



Iberian wolf's diet and its quality during breeding season: exploring the influence of zone, wolf groups, prey availability and individual factors

Isabel Barja^{1,2} · Ana Piñeiro¹ · Javier Talegón³ · Aritz Ruiz-González⁴ · Álvaro Navarro-Castilla^{1,2} · Amaia Caro⁴ · Toni Gago-Barja¹ · M. Carmen Hernández¹

Received: 7 August 2023 / Revised: 29 February 2024 / Accepted: 7 March 2024 / Published online: 20 March 2024

© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2024

Abstract

Large predators are essential in maintaining ecosystem functioning, and comprehending how their feeding habits change across natural and human-dominated landscapes is crucial to preserve biodiversity. In this study, the diet of Iberian wolves (*Canis lupus signatus*) during pup rearing season (July to September) has been studied in relation to prey abundance and putting emphasis in the analysis of the differences between zones, wolf groups and individual factors (age, sex and social status). For this, non-invasive monitoring was carried out in three zones of Spain where nine different wolf breeding groups were detected (Galicia, $n = 4$; Zamora, $n = 4$ and Valladolid, $n = 1$). Faecal samples were collected near breeding sites for dietary and genetic analyses, registering if it was or not a scent mark to know the social status of the individuals. Prey availability was determined by camera trapping or requesting the official census of wild prey in the study areas. We found differences in wolf's diet depending on the zone and the breeding group however, the diet did not vary depending on the age, sex and social status. In general, Iberian wolves mainly fed on wild ungulates (wild boar, roe deer and red deer), feeding on the most abundant prey, except for Baldriz group in Galicia which seems to be specialized in hunting roe deer. Domestic animals' consumption (sheep, goat, donkey, pig) was not high, but it occurred specially in agriculture and livestock areas (Ferrerías in Zamora and Valladolid) where wild prey were less available.

Significance statement

In this study, we shed light on the vital role of Iberian wolves in the ecosystem by investigating the dietary preferences during pup rearing season across different landscapes. Our research revealed differences in wolf diets based on geographic location and breeding groups, emphasizing their adaptability. We found that age, sex, and social status did not significantly influence their dietary preferences. Iberian wolves predominantly target wild ungulates, except for a specialized group in Galicia. Our study introduces a novel approach as it is the first to analyse wolf diet based on individual factors and using nitrogen analysis. Additionally, the study highlights previously overlooked dietary patterns of the Iberian wolf subspecies, despite its critical role in the ecosystem as a top predator. Furthermore, we emphasize the necessity of conserving wild ungulate populations to reduce livestock attacks by ensuring a stable supply of wild prey, promoting coexistence between humans and wolves.

Keywords *Canis lupus signatus* · Wild ungulates · Feeding habits · Diet quality · Nitrogen analysis · Rendezvous sites

Communicated by K. Eva Ruckstuhl.

✉ M. Carmen Hernández
mcarmen.hernandez@uam.es

¹ Unidad Zoología, Departamento de Biología, Universidad Autónoma de Madrid, C/ Darwin 2, Campus Universitario de Cantoblanco, 28049 Madrid, Spain

² Centro de Investigación en Biodiversidad y Cambio Global (CIBC-UAM) Universidad Autónoma de Madrid, C. Darwin 2, E-28049 Madrid, Spain

³ Llobu, Ecoturismo y Medio Ambiente, C/ Santa María 126, 49522 Mahide (Sierra de la Culebra), Zamora, Spain

⁴ Department of Zoology and Animal Cell Biology, University of the Basque Country (UPV/EHU), C/ Paseo de la Universidad 7, 01006 Vitoria-Gasteiz, Spain

Introduction

Wildlife population dynamics are determined by the complex interactions of multiple factors, including climatic conditions, predation, food availability and disease (Holmes 1995). Nutrition and reproduction are closely interlinked, when food requirements are not satisfactorily met, reproductive rates decline (Wade and Schneider 1992; Elmhagen et al. 2000; Allen and Ullrey 2004). Such decrease can affect population size (Fuller 1989; Stirling et al. 1999) and can have repercussions through entire ecosystems (Ripple and Beschta 2003; Hambäck et al. 2004; Hebblewhite et al. 2005). Understanding feeding ecology is crucial to comprehend the survival and productivity of animal populations (Barboza et al. 2008; Robbins 2012). Acquiring knowledge about diet quality and composition is critical not only in ecology and trophic studies but also in terms of economics and conservation.

The wolf (*Canis lupus*) is an important top predator which plays a fundamental role maintaining biodiversity in ecosystems (Wilmers and Getz 2005; Smith and Bangs 2009; Letnic et al. 2012) and the feeding ecology of this species has been extensively investigated across its European range (Salvador and Abad 1987; Papageorgiou et al. 1994; Meriggi et al. 1996; Sidorovich et al. 2003; Gazzola et al. 2005; Śmietana 2005; Valdmann et al. 2005; Ansoerge et al. 2006; Barja 2009a; Lanszki et al. 2012). Traditionally, wolves have been considered as generalist-opportunistic predators, selecting the most abundant and available prey (Ciucci 1994; Mattioli et al. 1995; Meriggi et al. 1996, 2011; Milanese et al. 2012). In areas where wild ungulates are numerous, wolves feed mainly on them (Jędrzejewski et al. 1992; Meriggi and Lovari 1996; Barja 2009a). Throughout southern Europe, in regions characterized by poor ecological conditions, wolf populations adapt to a diversity of food resources, such as livestock, fruits and small mammals (Castroviejo et al. 1975; Meriggi et al. 1991; Torres et al. 2015). Nonetheless, some studies have reported that wolves may show a clear food selection for particular prey species, even though these are less abundant than others (Potvin 1988; Dale et al. 1994; Meriggi et al. 1996; Kunkel et al. 2004). Studies carried out in the northern Iberian Peninsula show that the behaviour and preferences of the Iberian wolf (*Canis lupus signatus*) are closer to a facultative specialist than to an opportunist species (Barja 2009a), with a preference for specific and profitable wild prey over anything available. Prey availability can have a strong impact on reproductive success in many carnivore species (White and Ralls 1993; Fuller and Sievert 2001; Persson 2005), including wolves (Fuller 1989; Boertje and Stephenson 1992; Fuller et al. 2003). Reproduction is linked to a fundamental life-history trade-off

increasing energy requirements (Williams 1966; Calow 1979; Zera and Harshman 2001; Speakman 2008), thus, feeding habits of the group can be affected by this increase in trophic resources demand.

Wolves are social animals and groups are characterized as dominance hierarchies that consist of only one breeding couple sharing the leadership, the dominant male and female (Packard et al. 1983; Mech 1999). Maintaining dominance, which yields significant benefits (e.g., access to food and mates), results in increased reproductive success, but it comes at a cost as dominant individuals suffer higher levels of physiological stress than subordinates (Sands and Creel 2004; Barja et al. 2008a, b). Wolves also show cooperative breeding and hunting (Mech and Boitani 2007). Consequently, all members of the pack hunt and eat the same preys. However, dominant wolves eat first (Mech and Boitani 2007), gaining access to the most nutritional parts. Diet quality has significant repercussions on animal's physical condition (Loeb et al. 1991; Codron et al. 2007) and can be assessed through faecal indicators such as total nitrogen percentage (Aldezabal et al. 1993; Robbins et al. 2005; Baldwin and Bender 2009; Navarro-Castilla et al. 2023). This element is commonly used since proteins are biomolecules of high nutritional value, and it has been used to evaluate the nutritional quality of the diet of numerous mammalian species (Arman et al. 1975; Sakaguchi and Ohmura 1992; Sergiel et al. 2020).

The present study aims to analyse variations in feeding habits of wolf groups during the breeding season. In particular, trophic behaviour was examined in relation to prey availability, zone and wolf breeding group, social status, sex and age of individuals. (1) *Prey availability*: it is expected that the diet of wolves is affected by prey availability, which is related to the habitat type. As for prey selection, we expected that wolves selected the most vulnerable and profitable prey in each area, as they are facultative specialist predators (Barja 2009a). (2) *Zone and wolf group*: similarly, we predicted that trophic niche breadth would be broader in zones and groups with access to a higher variety of prey species because they can offer wider profitable dietary options for wolves. Particularly, we expected a broader niche breadth in Galicia compared to the other two zones (Zamora and Valladolid) as the first one is a protected area which should have a higher biodiversity. As for diet similarity, it is expected that wolf's diet would be more similar within zones and especially different in Valladolid compared to Galicia and Zamora, since habitat characteristics are dissimilar, which entails different prey presence and availability.

(3) *Individual factors*: since wolves are highly social animals that hunt together, we did not expect differences in the diet composition between sexes. Moreover, we predicted a correspondence between adults' and cubs' diets, since this

species exhibits cooperative breeding and therefore, they feed on the same preys. Furthermore, we addressed another key issue: how diet quality varies depending on social status. The diet composition of dominant and subordinate wolves should be similar. However, we expected dominant wolves to have more nourishing diets (i.e., protein-rich) due to feeding hierarchy, resulting in higher faecal nitrogen concentrations.

