

DEPARTMENT OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES

SPATIO-TEMPORAL PATTERNS OF THE WOLF AND ITS PREY IN A MEDITERRANEAN AREA

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Abstrakt

Från och med 1970-talet har populationer av topppredatorer som varg (Canis lupus) expanderat över hela Europa tack vare skyddslagstiftning och skapandet av många skyddade områden, det ökade överflödet av vilda klövdjur och andra faktorer. Återkomsten av dessa djur kan leda till olika konsekvenser ur såväl ekologisk som förvaltningssynpunkt. Stora rovdjur har rapporterats utöva en signifikant inverkan på ekosystemen genom att utlösa trofiska kaskader. I människopåverkade ekosystem, som är vanliga i Europa, som kännetecknas av mindre och spridda skyddade områden, kan närvaron och aktiviteten hos människor påverka förhållandet mellan rovdjur och bytesdjur, vilket påverkar topppredatorernas potential att påverka andra komponenter i ekosystemen. Den aktuella studien fokuserar på spatiotemporala distributionsmönster för vargen och dess huvudsakliga byte i ett skyddat kustområde i centrala Italien (Maremma Regional Park) med hjälp av kamerafångstmetodik. Som väntat visade vargen nattliga aktivitetsmönster, med toppar statistiskt associerade med aktivitetsrytmerna för vildsvin, med vilka ett anmärkningsvärt tidsöverlapp upptäcktes ($\Delta 4 = 0.91$). Daglig aktivitet observerades hos dovhjortarna, med ett medel-lågt överlapp med varg ($\Delta 4 = 0.53$). Resultaten stod i kontrast till fynden som erhölls på 1900-talet, det vill säga när vargen var frånvarande från området och dovhjortar visade nattlig och skymningsaktivitet. Resultaten skilde sig dessutom från vad som hittades 2017-2018, det vill säga ett par år efter vargens återkomst, då dygnsaktivitet endast rapporterades på platser som var mycket besökta av rovdjur. Rådjuren uppvisade aktivitetstoppar vid soluppgång och solnedgång, som vanligtvis rapporterats för denna art, vilket resulterade i ett lågt överlapp med varg ($\Delta 4 = 0.48$). Resultaten tyder på ett anti-predatoriskt svar hos dovhjortar i form av ändrat beteende över tid, medan inget stöd för rumslig undvikande upptäcktes.

Abstract

Starting from the 1970s, populations of top predators such as the wolf Canis lupus have been expanding across Europe thanks to the protective legislation and the creation of many protected areas, the increased abundance of wild ungulates and other factors. The return of these animals can trigger significant consequences from the ecological as well as management points of view. Large carnivores have been reported to exert a great impact on ecosystems by triggering trophic cascades. In anthropized ecosystems, common in Europe, they are characterized by smaller and scattered protected areas, the presence and activity of humans may influence predator-prev relationships, affecting the potential of apex predators to influence other components of ecosystems. However, the anti-predatory responses are not univocal, especially in fragmented contexts such as the European ones, were animal distribution overlap with human activities. The current study focuses on spatiotemporal distribution pattern of the wolf and its main prey in a costal protected area in central part of Italy (Maremma Regional Park) using camera trapping method. The wolf showed nocturnal activity patterns, with peaks statistically associated with the activity rhythms of the wild boar, with whom a remarkable temporal overlap was detected ($\Delta_4 = 0.91$). Diurnal activity was observed in the fallow deer, with a medium-low overlap with the wolf (Δ_4 = 0.53). Results contrasted with findings obtained in the 1900s, i.e., when the wolf was absent from the area and fallow deer showed nocturnal/crepuscular activity. Moreover, results were different from what found in 2017-2018, i.e., a couple of years after the stable return of the wolf, when diurnal activity was only reported in sites highly attended by predators. The roe deer showed activity peaks at sunrise and sunset, as typically reported for this species, resulting in a low overlap with the wolf ($\Delta_4 = 0.48$). Results suggest an anti-predatory response based on temporal avoidance by fallow deer, whereas no support for spatial avoidance was detected.

Introduction

According to the IUCN (International Union for the Conservation of Nature), during the last decades the populations of European large carnivores such as Eurasian lynx, the brown bear, the wolverine and especially the wolf are stabilizing or increasing thanks to multiple favorable factors (Chapron et al., 2014). Until a few decades ago, the wolf survived only in small and fragmented populations distributed in the Iberian Peninsula, along the Balkans and in Italy (Boitani et al., 1993), due to the persecution carried out by man. In the Italian peninsula, until the end of the 1800s, the presence of the wolf was still widespread throughout the territory, but the population reached its historical minimum around the seventies of the last century, when about 100 individuals were estimated (Boitani et al., 1975). Between 1970 and 1990 there was a considerable increase in the population quantified in about 500-1000 specimens (Gazzola et al., 2007). In fact, since the 1970s there has been a reduction of direct killing by humans because of legal protection, and an increase in habitats suitable for the species and its prev (Chapron et al., 2014; Ripple et al. al., 2014). The increase in the number of wolves in Italy has also been due to an increase of densities of its natural prey, i.e., wild ungulates. During the last decades, thanks to socio-economic changes and legislative regulations, ungulates have once again occupied a large part of the original territory. These mammals play a decisive role in ecosystems and in food webs, allowing the survival for other species, with particular reference to large carnivores. Then, since the 1990s, there has been a gradual but constant expansion of the area occupied by the wolf which has re-established a continuous distribution from the Apennines to the Alps (Galaverni et al., 2016; Lovari et al., 2016). The wolf is a species capable of using most of the terrestrial habitats, adapting to live in contexts ranging from areas with little or no anthropogenic disturbance to conditions of high human activity (The IUCN Red List of Threatened Specistes). This predator is characterized by a wide mobility, being able to move within 24 hours from 1-10km up to 17-38 km (Ciucci et al., 1997). Consequently, its home range can be very large and variable in size according to ecological and individual features, among which a major role is played by abundance and accessibility of prey. The wolf is a predator that tends to base its diet on medium sized-to-large mammals (Peterson et al., 2003; Newsome et al., 2016., Ferretti et al., 2019). When available, ungulates made its staple prey (Newsome et al., 2016); moreover, although wolves are generally described as generalist predators, they can select prey providing the best balance of benefits against costs. Thanks to the recent re-colonization of large sectors of the previous distribution range of this carnivore, predatorprey relationships are going be re-established after decades – or even centuries – of long absence. Thus, the study of predator-prey relationships in re-colonized areas is important to assess wolf ecological needs. to understand its interactions with other components of ecological communities, as well as to evaluate the impact it could have on certain human activities in more anthropized areas.

