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Research Article

Field work effort to evaluate biological parameters of interest for decision-making on the wolf (*Canis lupus*)

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Abstract

The grey wolf (*Canis lupus*) was extirpated from the Central System (Iberian Peninsula) in 1976, but the species recolonized the area by 2006. We monitored this new population from 2010 to 2018 using non-invasive sampling techniques; we determined its biological parameters and we described the necessary field work to obtain the required information for evidence-based decision-making regarding the management of wolf populations. Data collection was primarily based on the detection of wolf marking signs along sampling routes (e.g. dirt roads, trails, paths) and the scats, in particular, were used to delineate pack territories. Camera trapping was generally used to confirm pack size and reproduction. We detected a maximum of 13 wolf packs distributed in the study area during the eight years of monitoring; the mean pack size was 3.5 wolves. Reproduction always occurred when the mean pack size was at least 4 individuals by the end of winter (52.7%). We also determined that the scat-marked territory of breeding packs (i.e., those with $\geqslant$4 individuals) was >60 km^2 during the reproductive period. Overall, our results suggest that the low-cost monitoring methods commonly used to assess the status of wolf populations in Spain tend to overestimate both population size and reproductive success, suggesting the need for alternative methods.

Introduction

Given the difficulties inherent in monitoring large carnivores such as the grey wolf (*Canis lupus*), an elusive species, some of the data collection and statistical methods currently used to estimate abundance and distribution may yield erroneous results. The parameters of abundance and distribution are conditioned on an array of variables, since they are obtained by surveying large spatial areas using limited human and material resources (Ausband et al., 2014). The use of such surveying techniques can lead to overly optimistic conclusions, which may guide decisions that do not favour the conservation status of wolves or that impede the recolonization of historical territories (Quevedo et al., 2019; Fuller et al., 2003).

In some cases, decisions regarding wolf populations have been based on statistical analyses that were conditioned by the objectives of wildlife authorities, which require reports to determine how wolf populations should be managed, including their culling or hunting (e.g., by setting annual hunting quotas) (Quevedo et al., 2019; Holling, 1978). In countries where regional authorities (or states) are responsible for environmental management, there is a lack of cohesion in management measures across regions, with each applying different ones (Marucco and Boitani, 2012). In regions where the wolf is considered a problematic species, as occurs in Spain, management efforts are constantly criticized (Echegaray, 2014) because some within the community consider that population estimates are biased to justify political decisions or to satisfy the interests of certain sectors (see Hernández and González-Quirós, 2015, 2016; Sáenz de Buruaga et al., 2015).

The wolf is a rare apex predator. Proper monitoring of its populations requires field work to locate tracks and marks, which are in turn used to identify the spatial units delimited by different packs. Moreover, the assessment of a population requires extensive field work (Liberg et al., 2012) that extends beyond the historical territories of packs, in order to avoid missing packs and possible errors due to annual changes in territories. Monitoring that is not based on consecutive annual surveys of one area and that do not follow-up on the spatial units (Barrientos et al., 2010) cannot determine, without a significant margin of error, whether the population is increasing, decreasing, or stable. The outcome of using less reliable methods for decision-making is that population viability cannot be guaranteed (Nichols et al., 2008; Fuller et al., 2003).

Some of the methods currently used in Spain to estimate wolf populations and their biological parameters, which in turn impact decisions made by natural resource administrations in charge of the lethal control of wolves, consist of assessments based mainly on the number of wolf scats found per kilometre (km) along field routes that are surveyed

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typically over only one or two consecutive years. Recent assessments have been made for the wolf populations in the autonomous regions of Asturias (Hernández and González-Quirós, 2015, 2016) and Castilla y León (Sáenz de Buruaga et al., 2015). However, due to the short period of time covered by these studies and the low sampling effort, the resulting population estimates may not be sufficiently accurate. These methodological limitations and uncertainties, therefore, distort the status of these populations.

Given these issues, we conducted a data collection campaign over an eight-year period that was based mainly on the sampling of wolf scats, georeferencing of data, and camera trapping of wolf packs belonging to the population resulting from the recolonization of the Central System mountain ranges located in the central Iberian Peninsula. This study area constitutes the southwestern limit of the current distribution area of the wolf in Europe, as the species has been extirpated from southern Spain (as has been recognized by both national and international authorities, see e.g., Ministry for the Ecological Transition and the Demographic Challenge, https://www.miteco.gob.es/es/biodiversidad/temas/inventarios-nacionales/inventario-especies-terrestres/ieet_mamiferos_censo_lobo.aspx, and the Large Carnivore Initiative for Europe–IUCN/SSC Specialist Group https://www.lcie.org/Largecarnivores/Wolf.aspx). Using the data gathered, we determine the biological parameters of the studied wolf packs and establish more precise criteria with which to evaluate wolf populations and pack reproduction.

Materials and Methods

Study area

Field work was conducted in the Central System mountain ranges from 2010 to 2018. These ranges are located in the southern part of the provinces of Ávila and Segovia and the northern part of the provinces of Madrid and Guadalajara in the central Iberian Peninsula (Fig. 1).