Materials and methods

Study areas

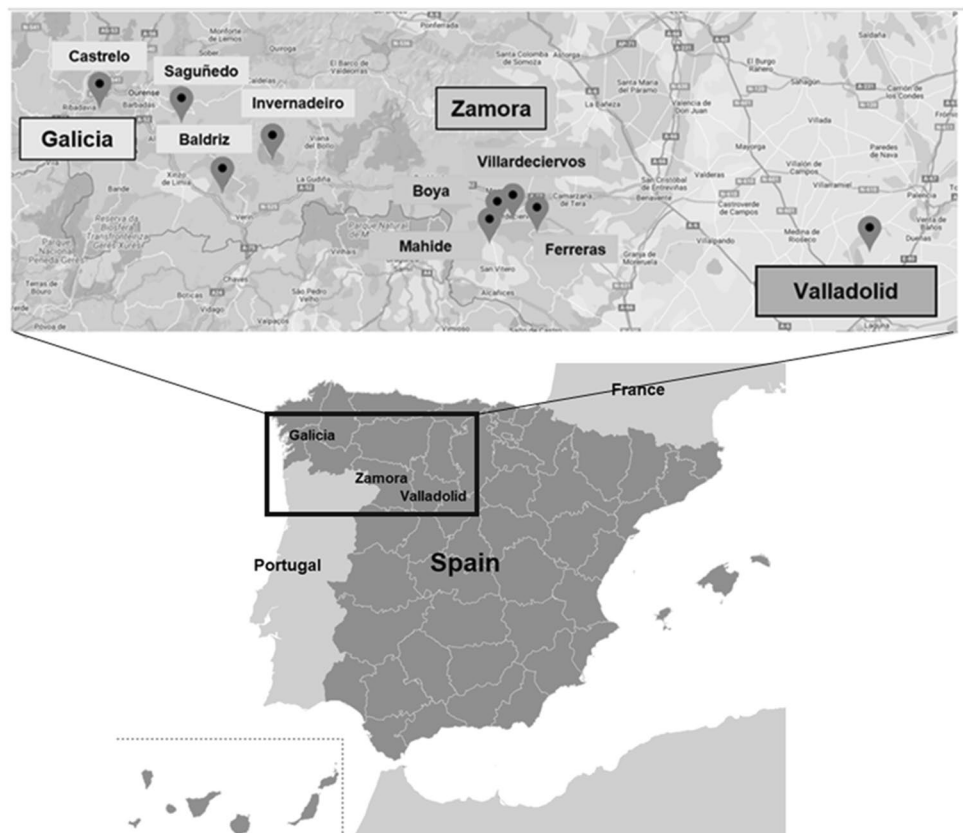
Fieldwork was carried out in the northwest of the Iberian Peninsula: Galicia, Zamora and Valladolid regions (Spain; Fig. 1) from 2008 to 2011 during pup caring months (July to October). These study areas were selected because the presence of Iberian wolf breeding groups was known in advance. In Galicia, the study location is a protected area of 5722 ha called Montes do Invernadeiro Natural Park. This area falls within an altitudinal range of 803–1707 m with a series of low mountains and deep valleys. The vegetation mainly consists in scrubland composed by heather (*Erica australis*), prickled broom (*Pterospartum tridentatum*) and sandling (*Halimium lasianthum*).

There are also deciduous forests in the valleys and along watercourses, characterised by oak (*Quercus robur*), birch (*Betula celtiberica*) and holly (*Ilex aquifolium*). Replanted Scot pine (*Pinus sylvestris*) forests are also common in the area. This region, especially the natural park, has a high density of wild ungulate prey such as roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*) and wild boar (*Sus scrofa*).

Study area in Zamora comprises the “Sierra de la Culebra” Regional Hunting Reserve. The Sierra de la Culebra is a mountain chain ranging from 800 to 1243 m of altitude. The vegetation is characterised by scrubland composed by heather (*Erica australis*). While natural forests are scarce and mostly found in the valleys, replanted Scot pine (*Pinus sylvestris*) forests are abundant in this reserve. As in Galicia, wolf preys primarily are roe deer (*C. capreolus*), red deer (*C. elaphus*) and wild boar (*S. scrofa*) (Barja 2009a; Vicente et al. 2000).

In Valladolid, the study region is dedicated to agriculture and livestock, being very flat and with very low tree density. There are no red deer in the area, and wild boars and roe deer are scarce. On the contrary, lagomorphs such as European wild rabbits (*Oryctolagus cuniculus*) and Iberian hares (*Lepus granatensis*) are abundant. There are also domestic pig (*S. scrofa domesticus*) farms in the study area where wolves can scavenge.

Fig. 1 Study area locations: Invernadeiro Natural Park, Saguñedo, Castrelo and Baldriz in Galicia, Boya, Ferreras, Mahide and Villardeciervos in Zamora and Valladolid



Wolf group detection and prey availability

Breeding wolf groups were spotted during May–June by the increase in faecal marking behaviour, which is more intense during breeding season (Barja et al. 2005). Faecal marking is carried out by the dominant pair and only in reproductively active groups (Barja et al. 2008a, b). Wolves use conspicuous substrates to deposit faeces to increase the effectiveness of the signal (Barja 2009b), this way wolves know which territories are occupied by other neighbouring groups. Later, breeding groups were confirmed by camera trapping in Galicia and by sightings in Zamora and Valladolid. A total of nine *rendezvous sites* (zones where pups are left from July to September while adults hunt, Mech and Boitani 2007) belonging to nine Iberian wolf breeding groups were located: four in Galicia (Invernadeiro Natural Park, Saguñedo, Castrelo and Baldriz), four in Zamora (Boya, Ferreras, Mahide and Villardeciervos) and one in Valladolid. Blinded methods could not be applied in this study because faecal samples were collected in the field from unidentified wolves.

To estimate wild ungulate prey availability (roe deer, red deer and wild boar) in Galicia, we set 12 camera trapping stations (model: Moultrie M-100) for one month (August) in each one of the four group *rendezvous site* locations. Cameras were set to take three pictures and one video to ensure prey detectability. We consider the same prey detection event the pictures and videos taken within a 30-minute time interval. We corrected the total number of photographic events between the number of days that cameras were active to set comparable conditions. In the case of Zamora and Valladolid, to know wild prey relative abundance we request to the Administration and the Hunting Reserve, respectively, the official census of wild prey. We also set a correction index for the census by dividing the abundance of wild ungulates by the total area (ha) occupied by each *rendezvous site*.

Faecal sample collection

Scats were collected during breeding months (June–September). For this, we surveyed transects along roads and firebreaks near the *rendezvous sites* walking and with a vehicle (10 km/h) for 15 days each month which were randomly selected. Transects were done early in the morning, when the probability to detect fresh faeces is higher due to wolf nocturnal and crepuscular habits (Barja et al. 2008a, b, 2018). Fresh scats were discriminated from old ones by the strong smell, a layer of mucus, and no signs of dehydration (Barja 2009a; Barja et al. 2008a, b; Martín et al. 2010). Wolf scats were discriminated from those of other carnivores by their size and shape, and wolf pup faeces were readily identified as they are significantly smaller compared to that of the adults. Scats of adult wolves were considered those above 20 cm in length and 2.5 cm in diameter. The excrements of

wolf pups are of a similar size to that of red fox faeces, but foxes do not enter *rendezvous sites* as they are potential prey for the wolves. From each scat we collected two subsamples: one for genetic analyses and another for nitrogen contents. Samples for genetic analyses were preserved in ethanol at -20°C whereas samples for nitrogen content were frozen at -20°C until laboratory analyses. Moreover, from each fresh scat detected, we collected hair and bone samples to conduct dietary analyses.

We also register if the scat was a scent mark or not, considering as scent marks only faeces deposited in conspicuous or elevated substrates or crossroads (Barja 2009b). In wolf groups only the dominant pair exhibit this marking behaviour (Barja 2009b; Barja et al. 2004, 2008a, b), hence, we considered that collected faeces with a marking function belonged to dominant wolves (Barja et al. 2008a, b).

Genetic analysis

To ensure that faecal samples belonged to Iberian wolves and not to other sympatric carnivores, we performed a genetic identification on the faecal samples collected in the field by sequencing mitochondrial DNA (mtDNA). We took a subsample of each faecal sample, placed it in tubes filled with ethanol (96%) and stored it at -20°C . For the extraction of the DNA, we used an extraction kit consisting in silica membranes and adapted to non-invasive samples (QIAamp DNA Stool Mini Kit, Qiagen). To identify the species origin of the samples, we sequenced a 440 bp fragment of the mitochondrial DNA control region following Vilà and colleagues (1999) methodology. We used the PCR (Polymerase Chain Reaction) technique and the universal primers Thr-L 15,926 and DL-H 16,340 for the amplification of the DNA. Then, we used gel electrophoresis to verify the success of the DNA amplification. In order to eliminate the primers and the excess of deoxynucleotides, we applied the alkaline phosphatase and exonuclease I (ExoSAP-IT) method for the cleaning and purification of the amplified product. Finally, the sequencing on this cleaned PCR product was conducted by the application of the commercial kit dRhodamine Terminator Cycle Sequencing Ready Reaction (Applied Biosystems) and an automatic sequencer ABI PRISM Model 3130 (Applied Biosystems). For the species identification, we compared the sequences obtained with reference sequences of dogs and wolves obtained in previous studies (Vilà et al. 1999; Randi et al. 2000; Pilot et al. 2010) and reference sequences deposited in the GenBank databases (<http://www.ncbi.nlm.nih.gov/>) using the BLAST 2.0 algorithm (<http://www.ncbi.nlm.nih.gov/BLAST/>).

We followed the method described in Seddon (2005) for the sex determination of the samples. By using the PCR technique, we amplified two specific canine markers: the DBX intron6 (249 bp), which identifies the X chromosome

in females and males, and the DBY intron7 (118 bp) for the Y chromosome in males. To verify the success of the DNA amplification, we conducted an electrophoretic migration of the amplified product in 1.5% agarose gels. Males were identified by the presence of two bands corresponding to the X and Y chromosomes, while females only presented the band of the chromosome X. All samples were processed in duplicate to cope with the low quantity and quality of DNA extracted from the faecal samples. When bands were faint or fuzzy and thus the identification by agarose gel was doubtful, samples were genotyped with two replicates using an automatic sequencer (ABI PRISM 3130, Applied Biosystems). We used the program GENEMAPPER version 4.0 (Applied Biosystems) to detect the fragments corresponding to the X and Y chromosomes.