In ecosystems, the ecological role of apex predators has an extremely important function (Ripple et al., 2004; Hebblewhite et al., 2005; Ripple et al., 2012; Ripple et al., 2014). In fact, the disappearance or reappearance of an apex predator could lead to various cascading effects on organisms belonging to lower trophic levels, including both plants and animals (Ripple et al., 2004). This process is called "trophic cascade", which occurs when an organism at the top of the food chain triggers effects on lower trophic levels through its direct impact on the density of its prey (through hunting) or, indirectly, triggering effects on prey behavior. The progressive return of this predator could be studied both on the ecological and at the socio-economic levels (Chapron et al., 2014; Ripple et al., 2014). The first aspect concerns the relationships that can be established between predators, competitors, prey, and the environment. The latter, on the other hand, can refer to the possible conflict that can arise between predator and anthropogenic activities, among which the most problematic consequence is the one relating to the damage caused by predation on livestock (Meriggi et al., 2011; Chapron et al., 2014).

A trophic cascade could occur in two different pathways: density mediation and behavioural mediation (Knight TM et al., 2005) Density mediated trophic cascades (DMTCs) require a numerical reduction of

herbivores caused by predation. Behaviourally mediated trophic cascades (BMTCs) involve nonlethal, antipredator responses of herbivores to the risk of predation (Schmitz et al., 2000). Behavioural and density mediation seems to have different effects on plants browsing-grazing dynamics: the first could lead to positive indirect effect on plant in area with high predation risk or high predator density, the second one instead lowering the density of the browsers could decrease more broadly the entire effect (Ford et al., 2014). Most of the literature about trophic cascades derives from studies conducted in North America, Canada and Africa. In particular, study areas where trophic cascades have been investigated are remarkably large in respect to the European context, and include a variety of landscapes, large portions of open areas and a relatively lower human activity. In large and heterogeneous landscapes, such as North American National Parks, herbivore species could be expected to have the opportunity to respond spatially to predation risk and thus avoid encounters with predators (Cusack et al., 2018, Berger et al., 2001, Hebblewhite et al., 2005, Crete et al. 1999). Conversely, the European context is highly anthropized and wolf packs have already re-colonized areas close to anthropic activities, where there is intensive forestry activities and presence of humans, as it occurs in countries like Germany, Italy, Scandinavia, Netherlands and Denmark (Kuijper et al., 2016). In anthropogenic landscapes, humans strongly affect the abundance and guality of food resources for prey, mesopredators and apex predators. In fact, during the last thirty years, the amount of food consciously or unconsciously provided by humans, in all the possible ways from agriculture to catering (so called food subsidies), has increased greatly (Kuijper et al., 2016). There are examples in Europe where there is some level of legal harvest of carnivores to reduce the conflict with human activities. In anthropized ecosystems, wolves may be expected to make a substantial use of anthropogenic food such as garbage (Boitani, 1992; Ciucci et al., 1997) or especially livestock (Ciucci et al., 2020, Newsome et al., 2015). On the other hand, human dominated landscapes could limit sensibly the possibility for wild ungulate to roam and adapt spatially their behaviour to the ecological pressure of apex predators. In such conditions, predatory behaviour could be expected to be affected, thus influencing the potential for trophic cascades (Hebblewhite et al., 2005, Kuijper et al., 2016). Hence, the human factor and the little space available to freely roam could lead to behavioural responses different from spatial avoidance, such as temporal avoidance, increase of vigilance activity, and/or increasing group size. Relationships in ecosystems are not always stable, but they change and can shift throughout the years. Therefore, inter-annual studies would be necessary to assess the complex ecological relationships existing between predator and prey, as well as anti-predatory responses.

Increasing the knowledge on interactions between predator and prey is important for an appropriate conservation of ecosystems and for the development of adequate management plans for habitats and species of conservation interest (Hebblewhite et al., 2005; Ripple et al., 2012; Ripple et al., 2014). Since the large carnivores are at the top of the food chain, it has been hypothesized that the prey-predator dynamics may involve direct or indirect control by these predators over the herbivore community, influencing their behavior, ecology, and distribution and therefore the dynamics of the entire ecosystem (Hebblewhite et al., 2005; Ripple et al., 2014; Rossa et al., 2021). However, the predation mechanism is influenced by various other factors concerning the area and densities i.e., accessibility, richness or use of the space by prey (Ferretti et al., 2019a), and therefore, overall, the selective choice of the pre follows the principle of profitability, where the relationship between costs and benefits is a balance between the maximum energy input and the energy expended to obtain it (Stephens et al., 1986). On the other hand, evolving together with the predators, prey is expected to develop over the time anti-predatory tactics useful to avoid the encounter with the predator and therefore surviving. These responses are identifiable. thanks to the so-called "ecology of fear" (Ripple et al., 2004; Ripple et al., 2014), as a consequence of intense predatory pressure, which can cause changes in the behavior of the prey. Examples of such change could be in the use of space (habitat) or in the temporality and foraging modalities (Theuerkauf et al., 2003; Ripple et al., 2004; Ripple et al., 2012).