The study area encompasses about 435000 hectares, and includes high mountain ecosystems that alternate with deep valley ones, with an altitude range from 850 to 2200 metres above sea level. Areas with native Pyrenean oak and evergreen oak groves (*Quercus* spp.), pine forests (*Pinus* spp.), and large areas of brush (e.g. *Cistus* spp., *Genista* spp., *Erica* spp., among others) are common, with typical Mediterranean habitats alternating with alpine habitats. The entire territory is subject to human use (e.g., forestry, hunting, farming, livestock raising, or tourism) with different degrees of intensity or exploitation found in different areas.

According to the 2018 land use map of the European CORINE Land Cover Programme (https://land.copernicus.eu/pan-european/corine-land-cover/clc2018?tab=metadata), the percentage of land cover types occupying the area is 87.43% for forest and semi-natural areas, 8.10% for agricultural areas, and 0.23% for artificial surfaces. Within the agricultural areas, pastures occupy 4.11% of the land, and within the forest and semi-natural areas, sclerophyllous vegetation occupies 23% of the land; coniferous forests, 21.43%; natural grasslands, 18.64%; broad-leaved forests, 10.54%; and moors and heathland, 5.05%.

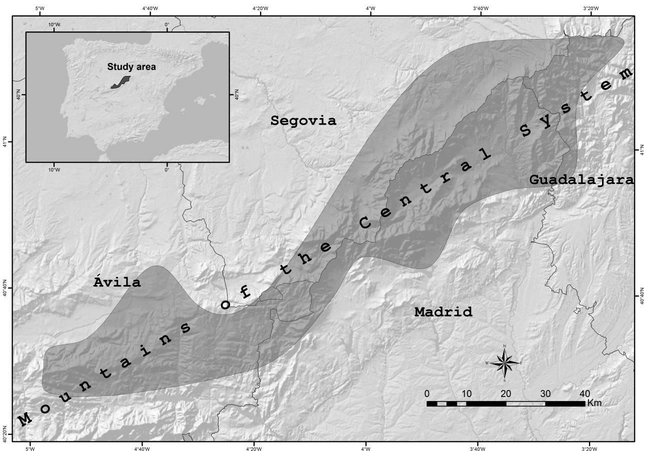

Figure 1 – Map showing the study area in the central Iberian Peninsula. The Central System, which mainly includes the mountain ranges of Ayllón, Guadarrama, Malagón, and Paramera, spans the provinces of Ávila, Madrid, Segovia, and Guadalajara in Spain.

Despite the abundance of cattle in the study area, the main food category consumed by wolves is wild ungulates, in particular the wild boar (*Sus scrofa*) and the roe deer (*Capreolus capreolus*). Other consumed species were the Spanish ibex (*Capra pyrenaica*) and the red deer (*Cervus elaphus*) (unpublished data).

Data collection

In carrying out the field research, we observed all of the ethical principles related with scientific integrity and good practices included in the documents prepared by the Spanish National Research Council (CSIC, its acronym in Spanish), the Ministry of Science and Innovation, and various universities in Spain (https://www.csic.es/es/el-csic/etica/Integridad-cientifica-y-buenas-practicas). Following recommendations to conduct research without causing harm to the study animals, we used non-invasive techniques to avoid disturbing the wolves. Field work was not conducted near the den area during the period before or immediately after pups were born. Sampling was carried out respecting wolf marks and with as few disturbances as possible by minimizing the repetition of itineraries and the number of visits to camera traps to download data. To facilitate this, cameras were equipped with high capacity memory cards and long-lasting batteries.

Prior to this study, no scientific information was available for the population resulting from the recolonization of the Central System. To initiate the field work and locate wolf packs, we gathered information from various sources, including those reporting livestock attacks, news from reliable sources, sightings, and wolf deaths due to hunting or other causes. To locate wolf marks (mainly scats, tracks, and scratches), we planned sampling routes that could be traversed on foot. Mitochondrial DNA from scats was analysed for some wolf packs at the beginning of the study to confirm that they were indeed from wolves.

Once the wolf packs were located, the main sampling effort was carried out during late spring, summer, and early autumn by walking routes of 10 to 20 km that followed dirt roads, trails, and paths. The sampling was reinforced by short, focused linear transects. The number of scats recorded in this study reflects those that were found on the day each route was initially sampled. When routes were repeated, any new scats recorded were not added to the number reported here. Sampling was similar for all packs as the entire study area has the same level of human use, and a similar density of roads and trails. Routes were surveyed beyond the last recorded scats to confirm the limits of the marked territory. Consequently, our route design tended to be flexible, and adapted to the territory of each pack whose area had been entirely covered by routes. This allowed us to obtain a fairly complete map of georeferenced marks for the different packs each year.

Identification of signs of wolf activity was based on scat location, disposition, dimensions, and content of wild ungulates and domestic cattle (see Echegaray and Vilà, 2010; Spaulding et al., 2000), as well as previous knowledge of wolf biology in other areas (Cuesta et al., 1991; Castroviejo et al., 1981). Each wolf mark found was photographed, using a ruler as a scale, and its location was geographically referenced using a Garmin GPSMAP 62st for the spatial analysis. Scats constitute the main element with which wolves mark prominent areas of their territory (Barja et al., 2004, 2005; Zub et al., 2003). In this study, we included only recent, scented scats, that is, scats with little to no disaggregation. Recent scats crushed in the tire section of dirt roads, trampled by cattle, or altered by insects were also included. Scratch marks were not taken into account in the count of the number of marks/km because they are very scarce in the study area.