Diet analysis

Wolf diet was determined by identifying guard hairs as well as bone remains in the scats. From each scat, we collected sample hairs from all the different groups found, as macroscopic discrimination between groups is feasible in the field. Due to wolf feeding habits, we consider that the presence of hairs from a certain species implies they have hunted down that prey and the group has consumed it entirely, as Barja (2009a) found that 98.5% of Iberian wolf scats contained only remains of one prey species. Then, in the laboratory, we prepared cuticle slides using hair spray as medium (Barja 2009a) to use cuticle patterns for species identification (Teerink 1991; Barja et al. 2021). We used a microscope (Olympus 400X) to compare the cuticle patterns found in the samples with those in reference manuals (Teerink 1991; Barja et al. 2021) and with reference hairs collected in the study area (Barja 2009a; Barja et al. 2021). Bone remains were identified using dichotomic keys and by comparing with a reference collection.

Elemental nitrogen analysis

Since total nitrogen content of faeces seems to be a good indicator of protein ingestion and hence, diet quality (Aldezabal et al. 1993; Robbins et al. 2005; Baldwin and Bender 2009; Navarro-Castilla et al. 2023), we analysed nitrogen contents of wolf faeces to evaluate nutritional condition (protein intake) of individuals.

Frozen faecal samples were dried in the laboratory oven at 90°C until they exhibited a constant weight, which took 24 h. Following, using liquid nitrogen, we pulverized the samples in a mortar and 1 g of each pulverized sample was stored and later analysed at the Research Support Central Services (SCAI - University of Málaga, Spain). Total faecal nitrogen was determined by carrying out the elementary chemical analysis on a PERKIN-ELMER 2400 CHN elemental analyser, using the

classical Pregl-Dumas method according to Sergiel et al. 2020. Faecal nitrogen content is presented as g N/100 g dry faeces.

Data analysis

The composition of the diet was expressed in terms of frequency of occurrence (the total number of times that each prey species appeared in faecal samples) and the percentage of consumed biomass. Since the energy provided by each prey is different depending on its weight, the consumed biomass of each prey species was estimated by multiplying its frequency of occurrence by that prey mean weight, considering both adult and juvenile weights (*C. capreolus* 15.8 kg; *C. elaphus* 57.5 kg; *S. scrofa* 48.5 kg; *C. aegagrus* 15.7 kg; *O. aries* 16.8 kg; *E. africanus asinus* 100.0 kg; *S. scrofa domesticus* 80.0 kg; *O. cuniculus* 1.2 kg; Urios 1995; Llana et al. 1996; Blanco et al. 1998; Mateos-Quesada 2002; Soffiantini et al. 2006; Barja 2009a; Meriggi et al. 2015). When species identification was not possible, consumed biomass was estimated using the mean weight of the corresponding member species of that group (unidentified ungulate: 40.6 kg, mean between *C. capreolus*, *C. elaphus* and *S. scrofa* mean weights). Identification problems only arose with small or deteriorated fragments of the hair that cannot allowed us to distinguish between wild ungulate species. Domestic species were possible to identify by macroscopic characteristics of the hair.

To analyse the relationship between prey consumption and wild ungulate availability in each wolf breeding group, we recorded the total number of times that each prey species appeared in faecal samples (ObsF). Since the number of scats collected in each group was different, ObsF were corrected using the following equation:

$$(ObsF^* = ObsF * I_c)$$

where ObsF* is the corrected frequency and I_c is the correction index:

$$\left(I_c = \frac{N_m}{N} \right)$$

I_c index was calculated by dividing the number of faecal samples collected in each breeding group (N_m) by the mean number of faecal samples collected in all groups (N).

To estimate wild ungulate prey availability, we calculated the expected frequencies:

$$\%Esp = \frac{D_i \cdot 100}{\sum D_i}$$

where D_i corresponded to each prey species availability in each group and D_i the total ungulate availability in each group. Expected frequencies (ExpF) were calculated as:

$$ExpF = \frac{\Sigma Obs.F^* \cdot \%Exp}{100}$$

Jacobs (1974) prey selection index was used to calculate wolf ungulate preferences:

$$D = \frac{r - p}{r + p - 2pr}$$

where r is the contribution of each ungulate species in relation to the total number of prey and p is the abundance of that prey in that study area. D can take values between from -1 to $+1$, -1 implies a negative selection, 0 no selection and $+1$ positive selection.

Moreover, we used Levins (1968) index (L) to estimate trophic niche breadth:

$$B = \frac{1}{\Sigma P_i^2}$$

where P_i is the contribution of each prey to total biomass ingested in each wolf group. B values next to 1 indicate a highly specialised diet whereas larger values indicate an opportunistic trophic behaviour. Since data was not normally distributed, we used non-parametric Kruskal-Wallis test to compare differences between zones (Galicia / Zamora / Valladolid) and Kolmogorov-Smirnov test to compare differences between wolf groups.

To analyse diet similarity, we used Pianka (1973) index:

$$\alpha_{gz} = (\Sigma P_g \cdot \Sigma P_z) \cdot \left[(\Sigma P_g)^2 \cdot (\Sigma P_z)^2 \right]^{0.5}$$

where α_{pz} would be the similarity between wolf breeding groups in Galicia and Zamora, P_g the contribution of one prey species to the total biomass ingested in Galicia and P_z the contribution of one prey species to the total biomass ingested in Zamora. This index was calculated by comparing all study areas between them. Values close to 0 indicate the minimum niche overlap. To test differences between zones (Galicia / Zamora), we used an ANOVA test and a Student's t -test to compare differences between.

To compare wild ungulate abundance within zones, we used a t -test. Since data was not normal distributed, we used non-parametric Chi-square (χ^2) tests to check the independence between the observed and expected prey presence in diet depending on the wolf breeding group, age and social status. To analyse differences in the type of prey consumed between different groups (zone, wolf breeding group, social status, sex and age) we used contingency table analysis. We used Pearson χ^2 when the table had less than 20% of the expected frequencies > 5 . In contingency tables where more than 20% of the expected frequencies

were < 5 , the Monte Carlo's exact test was used. In 2×2 tables where $df = 1$, we used Yates's continuity correction. In 2×2 tables we used Fisher exact test and χ^2 of Pearson for the rest of the cases.

To analyse differences in total faecal nitrogen (%) between breeding groups, sexes, dominant and subordinate individuals we performed non-parametric Kruskal-Wallis and Mann-Whitney tests because data did not fit normal distribution, not even transformed. We only had data of faecal nitrogen contents for Galicia and Valladolid wolf breeding groups because we did not receive any funding to carry out the analysis in Zamora's wolf groups.

Data are represented as mean and/or median \pm standard error (SE), quartiles are given also for nitrogen analyses since data was not normally distributed. The software used to perform the statistical analysis was SPSS 23.0 for Windows (SPSS Inc, Chicago, IL, USA).

Results

Genetic analysis

We successfully identified as wolf scats 63 of the 105 faecal samples, 39 samples did not amplify and 3 belonged to red foxes (*Vulpes vulpes*). Of the total number of samples, we analysed 84 to determine the sex, the 63 samples genetically identified as wolf and 21 of the non-amplified samples that we knew unequivocally to be wolf (by camera traps), resulting in 21 females and 22 males; the rest did not amplify.

Wild ungulate abundance (prey availability)

The abundance of wild ungulates in Zamora and Galicia was similar. However, in Valladolid wild ungulate availability was low, being the main prey available the rabbit (Table 1). In Valladolid, wild boar was the most abundant wild ungulate (75.0%), as well as in Galicia (59.5%), however, red deer was the most abundant in Zamora (86.8%; Table 1). In Galicia, Baldriz was the region with the highest availability of wild boar (81.0%) followed by Castrelo (61.2%), Saguñedo (58.8%) and Invernadeiro (39.1%; $t = 6.99$; $df = 3$; $p = 0.06$). Roe deer was more abundant in Castrelo and Saguñedo (38.8% and 31.2%, respectively) following by Baldriz (19.0%) and Invernadeiro (7.1%; $t = 3.45$; $df = 3$; $p = 0.04$). In Invernadeiro, red deer was the most abundant ungulate (53.9%), being absent in Castrelo and Baldriz (Table 1). In Zamora, red deer was the most abundant ungulate: Boya and Villardeciervos (89.1%), Mahide (86.6%) and Ferreras (77.4%; $t = 23.72$; $df = 2$; $p = 0.02$; Table 1). Data belonged to the hunting census carried out by the rangers during rutting season, but they did not have census available for wild boars, being present in the area.

Table 1 Wild ungulate availability for each wolf group

Wolf breeding group	Wild ungulate availability						Correction index	Method
	<i>Sus scrofa</i>		<i>Capreolus capreolus</i>		<i>Cervus elaphus</i>			
	Abundance (number of individuals)	Corrected abundance	Abundance (number of individuals)	Corrected abundance	Abundance (number of individuals)	Corrected abundance		
GALICIA								
Invernadeiro	66.0	0.05	12.0	0.01	91.0	0.08	1.212	Camera trapping
Saguñedo	64.0	0.11	34.0	0.06	11.0	0.02	603	
Castrelo	52.0	0.08	33.0	0.05	-	-	679	
Baldriz	128.0	0.44	30.0	0.10	-	-	294	
ZAMORA								
Boya	-	-	19.0	0.00	155.5	0.02	8.384	Reserve census
Ferreras	-	-	19.0	0.00	65.0	0.01	11.228	
Mahide	-	-	19.0	0.00	122.5	0.01	8.925	
Villardecier-vos	-	-	19.0	0.00	155.50	0.02	8.384	
VALLADOLID	6.0	0.00	2.0	0.00	-	-	2.850	Hunting census

Correction index in Galicia was obtained by dividing the total number of photographic events between the number of days that cameras were active. In the case of Zamora and Valladolid, the correction index was obtained by dividing the abundance of wild ungulates by the total area (ha) of each location

Frequency of occurrence and ingested biomass: differences between zones and groups

Wolf's diet was different depending on the zone considered: Galicia, Zamora or Valladolid ($\chi^2 = 130.32$; $df = 18$; $p = 0.001$, $N = 405$). We also found that wild ungulate consumption was dependent on ungulate availability in all wolf groups (Table 3; $\chi^2 = 40.11$; $df = 10$; $p = 0.05$, $N = 405$).