The case considered in this study concerned the wolf as a large carnivore in a recently recolonized Mediterranean coastal area, the Maremma Regional Park (central Italy). Previous work, conducted at an initial stage of the wolf re-settlement, showed that the main local prey of the wolf was the fallow deer Dama dama, followed by the wild boar Sus scrofa and the roe deer Capreolus capreolus, while the livestock (mainly cattle and sheep which are rarely preyed upon) was used occasionally (Ferretti et al., 2019a). Fallow deer and wild boar were the most preved species in summer, while in winter an increase in the use of alternative prev has been detected, such as meso-mammals such as the nutria Myocastor covpus (Ferretti et al., 2019a). What is particularly interesting is the way the wolf selects its three main preys, which does not seem to reflect their availability. The fallow deer was in fact the most selected species, the wild boar the species used according to its availability and the roe deer the least predated species (Ferretti et al., 2019a). This result was explained as the outcome of the number of encounters between prey and predators, which is expected to be greater in gregarious species (e.g., fallow deer) than in solitary species (e.g., roe deer), or in the active defense of prey during predation, as found in wild boar (Hebblewhite et al., 2002). At the same time, camera trapping showed that the main prey, i.e., the fallow deer, had clearly diurnal temporal activity patterns in sites highly attended by wolves, being nocturnal/crepuscular in sites with low wolf use (Rossa et al., 2021; see also Zanni et al., 2021 and Niglio 1995, for a wolf-free site and our study area in times when wolves were absent, respectively). These results suggested a behavioral response based on temporal avoidance. Preliminary studies were based on a small sample of camera trapping locations and guite restricted in spatial coverage (21 locations across a c. 30 km² area used by a single pack, Rossa et al., 2021). It is thus necessary to replicate and extend the study to verify (i) whether the temporal response of fallow deer is confirmed after several years, (ii) whether a spatial response also occurred, and (iii) the spatial-temporal relationships between the wolf and the other two ungulate species.

Thus, this thesis proposes to give an evaluation of the spatial and temporal mechanisms of interaction between the wolf and its three main preys at a temporal lag of *c*. five years after wolf re-colonization (cf. Rossa et al., 2021). In anthropized systems, the presence of anthropic structures would be expected to limit the opportunity for large herbivores to modify their movements to spatially avoid predators over vast landscapes (Kuijper et al., 2016). If so, temporal responses – rather than spatial ones – may be expected to primarily occur, leading to temporal shifts of activity to times of day when the probability of encounters with predators is reduced (Kohl et al., 2018; Palmer et al., 2021). Accordingly, I would expect that temporal responses be more likely to occur than spatial ones, in my study system. Consistently with previous findings, I would expect a negative temporal association between the fallow deer and the wolf (Rossa et al., 2021). Furthermore, I would expect that the wolf be spatially associated with its local major prey, i.e., the fallow deer and the wild boar (Ferretti et al., 2019a).

Aim

The purpose of my thesis was to evaluate the spatial and temporal relationships among the wolf and its main prey (fallow deer, wild boar and roe deer) throughout a whole year in the Maremma Regional Park (April 2020-March 2021), and to compare results with findings of a preliminary study conducted in an initial stage of wolf recolonization (Ferretti et al. 2019a; Rossa et al. 2021).

In particular, I wished to address the following issues:

- temporal rhythms of activity of the wolf and those of ungulates at the seasonal scale.
- temporal overlap between the wolf and its prey, to evaluate the potential for anti-predator responses based on temporal avoidance of the predator.
- spatial relationships between the wolf and its prey, to test the potential for spatial association *vs.* avoidance patterns.

Material and methods

Study area



Fig.1: Map of the study area and the camera trap locations (red dots). Study site is 90 km².

The research was conducted in the Maremma Regional Park, a coastal area in central of Italy (c. 90 km²) protected since 1975 by Regional Law n. 65. The local climate is Mediterranean, characterized by hot dry summers and wet winters. The temperatures during the entire year range from 9°C in January to 24°C in August, and monthly rainfall ranges from 9.3 mm in July to 81.8 mm in November (Ferretti et al. 2021). The protected area is located on the Tyrrhenian coast of southern Tuscany in the province of Grosseto, including municipalities of Grosseto, Magliano in Toscana and Orbetello.

The Park is characterized by different habitats: a substantial portion is occupied by the Uccellina Hills, with the highest peak of 417m a.s.l. (Poggio Lecci), which cover the central-southern part of the entire area. The Pinewood of Alberese is another significant sector, including a pinewood planted during the 18th by the Lorena's which stands between the Uccellina hills, and the Trappola swamp and the Ombrone mouth. The marshy area which occupies the northernmost part of the park it is characterized by the presence of wetlands, meadows, and open fields.

Regarding the vegetation of the entire area, it is mainly characterized by Mediterranean sclerophylly scrub wood (58%), including three main wood types: oakwood, mainly holm oak *Quercus ilex* trees; scrub wood, principally holm oak and strawberry tree *Arbutus unedo*; garrigue, with bushes (mainly holm oak, rosemary *Rosmarinus officinalis*, juniper *Juniperus spp*.and rockrose *Cistus* spp.). The northern part of the area contains the pinewood (10%, mainly domestic pine *Pinus pinea*), abandoned olive groves and pastures (15%), set-aside grassland (4%), and crops (12%), mainly cereals and sunflower.