The patterns and characteristics of wolf tracks and scats are different from those of dogs; therefore, we used their associations to distinguish them. Furthermore, in the study area, there are generally no wild or free-roaming dogs. On occasion, there are dogs that travel with their owners in specific areas; however, their tracks can usually be associated with those of their owner. There are also cattle owners who drive dogs

Table 1 – Information on the sampling effort conducted in the different wolf pack territories during the study period by year, and the total number of wolf scats found for each pack by year.

Wolf pack	Year	No. of routes	No. of km surveyed	Mean route distance (km)	No. of wolf scats recorded
Guadalajara 1	2010	16	138.3	8.6	141
	2011	7	64.6	9.2	33
	2012	5	60.5	12.1	64
	2014	2	19.0	9.5	7
	2018	15	111.0	7.4	68
Mean (SD)		9(6)	78.7(46.6)	9.36(1.73)	63(50)
Guadalajara 2	2016	14	124.8	8.9	62
	2017	13	140.0	10.7	105
Mean (SD)		13.5(1)	132(10.7)	9.8(1.27)	83.5(30)
Madrid 1	2010	19	187.3	9.8	55
	2011	13	141.0	10.8	143
	2012	16	125.7	7.8	31
	2013	8	104.7	13.08	25
	2014	8	75.4	9.4	21
	2017	30	300.0	10.0	98
	2018	23	300.0	13.04	60
Mean (SD)		16.7(8)	176.3(91.1)	10.56(1.93)	61.8(45)
Madrid 2	2015	9	119.0	13.2	28
	2017	8	94.9	11.8	105
	2018	3	20.0	6.6	24
Mean (SD)		6.6(3)	78(51.6)	10.53(3.47)	52.3(46)
Madrid 3	2017	6	117.7	19.6	52
	2018	7	75.0	10.7	71
Mean (SD)		6.5(1)	96.3(30.2)	15.1(6.29)	61.5(13)
Segovia 1	2010	13	124.4	9.5	102
	2011	10	141.6	14.16	90
	2012	7	89.5	12.7	18
	2013	7	86.5	12.3	42
	2014	2	12.0	6.0	11
	2017	7	102.4	14.6	60
	2018	4	82.7	20.6	81
Mean (SD)		7.1(4)	91.3(41.1)	12.8(4.53)	58(35)
Segovia 2	2011	8	80.0	10	43
	2014	6	31.2	5.2	4
	2018	5	75.8	15.1	29
Mean (SD)		6.3(2)	62.3(27)	10.1(4.95)	25(20)
Segovia 3	2015	5	63.2	12.6	10
Segovia 4	2013	3	45.4	15.1	15
Segovia 5	2017	10	166.7	16.6	69
	2018	8	131.1	16.3	33
Mean (SD)	9(1)	148.9(25.2)	16.45(0.21)	51(25)	
Ávila 1	2015	14	95.9	6.8	20
Ávila 2	2015	6	102.0	17.0	25
Ávila 3	2016	10	74.7	7.47	33

in vehicles to the location of their cow herds. Though these dogs may defecate in these spots, they do not roam free in the mountains. In the Sierra de Ayllón range in Guadalajara, there are some flocks of grazing sheep herded by mastiffs, but these dogs are confined to a few localities. With respect to hunting dog scats, wolf scat sampling was carried out mainly in summer when the excrements of hunting dogs are already old as hunting drives typically end in February. Also, hunting dogs usually defecate at gathering points before being released for a hunt, which can be easily identified by the high number of scats at these places. Moreover, in general, dog scats tend to be granular and lack bones as their diet consists of dog feed. Therefore, we were confident that the tracks and scats sampled were indeed from wolves.

Camera trapping data was also used to verify the presence of wolves. We incorporated the camera trapping technique in our study in the autumn of 2012. The use of camera traps greatly facilitates the monitoring of the specific structure and dynamics of wolf packs (Galaverni et al., 2011; Balme et al., 2009). Camera traps (Reconyx HC-600, Browning, VicTsing) were installed on trails and paths with recent wolf tracks and marks, particularly in the summer months (July–September). We focused primarily on route sections widely used by the wolves (e.g., those with 10 or more scats per km that connected feeding areas with a den site, or trails with tracks and scats of juvenile wolves). Cameras were also placed on trail sections with few scats far from den sites when needed for the study, such as to determine which individuals were active in those locations. Cameras were positioned to obtain images of the wolves in lateral view, and faced north to avoid the direct effect of the sun, at a distance of 1 to 2 metres from the side of the trail, slightly elevated above ground level and hidden by vegetation.

The number of wolves in each pack and the number of juveniles were estimated by analysing image sequences of individuals passing one after another in a line in front of the cameras at intervals of less than a second to a few seconds, and in some cases through the identification of individuals by their pelage, which, in summer, are highly conspicuous and stable, and other morphological characteristics. Dir-

Table 2 – Descriptive statistics of the variable wolf pack size by year of study.