In Galicia, we found statistically significant differences in the diet of all groups ($\chi^2 = 34.84$; $df = 18$; $p = 0.01$; $N = 114$), being wild ungulates, particularly roe deer (29.4% FO) and wild boar (43.7% FO), the main prey of wolves (Table 2). However, Invernadeiro and Saguñedo groups mainly preyed upon wild boar and red deer, whereas Castrelo and Baldriz groups predominantly consumed wild boar and roe deer. Domestic ungulates were not as abundant as wild prey in wolf's diet, but goat's (*Capra aegagrus*) remains were found in wolf's scats (Table 2). Considering the biomass ingested, the wild boar was the species that most contributed to the wolf's diet, followed by red deer in Invernadeiro and Saguñedo, and roe deer in Castrelo and Baldriz (Table 2). In all groups the wild boar was the prey that appeared most frequently, followed by red deer and roe deer in Invernadeiro, red deer, roe deer and goat in Saguñedo and roe deer in Castrelo and Baldriz (Table 2).

In Zamora, we also found statistically significant differences in the trophic ecology between wolf's groups ($\chi^2 = 83.17$; $df = 21$; $p = 0.001$; $N = 86$) and the diet was more varied than in Galicia, including different prey species

such as roe deer (2.9% FO), red deer (4.9% FO), unidentified ungulates (18% FO), wild boar (38.2% FO), sheep (*Ovis aries*; 5.9% FO) and donkey (*Equus africanus asinus*; 7.8% FO). In Boya, donkey and red deer were the preys most consumed by wolves, both in frequency of occurrence and total biomass (Table 2). In Ferreras group, wild boars and donkeys were the prey that most contributed to the total biomass ingested, while sheep were also frequently found in wolf scats. Wild boars and unidentified ungulates were the preys that most contributed to the biomass ingested and the most frequently found in Mahide group. Finally, in Villardeciervos group, the wild boar was the main prey, both in frequency of appearance and biomass ingested (Table 2).

The wolves of Valladolid group also showed a varied diet. Wild boars (47% FO) and rabbits (11.2% FO) were the prey most frequently found, but we also found roe deer (10.3% FO), red deer (4.9% FO), pigs (6.2% FO) and goats (3.1% FO). Domestic pigs and wild boars were the prey species which most contributed to the total biomass ingested (Table 2).

Prey selection, trophic niche breadth and diet similarity in the wolf breeding groups

The majority of wolf groups did not positively select wild boars nor other wild ungulates according to Jacob's prey selection index. Only Ferreras group in Zamora positively selected roe deer, being this species negatively selected in Mahide and Villardeciervos groups. Moreover, red deer was

Table 2 Frequency of occurrence (FO) in wolf scats and total biomass ingested (B) of each prey species in Galicia (A), Zamora (B) and Valladolid (C) wolf breeding groups

A		Galicia (n = 119)							
		Invernadeiro N = 29		Saguñedo N = 11		Castrelo N = 41		Baldriz N = 38	
Prey species		FO	B	FO	B	FO	B	FO	B
Wild ungulates									
<i>Capreolus capreolus</i>		0.14	2.21	0.18	2.84	0.34	5.37	0.39	6.16
<i>Cervus elaphus</i>		0.17	9.78	0.18	10.35	-	-	-	-
<i>Sus scrofa</i>		0.48	23.28	0.36	17.46	0.46	22.31	0.39	18.92
<i>Unidentified ungulate</i>		0.07	2.84	-	-	-	-	-	-
Domestic ungulates									
<i>Capra aegagrus</i>		0.07	1.10	0.18	2.83	0.10	1.57	0.18	2.83
Other preys									
<i>Oryctolagus cuniculus</i>		0.03	0.04	0.09	0.11	-	-	-	-
B		Zamora (n = 102)							
		Boya N = 17		Fereras N = 9		Mahide N = 47		Villardeciervos N = 29	
Prey species		FO	B	FO	B	FO	B	FO	B
Wild ungulates									
<i>Capreolus capreolus</i>		0.12	1.90	0.11	1.74	-	-	-	-
<i>Cervus elaphus</i>		0.29	16.68	-	-	-	-	-	-
<i>Sus scrofa</i>		0.18	8.73	0.22	10.67	0.43	20.86	0.48	23.28
<i>Unidentified ungulate</i>		-	-	-	-	0.36	14.62	0.07	2.84
Domestic ungulates									
<i>Capra aegagrus</i>		-	-	-	-	0.11	1.73	0.03	0.47
<i>Ovis aries</i>		-	-	0.22	3.70	-	-	0.07	1.18
<i>Equus africanus asinus</i>		0.29	29	0.11	11	0.02	2	0.03	3
C		Valladolid N = 65							
Prey species		FO	B						
Wild ungulates									
<i>Capreolus capreolus</i>		0.10	1.58						
<i>Cervus elaphus</i>		0.04	2.30						
<i>Sus scrofa</i>		0.47	22.80						
Domestic ungulates									
<i>Capra aegagrus</i>		0.03	4.80						
<i>Sus scrofa domestica</i>		0.06	4.80						
Other prey									
<i>Oryctolagus cuniculus</i>		0.11	0.13						

negatively selected in Ferreras, Mahide and Villardecierros wolf groups (Table 3).

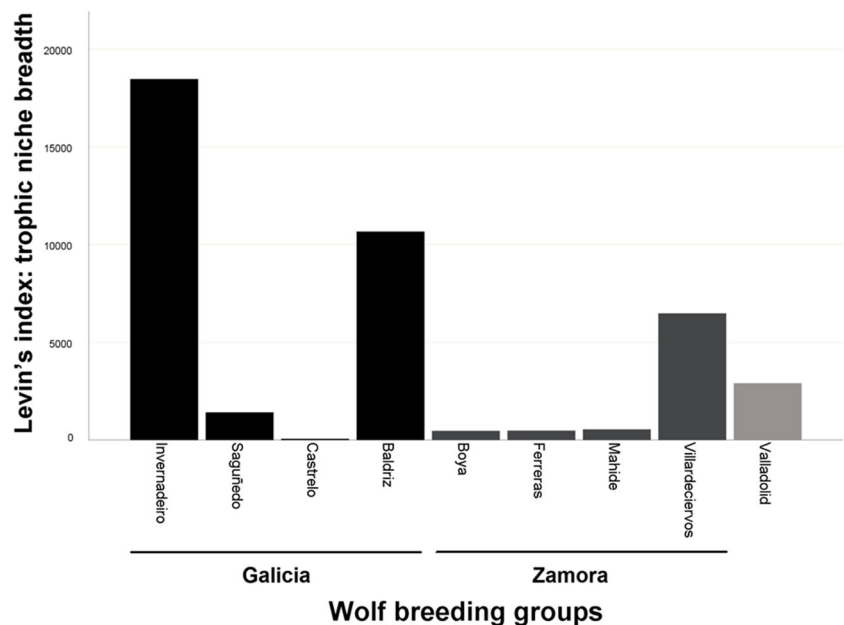
The highest trophic niche breadth was found in wolf groups inhabiting Galicia ($L = 7651.83$), followed by Valladolid ($L = 2910.2$) and Zamora's groups ($L = 1993.25$). In Galicia, Invernadeiro group exhibited the most diverse diet ($L = 18470.0$), followed by Baldriz ($L = 10659.0$; Fig. 2). Regarding Zamora's wolves, it was Villardecierros group the one with the highest trophic niche breadth ($L = 6474.0$; Fig. 2). Niche breadth differences were statistically

significant between wolf groups ($K = 0.273$; $df = 8$; $p = 0.05$) but not between zones ($K = 0.750$; $df = 1$; $p = 0.386$).

We also found differences comparing diet similarity between wolf groups ($T = 7.858$; $df = 11$; $p = 0.01$). According to Pianka's index, wolf's diet was more similar between Galicia and Zamora ($\alpha = 0.23$) than Galicia-Valladolid ($\alpha = 0.14$) and Zamora-Valladolid ($\alpha = 0.10$). Pianka's index was higher in Galicia's groups than in Zamora's ones ($F = 33.820$; $df = 1$; $p = 0.01$). In Galicia, wolves of Baldriz-Castrelo and Invernadeiro-Castrelo had the highest diet

Table 3 Observed frequencies (the total number of times that each prey appeared in wolf faecal samples) vs. expected frequencies (prey species availability) depending on each wolf groups

	GALICIA					
	<i>S. scrofa</i>		<i>C. capreolus</i>		<i>C. elaphus</i>	
	Obs	Exp	Obs	Exp	Obs	Exp
Invernadeiro	11.09	12.41	3.17	2.26	3.96	17.11
Saguñedo	9.11	20.54	4.56	10.91	4.56	0.32
Castrelo	10.49	19.45	7.73	12.32	-	-
Baldriz	112.89	231.70	112.89	54.30	-	-
	ZAMORA					
	Obs	Exp	Obs	Exp	Obs	Exp
Boya	5.47	-	9.11	3.46	3.64	28.32
Ferreras	12.15	-	0.00	7.19	6.07	24.59
Mahide	18.22	-	0.00	4.27	0.00	27.52
Villardeciervos	18.22	-	0.00	3.46	0.00	28.32
	VALLADOLID					
	Obs	Exp	Obs	Exp	Obs	Exp
	13.47	23.84	4.75	7.95	0.00	-

Fig. 2 Trophic niche breadth comparison using Levin's index in all breeding wolf groups: Galicia (Invernadeiro Natural Park, Saguñedo, Castrelo and Baldriz), Zamora (Boya, Ferreras, Mahide and Villardeciervos) and Valladolid

similarities (Fig. 3). In Zamora, we found that Ferreras-Villardeciervos groups exhibited more diet overlapping (Fig. 3).

