patterns, when living socially segregated, even when using the same area (spring, summer).

In ***Chapter 2***, we took under investigation the territorial period of male roe deer only. We investigated the spatial behaviour and activity rhythms of VHF-radio-collared males during the territorial period simultaneously. Territorial behaviour was up to now researched mostly on the basis of spatial use patterns (e.g. Linnell & Andersen, 1998), and on the basis of behavioural observations during the territorial or reproductive period, like territorial barking phenomenon (e.g. Reby et al., 1999), territory marking behaviour (e.g. Johansson & Liberg, 1996) or aggression (e.g. Wahlström, 1994; Hoem et al., 2007) and mating acts (e.g. Börger, 2006). As direct behavioural observations can be assessed only in open, non-forested landscape, usually agricultural and mosaic areas, we could not use such techniques in our study area, given that it is covered in forests in 85%. Thus, we combined the spatial use data with the activity levels of radio-collared males for the first time (but see Batard, 2010 for a study on territory establishment phase of roe deer bucks).

On the basis of spatial data clustering we managed to distinguish three groups of males. One was group of old males that showed spatial patterns of non-territoriality, wandering over large areas, including over the territories of several other radio-collared males. One group included males that possessed very small home ranges in all three bimonths, suggesting they may be territorial, but we can not exclude the possibility that the group included also males with other strategies than territoriality. Namely, peripherals and satellites could express similar spatial patterns due to spatial restriction by territorial neighbours or necessity to obtain a refuge (see Liberg et al., 1998). Actually, a yearling was included in this group, but its extremely low activity levels throughout the period and occupation of the area of another territory-holder clearly indicated its sub-ordinate and "low profile" behaviour for being tolerated by his territorial host. The last group included males that managed to defend their territory later than other males, as they arrived in the area of their dominance in second bimonth only (May-June). Also Melis et al. (2004) reported on males that occupied the same home range over the year ("annually site- faithful") in contrast to males, which used different areas during territorial period than during the cold months ("seasonally site-faithful") and concluded that being resident throughout the year could be a privilege for territory holding in comparison to late-coming territory holders. Nevertheless, one of these males in our study was 3 years old and due to its extremely high activity levels throughout the territorial period we believe it defended its first territory (see ***Chapter 2*** for alternative possibilities). Moreover, we confirmed, that not all 3-year olds were able to hold a territory in this population, which meant that there is a certain part of population that defend the first territories at four years of age (Liberg et al., 1998). To evaluate, if this is a common or a rare case given an average density of 31,0 ± 1,9 deer/km^2, certain environmental structure, and inter-specific relationships, further studies on a larger sample size would be needed.

Our preliminary results suggested that connection between spatial use patterns and activity patterns is not simple and/or direct. The clusters of spatial use affected variation in activity levels just partly, indicating possible inter-individual variation from this aspect, too. Most likely factors like environmental variation (see *Chapter 1*), territory structure, neighbours and non-territorial males, population factors, which were not controlled for in this study, influenced the relationship between spatial behaviour and activity rhythms.

However, the temporal aspect of activity patterns revealed an important insight into male territoriality and reproductive strategy. Activity levels of monitored males were the highest in the first bimonth (March-April), after which a trend of a graduate decrease was indicated, until the activity level didn't stabilise from June on. In contrast, one might expect occurrence of elevated activity levels also/only during the reproductive period, or during the whole territorial period, given that the markings and aggressive encounters were reported to occur during the whole territorial period (Johansson & Liberg, 1996; Hoem et al., 2007). However, the importance of territory establishment period for roe deer males was emphasised from observed results, supporting the findings of Batard (2010). Such pattern of activity also supported the "low-risk low-gain" strategy of roe deer males, proposed by Linnell and Andersen (1998), and supported with several subsequent studies (e.g. Melis et al., 2004; Vanpé et al., 2009). It states that "always stay" tactics, highly stable territorial net in the landscape, high site fidelity to the territories and extremely long tenures compensate for relatively low energetic investment in mating made each year. Among others, scattered and solitary distribution of roe deer females (Linnell & Andersen, 1998) that might not be distributed as an ideal free distribution (Vanpé et al., 2009; but see Wahlström & Kjellander, 1995) makes them a resource difficult to obtain and/or control. There is therefore little to be gained by excessive energy expenditure on mating within a given season (Owen-Smith, 1977). To support this view, due to high fidelity to the site of first-established territory, thus defending the same territory for several years (or a lifetime), males do not seem to compete for resource rich sites very much (Vanpé et al., 2009). Additionally, high site-dependent dominance of males ensures quite stable territory-ownership net in the landscape, similar as was reported by Hoem et al. (2007), where even due to lost fight no territory loss occurred. Such observations suggested that male territoriality in roe deer does not conform entirely to the general characteristics of resource defence polygyny mating system. When the issue was researched with molecular tools, Vanpé et al. (2009) indeed confirmed that on the continuum from monomorphic and monogamous species with territorial mating systems to highly dimorphic and polygynous species with dominance-rank based mating systems roe deer occupies a position closer to monomorphic and monogamous territorial ungulates. The authors evidenced that in line with the low level of sexual size dimorphism of the species and thus similar life-history traits in both sexes, low variance in male breeding success indicates a weakly polygynous mating system with low opportunity for sexual selection.

To conclude, we believe that such kind of combination of radio-tracking can reveal a promising technique for investigations on behavioural aspects of annual life-cycles and life-history traits of certain species. However, in our case we faced a limitation of our data collection as not having data of more precise temporal frame at our disposal (see *Chapter 2*). More frequent data collections will be needed in order to obtain more accurate variation in activity levels and spatial movements through the progressive territorial period, enabling more accurate evaluation of certain aspects of territorial behaviour in male roe deer.