	Mean size			
Year	Mean	Std. deviation	Minimum	Maximum
2010	4.3	1.61	2.5	5.5
2011	3.4	1.18	2.5	5.0
2012	2.2	0.29	2.0	2.5
2013	2.7	0.75	2.0	3.5
2014	2.5	1.08	1.5	4.0
2015	4.5	0.91	3.5	5.5
2016	3.8	0.35	3.5	4.0
2017	3.8	1.92	1.5	7.0
2018	4.0	1.63	1.5	6.0
Total	3.5	0.91	1.5	7.0

ect observations of wolves, adult and juvenile tracks, juvenile scats, images of gravid or lactating females, adult and juvenile roadkill, and other data on dead wolves were also used to detect the recent presence of wolves, count individuals and/or confirm the occurrence of reproduction.

The use of camera traps has been described as expensive and logistically demanding (Ausband et al., 2014; Swann et al., 2004), however, in our study, we never needed more than four cameras per wolf pack. We also only had to make weekly or monthly visits to download data from the cards and change the batteries. The camera trap images allowed us to not only clarify doubts about whether one versus two packs were present in an area but also determine the distances travelled by breeding females within their territory during the breeding period. For instance, using the images we obtained of three breeding females from different packs, we determined they travelled a distance as great as, respectively, 14, 18, and 19 km from the den.

Sampling effort

During the study period, a total of 3824 km was sampled on foot. This total was distributed among 347 routes in the mountainous areas of the four provinces in which the Central System spans. The number of routes travelled, number of km sampled, average distance of each route and number of scats recorded for each pack and year has been provided, as has the mean and standard deviation of these data for the set of years each pack's territory was surveyed (Tab. 1).

Statistical analyses

The purpose of the statistical analyses in this study was to predict reproduction in wolf packs using the collected variables in order to identify the most decisive variable. Three primary analyses were performed with the data. First, a categorical principal components analysis (CATPCA) was performed on the independent variables (i.e., number of transects, distance surveyed, and number of scats found) to determine their relationship with mean pack size. This analysis is based on optimal scaling to avoid problems related to non-normality (Gaussianity). Using this data, a binary logistic regression analysis was then performed to predict the relationship between selected independent variables and reproduction, the binary dependent variable. Reproduction was the dependent variable as it is the factor that most influences the spatial and temporal use of a territory by wolves (Roque et al., 2001). For the stepwise regression, we used the forward selection (conditional) approach to select the optimal model. Finally, this categorical dependent variable (with a value of 0 or 1, depending on the absence or presence of reproduction) was used in decision tree analyses to predict the probability of reproduction. This procedure creates a tree-based classification model by classifying cases into groups of the dependent variable based on the values of the independent variables. In this case, the independent variables were considered those related to each pack and year (i.e., number of scats, number of transects, km surveyed, maximum and minimum pack size, mean size, and area of the territory). Specifically, we used the Classification and Regression Trees (CART) method, which splits the data (finding the optimal cut point) into segments that are as homogeneous as possible with respect to the dependent variable. With this method, it is possible to force the first variable, with the subsequent variables being chosen in the following steps, provided they were discriminatory and no multicollinearity issues arose during the stepwise sequential process. To detect and control for multicollinearity, we (1) analysed the independent variables through a CATPCA, and (2) resolved multicollinearity in the models using a stepwise variable selection method in the logistic regression and decision tree analyses.

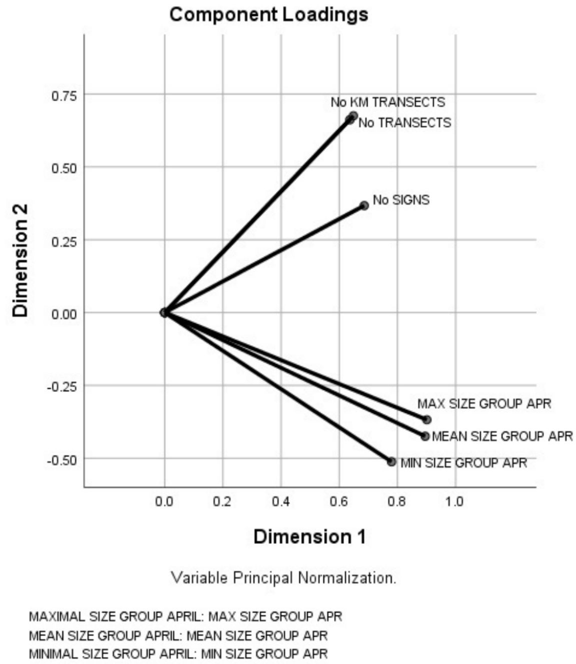

Figure 2 – Categorical principal components analysis.

