

1 ORIGINAL PAPER

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3 **Towards a standardized index of European rabbit abundance in Iberian**

4 **Mediterranean habitats**

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6 J. Fernandez-de-Simon · F. Díaz-Ruiz · F. Cirilli · F.S. Tortosa · R. Villafuerte · M.

7 Delibes-Mateos · P. Ferreras

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9 J. Fernandez-de-Simon (corresponding author) · F. Díaz-Ruiz · R. Villafuerte · M.

10 Delibes-Mateos · P. Ferreras

11 Instituto de Investigación en Recursos Cinegéticos, IREC (CSIC-UCLM-JCCM),

12 Ronda de Toledo s/n, 13071, Ciudad Real, Spain

13 Phone: +34 926 295 450

14 Fax: +34 926 295 451

15 e-mail: [javier.fernandez@uclm.es](mailto:javier.fernandez@uclm.es)

16

17 F. Cirilli · F.S. Tortosa

18 Department of Zoology, University of Cordoba, Campus de Rabanales Ed. Darwin,

19 14071 Córdoba, Spain

20

21 **Abstract** European rabbits *Oryctolagus cuniculus* are a keystone species in Iberian  
22 Mediterranean ecosystems. Studies over the past few decades have revealed drastic  
23 declines in many rabbit populations. However, the reliability of methods for estimating  
24 rabbit abundance, particularly when at low numbers, is not well understood. Further,  
25 better standardization of these methodologies would allow abundance estimates to be

1 more reliably compared between areas and periods. Consequently, we compared several  
2 frequently used methods of estimating rabbit abundance and assessed the advantages  
3 and disadvantages of each. During the summers of 2008 and 2009, in 11 localities of  
4 central-southern Spain we undertook (a) driving transect counts of rabbits, either at dusk  
5 or at night, (b) linear transects on foot recording rabbit signs, (c) cleared-plot pellet  
6 counts at permanent plots, and (d) standing crop counts, both with and without habitat  
7 stratification. Density estimated at night from driving transects using the Distance  
8 Sampling method (the reference method against which all other indices were  
9 compared), varied from 0 to 2.69 rabbits ha<sup>-1</sup>. Most pellet-count indices were  
10 significantly related to the reference method. In particular, cleared-plot pellet counts in  
11 permanent plots corrected for pellet persistence showed the best correlation with the  
12 reference method. In contrast, latrine counts were not related to the reference method  
13 index, and we recommend against their use. A standard methodology based on cleared-  
14 plot pellets counts could be used to monitor rabbit abundance on a large scale,  
15 particularly where there is an urgent need for management and conservation of rabbits,  
16 and their associated endangered predators.

17 **Keywords** Abundance indices · Density estimates · Lagomorphs · *Oryctolagus*  
18 *cuniculus* · Reference method · Standardization

19

## 20 **Introduction**

21 European rabbits *Oryctolagus cuniculus* are a key species in Iberian Mediterranean  
22 ecosystems (Delibes-Mateos et al. 2007; Valverde 1967), being consumed by more than  
23 40 predator species (Delibes and Hiraldo 1981; Delibes-Mateos et al. 2008a). Rabbits  
24 also act in this area as ecosystem engineers, because of their effect in vegetation  
25 (creation of open areas, preservation of plant species diversity, increased plant growth