When the park was established in 1975 there were no signs of the presence of the wolf (Arrigoni et al., 1976) but during 2006 and 2008 a pack of wolves with sings of admixture with dogs was detected (Gallo et al., 2015). Until 2015 sings of presence of large predators were not found suggesting local extinction

(Ferretti et al., 2019b), when it was documented, in the Uccellina's hills area, the presence of a pack of wolves whose identity was verified by genetic analysis (carried out by ISPRA, i.e., the Italian National Wildlife Institute)(Ferretti et al., 2016, 2018;Fazzi et al., 2018).Two years later, a second pack formed up north of the Ombrone river (Fazzi et al., 2018;Ferretti et al., 2018), whereas a third pack was reported in 2019-2020.Apart from wolves and its main preys (the fallow deer, the wild boar, and the roe deer), there are many other species of mammals: the crested porcupine *Hystrix cristata*, the introduced coypu *Myocastor coypus*, the European brown hare *Lepus europaeus*, the red fox *Vulpes vulpes*, the Eurasian badger *Meles meles*, the wildcat *Felis silvestris*, the stone marten *Martes foina*, the pine marten *Martes martes*, and a few species of smaller mammals. Livestock includes free-ranging cattle and horses, which roam in sectors of pinewood and abandoned olive groves and pastures, as well as two sheep herds in localized sectors of the agricultural area. Population control of fallow deer (selective culling), i.e., a non-native species in Italy, and wild boar (selective culling and trapping/removal) is carried out by the park agency to limit negative effects of these ungulates on habitats and species of conservation interest, on cultivations, as well as to reduce competition with native roe deer (Ferretti et al., 2011).

Data collection

The spatial-temporal patterns of the wolf and its prey were analyzed through intensive standardized camera trapping: for the purpose of this work, I used data covering a full year and collected between April 2020 and March 2021. This study was conducted with the help of 60 camera traps located within a sampling grid (cells size: 1 × 1 km) laid over to the non-agricultural part of my study area through a Geographic Information System (G.I.S., 1 location per suitable cell). Camera traps were deployed on animal trails, paths and/or forest roads suitable to detect mammals (Ferretti et al., 2021; Rossa et al., 2021). All locations were monitored continuously during the study period. Camera traps (models: Owlzer Guard-Z2, Comitel Guard 1 and Guard Micro-2, Ir-PlusHD, Ir-Plus110), triggered by passive InfraRed sensor (PIR) with a trigger time \leq 1 second, we put on site and set up to record videos lasting 30 seconds, with an interval of 1 second between two consecutive videos. They were active 24 hours a day as well, to get better evaluation of the activity rhythms of the species, are set on the solar time (Rovero et al., 2013). We preferred to use the IR at 940m Nm (No glow-led) to reduce the disturbance caused to animals, despite the lower guality of the videos at night (Calaridge et al., 2005, Wearn & Glover-Kapfer, 2017). All the camera traps were equipped with an external battery (contained in a hermetically sealed structure and connected to the camera through a cable) and a 16/32GB SD memory card, both of which are checked and replaced approximately every 4 weeks. The recorded videos were then downloaded and subsequently viewed by observers. Then, all the information obtained were uploaded on a Microsoft Excel database including date, time, sequence identification code of the video, location identification code, number of individuals appeared in the video, species, operator's initials.

Temporal patterns of predator and prey

The first major step for my study consisted in the investigation of temporal activity patterns of both the wolf and its prey, to understand in detail what are the daily habits throughout the four seasons. This primary part of the study was carried out using the Kernel density estimation method (Ridout et al., 2009) through the R Studio software (4.0.4). From this analysis, using time as a continuous and circular variable, density functions were obtained: by plotting time in continuous form on the abscissa axis and the density function in the ordinates (Ridout et al., 2009; Meredith et al., 2014; Lund et al., 2017; Marcus, 2021). The result obtained is explicable with a graph whose area indicates the likelihood of observing the activity of the focal species in each time period (Ferretti et al., 2019, Rossa et al., 2021) To make sure that inference on results was robust, the degree of uncertainty of the estimate of activity rhythms was evaluated, statistically calculated through random resampling with replacement and relative 95% confidence interval

(bootstrap, n = 1000 resampling; Davis et al., 2012; Mori et al., 2020; Rossa et al., 2021). The last phase, on the other hand, involved the non-parametric calculation of the temporal overlap coefficient Δ , which is a standard index commonly used to evaluate temporal relationships between species through the estimate of the overlap of their activity models (Cusack et al., 2018). The value of the coefficient has a range that can varies from 0 (in the absence of overlap) to 1 (complete overlap) (Linkie et al., 2011). Values of the coefficient \leq 0.5 indicate "low" overlap, while values> 0.75 identify a "high" overlap (Monterroso et al., 2014).

To reduce the risk of evaluating data referring to more than one shoot close in time of the same individual(s), the videos of the same species captured in less than 30 minutes in the same location were considered as a single "event", with the time of the first recording as the detection time (Tobler *et al.*, 2008; Lucherini et al., 2009; Monterroso *et al.*, 2014; Torretta *et al.*, 2016, 2017; De Satgé *et al.*, 2017; Ferretti et al., 2019, 2021). All these analyses were conducted at the seasonal scale (spring: April-June; summer: July-September; autumn: October-December; winter: January-March). Data were analyzed through the R packages 'overlap' and 'activity' (Ridout et al., 2009).