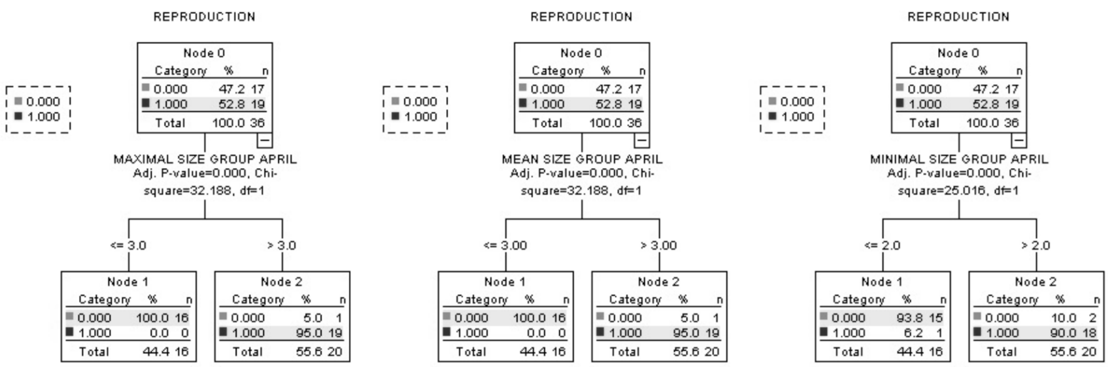

Figure 3 – Decision trees for reproductive success.

Table 3 – Reproductive success by year over the duration of the study period (N: number of packs; Mean: percentage of reproductions).

					95% C.I. for the Mean			
Year	**N**	**Mean**	**Std. deviation**	**Std. error**	**Lower limit**	**Upper limit**	**Minimum**	**Maximum**
2010	3	0.67	0.577	0.333	-0.77	2.10	0	1
2011	4	0.50	0.577	0.289	-0.42	1.42	0	1
2012	3	0.00	0.000	0.000	0.00	0.00	0	0
2013	3	0.33	0.577	0.333	-1.10	1.77	0	1
2014	4	0.25	0.500	0.250	-0.55	1.05	0	1
2015	4	1.00	0.000	0.000	1.00	1.00	1	1
2016	2	1.00	0.000	0.000	1.00	1.00	1	1
2017	6	0.50	0.548	0.224	-0.07	1.07	0	1
2018	7	0.57	0.535	0.202	0.08	1.07	0	1
Total	36	0.53	0.506	0.084	0.36	0.70	0	1

Results

The study area was sampled in its entirety over the eight-year study period. A total of 13 packs/groups were detected over the duration of the study, and 36 data sets were obtained. A data set refers to the data gathered for a single wolf pack/year. The distribution of packs by province was Segovia (5), Ávila (3), Madrid (3), and Guadalajara (2). The wolf packs occupied 208400 of the total 435000 hectares comprising the study area, representing 47.90% of the total area. A total of 1878 wolf scats were found and georeferenced for the 13 packs studied (Tab. 1).

For some packs, we have data spanning several years. However, for others, successive annual monitoring could not be performed because of the temporary disappearance of wolves due to being hunted or roadkill. Monitoring continued in the years when packs were re-established and became linked again to a defined territory. However, some packs were only monitored for one year. Increased persecution of wolves by illegal hunting occurs mainly in the years they breed, as their presence is more evident, which ultimately leads to a reduction in the number of individuals. In fact, we observed that, for several of the packs whose territories coincide with livestock areas, and where human attitudes towards the wolf are not favourable, the first wild boar drives of autumn in some of the years occurred on hunting grounds that included the wolf den area (personal observations). The number of scats found for these packs in the year following was much lower than the preceding one, indicating fewer wolves in the pack. Consequently, there are cycles of decline and recovery as reflected by the annual data on number of scats, pack averages during the study period and the high standard deviation values (Tab. 1).

Likewise, the annual variation in the number of images obtained by camera trapping over the study period was mainly due to the same reason as above, the temporary disappearance of some packs, although the level of success in camera placement and other circumstances such as cameras being displaced by wild boars or cattle or the disappearance of cameras also influenced the number of images obtained per year. For the packs GU1, M1, and SG1, the mean and standard deviation of the number of images obtained in the different years are 143.5 ± 191.6, 62.5 ± 16.2 and 31.6 ± 21.2, respectively. For the other packs, the number of images obtained annually ranged between 15 and 202 (68.1 ± 64.08), except for M2 for which 1148 images were obtained in 2015 due to one of the cameras being placed near a cattle carcass.

Camera trapping effectiveness

Despite the variation in the number of images obtained per year, the incorporation of the camera trapping technique in our study proved to be extremely useful. The effectiveness of the detection of reproduction and population counts increased from 77.7% and 66%, respectively, between 2013 and 2015, to 100% for both variables in 2017 and 2018. Mainly by using this technique, we confirmed that reproduction occurred 19 times over the duration of the study period.

Relationship among variables

The overall results of the statistical analysis demonstrate that the analysed variables, which accounted for 85.4% of the variance, indicating a strong relationship among them, are observable elements that can be used to predict the presence of reproduction and the degree of field work necessary to be able to draw accurate conclusions about the conservation status of the wolf population in the Iberian Peninsula.

The most decisive variable affecting wolf population dynamics was mean pack size, as well as the minimum and maximum values estimated for this variable. Counting the number of wolves at the end of the breeding season (September/October) to estimate mean pack size would overlook the impact of the high annual mortality of pups and dispersed juveniles (Lovari et al., 2007; Jedrzejewska et al., 1996). Therefore, we considered the end of winter (March/April) as the most appropriate time to assess mean pack size per year. Our data showed that the mean pack size in the Central System was 3.5 wolves during the study period (Tab. 2).