In ***Chapter 3*** we examined the relationship between genetic relatedness and spatial distance in order to evaluate the extent, to which roe deer in Alpe di Catenaia behaved in a manner consistent with the hypothesis on female biased dispersal in resource defence mating system (Greenwood, 1980). We assessed genetic distance using pairwise relatedness among individual roe deer that were monitored through radio-tracking. The Mantel test revealed no correlation between spatial distance and relatedness for the set of radio-collared roe deer in none of the seasons and this held true for both sexes. Thus, the results did not support the prediction, that closely related roe deer established home ranges in vicinity. Genetically dissimilar individuals might so occur in close spatial proximity (Comer et al., 2005). Both sexes thus seemed to have very similar genetic structure in the population, which did not agree with a sex-biased dispersal model. The results thus did not support Greennwood's (1980) prediction on female biased dispersal in ungulates with resource defence mating strategy. Similarly, also the study from mosaic landscape in France confirmed that there was no female biased dispersal in the population (Coulon et al., 2006). The authors actually suggested that the opposite might be true, so that a slight male bias in dispersal could be present in that population. Our results also provided no support for the alternative prediction, that closely related females might form overlapping home ranges (Porter et al., 1991).

Actually, our findings coincided with the predictions of Wahlström and Liberg (1995) on density-dependent dispersal in roe deer. They predicted, that at intermediate to high population densities there should be no sex difference in dispersal. This scenario could explain our observed data (no difference between sexes and no correlation between relatedness and geographic distance), as due to high dispersal rate of both sexes the gene shuffling in the population could limit the genetic structure of the population. However, our observed pattern might be favoured due to small geographical scale sampled and due to highly forested study area, which might influence dispersal patterns.

On the other hand, in ***Chapter 4*** we turned our interest to relationships between a large herbivore and forest vegetation. We were interested in both sides of the aspect: how roe deer influenced vegetative regeneration of two economically and ecologically very important broadleaved tree species by browsing, and how coppicing as silvicultural technique influenced roe deer spatial behaviour. The first issue was investigated through analytic and laboratory approach of detailed measuring of biomass of newly grown vegetative sprouts in fenced and non-fenced plots (in both Turkey oak *Quercus cerris* and chestnut *Castanea sativa* coppice) and also with quick estimates of percentage of browsed sprouts in each plot (damage ranking), in order to assess a browsing index. The impact of coppicing on roe deer spatial behaviour was investigated by spatial monitoring of radio-collared roe deer.

The outcomes clearly indicated that roe deer browsed the newly grown vegetative sprouts after the coppicing. Also a study in France showed that roe deer preferred herbs and young leaves and buds of deciduous trees during spring and summer (Cransac et al., 2001). However, roe deer browsing in our study affected the regeneration of Turkey oak coppice to a much larger degree than that of chestnut coppice. All the parameters measured in the study (height and collar diameter of the sprouts, stool biomass) indicated more severe browsing on the Turkey oak during all four years, while browsing hardly affected chestnut stools. Speculatively, this process could lead to interfering in the competition between Turkey oak and chestnut in the first years after coppicing. For example, in Netherlands oaks were outcompeted by beech in the forest canopy due to differential ungulate browsing pressure (Kuiters & Slim, 2002). Such different browsing effect might be due to different olfactory/gustatory attraction of the two tree species for the roe deer. For instance, for several large herbivore species the selective feeding on preferred plant species or certain plant parts has been reported (Tixier et al., 1998; Cransac et al., 2001; Jarni et al., 2004; Verheyden-Tixier et al., 2008). The selection was usually connected with the nutrient content in the browse/graze (selection for plants, high in soluble sugars; Verheyden-Tixier et al., 2008; Bergquist et al., 2003), temporal scale (seasons) and biological aspects of the animal (sex, age, reproductive status; Cransac et al., 2001), and with the openness of habitat (canopy cover) and perceived closeness to human settlements (proximity being mostly avoided; Mysterud et al., 1999). Moreover, it must be taken into account, that due to faster growth chestnut sprouts escaped the maximum reach height of roe deer already after one year, unlike the Turkey oak sprouts.

Results of a detailed laboratory analyses corresponded to a browsing index, as it showed the same trend as the analytical measurements. Even if the browsing index was decreasing from the first year after the coppicing towards the fourth in both Turkey oak plots and chestnut plots, it was three times higher for Turkey oak coppice than for chestnut coppice, and even approaching zero in the latter. The outcome suggested that such browsing index could be used for long-term monitoring of the roe deer browsing effects, as for assessing the browsing effects on larger spatial scales analytic approach can not be used due to time and cost expenditures (see Morellet et al., 2003).

In this study it was also evidenced that roe deer increased the use of coppice areas both during and after logging in comparison with the use of these areas before the coppicing. The outcome thus suggested that this silvicultural technique created a favourable habitat for roe deer. Arguably, roe deer found favourable environmental conditions in coppice stands from several points of view. Firstly, large quantities of biomass that accumulated on the forest floor during the wood harvest represented newly available food resource (e.g. crown foliage). Secondly, after the forest works the vegetation density increased due to new growth of sprouts from the stools, which decreased the visibility in the stands. Such environment provided good cover for roe deer in terms of both safe resting places and hiding places for fawns as part of anti-predator strategy of roe deer females with fawns (Mysterud & Ims, 1999; Bongi et al., 2008). Forest management plans should thus account for this effect of coppicing technique on roe deer behaviour. Similar effects of management of natural resources such as silvicultural techniques on behaviour of large herbivores were brought to attention in other studies, as well (e.g. Mysterud & Ǿstbye, 1999; Morellet et al., 2007).

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