Spatial analysis

Regarding the spatial relationships between wolf and prey, I evaluated whether (*i*) spatial patterns of the wolf were associated with those of its prey, and (ii) spatial patterns of ungulates suggested a potential avoidance of their predator. To this purpose, I used generalized linear mixed models (GLMMs, Crawley, 2007), fitting a set of models for each focal species (wolf, wild boar, fallow deer, roe deer). As a first step, I checked the distribution of the putative response variables, i.e., the number of detections of wolf, wild boar, fallow deer, and roe deer, in each location and month, to identify the most suitable error distributions for models. Since I worked with count data that did not show a not normal distribution, containing only zero and positive integers. I modeled the response variables with a negative binomial distribution (Crawley, 2007). I continued building a first set of preliminary models for each species including predictors that were biologically relevant and potentially associated with the detection probability of the species, i.e. the height at which the camera was deployed, percentage shrub cover in a 10 m radius around the camera trapping site (estimated visually in the field), percentage canopy cover in a 10 m radius around the camera trapping site (estimated visually in the field), and the camera model. I added the log (number of days with the camera working) as an offset variable to standardize the detection rate according to the sampling effort, and to detect if these variables influence the detection rate. location and month. The ID code of all locations was inserted as a random effect in all the models to control for repeated detections in the same camera location throughout the different seasons. Since none of these predictors turned out to influence the detection rate of my focal species, I excluded them from the following analyses.

After this process, for each species I fitted a global model including the following predictors: prey (for the wolf) or predator (for prey) detection rates, habitat (pinewood; oakwood; shrub wood; ecotone and open areas), people detection rate, season (spring; summer; autumn; winter). For each species, detection rates were calculated for each location as the ratio of the number of detections over the number of the days when the camera was working. Offset variables and random effects were used as above. Considering detection rates allows to evaluate the rate of use of each single location by our focal species, thus providing a finer information on the interspecific relationships of site-specific intensity of activity in respect to that provided by analytical approaches based on detection/non detection surveys (see e.g., Cusack et al., 2016).

Then, using the package 'MuMIn' (Bartón, 2015), a selection model was performed to select the simplest model with lowest AIC (Akaike's Information Criterion); moreover, I selected for inference those models showing Δ AIC value < 2 with respect to the best one. Moreover, I checked the validity of the models using the "DHARMa" Package, visually analyzing the residuals.

Results

During the study period, the number of video suitable for the analyses was 20680 (n = 1523, wolf; n = 5441, wild boar; n = 5424, fallow deer; n = 589, roe deer; n = 8292, people). The temporal analysis of the activity patterns has shown that wolf activity rhythms were mainly concentrated during the night, with a peak of activity at dusk and an evident avoidance of daytime hours (Fig. 2). The graphs show small seasonal variations, with an apparent increase of activity at dawn and sunset in autumn and winter in respect to summer and spring, when the mobility during the day was apparently negligible (Fig. 2).

The wild boar activity rhythms showed a pattern very similar to that of the wolf, with nocturnal activity, and reduced activity during the day, and a peak of activity at sunset. At the seasonal level, in spring and especially in summer the diurnal activity was lowered with a high peak of activity at sunset; in winter the diurnal activity but always with a preference for the nighttime.

Fallow deer results revealed a lower frequency of activity at night and a consequent greater activity throughout the day. The pattern highlights in particular two peaks of activity, one at sunrise and one at sunset.

During spring and summer, the daily activity pattern of the fallow deer remained almost constant and relatively low compared to winter and autumn, when diurnal activity became more intense, always with a peak at sunrise and sunset.

As for the roe deer, the peak in the frequency of activity occurred at dawn, with relatively low values interspersed with peaks of lesser intensity during the rest of the day. The only remarkable seasonal variation occurred during the summer when, unlike the other seasons, the daily activity was very low throughout the day with much lower peaks at sunrise and sunset.

As expected, people were detected during the day, with a clear peak at around midday (Fig. 2).



Fig. 2: This figure represents the temporal activity patterns of all the species considered in the Maremma Regional Park, assessed through camera trapping and estimated through Kernel density estimation, obtaining density functions related to time as a continuous and circular variable (March 2020-April 2021; from up to down: wolf, wild boar, fallow deer, roe deer, people). Colored lines represent bootstrapped estimates of activity patterns; dashed black lines represent 0.95 confidence intervals. Black rectangles indicate the nighttime and the grey zone show the times of day concerning dawn and dusk, considering the minimum/maximum day with sunrise/sunset in each season.

Temporal Overlap

Temporal overlap of the wolf and the wild boar was remarkably high, showing the highest peak in winter (Fig.4) with a coefficient of $\Delta_4 = 0.90$ and a 95% confidence interval between 0.85 and 0.93. At the seasonal scale, the temporal overlap between the wolf and the wild boar was lowest in spring $\Delta_4 = 0.80$ and a 95% confidence interval between 0.75 and 0.87; moreover, the overlap coefficient in summer was $\Delta_4 = 0.86$ and in autumn $\Delta_4 = 0.84$

On the contrary the temporal overlap between the wolf and the two species of deer was "moderate" (*sensu* Monterroso et al., 2014), being slightly higher for the fallow deer and lower for the roe deer (Fig.3). The temporal overlap between the wolf and the fallow deer showed the greatest interspecific overlap coefficient in spring $\Delta_4 = 0.76$ and a 95% confidence interval between 0.72 and 0.83, indicating an intermediate interspecific overlap and a good reliability of the result, with the lowest peak of overlap in autumn $\Delta_4 = 0.50$ with 95% confidence interval between 0.48-0.56; in summer the overlap was $\Delta_4 = 0.57$ and in winter 0.59. As for the overlap between wolf and roe deer, it proved to be low in winter $\Delta_4 = 0.54$, confidence intervals = 0.0.50 - 0.64), with the highest peak is on summer (Fig.4). ($\Delta_4 = 0.68$, confidence intervals = 0.61-0.76), additionally in autumn the temporal coefficient was $\Delta_4 = 0.55$ and in spring $\Delta_4 = 0.61$.