Influence of individual factors in wolf diet

We did not find statistically significant differences in the wolf's diet depending on age (frequency of occurrence of each prey: $\chi^2 = 39.00$; $df = 14$; $p = 0.08$; $N = 265$). In both adults and pups, wild boar was the prey that most frequently appeared, followed by roe deer in adults and unidentified ungulated in pups (Table 4). Parallely, differences in the wolf's diet depending on the sex (frequency of occurrence percentage of each prey: $\chi^2 = 24.00$; $df = 16$; $p = 0.24$,

$N = 43$; Table 4) and social status were not statistically significant (frequency of occurrence percentage of each prey: $\chi^2 = 29.75$; $df = 14$; $p = 0.23$, $N = 148$; Table 5).

Diet quality by nitrogen analysis

Nitrogen analysis in wolf scats showed that there were no differences in diet quality of wolf breeding groups depending on the zone (Galicia mean 5.29 ± 0.44 SE, median 4.55, $P_{25} = 3.53$, $P_{75} = 6.13$ g N/100 g dry faeces; Valladolid mean 4.45 ± 0.42 , median 3.70, $P_{25} = 2.70$, $P_{75} = 5.50$ g N/100 g dry faeces; $F = 1.697$, $df = 1$, $p = 0.19$, $N = 91$). Moreover, differences in diet

Fig. 3 Diet similarity between wolf groups in Galicia (Invernadeiro Natural Park, Saguñedo, Castrelo and Baldriz) and Zamora (Boya, Ferreras, Mahide and Villardeciervos) using Pianka's index

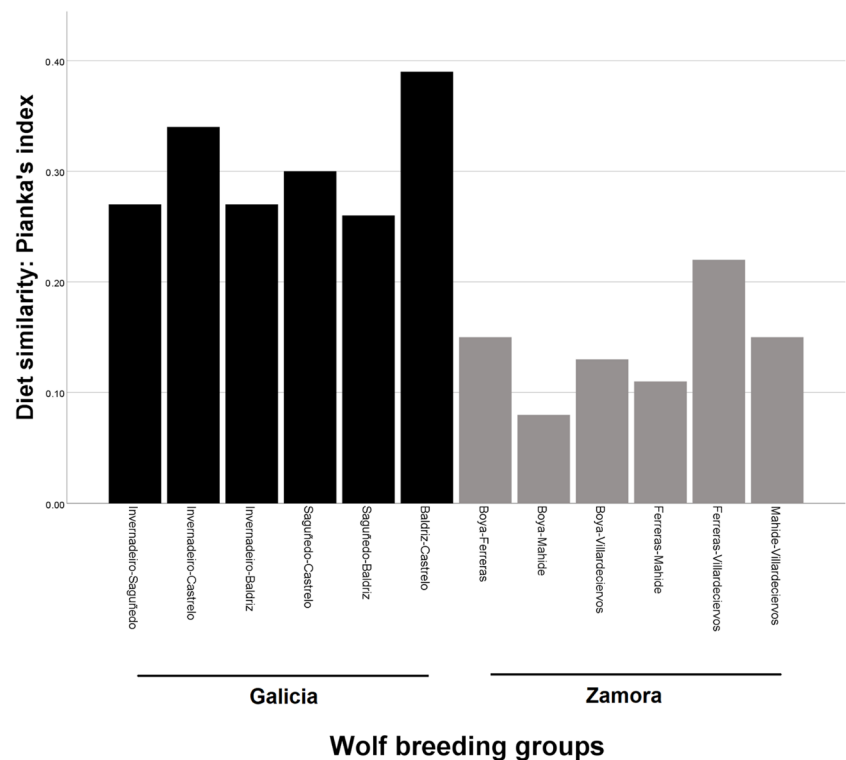


Table 4 Frequency of occurrence (FO) of each prey in wolf scats and total biomass ingested (B) of each prey depending on wolf's sex and age in all nine wolf breeding groups

Prey species	Adults N=212		Pups N=53		Males N=22		Females N=21	
	FO	B	FO	B	FO	B	FO	B
Wild ungulates								
<i>Capreolus capreolus</i>	0.19	20.57	0.06	1.51	0.23	6.52	0.32	5.52
<i>Cervus elaphus</i>	0.05	13.58	0.04	2.72	0.04	2.72	0.03	1.36
<i>Sus scrofa</i>	0.42	82.54	0.36	17.62	0.46	24.11	0.29	9.27
<i>Unidentified ungulate</i>	0.05	9.29	0.21	10.22	-	-	-	-
Domestic ungulates								
<i>Capra aegagrus</i>	0.08	21.49	0.09	6.32	0.11	7.58	0.09	3.79
<i>Ovis aries</i>	0.02	5.94	0.00	-	-	-	-	-
<i>Equus africanus asinus</i>	0.03	11.54	0.04	3.85	-	-	-	-
<i>S. scrofa domesticus</i>	0.05	24.74	0.08	9.90	0.09	12.37	0.15	12.37
Other prey								
<i>Oryctolagus cuniculus</i>	0.10	6.318	0.09	1.44	0.09	1.44	0.12	1.15

quality between sexes and age were also non-statistically significant (males mean $5.50 \pm \text{SD } 0.63$, median 4.55, $P_{25} = 2.90$, $P_{75} = 6.30$ g N/100 g dry faeces; females mean 5.12 ± 0.64 , median 4.70, $P_{25} = 2.80$, $P_{75} = 7.80$ g N/100 g dry faeces; $U = 356.50$, $df = 1$, $p = 0.94$, $N = 57$; adults mean 5.07 ± 0.38 , median 4.30, $P_{25} = 2.90$, $P_{75} = 5.97$ g N/100 g dry faeces; pups mean 4.62 ± 0.42 , median 4.50, $P_{25} = 3.60$, $P_{75} = 5.70$ g N/100 g dry faeces;

$U = 539.00$, $df = 1$, $p = 0.80$, $N = 91$). Finally, nitrogen content in wolf's faeces did not differ between dominant and subordinate individuals (dominant mean 5.71 ± 0.95 , median 4.76, $P_{25} = 3.92$, $P_{75} = 5.85$ g N/100 g dry faeces; subordinates mean 5.47 ± 0.77 , median 4.90, $P_{25} = 3.90$, $P_{75} = 6.30$ g N/100 g dry faeces), the differences were not statistically significant ($U = 117.5$, $df = 1$, $p = 0.89$, $N = 31$).

Table 5 Frequency of occurrence (FO) of each prey in wolf scats and total biomass ingested (B) of each prey depending on wolf's social status

Prey species	Dominant N=48		Subordinates N=100	
	FO	B	FO	B
<i>Capreolus capreolus</i>	0.23	5.52	0.09	4.52
<i>Cervus elaphus</i>	0.04	2.72	0.06	8.15
<i>Sus scrofa</i>	0.46	20.40	0.50	46.37
<i>Capra aegagrus</i>	0.10	6.32	0.06	7.58
<i>Ovis aries</i>	0.02	1.48	0.03	4.45
<i>Equus africanus asinus</i>	0.06	5.77	0.05	9.62
Unidentified ungulate	0.04	1.86	0.18	16.72

Discussion

Prey availability and diet differences between zones and breeding wolf groups

We found that the wolf's diet in the study areas was strongly dependent on prey availability, being wild ungulates the central core of their diet, as it has been described in previous studies characterizing wolves as generalist-opportunistic predators through its European range (Ciucci 1994; Mattioli et al. 1995; Meriggi et al. 1996, 2011; Milanesi et al. 2012). However, some wolves, such as the breeding group studied in Baldriz (Galicia), consumed much more roe deer than it was expected due to its abundance, suggesting that some wolf groups show marked preferences for certain prey species regardless its abundance (Potvin 1988; Dale et al. 1994; Meriggi et al. 1996; Kunkel et al. 2004). Therefore, these results could indicate that some wolf breeding groups may be specialised in hunting specific prey species (Barja 2009a). Moreover, these findings could be also determined by the fact that during the breeding season of wolves, roe deer and wild boars also reproduce, and wolves have been reported to prey upon juvenile roe deer and wild boars (Blanco et al. 1998; Rigg and Gorman 2004; Barja 2009a), because they are an easy target compared to adult prey.

Moreover, wolf's diet was different depending on the zone (i.e., Galicia, Zamora or Valladolid) and the breeding group studied, being wild boars and cervids the main prey in their diet. In Galicia, the variation in the wolf's diet between breeding wolf groups correlated with prey availability, except for Baldriz's group, which fed on roe deer almost four-times more than expected, which, as we mentioned above, could be a local feeding specialisation of this particular wolf group. Invernadeiro and Saguñedo groups consumed both roe deer and red deer, while Castrolo and Baldriz wolves did not feed upon roe deer, probably because its scarcity but also because it's a

more difficult prey to kill (Barja 2009a). As for domestic ungulates, some goat remains were found, but in general, wild boar and wild cervids were the main preys consumed, which corroborates that livestock consumption by wolves is not generalised in Galicia (Barja 2009a).