1 by inducing soil fertility), but also because they provide feeding resources (latrines) for  
2 many invertebrate species, and nest sites and shelter (burrows) for vertebrates and  
3 invertebrates (Delibes-Mateos et al. 2008a; Gálvez et al. 2009). Rabbits are also a  
4 popular small game species (Angulo and Villafuerte 2003). Although considered  
5 harmful pest species in other parts of the world (Lees and Bell 2008; Thompson and  
6 King 1994; Williams et al. 1995), European rabbits are only regarded as an agricultural  
7 pest in a few localized areas in Spain (Barrio et al. 2010b; Ríos 2010). Rabbit numbers  
8 on the Iberian peninsula have declined over recent decades, due mainly to habitat loss  
9 (Delibes-Mateos et al. 2010) and the occurrence of two viral diseases, myxomatosis  
10 during the 1950s (Muñoz 1960) and rabbit hemorrhagic disease (RHD) at the end of the  
11 1980s (Villafuerte et al. 1995). Since the RHD outbreak in Doñana National Park  
12 (southwestern Spain), rabbit numbers have declined by an estimated 90% (Moreno et al.  
13 2007). Most populations in Spain have continued to decline (Delibes-Mateos et al.  
14 2008b), resulting in significant economic and ecological consequences for Iberian  
15 Mediterranean ecosystems (Delibes-Mateos et al. 2008a). Monitoring rabbit populations  
16 is currently a major challenge for conservation, making the development of widely  
17 applicable and reliable monitoring methods particularly important (Delibes-Mateos et  
18 al. 2009). For instance, the Iberian lynx (*Lynx pardinus*) is a critically endangered  
19 predator specialist on rabbits (Ferrerías et al. *in press*) and it has been calculated that the  
20 autumn mean rabbit density required for Iberian lynx residence is 1 rabbit ha<sup>-1</sup>, while  
21 the spring mean rabbit density required for reproduction is 4.6 rabbits ha<sup>-1</sup> (Palomares et  
22 al. 2001). Hence, large and cost-effective spatial monitoring projects to select areas  
23 suitable for lynx re-establishment are dependent on reliable and easy-to-perform indices  
24 of rabbit abundance (Ferreira and Delibes-Mateos *in press*).

1 Direct and indirect methods are currently used to estimate rabbit abundances and  
2 population trends. Direct methods are based on surveys or counts of the animals, while  
3 indirect methods are based on the monitoring of animal signs. One of the most  
4 commonly used direct methods is counting individuals along linear transects, which can  
5 provide absolute estimates of density (Barrio et al. 2010a; Martins et al. 2003;  
6 Palomares 2001; Palomares et al. 2001) by using the Distance Sampling method  
7 (Buckland et al. 1993). The accuracy of this method is dependent on several  
8 assumptions (e.g., objects on the line or point are detected with certainty, objects do not  
9 move, measurements are exact; Thomas et al. 2010) and these may not always be met.  
10 A considerable sampling effort is also required to obtain sufficient number o sightings  
11 to reliably estimate density in low density populations (Newey et al. 2003). One  
12 alternative to the Distance Sampling method is the kilometric abundance index  
13 (sightings  $\text{km}^{-1}$ ; Beltrán 1991; Moreno et al. 2007; Williams et al. 2007), which is  
14 correlated with population density (Barrio et al. 2010a; Palomares et al. 2001) and  
15 provide data for a range of abundances. Rabbit counts have also several drawbacks (see  
16 e.g., Martins et al. 2003; Twigg et al. 1998; Villafuerte et al. 1993). Other direct  
17 methods used to estimate densities are based on live trapping, mostly by means of  
18 capture-recapture (e.g., Ballinger and Morgan 2002; Marchandeanu et al. 2006) and the  
19 minimum number of individuals known to be alive (MNA, King and Wheeler 1985;  
20 Wood 1988).

21 Indices from rabbit signs are alternatives to direct methods. Indices based on  
22 counts of warrens are widely used (Myers et al. 1975) and can be corrected for distances  
23 traversed during these counts (Palomares 2001). Pellet counts per unit area can also be  
24 used to estimate abundance (Cabezas and Moreno 2007; Delibes-Mateos et al. 2008b;  
25 Moreno and Villafuerte 1995), as can latrines per unit of distance (Calvete et al. 2006).

1 The standing crop count method involves counting pellets during only one visit,  
2 enabling a large area to be sampled. Nevertheless, to estimate absolute densities it is  
3 necessary to correct for defecation rates and pellet persistence (Putman 1984). The  
4 cleared-plot count method involves counting pellets that accumulate over a period of  
5 time in plots from which pellets had been previously removed (e.g., Palomares 2001). It  
6 is important to identify the period during which pellet accumulation and persistence is  
7 highest in order to reduce the zero counts in low density populations and the effect of  
8 pellet decay in pellet counts. This method is time consuming and labor intensive and it  
9 is necessary to optimize the time of visits (Massei et al. 1998). This method can provide  
10 reliable estimates of abundance when animal densities are low (Murray et al. 2002), a  
11 situation limiting the accuracy of other methods. Other signs, such as scrapes and  
12 tracks, are rarely used to obtain indices of rabbit abundance (but see Twigg et al. 2001).