Fig. 3: Coefficients of interspecific overlap (Δ_4) of temporal activity patterns between the wolf, ungulates and people with 95% confidence intervals at the seasonal scale).



Fig.4: Temporal overlap of activity pattern on seasonal scale. The blue area represents the overlap time spent among the species.

Spatial analysis

The model selection procedure led to select only the best model for wolf detection rates (Table 1). In particular, the wolf detection rate increased along with wild boar and fallow deer detection rates (Table 2; Fig. 5), Instead the wolf detection rate decreased with the increase of the roe deer detection rate (Table 2; Fig. 5). Moreover, the wolf was detected more often in ecotone/open habitats than in the other habitats (Table 2; Fig. 6).

As for wild boar spatial patterns, the best model was selected (Table 1). In particular, wild boar was detected more often in the open habitat (Table 2; Fig. 5).

As for fallow deer spatial patterns, the best model was selected (Table 1). Fallow deer detection rates were concentrated in the open habitat

As the roe deer spatial patterns, the best model was selected and this cervid was detected the most in the shrub habitat (Table 2; Fig. 5).

Table.1: Factors influencing the passage frequency of wolf, wild boar, fallow deer and roe deer evaluated by GLMMs: summary of the selection of the models.

Species	Model	Predictors	Κ	LogLik	AICc	ΔΑΙC	Weight
		Fallow deer + Roe deer + Wild boar +					
Wolf	Best	People + Habitat	11	-1.060.825	2.144.095	0	1.000
Wild							
boar	Best	Wolf + Season	8	-1754024	3524288	0	1.000
Fallow							
deer	Best	Wolf + Season + Habitat	11	-1.603.100	3.228.644	0	1.000
Roe							
deer	Best	Habitat	7	-6850770	1384341	0	1.000

Table.2: Factors affecting the frequency of passage of wolf, wild boar and fallow deer evaluated by GLMMs.

 Variables included in the selected models are shown, together with their relative coefficients, standard error, 0.95 confidence intervals and p-values.
 Confidence interval

(0.95)

						Lower	Upper	
Species	Model	Predictors	В		S.E.	mean	mean	P-value
Wolf	Best	Intercept	-3.352		0,222	-3.787	-2.917	<.001
		F.Fallow deer	0.129		0.066	0.000	0.259	0.050
		F.Wild boar		0.197	0.063	0.074	0.320	0.002
		F.Roe deer	-0.121		0.069	-0.256	0.014	0.078
		People		0.162	0.064	0.037	0.286	0.011
		Habitat(Open)	1.549		0.333	0.896	2.203	<.001
		Habitat(Pinewood)		0.834	0.354	0.140	1.528	0.019
		Habitat(Shrub)		0.432	0.328	-0.210	1.074	0.187
Wild boar	Best	Intercept	-1.427		0.182	-1.784	-1.070	<.001
		F.Wolf		0.120	0.054	0.015	0.226	0.025
Fallow deer	Best	Intercept	-1.101		0.320	-1.728	-0.473	<.001
		Wolf		0.115	0.055	0.008	0.222	0.035
		Habitat(Open)	-0.61		0.473	-0.989	0.866	0.897
		Habitat(Pinewood)	-0.918		0.492	-1.883	0.047	0.062
		Habitat(Shrub)	-0.911		0.460	-1.813	-0.008	0.048
		Season(Summer)		-0.376	0.188	-0.744	-0.008	0.045
		Season(Autumn)		0.141	0.186	-0.223	0.505	0.449
		Season(Winter)	-0.555		0.187	-0.921	-0.189	0.003
Roe deer	Best	Intercept	-3.232		0.263	-3.748	-2.715	< .001
		Habitat [Open]	-1.236		0.420	-2.059	-0.414	0.003
		Habitat [Pinewood]	-1.627		0.476	-2.560	-0.695	< .001
		Habitat [Shrub]	0.024		0.370	-0.701	0.750	0.948



Fig.5: Wolf detection rate (number of detections per day) in relation to fallow deer, wild boar and roe deer detection rates. The blue lines indicate the relationships between variables estimated through Generalized Linear Mixed Models; the shaded area indicates the 0.95 confidence intervals of the estimated relationships.



Fig.6: Detection rate (number of detections per day) for each species in relation to the habitat. Relationships estimated through Generalized Linear Mixed Models and relevant 0.95 confidence intervals are shown.

Discussion

The results of this study conducted on the interspecific relationships between the wolf its main prey confirmed primarily the predator nocturnal activity (Ciucci et al., 1997; Rossa et al., 2021, for my study area) with evident peaks at dawn and dusk throughout all the four seasons across the period of study (April2020-March2021). The fallow deer showed a remarkably high diurnal activity and low nocturnal activity, their highest peaks were found during the cold seasons (Autumn and winter). Moreover, the nighttime activity pattern of this cervid resulted to be remarkably higher in periods in which the wolf was absent from the study area (Nigli 1995), or during 2017-2018 when the stable presence of the wolf was the its initial stage (Rossa et al., 2021) and/or sites in which the wolf was not present at high density (Zanni et al ., 2021). Across the whole period of study spatial partitioning seemed to not play a considerable role in predator prey ecology, our findings showed a consistent spatial overlap of the wolf and its main prey expect for the roe deer, on the other hand our results suggested a temporal avoidance of the predator by the fallow deer. The high degree of spatial overlap could be due to the poor dispersal opportunity. the high prey densities, and accessibility. The wolf showed a substantial temporal and spatial overlap with wild boar, as well as a spatial overlap with the fallow deer using the most the open habitat where these species are more abundant. This result may bear implications for its predation habits, enhancing its probability to encounter these ungulates that constitute its main prey in my study area (Ferretti et al., 2019). As for the roe deer, both the temporal and spatial overlap with the wolf were very low compared to the other prey used (see also Rossa et al., 2021), consistently with the low use of this prey by the wolf, in my study area (Ferretti et al., 2019). The prey species could develop anti predators' responses aim to increase individual survival rate. Over a certain