The diet of wolves was particularly diverse in Zamora compared to Galicia and Valladolid, including mostly wild boars and wild cervids but also donkeys and sheep. It is worth to mention that donkeys were provided by hunting reserves to lure wolves and facilitate their hunting. The availability of this vulnerable prey could explain why cervid consumption was lower than expected, especially in Mahide and Villardeciervos groups. The wolf breeding groups studied in Zamora occupied a territory where wolf hunting have been allowed until recently. Hunting wolves often leads to the disruption of their pack because the breeding pair is most likely to be killed as they lead the other members of the group. The destabilisation of the group may lead to livestock attacks (Wielgus and Peebles 2014; Fernández-Gil et al. 2016) as younger and inexperienced individuals attack cattle because they lack key hunting skills. However, future studies should address how feeding habits of Sierra de la Culebra wolves changed since hunting has been prohibited as all Spanish wolf populations have been included in the Spanish Catalog of Endangered Species (MITECO TED/980/2021) and they do not provide donkeys in feeding points anymore.

As for the wolf breeding group studied in Valladolid, it showed a diet mainly based on wild boar, rabbits, roe deer and domestic pigs. This group lives in an agricultural and livestock farming area characterised by a low availability of wild ungulates and a high availability of rabbits. Wolves showed an opportunistic trophic behaviour, feeding on lagomorphs (which were the prey most frequently found in scats) while pig remains discarded by nearby farms contributed the most to the total biomass consumed by this group. In general, the diet of Iberian wolves followed the same pattern that the review of Zlatanova et al. 2014 described for European wolves: wolf groups living in more natural areas with access to wild ungulates chiefly consume wild prey, whereas wolves living in anthropogenic habitats with limited access to wild prey include more livestock in their diet, as well as smaller prey such as lagomorphs.

It is necessary to mention that one of the main limitations of this study was that it was not possible to estimate prey abundance using the same methodology in all the zones. In other words, for Galicia, we used camera traps to estimate prey abundance, while for Zamora and Valladolid, we relied on hunting censuses. Therefore, absolute numbers of prey available may not be directly comparable between zones. Nevertheless, we emphasize the proportional contribution of each prey species to the overall available prey. We strongly believe that prey composition would remain similar

even camera traps were underestimating the total amount of prey available.

Trophic niche breadth and diet similarity among zones and wolf breeding groups

The diet of wolves was more diverse in Galicia probably because it is a well-preserved zone which possess a high availability wild prey. Wolves living in Galicia inhabit Invernadeiro Natural Park and surroundings, where a large part of this study area is protected with low human perturbations, and therefore wild prey density is high. Hence, it was expected that wolves in this region focused on feeding wild prey. In the case of Invernadeiro, the trophic niche breadth was the highest of Galicia because this group lives inside the most remote and protected area, where biodiversity is expected to be higher than the groups living in the park surroundings. Wolf groups inhabiting Zamora also possesses a diverse diet, probably because it includes a high variety of both wild and domestic prey. On the contrary, Valladolid's wolf group inhabits an area heavily transformed by human activity, which implies lower biodiversity and wolves have adapted to feed on rabbits and scavenge farm carcasses.

Furthermore, feeding habits were more similar between Galicia and Zamora because habitat characteristics of Valladolid were particularly different, as it is a strongly modified landscape by human agricultural and livestock farming activities. Considering each zone, diet similarity among breeding wolf groups was explained by prey availability. For instance, wolves of Baldriz and Castrelo had the highest similarity index in Galicia because they mainly fed upon roe deer and wild boar, being red deer not available for this groups. In Zamora, on the contrary, Ferreras and Villardeciervos groups consumed red deer as the main prey, followed by roe deer because they were the most abundant.

Influence of individual factors in wolf diet

Wolf's diet did not vary depending on individual factors such as age, sex and social status. Since wolves are cooperative hunters, it was expected that all the members of the group fed on the same preys, regardless its sex, age or social status (Valdmann et al. 2005; Žunna et al. 2009). However, previous studies have found some differences. For instance, Očenjak et al. (2020) found that female Croatian wolves consumed more birds, rodents, and dogs than males. And Mysłajek et al. (2019) showed that Polish wolf pups feed upon beavers much more than adult wolves. The diet of Iberian wolves depending on individual factors had not been studied until now and this is the first report showing that all the members of the group have the same diet.

Diet quality by nitrogen analysis

Nitrogen contents of wolf scats did not differ between study areas, which suggests that diet quality is similar among wolf groups despite dietary composition differences. As we expected, we did not find a significant variation in diet quality depending on the age nor sex, because wolves hunt together and consume the same prey. However, contrary to our prediction, we could not show any differences in wolf's faecal nitrogen contents depending on the social status. Iberian wolf packs are characterised by being rather small family groups composed by the breeding pair and their offspring (Barrientos 2000; Fernández-Gil et al. 2020). Perhaps, because groups are reduced compared to wolf packs inhabiting other regions, which could reach up to 20 individuals (Stenglein et al. 2011), competition for feeding resources between the members of the family might not be that strong, leading to a more equal diet quality among all members of the group.

Conservation and management implications

This study highlights that when wild prey is available, wolves consume livestock minimally. Therefore, to prevent attacks on livestock and mitigate human-wolf conflict, it is crucial to implement measures that either maintain or bolster wild ungulate populations. Environmental interventions should focus on providing grazing areas and but also vegetation that offers shelter to prey, emphasizing the importance of proper administration and forestry management. Another key aspect for fostering coexistence between livestock farming and wolf populations are adequate livestock husbandry practices. If livestock is guarded, the chances of getting attacked by wolves decrease. As we mentioned, wolves hunt on profitable and vulnerable prey, thus, if the access dangerous, they won't take the risk (Bruns et al. 2020; Barja et al. 2023). Hence, leaving free-ranging flocks unattended by shepherds or livestock-guarding dogs is not recommended. Enhanced husbandry practices, such as the use of fences, bringing herds into the village or other shelters at night, and employing guarding dogs, should be incentivized by the government.

In conclusion, in this study we reported that Iberian wolf groups during breeding season showed an opportunistic feeding behaviour because their diet was basically explained by prey availability. However, some wolf groups prey upon roe deer more than it was expected, which indicates a local feeding specialisation. We also confirmed that all members of the group consumed the same preys and had a similar diet quality, even between dominant and subordinate individuals. This may imply that, due to the overall small pack size, all individuals are allowed to access and exploit the resources and, in consequence, the feeding hierarchy is not

that strong in Iberian wolves compared to their American relatives (Mech and Boitani 2007). This is the first study that examines in detail the importance of individual factors (social status, sex and age) on Iberian wolf's diet along with differences between wolf groups and zones. Furthermore, the analysis of diet quality using nitrogen analysis is also a completely new approach, providing us with results about differences in the diet of wolves according to social status. We believe that this unique focus on the Iberian wolf contributes valuable insights to the broader understanding of wolf ecology and behaviour. Moreover, our results entail a novel approach by examining the dietary patterns of the Iberian wolf subspecies, which has not received the same level of attention in previous research despite being a keystone, highly sensitive and scarce species. We believe that this unique focus on the Iberian wolf provides valuable contributions to the broader understanding of wolf ecology and behavior. Our study also stresses the importance of wild ungulate populations conservation to boost the coexistence between humans and wolves, as it is a key strategy to decrease livestock attacks by wolves because they will hunt wild prey as long it is available. Finally, future studies will be necessary to determine if these dietary patterns (such as the absence of differences depending on individual factors) can be extrapolated to the populations of wolves inhabiting other European regions.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00265-024-03457-4>.

Acknowledgements The authors wish to thank the Xunta de Galicia, the Junta de Castilla y León and the Reserva de Caza de la Sierra de la Culebra for the permits granted to carry out this study. Also, to all the gamekeepers for their collaboration, specially to Tomás, Ricardo, Paco, Ruben and Roberto. During part of this study, IB was granted a postdoctoral contract by the Universidad Autónoma de Madrid. We would also like to express our sincere gratitude to the reviewers for their valuable time and insightful comments on our manuscript.

Author contributions Conceptualization and design: IB; Field work: IB, AP, JT, TG-B; samples analysis for diet: IB; preprocessing of faecal samples to evaluate diet quality: AN-C (The total N % was analyzed in the SIDI); genetic analysis: AR-G, AC; data analysis: MCH, IB; wrote the paper: MCH, IB. The field work of the project was self-sponsored by IB.

Data availability The data that support the findings of this study are available in the [supplementary material](#).

Declarations

Ethical statement This study employed non-invasive methodologies in the examination of the wolf's diet. This research was performed in compliance with all applicable laws and rules set forth by Xunta de Galicia, the Junta de Castilla y León and the Reserva de Caza de la Sierra de la Culebra. All the necessary permits for faecal sample collection and camera trapping were in hand when the research was conducted. All applicable international, national, and/or institutional guidelines for the research of wild animals were followed.

Competing interests We declare that we have no competing interests.