The CATPCA performed to discriminate the relationship between the variables assessed during the sampling effort shows that the pack size variables are more related to the number of scats found than to the transect distance surveyed. All of the relationships were positively correlated, meaning that the larger the number of scats found and the greater the sampling effort (number of transects and km surveyed), the larger the pack size (Fig. 2).

Reproductive success

According to the binary logistic regression analysis, mean pack size (the positive coefficient in the equation) best predicted reproductive success (see Tab. 2 and 3), which was positively correlated with a larger mean pack size, as shown in the CART decision tree analysis used to predict the interaction of each pack size variable (mean, maximum, and minimum) with reproductive success (Fig. 3). In order to meet the conditions for reproductive success and to maintain or increase the size of wolf packs, a minimum reference pack size of three members

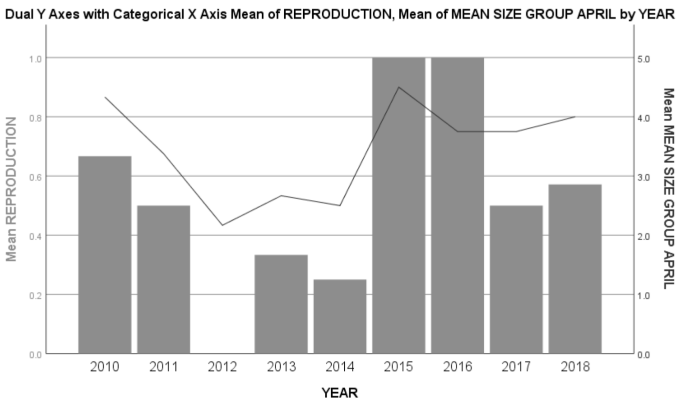

Figure 4 – Relationship between reproductive success and mean pack size.

was required, as reproduction was nearly guaranteed in packs with at least four members (95%). Among the packs with fewer than three individuals, reproduction was successful in only one of the 36 cases. A minimum or mean size of four individuals per pack occurred in packs with a maximum size of five or more adult members. These pack sizes, which were initially evident as a condition for reproduction, were not always achieved due to the impact of human interference on the wolf population. Indeed, packs of these sizes are relatively rare in the study area. The tendency toward small pack sizes contributes to the unfavourable conservation status of the wolf in this area.

Regarding the differences in reproductive success by year during the study period (2010–2018), the analysis of the overall data obtained from the 36 data sets revealed that the mean rate of reproductive success was 52% (19 positive cases versus 17 negative ones) (Tab. 3). This figure differs greatly from the estimates of reproductive success reported generally for packs in the Iberian region (see discussion). However, during the 2015–2016 period, mean pack sizes were larger compared with other periods, resulting in a reproductive success rate of 100% in the study area.

As for the relationship between reproductive success and mean pack size by year, the peak values of mean pack size coincided with the highest mean reproductive success rates, and in the years that mean pack size decreased in the overall population, so did reproductive success (Fig. 4, see also Tab. 2 and 3). Although year-over-year differences did not have a significant effect on reproduction, in both 2015 and 2016, the mean value was 1, which was significantly higher than those values in other years. This maximum value corresponded to a period of larger pack sizes. Reproductive success values for the 2010–2011 and 2017–2018 periods were close to the overall mean value for the entire study period, as the wolf packs maintained the same mean size in relation to the overall set. In 2012 and 2014, there was a sharp decrease in mean pack size (see Tab. 2), likely due to casualties related to contact with humans. Reproduction values recovered modestly in subsequent years.

Optimal sampling effort

We related the number of scats found on the sampling routes to the reproductive outcome of packs and determined that ±57 scats found for a single pack of wolves within its territory during the summer season (May to October) corresponded to an 80% probability of reproduction. This probability rose to 90% when 70 or more scats were found. Targeted sampling to find scats and to delimit each pack's territory, combined with modern monitoring methods such as camera trapping, allowed us to minimize uncertainty and obtain accurate estimates to predict reproductive success.

We found a clear relationship between the extent of sampling effort (the number of transects and total km surveyed per pack) and the reliability of the data. Once a pack territory has been delimited, surveying at least 38.3 km of transects can guarantee the detection of reproduction with a probability of 59%; surveying 95.4 km increases that probability to 68% (Fig. 5). The relationship between the number of transects and km surveyed and the number of scats found was clearly positive (i.e., increasing the sampling effort increases the probability of success in detecting reproduction). Furthermore, both of these variables were linked to pack size.

Regarding this last point, when delimiting the territorial area of a wolf pack in the field, we concluded that both the area occupied during the reproduction period (main centre of activity) and the borderline areas had to be sampled during the surveys until no scats were found, unless we detected clear signs of spatial separation from other packs based on other criteria. A positive correlation was observed between territory size (surface area) and all the other variables, especially pack size. A pack of wolves occupying a territory of 60 km^2 or more showed a successful reproduction outcome in 87.5% of the cases (Fig. 6). However, the territorial variable should be analysed with caution because different ecological and human-mediated constraints can be found in territories of similar size or with similar characteristics (e.g., availability of wild prey, access to food resources of human origin, adequate refuge areas, existence of nearby packs).