amount of time, they can change their temporal activity pattern choosing to be more active at specific time of the day when the predator is less active or change their spatial behavior avoiding high risky site where the wolf is present at high densities. Nevertheless, the pattern previously mentioned could remain stable or vary and thus showing fluctuation over the years.

Wild boar lack of tapethum lucidum (Alina et al., 2008) which is a thin layer beneath the retina reflecting the light, thus they have been suggested to prefer roaming during brighter nights (Gordigiani et al., 2021). At the same time, also under these conditions wolves could easily ambush them: in fact, these carnivores have been shown to increase their hunting efficiency during brighter night (Theuerkauf et al. 2003). A recent meta-analysis showed that the wild boar is the most frequently used prey by wolves, in Italy (Mori et al., 2017). It has been suggested that these suids have specific characteristics that make them suitable prey to wolves: they are large - thus being a substantial prey – and are easily detectable because they move in large groups, they are very noisy during their foraging activity, because of grunts, and they have a strong and recognizable smell (Cahill et al. 2003; Massei et al. 2014). Since no evidence was found supporting a temporal and/or temporal avoidance of the predator by wild boar, other anti-predator strategies would be expected to be adopted by these ungulates. In particular, wild boar may use strategies based on group defense or dilution effect/increased predator detection in large groups: future work should test for it. The analyzed data did not provide support to wild boar implementing an anti-predatory response based on spatial and/or temporal avoidance of the wolf (Creel et al., 2014); in fact, the activity rhythms of these two species were very similar. It is notable a remarkable shift of activity peaks throughout the year for this ungulate being much more relevant in spring and summer and lower during autumn and winter.

On the other hand, divergence and limited temporal association occurred between the wolf the fallow deer, which showed opposite rhythms of activity that extend throughout the times of day with broad daylight. This is also confirmed by the level of space-time overlap, which is higher between wolf and wild boar and at the same time lower with wolf-deer and wolf-roe deer. All this is in agreement with the existing literature on the Maremma Regional Park, which highlights how the food habits of the wolf are mainly based on wild ungulates and how, among these, wild boar and fallow deer are the most used (Ferretti et al., 2019, 2021).

This study highlights the possibility that groups of ungulates in the rooted presence of a certain predator may over time behaviorally adapt to this pressure, changing their activity pattern to avoid undesired encounters (Hebblewhite, et al., 2002). For what concern this ungulate, there seem to have been changes in their rhythms of activity thanks to the comparison with previous data (Fig.7). relating to periods in which the wolf was not

present (Niglio, 1995) or had recently settled in the park (Rossa et al., 2021) that suggest an active anti-predatory response of these animals to the constant pressure exerted by the wolf. In fact, these deer showed nocturnal / crepuscular activity rhythms in the absence of the wolf (Niglio, 1995). Rossa et al. (2021), on the other hand, found a very widespread and well-structured diurnal activity only in sites highly used by the wolf. On the contrary, the results of my study show a widespread diurnal activity, consistent with a by now consolidated presence of the predator.



Fig.7: Temporal activity patterns of medium-sized and large mammals (September 2017–August 2018). Colored lines represent bootstrapped estimates of activity patterns; dashed black lines represent 95% confidence intervals (Rossa et al., 2021).

Moreover, these results confirm the relations with the previous study carried out in the park which showed that the highest detection rate of wolf was found in area with the highest densities and detection rate of fallow deer underlying that spatial response was not detected now nor before. This pattern could lead to change in the wolf diet and/or in change in wolf temporal pattern which could respond at the ungulate's changes: further studies on this specific interaction are needed.

The main driven force of predator prey interactions are the attraction of the predator and the avoidance of the prey (Sih 1984), these two processes could vary in their degree of intensity, form and they directly depend on the characteristics of the ecosystem, thus due to this possible variation the outcome could lead to a low spatial-temporal overlap even if the interaction exist (Cusack et al.,2016). The poor spatial-temporal synchronization of the wolf with the roe deer is consistent with the reduced use of this ungulate by the wolf in my study area compared with the use of the others two ungulate in the research. However, it is worth to say that the roe deer occurs at low density in the Maremma Regional Park, where it competes with the fallow deer for foraging areas (Ferretti et al., 2010, Ferretti et al., 2011). In particular, the latter has been reported to actively chase the former away, or displace it, forcing roe deer not to use open habitat (Ferretti et al., 2011). Since the roe deer tends to avoid sites highly attended by fallow deer (Ferretti et al., 2021), the negative relationship between wolf spatial patterns and those of the roe deer is likely to be determined by the former being more active in sites avoided by the latter, this interpretation yield that the spatial analysis of the roe deer and wolf are inconsistent showing the

detections rate of the wolf decreasing with the increase of the roe deer detections; the possible explanation is that the roe deer used the most habitat that the wolf did not use across the study period. Sympatric animals may show such behavioral patterns as the differential use of space and/or time to avoid competitive encounters (Zanni et al., 2020). Further studies should assess which ecological pressure affects more this ungulate, e.g., predation, competition or climatic factors (Ferretti et al., 2021).