References

- Aldezabal A, Garin I, García-González R (1993) Concentración de nitrógeno faecal en ungulados estivantes en Los pastos supraforestales del Parque Nacional De Ordesa Y Monte Perdido. *Pastos* 1:101–114
- Allen ME, Ullrey DE (2004) Relationships among nutrition and reproduction and relevance for wild animals. *Zoo Biol* 23:475–487
- Ansorge H, Kluth G, Hahne S (2006) Feeding ecology of wolves *Canis lupus* returning to Germany. *Acta Theriol* 51:99–106
- Arman P, Hopcraft D, McDonald I (1975) Nutritional studies on east African herbivores. 2. Losses of nitrogen in the faeces. *Brit J Nutr* 33:265–276
- Baldwin RA, Bender LC (2009) Foods and nutritional components of diets of black bear in Rocky Mountain National Park, Colorado. *Can J Zool* 87:1000–1008
- Barboza PS, Parker KL, Hume ID (2008) Integrative wildlife nutrition. Springer, Berlin
- Barja I (2009a) Prey and prey-age preference by the Iberian wolf *Canis lupus signatus* in a multiple-prey ecosystem. *Wildl Biol* 15:147–154
- Barja I (2009b) Decision making in plant selection during the faecal-marking behavior of wild wolves. *Anim Behav* 77:489–493
- Barja I, de Miguel FJ, Bárcena F (2004) The importance of crossroads in faecal marking behaviour of the wolves (*Canis lupus*). *Naturwissenschaften* 91:489–492
- Barja I, Miguel FJ, Barcena F (2005) Faecal marking behavior of Iberian wolf in different zones of their territory. *Folia Zool* 54:21
- Barja I, Silván G, Illera JC (2008a) Relationships between sex and stress hormone levels in feces and marking behavior in a wild population of Iberian wolves (*Canis lupus signatus*). *J Chem Ecol* 34:697–701
- Barja I, Silván G, Rosellini S, Piñeiro A, Illera MJ, Illera JC (2008b) Quantification of sexual steroid hormones in feces of Iberian wolf (*Canis lupus signatus*): a non-invasive sex typing method. *Reprod Domest Anim* 43:701–707
- Barja I, Hernández MC, Gago-Barja T, Navarro-Castilla A (2018) Iberian wolf activity patterns: from understanding to conservation. In: *Proceedings of the International Congress of the Spanish Society of Ethology and Evolutionary Ecology*, Mieres, Spain (abstract)
- Barja I, Hernández MC, Navarro-Castilla Á (2021) Manual de Los patrones macroscópicos y cuticulares del pelo en mamíferos de la península ibérica. Universidad Autonoma de Madrid, Madrid, Spain
- Barja I, Navarro-Castilla Á, Ortiz-Jiménez L et al (2023) Wild ungulates constitute the basis of the diet of the Iberian wolf in a recently recolonized area: wild boar and roe deer as key species for its conservation. *Animals* 13:3364
- Barrientos LM (2000) Tamaño y composición de diferentes grupos de lobos en Castilla y León. *Galemys* 12:249–256
- Blanco JC, Alcántara M, Ibañez C, Aguilar A, Grau E, Moreno S, Babamtiñ I, Jordan G, Villafuerte R (1998) Guía De Campo De Los mamíferos de España. GeoPlaneta, Barcelona
- Boertje RD, Stephenson RO (1992) Effects of ungulate availability on wolf reproductive potential in Alaska. *Can J Zool* 70:2441–2443
- Bruns A, Waltert M, Khorozyan I (2020) The effectiveness of livestock protection measures against wolves (*Canis lupus*) and implications for their co-existence with humans. *Global Ecol Conserv* 21:e00868

- Calow P (1979) The cost of reproduction—a physiological approach. *Biol Rev* 5:23–40
- Castroviejo J, Palacios F, Garzon J, Cuesta L (1975) Sobre la alimentación de los cánidos ibéricos. In: Proceedings of the XII Congress of the International Union of Game Biologists. IUGB, Lisboa, pp 39–46
- Ciucci P (1994) Movements, activities and resources of the wolf (*Canis lupus*) in two areas of the central-northern Italy. PhD thesis, Università di Roma La Sapienza
- Codron D, Lee-Thorp JA, Sponheimer M, Codron J, de Ruiter D, Brink JS (2007) Significance of diet type and diet quality for ecological diversity of African ungulates. *J Anim Ecol* 76:526–537
- Dale BW, Adams LG, Bowyer RT (1994) Functional response of wolves preying on barren-ground caribou in a multiple-prey ecosystem. *J Anim Ecol* 63:644–652
- Elmhagen B, Tannerfeldt M, Verucci P, Angerbjörn A (2000) The arctic fox (*Alopex lagopus*): an opportunistic specialist. *J Zool* 251:139–149
- Fernández-Gil A, Naves J, Ordiz A, Quevedo M, Revilla E, Delibes M (2016) Conflict misleads large Carnivore management and conservation: brown bears and wolves in Spain. *PLoS ONE* 11:e0151541
- Fernández-Gil A, Quevedo M, Barrientos LM, Nuño A, Naves J, de Gabriel M, Ordiz A, Revilla E (2020) Pack size in humanized landscapes: the Iberian wolf population. *Wildl Biol* 2020:1–9
- Fuller TK (1989) Population dynamics of wolves in north-central Minnesota. *Wildl Monogr* 105:3–41
- Fuller TK, Sievert PR (2001) Carnivore demography and the consequences of changes in prey availability. In: Gittleman JL, Funk SM, MacDonald DW, Wayne RK (eds) *Carnivore Conservation*. Cambridge University Press, Cambridge, pp 163–178
- Fuller TK, Mech LD, Cochrane JF (2003) Wolf population dynamics. In: Mech LD, Boitani L (eds) *Wolves: Behavior, ecology, and conservation*. University of Chicago Press, Chicago, pp 161–191
- Gazzola A, Bertelli I, Avanzinelli E, Tolosano A, Bertotto P, Apollonio M (2005) Predation by wolves (*Canis lupus*) on wild and domestic ungulates of the western Alps, Italy. *J Zool* 266:205–213
- Hambäck PA, Oksanen L, Ekerholm P, Lindgren Å, Oksanen T, Schneider M (2004) Predators indirectly protect tundra plants by reducing herbivore abundance. *Oikos* 106:85–92
- Hebblewhite M, White CA, Nietvelt CG, McKenzie JA, Hurd TE, Fryxell JM, Bayley SE, Paquet PC (2005) Human activity mediates a trophic cascade caused by wolves. *Ecology* 86:2135–2144
- Holmes JC (1995) Population regulation: a dynamic complex of interactions. *Wildl Res* 22:11–19
- Jacobs J (1974) Quantitative measurement of food selection. *Oecologia* 14:413–417
- Jędrzejewski W, Jędrzejewska B, Okarma H, Ruprecht AL (1992) Wolf predation and snow cover as mortality factors in the ungulate community of the Białowieża National Park, Poland. *Oecologia* 90:27–36
- Kunkel KE, Pletscher DH, Boyd DK, Ream RR, Fairchild MW (2004) Factors correlated with foraging behavior of wolves in and near Glacier National Park, Montana. *J Wildl Manage* 68:167–178
- Lanszki J, Márkus M, Újváry D, Szabó Á, Szemethy L (2012) Diet of wolves *Canis lupus* returning to Hungary. *Acta Theriol* 57:189–193
- Letnic M, Ritchie EG, Dickman CR (2012) Top predators as biodiversity regulators: the dingo *Canis lupus dingo* as a case study. *Biol Rev* 87:390–413
- Levins R (1968) *Evolution in changing environments: some theoretical explorations*. Princeton University Press, Princeton
- Llaneza L, Fernández A, Nore C (1996) Dieta Del lobo en dos zonas de Asturias (España) que difieren en carga ganadera. *Doñana Acta Vertebr* 23:201–213
- Loeb SC, Schwab RG, Demment MW (1991) Responses of pocket gophers (*Thomomys bottae*) to changes in diet quality. *Oecologia* 86:542–551
- Martín J, Barja I, López P (2010) Chemical scent constituents in feces of wild Iberian wolves (*Canis lupus signatus*). *Biochem Syst Ecol* 38:1096–1102
- Mateos-Quesada P (2002) *Biología y comportamiento del Corzo Ibérico*. PhD thesis, Universidad de Extremadura
- Mattioli L, Apollonio M, Mazzarone V, Centofanti E (1995) Wolf food habits and wild ungulate availability in the Foreste Casentinesi National Park, Italy. *Acta Theriol* 40:387–402
- Mech LD (1999) Alpha status, dominance, and division of labor in wolf packs. *Can J Zool* 77:1196–1203
- Mech LD, Boitani L (eds) (2007) *Wolves: behavior, ecology, and conservation*. University of Chicago Press, Chicago
- Meriggi A, Lovari S (1996) A review of wolf predation in southern Europe: does the wolf prefer wild prey to livestock? *J Appl Ecol* 33:1561–1571
- Meriggi A, Rosa P, Brangi A, Matteucci C (1991) Habitat use and diet of the wolf in northern Italy. *Acta Theriol* 36:141–151
- Meriggi A, Brangi A, Matteucci C, Sacchi O (1996) The feeding habits of wolves in relation to large prey availability in northern Italy. *Ecography* 19:287–295
- Meriggi A, Brangi A, Schenone L, Signorelli D, Milanesi P (2011) Changes of wolf (*Canis lupus*) diet in Italy in relation to the increase of wild ungulate abundance. *Ethology Ecol Evol* 23(3):195–210
- Meriggi A, Dagradi V, Dondina O, Perversi M, Milanesi P, Lombardini M, Raviglione S, Repossi A (2015) Short-term responses of wolf feeding habits to changes of wild and domestic ungulate abundance in Northern Italy. *Ethol Ecol Evol* 27:389–411
- Milanesi P, Meriggi A, Merli E (2012) Selection of wild ungulates by wolves *Canis lupus* (L. 1758) in an area of the Northern Apennines (North Italy). *Ethol Ecol Evol* 24:81–96
- Mysłajek RW, Tomczak P, Tolkacz K, Tracz M, Tracz M, Nowak S (2019) The best snacks for kids: the importance of beavers *Castor fiber* in the diet of wolf *Canis lupus* pups in north-western Poland. *Ethol Ecol Evol* 31:506–513
- Navarro-Castilla Á, Hernández MC, Barja I (2023) An experimental study in wild wood mice testing elemental and isotope analysis in faeces to determine variations in food intake amount. *Animals* 13:1176
- Octenjak D, Pađen L, Šilić V, Reljić S, Vukičević TT, Kusak J (2020) Wolf diet and prey selection in Croatia. *Mammal Res* 65:647–654
- Packard JM, Mech LD, Seal US (1983) Social Influences on Reproduction in Wolves. In: Carbyn LN (ed) *Wolves in Canada and Alaska: their status, biology, and management*. Proceedings of the Wolf Symposium held in Edmonton, Alberta. Canadian Wildlife Service Report Series 45, Edmonton, Alberta, pp 78–85
- Papageorgiou N, Vlachos C, Sfougari A, Tsalchidis E (1994) Status and diet of wolves in Greece. *Acta Theriol* 39:411–416
- Persson J (2005) Female wolverine (*Gulo gulo*) reproduction: reproductive costs and winter food availability. *Can J Zool* 83:1453–1459
- Pianka ER (1973) The structure of lizard communities. *Annu Rev Ecol Syst* 4:53–74
- Pilot M, Branicki W, Jędrzejewski W, Goszczyński J, Jędrzejewska B, Dykyy I, Shkvyrya M, Tsingarska E (2010) Phylogeographic history of grey wolves in Europe. *BMC Evol Biol* 10:104
- Potvin F (1988) Wolf movements and population dynamics in Papineau-Labelle reserve, Quebec. *Can J Zool* 66:1266–1273
- Randi E, Lucchini V, Christensen MF, Mucci N, Funk SM, Dolf G, Loeschke V (2000) Mitochondrial DNA variability in Italian and east European wolves: detecting the consequences of small population size and hybridization. *Conserv Biol* 14:464–473