According to our results on wolves in the Central System, packs must have at least four individuals and an available territory of at least 60 km^2 to ensure reproduction. In addition, we found that the sampling effort required to obtain data reliable enough to evaluate reproductive success was to survey, on foot, at least 38 km of transects in selected and non-predefined areas in order to collect at least 57 scats and delimit a marked territory of at least 60 km^2. Given that all the correlations between the variables were positive, the greater the value of the variable studied (e.g., greater sampling effort or more accurate results), the more likely it is that reproduction can be verified (e.g., the probability of detecting reproduction increases to 90% when more than 70 scats are found).

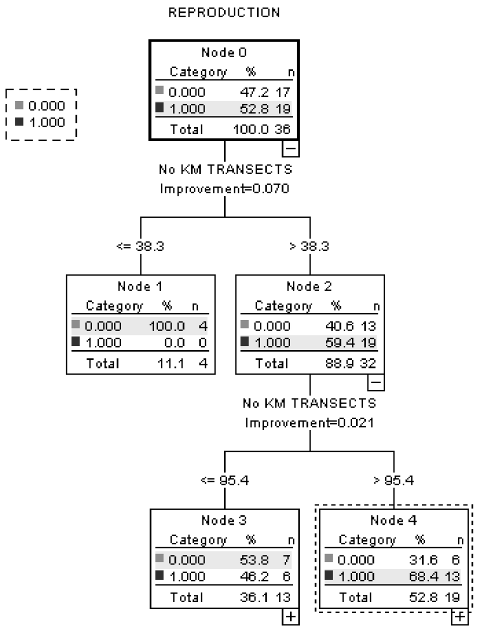

Figure 5 – Decision tree for reproductive success in relation to sampling effort (km of transects surveyed).

Discussion

According to the mortality records available in the collections at the National Museum of Natural Sciences of Madrid (MNCN–CSIC), the last wolf from the historical population of the Central System was hunted down in the Sierra de Ayllón range in 1976. The mountains of the Central System were subsequently recolonized by wolves from north of the Duero River. According to the MNCN records, evidence of this recolonization was first documented for the Sierra de Guadarrama range (in the province of Segovia) in 2006.

The new wolf population of the Central System is located south of the Duero River. In this region, the species has the highest category of protection granted by the European Union: it is a species of community interest, a priority species, and a species in need of strict pro-

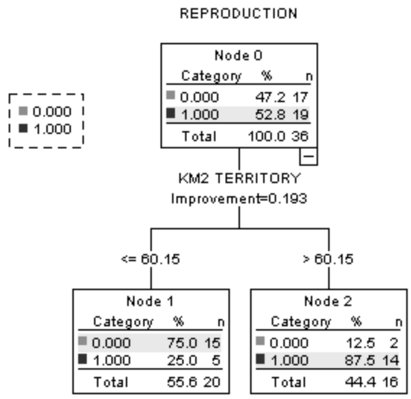

Figure 6 – . Decision tree for reproductive success in relation to territory size.

tection. This wolf population is of great importance because it represents the southwestern-most population in the distribution area of the wolf in Europe. Therefore, it represents an ideal population to monitor and study in order to track the potential recolonization of more southern areas of the Iberian Peninsula. Though there are studies of wolf populations in other areas of expansion or recolonization in southwestern Europe, such as in the French Alps (Duchamp et al., 2012) or Italy (Marucco et al., 2012), in Spain, until now, only historical populations located north of the Duero River, such as in Galicia and Asturias, have been the focus of scientific studies.

The level of field work dedicated to monitoring wolf populations generally tends to be insufficient, with some authors claiming that studied populations are saturated, or logistical limitations. By following up to 13 wolf packs over a period of eight years, we conclude that the difficulties associated with tracking large, elusive, and highly mobile carnivores like the wolf (Ausband et al., 2010) are not insurmountable. We found that the level of the sampling effort largely determines the success of data interpretation. Therefore, as demonstrated by our study, it is important to obtain sufficient field information so that the statistical analysis of the data yields reliable results.

Some previous studies assessing the conservation status of wolves in Spain in order to justify their control and/or exploitation simply do not include sufficient field information. Here, we highlight one example. In 2015 and 2016, the status and the number of breeding units of the wolf population in Asturias was assessed (Hernández and González-Quirós, 2015, 2016) to justify the program of wolf control actions planned for 2017–2018 that was being promoted by the General Directorate of Natural Resources of the Autonomous Government of the Principality of Asturias. This assessment included the analysis of 44 wolf packs. In the 2015 study, 185 routes were surveyed in summer, covering a total of 756 km, for an average of 2.80 routes and 11.4 km per pack. In the 2016 study, 199 routes and a total of 832 km were surveyed in summer, for an average of 3 routes and 12.6 km per pack. Compared with our results, these averages represent only about a third of the effort needed to have a 59% probability of detecting reproduction. Eight times the average effort made in the Asturias assessment would be need to increase that probability to 68%. Based on our data, the level of sampling effort commonly completed to support the evaluations performed or commissioned by administrative agencies is not only very low but also insufficient to draw reliable conclusions.