Moreover, predator and prey interactions could occur even with a low temporal and/or spatial overlap (Cusack et al., 2016) mostly in a relatively small, protected area such the one used to carry out the study.

Further work would help evaluating whether predator avoidance by prey occurs at finer spatial and/or temporal scales. Courbin et al. (2015) showed that zebras moved away from the areas where they found lions two hours after the encounter. Conversely, Cusack et al. (2018) found no evidence of negative spatial or temporal avoidance of wolves by wapiti in Yellowstone. Analyses of camera trapping data at finer temporal scales (e.g., daily detection rates or time-to-encounter analyses: Karanth et al., 2017; Zalewska et al., 2021) or GPS telemetry of a large sample of individuals (Cusack et al., 2018) would be necessary to improve understanding of mechanisms of interactions acting at shorter temporal – or finer spatial – scales than those adopted in my study. Resource partitioning is not always distributed along clear space and time axes (Müller et al., 2022) and it varies with different areas and ecosystem making these interactions context dependent.

In conclusion, the results obtained from this study showed that the behavioral and space-time habits of the wolf were closely related to those of its main prey and that only the fallow deer has shown a detachable behavioral response. Moreover, it is plausible to think that the prey species could not have developed noticeable spatial response due to the small size of the park (90 km²) and because the protected area is surrounded by high level of human activity making it almost impossible to them to freely use that space avoiding the predator (Kuijper et al., 2016).

Conclusion

The fallow deer has apparently reacted to predatory pressure by changing its rhythms of activity (see Rossa et al., 2021), while the wild boar did not seem to have developed anti-predatory strategies based on spatial and / or temporal avoidance (Cusack et al., 2018). The roe deer, on the other hand, would seem to have responded with a spatial adaptation, choosing areas with a lower rate of encounter with the wolf, although subsequent analyzes are necessary to verify this hypothesis and fill the uncertainty of the data caused by the small sample of observations for this ungulate.

My results match the outcome that was highlighted by the previous research by Ferretti et al. (2019) and Rossa et al. (2021): the most used prey by the wolf is the wild boar, showing the highest space-time overlap with the predator.

It is important to point out that this study was conducted in an anthropized area showing remarkable limitations to possibilities for prey to largely modify their spatial patterns to avoid predators. This is probably a common scenario to other study areas in European anthropized ecosystems, distant from the one studied in other continents (Crête et al., 1999; Cozzi et al., 2012; Ripple et al., 2015). In fact, the characteristics of the territory, its ecological connectivity, the high human densities, and the fragmentation make the study of predator-prey dynamics highly context-dependent (Ausilio et al., 2021). Accordingly, it is plausible that the influence of humans on predator-prey dynamics in anthropized ecosystems could limit the potential for apex predators to trigger trophic cascades (Kuijper et al., 2016), although this aspect should specifically be tested for my study area.

Future research directions should investigate the possible changes in the wolf diet in response to fallow deer switch in temporal activity, and how it could affect the densities of wild ungulates. The possible alternative behavioral responses of the wild boar not yet tested should be evaluated (e.g., increased group size). Moreover, the possible consequences on the ecosystem should be tested. To discover this and other ecological dynamics that are established in this area recently colonized by the wolf it is necessary to continue the monitoring and analysis as predator prey populations change in numbers. Continuing to study these dynamics on a multi-year scale is vital both from an evolutionary and conservation perspective in order to enrich scientific knowledge on interspecific relationships in general, and more specifically on those relating to predator-prey dynamics.

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What's the relevance of this research?

Large carnivores such as wolves can have a significant impact on ecosystems, they are able to influence the entire chain by triggering trophic cascades, especially when they have been absent or very scarce for decades. Prey species such as ungulates could adopt antipredator responses based on temporal or spatial avoidance, could increase the group size and/or decrease the time spent foraging to enhance vigilance to better detect the predator. In the relationship between predator prey over the time, the latter will try to increase the effectiveness of its hunting as much as possible and therefore the possibility to detect and kill the prey by rationalizing its energy, while on the contrary the prey will try to avoid the encounter with the predator and thus raising his chances of survival. It is difficult to assess the impact of certain carnivores on the various aspects of an ecosystem, since each action corresponds to a consequence that is hardly unique and depends on a multitude of factors that are intertwined with each other and thus are context dependent.

Aim of the study

The objective of this research was to evaluate the temporal and spatial responses of wild ungulates towards the wolf predatory pressure and investigate their possible changes thanks to the previous study done at the park.

How was the study carried out?

This study was conducted during a year of intense camera trapping method which involved the use of 60 camera trap locations and the information of the videos from the cameras were reported on a database (Excel) and analyzed in two major steps using R Studio (4.0.4).

What did we find?

From the analysis of these data, it seems that the only one to adopt an anti-predatory response identifiable with that of space / time was the fallow deer which have shown an intense daytime activity and almost completely absent activity at night, contrary to what was reported by previous studies done in the park when the wolf was not stable, and this ungulate used to be more mobile during the night. While the wild boar does not seem to have implemented any spatial or temporal avoidance, the only anti-predatory response that can be detected is the active protection of the offspring and the group. As for the Roe deer it is a wolf prey, but little used compared to the first two, it occupies less comfortable areas such as open grazing areas but instead it uses more rocky hills and dense scrub areas.

Future studies?

To assert which ecological pressure makes affect the roe deer more behavior the most, if the predatory pressure exerted by the wolf or that of the fallow deer, specific study is needed; moreover, continuous, and multiple year scale studies are vital to better understand these interactions where the human activities are very high and could therefore affect these relations.