13         Studies monitoring the abundance and trends of rabbit populations in the Iberian  
14 Peninsula have used most of the methods described above (Calvete et al. 2006; Delibes-  
15 Mateos et al. 2008b; Moreno et al. 2007; Williams et al. 2007). Unfortunately, due to  
16 the variety of methods used, it is not always possible to compare results (i.e. rabbit  
17 abundances) among studies (Delibes-Mateos et al. 2009). Several studies have  
18 attempted to standardize methodologies by comparing indices with a reference method,  
19 which provides less biased estimates of population size and absolute densities, often  
20 using live trapping indices (Ballinger and Morgan 2002; Marchandeanu et al. 2006;  
21 Wood 1988). However, the high costs in human effort, time and logistical resources  
22 prevent the use of live trapping in wide-scale studies, in many localities and in  
23 medium/long-term monitoring programs. As a result most studies have been based on  
24 rabbit counts (Barrio et al. 2010a; Myers et al. 1975; Palomares 2001; Palomares et al.  
25 2001), though as the same methods are not simultaneously performed in the same study

1 areas, the optimal method for specific conditions and objectives is unclear. Moreover,  
2 fewer studies have used a wider approach than using a locality scale. We therefore  
3 sought to compare several commonly used methods, and assessed the advantages and  
4 disadvantages of each. We propose common and comparable methodologies for  
5 assessing rabbit abundances according to the objectives and scale of each study.

6

## 7 **Material and methods**

### 8 **Areas and periods of study**

9 Field work was performed in 11 localities of central-southern Spain (Fig. 1), with  
10 different rabbit abundances but similar habitat structures and climate. All localities had  
11 a Mediterranean climate, characterized by wet, mild winters and warm, dry summers  
12 with marked drought periods. Habitats were composed mainly of Mediterranean  
13 scrubland, pastures, croplands, ‘dehesas’ (savanna-like formations that combine  
14 pastures with intermittent cereal cultivation in park-like oak woodlands; Blondel and  
15 Aronson 1999) and tree plantations. We used the same methods (Table 1, further details  
16 below) to estimate rabbit abundance in each locality during the summers (June–August)  
17 of 2008 (localities 1–8) and 2009 (localities 9–11). For some indices (density estimates  
18 at night, DEN-N; kilometric abundance index at night, KAI-N; cleared-plot pellet  
19 counts corrected and uncorrected by persistence, COR and UNC respectively, see below  
20 and Table 1) and localities (1–8), data were also collected in the winter–spring  
21 (December-May) seasons of 2007–2009 and in summer 2007.

22

### 23 **Driving transect counts**

24 Rabbits are active during twilight and at night, with two activity peaks that coincide  
25 with sunrise and sunset (Díez et al. 2005). However, rabbit counts at night provide more

1 precise estimates than at dusk (Barrio et al. 2010a). We counted rabbits at dusk (starting  
2 one hour before sunset) and at night (starting two hours after sunset) in good weather  
3 conditions (no strong winds and no rainfall). Surveys were undertaken at each locality  
4 and at each of these periods of the day (dusk and night) for three consecutive days,  
5 unless climatic or logistic circumstances prevented so. Transects were travelled in an  
6 all-terrain pick-up at a speed of 15 km/h along dirt tracks varying in minimum length  
7 (mean  $\pm$  SE = 14.04  $\pm$  1.61 km) and traversing different habitats with good visibility.  
8 Most of the sightings were observed within 100 m, though some observations were  
9 exceptionally obtained up to 200 m approximately. One observer stood at the trunk of  
10 an all-terrain pick-up observing the 180° area ahead. At night, a 100-W halogen  
11 spotlight was used. The distance (m) of each rabbit from the observer was measured  
12 using a telemeter, and the angle between the transect line and the line from the observer  
13 to the animal was measured using a compass. We calculated an average kilometric  
14 abundance index (rabbits seen km<sup>-1</sup>, KAI-N and KAI-D) at each locality by pooling the  
15 data obtained from the three replicates, and we estimated the rabbit density (rabbits ha<sup>-1</sup>,  
16 DEN-N and DEN-D) with the Distance Sampling method (Buckland et al. 1993), using  
17 the Fourier series estimator as detection function in TRANSECT software (Burnham et  
18 al. 1980).