On the other hand, some of the variables shown to be highly informative in our study are not commonly used in wolf assessments. These include the average number of individuals before reproduction, although this data was considered important by Lake et al. (2013); the area or extension of marked territory at the time of reproduction; and the delimitation of a pack's territory from that of adjacent packs. By contrast, the number of wolf marks/km is often used in assessments. However, this variable is not determinant since we observed high values in non-breeding packs, and it is dependent on whether or not the routes surveyed pass through refuge areas or trails heavily used by wolves.

In addition, it should be borne in mind that, since the wolf is a species of community interest in European Union countries, decisions on the exploitation of its populations or the derogation of the provisions provided by the EU's strict protection system can only be made after evaluating whether the conservation status of the population is favourable, something that is essential but not done. The three predictions established by the Habitats Directive (Directive 92/43/EEC) are whether the population dynamics data, the evolution of the geographical distribution, and the extension of habitat make it possible to predict that there will be a viable wolf population in the long term. However, a two-year population assessment does not replace a study of population dynamics, which requires, at the least, monitoring for 10 to 15 years. Therefore, decisions on wolf control are being made in Europe without relevant studies first being conducting to determine whether the conservation status of the wolf population is favourable or not.

Trend of the wolf population studied

Our investigation reveals that the population that recolonized the Central System of Spain already shows symptoms of general stagnation and decline in certain areas. For example, in the two territories studied in the province of Guadalajara, the population dynamic is one of recurrent real-time settlement and extinction. Throughout the study area, there are very few packs with regular reproductive activity that could be considered stable over the years to serve as a source of young wolves that can expand to other territories.

In our analysis of the 36 data sets, we observed a mean number of 3.5 wolves per pack during the study period based on data gathered in winter prior to the reproductive season (March-April). This mean value was lower than the numbers estimated by other authors for packs in other areas under similar ecological conditions: 3.8 to 4.4 wolves/pack in the Cantabrian Mountains (Fernández-Gil, 2014; Fernández-Gil et al., 2020); 4.5 wolves/pack in Portugal (Pimenta et al., 2005); 5 wolves/pack, with an added peripheral individual, in Italy (Lovari et al., 2007); and 3.6 wolves/pack in Poland (Jedrzejewska et al., 1996). These values differ greatly from the estimated 8 to 11 wolves per pack reported for other Spanish populations (Sáenz de Buruaga et al., 2015).

The reproduction rate in our study area was relatively low (52.7%). This rate is much lower than estimates obtained by assuming that every wolf pack is reproductive (Llaneza and Blanco, 2005). However, not all wolf packs reproduce successfully: it is generally accepted that a minimum of 20% of packs in the Iberian region either do not reproduce or have reproductive failures (Barrientos et al., 2010). At a rate of 52.7%, the annual renewal of the wolf population in this region is precarious. The low reproductive success rate in the Central System is likely related to the high mortality and low density of wolves in this region. Mortality is generally overlooked in wolf management (Álvares et al., 2010); however, if we assume that pup mortality (Barrientos, 2000; Jedrzejewska et al., 1996; Valverde and Hidalgo, 1979) and mortality due to human intervention, such as poaching or accidental kills (Jedrzejewska et al., 1996), are high, the mortality rate may exceed 35% per year (Fernández-Gil et al., 2010). An elevated mortality rate leads to population stagnation or zero growth (Blanco and Cortés, 2001; Fuller, 1989, 1995), which seriously undermines a species' potential to expand.

Determinant variables

We observed that mean pack size influenced the wolf population studied in a variety of ways, aside from marking patterns (see Zub et al., 2003). Mean pack size was the most decisive variable of all the ones analysed: it determines the intensity of territorial marking (scats) and the probability of reproductive success, and also influences the level of sampling effort (number of transects surveyed and km walked) researchers must invest in order to obtain accurate results. Consequently, the small mean pack sizes observed in the Central System study area negatively influence reproduction rates and the stability of the wolf population or its potential to grow. Increased mortality due to culling (by derogations of Article 12 of Directive 92/43/EEC) and poaching, which are recurring situations in the study area, have led to local wolf extinctions.

Although a stronger correlation was observed between the pack size variables and the number of scats found than the transect distance surveyed, the relationships among all the variables clearly reflect the fact that a larger pack deposits more scats and occupies more territory. These results also highlight the importance of having an adequate level of sampling effort in the delimitation of territories, as territory size was also positively correlated with all the other variables, particularly pack size.

We propose that the number of wolf marks/km (kilometric scat abundance index) should only be used as a guide for locating breeding areas or for interpreting the intensity of territorial use by wolves. We advise against using this measure to predict something as consequential as wolf density or reproductive activity. In fact, during the course of our field work, we observed several cases of non-breeding wolf packs marking preferred tracks with the same intensity as breeding packs.

The methodology and the recommended level of field work per pack suggested in this study, combined with camera trapping and/or other direct or indirect methods, can be applied, with the necessary precautions, to other local or regional wolf populations in areas of expansion or on the margins of distribution areas, as well as in areas that harbour historical populations. As shown here, the proposed methodology, and the data obtained with it, can yield reliable assessments of the current status of populations. It can also be used repeatedly and reliably for long-term population dynamic studies. One of the main benefits of this approach is that it would provide accurate information for evidence-based decision-making regarding the management of wolf populations to ensure the conservation of the species.

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