- Rigg R, Gorman M (2004) Spring-autumn diet of wolves (*Canis lupus*) in Slovakia and a review of wolf prey selection. *Oecologia Mont* 13:30–41
- Ripple WJ, Beschta RL (2003) Wolf reintroduction, predation risk, and cottonwood recovery in Yellowstone National Park. *For Ecol Manag* 184:299–313
- Robbins CT (2012) *Wildlife feeding and nutrition*. Elsevier, Amsterdam
- Robbins CT, Felicetti LA, Sponheimer M (2005) The effect of dietary protein quality on nitrogen isotope discrimination in mammals and birds. *Oecologia* 144:534–540
- Sakaguchi E, Ohmura S (1992) Fibre digestion and digesta retention time in guinea-pigs (*Cavia porcellus*), degus (*Octodon degus*), and leaf-eared mice (*Phyllotis darwini*). *Comp Biochem Physiol A* 103:787–791
- Salvador A, Abad PL (1987) Food habits of a wolf population (*Canis lupus*) in León province, Spain. *Mammalia* 51:45–52
- Sands J, Creel S (2004) Social dominance, aggression, and faecal glucocorticoid levels in a wild population of wolves, *Canis lupus*. *Anim Behav* 67:387–396
- Seddon JM (2005) Canid-specific primers for molecular sexing using tissue or non-invasive samples. *Conserv Genet* 6:147–149
- Sergiel A, Barja J, Navarro-Castilla Á, Zwijsacz-Kozica T, Selva N (2020) Losing seasonal patterns in a hibernating omnivore? Diet quality proxies and faecal cortisol metabolites in brown bears in areas with and without artificial feeding. *PLoS ONE* 15:e0242341
- Sidorovich VE, Tikhomirova LL, Jedrzejewska B (2003) Wolf *Canis lupus* numbers, diet and damage to livestock in relation to hunting and ungulate abundance in northeastern Belarus during 1990–2000. *Wildl Biol* 9:103–111
- Śmietana W (2005) Selectivity of wolf predation on red deer in the Bieszczady Mountains, Poland. *Acta Theriol* 50:277–288
- Smith DW, Bangs EE (2009) Reintroduction of wolves to Yellowstone National Park: history, values, and ecosystem restoration. In: Hayward MW, Somers MJ (eds) *Reintroduction of Top-Order predators*. Wiley-Blackwell, Oxford, pp 92–125
- Soffiantini CS, Lazzini C, Sabbioni A, Zanon A, Beretti V (2006) Growth curves in roe deer population of Massa-Carrara province [Toscana]. *Ann Fac Med Vet Univ Parma* 26:319–334
- Speakman JR (2008) The physiological costs of reproduction in small mammals. *Phil Trans R Soc B* 363:375–398
- Stenglein JL, Waits LP, Ausband DE, Zager P, Mack CM (2011) Estimating gray wolf pack size and family relationships using noninvasive genetic sampling at rendezvous sites. *J Mammal* 92:784–795
- Stirling I, Lunn NJ, Iacozza J (1999) Long-term trends in the population ecology of polar bears in western Hudson Bay in relation to climatic change. *Arctic* 52:294–306
- Teerink J (1991) *Atlas and identification key hair of West-European mammals*. Cambridge University Press, Cambridge
- Torres RT, Silva N, Brotas G, Fonseca C (2015) To eat or not to eat? The diet of the endangered Iberian wolf (*Canis lupus signatus*) in a human-dominated landscape in central Portugal. *PLoS ONE* 10:e0129379
- Urios V (1995) *Eto-ecología de la depredación del lobo Canis lupus signatus en el NO de la Península Ibérica*. PhD tesis, Universidad de Barcelona
- Valdmann H, Andersone-Lilley Z, Koppa O, Ozolins J, Bagrade G (2005) Winter diets of wolf *Canis lupus* and lynx *Lynx lynx* in Estonia and Latvia. *Acta Theriol* 50:521–527
- Vicente JL, Rodríguez M, Palacios J (2000) Gestión Del lobo ibérico (*Canis lupus Signatus* Cabrera, 1097), en la reserva regional de caza Sierra De Culebra (Zamora)'. *Galemys* 12:181–199
- Vilà C, Amorim R, Leonard A, Posada D, Castroviejo J, Petrucci-Fonseca F, Crandall A, Ellegren H, Wayne K (1999) Mitochondrial DNA phylogeography and population history of the grey wolf (*Canis lupus*). *Mol Ecol* 8:2089–2103
- Wade GN, Schneider JE (1992) Metabolic fuels and reproduction in female mammals. *Neurosci Biobehav Rev* 16:235–272
- White PJ, Ralls K (1993) Reproduction and spacing patterns of kit foxes relative to changing prey availability. *J Wildl Manage* 57:861–867
- Wielgus RB, Peebles KA (2014) Effects of wolf mortality on livestock depredations. *PLoS ONE* 9:e113505
- Williams GC (1966) Natural selection, the costs of reproduction, and a refinement of lack's principle. *Am Nat* 100:687–690
- Wilmers CC, Getz WM (2005) Gray wolves as climate change buffers in Yellowstone. *PLoS Biol* 3:e92
- Zera AJ, Harshman LG (2001) The physiology of life history trade-offs in animals. *Annu Rev Ecol Syst* 32:95–126
- Zlatanova D, Ahmed A, Valasveva A, Genov P (2014) Adaptive diet strategy of the wolf (*Canis lupus* L.) in Europe: a review. *Acta Zool Bulg* 66:439–452
- Žunna A, Ozoliņ J, Pupila A (2009) Food habits of the wolf *Canis lupus* in Latvia based on stomach analyses. *Est J Ecol* 58:141

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.

Terms and Conditions

Springer Nature journal content, brought to you courtesy of Springer Nature Customer Service Center GmbH (“Springer Nature”).

Springer Nature supports a reasonable amount of sharing of research papers by authors, subscribers and authorised users (“Users”), for small-scale personal, non-commercial use provided that all copyright, trade and service marks and other proprietary notices are maintained. By accessing, sharing, receiving or otherwise using the Springer Nature journal content you agree to these terms of use (“Terms”). For these purposes, Springer Nature considers academic use (by researchers and students) to be non-commercial.

These Terms are supplementary and will apply in addition to any applicable website terms and conditions, a relevant site licence or a personal subscription. These Terms will prevail over any conflict or ambiguity with regards to the relevant terms, a site licence or a personal subscription (to the extent of the conflict or ambiguity only). For Creative Commons-licensed articles, the terms of the Creative Commons license used will apply.

We collect and use personal data to provide access to the Springer Nature journal content. We may also use these personal data internally within ResearchGate and Springer Nature and as agreed share it, in an anonymised way, for purposes of tracking, analysis and reporting. We will not otherwise disclose your personal data outside the ResearchGate or the Springer Nature group of companies unless we have your permission as detailed in the Privacy Policy.

While Users may use the Springer Nature journal content for small scale, personal non-commercial use, it is important to note that Users may not:

1. use such content for the purpose of providing other users with access on a regular or large scale basis or as a means to circumvent access control;
2. use such content where to do so would be considered a criminal or statutory offence in any jurisdiction, or gives rise to civil liability, or is otherwise unlawful;
3. falsely or misleadingly imply or suggest endorsement, approval, sponsorship, or association unless explicitly agreed to by Springer Nature in writing;
4. use bots or other automated methods to access the content or redirect messages
5. override any security feature or exclusionary protocol; or
6. share the content in order to create substitute for Springer Nature products or services or a systematic database of Springer Nature journal content.

In line with the restriction against commercial use, Springer Nature does not permit the creation of a product or service that creates revenue, royalties, rent or income from our content or its inclusion as part of a paid for service or for other commercial gain. Springer Nature journal content cannot be used for inter-library loans and librarians may not upload Springer Nature journal content on a large scale into their, or any other, institutional repository.

These terms of use are reviewed regularly and may be amended at any time. Springer Nature is not obligated to publish any information or content on this website and may remove it or features or functionality at our sole discretion, at any time with or without notice. Springer Nature may revoke this licence to you at any time and remove access to any copies of the Springer Nature journal content which have been saved.

To the fullest extent permitted by law, Springer Nature makes no warranties, representations or guarantees to Users, either express or implied with respect to the Springer nature journal content and all parties disclaim and waive any implied warranties or warranties imposed by law, including merchantability or fitness for any particular purpose.

Please note that these rights do not automatically extend to content, data or other material published by Springer Nature that may be licensed from third parties.

If you would like to use or distribute our Springer Nature journal content to a wider audience or on a regular basis or in any other manner not expressly permitted by these Terms, please contact Springer Nature at

onlineservice@springernature.com