19

## 20 **Linear transects on foot**

21 Four-km transects were walked by two observers traversing areas favorable to rabbits,  
22 mainly those ecotones between Mediterranean scrubland and pastureland or cropland  
23 (Delibes-Mateos et al. 2008b). The variables assessed along the transect were rabbits  
24 (rabbits seen km<sup>-1</sup>, RAB), latrines (latrines km<sup>-1</sup>, LATR), scrapes (scrapes km<sup>-1</sup>, SCR)  
25 and warrens (warren entrances km<sup>-1</sup>, WARR) indices. For the latter index, all entrances

1 (either active, inactive or unknown) were considered, since > 90 % of the entrances  
2 observed were active, with little difference in indices using only active entrances or total  
3 entrances. A pellet index (pellets m<sup>-2</sup>, PEL) was obtained from 40 circular plots of 0.5  
4 m<sup>2</sup> each, regularly distributed along the transect (one plot per 100 m). A relative density  
5 index (RDI) was obtained as the first axis score from a Principal Component Analysis  
6 (PCA, Zar 1984) of four raw variables that were highly correlated (LATR, WARR,  
7 RAB and SCR) following Delibes-Mateos et al. (2008b) and Villafuerte et al. (1998).

8

### 9 **Local pellet counts in permanent plots**

10 A 30x90 m grid was set in the area with the highest rabbit abundance at each locality,  
11 identified from the four-km transect on foot (see above). Every month, we performed  
12 cleared-plot pellet counts on the sampling areas, which consisted of 40 plots sited as  
13 four parallel lines of 10 plots each (Fernandez-de-Simon et al. *submitted*). The distance  
14 between each plot and line was 10 m, resulting in a regular sampling grid. After  
15 counting all pellets within the 0.5 m<sup>2</sup> circular plot centered at a wooden stake, we  
16 cleared the plot and, approximately one month later, we again counted the pellets in  
17 each plot. For each month, we obtained an uncorrected daily pellet accumulation rate  
18 (UNC) by calculating the average number of pellets m<sup>-2</sup> day<sup>-1</sup> (e.g., Catalán et al. 2008).  
19 We also estimated the persistence of rabbit pellets for each month and locality by  
20 placing 10 pellets marked with nail polish in each of five plots, (n = 50 marked pellets  
21 per locality per month, Fernandez-de-Simon et al. *submitted*). Remaining marked pellets  
22 were counted one month later, and daily persistence rates were calculated by assuming a  
23 constant daily decay between counts. The daily pellet accumulation rate corrected for  
24 persistence (COR) was calculated according to Palomares (2001), using the formula:

25



1 
$$\text{COR} = \text{UNC} (\text{DPR}-1) / (\text{DPR}^{\text{nd}} - \text{DPR})$$

2

3 where DPR = daily persistence rate of pellets, and nd = number of days elapsed since  
4 the last count.

5 We used the data from the month closest to the night counts dates, as the density  
6 estimates at night (DEN-N) were used as the reference method (see below). We also  
7 measured the standing crop counts (pellets m<sup>-2</sup>, STA) in this high density area by  
8 counting dung pellets within 40 0.5 m<sup>2</sup> circular plots interspersed halfway among the  
9 cleared-plots within the same line.

10

### 11 **Stratified pellet counts**

12 We walked seven 400-m transects in each locality that contained 40 standing crop  
13 counts in regularly distributed 0.5 m<sup>2</sup> circular plots (one plot every 10 m). Transects  
14 were stratified among the habitats according to the proportion of the area occupied by  
15 each habitat type in each locality. The rabbit abundance index was determined by  
16 calculating the average number of pellets m<sup>-2</sup> (STR, Table 1) for each locality.

17

### 18 **Statistical analyses**

19 Density estimates at night (rabbits ha<sup>-1</sup>, DEN-N) was considered the reference method  
20 for estimating rabbit density when comparing the different techniques (Barrio et al.  
21 2010a). The Distance Sampling method has been used previously to estimate rabbit  
22 density (Barrio et al. 2010a; Martins et al. 2003; Palomares 2001; Palomares et al.  
23 2001). Linear regression was used to test the ability of the indices of rabbit abundance  
24 (independent variables) to predict DEN-N (dependent variable). First, we calculated  
25 linear regression using the data from the summers of 2008 and 2009, when all methods

1 were assessed at each locality. All regressions were forced through zero intercept, as the  
2 indices should be zero when the rabbit density is zero. The degree of fitting was  
3 assessed using the coefficient of determination ( $r^2$ ). Relationships were considered  
4 significant when  $P < 0.05$ . Afterwards, we tested whether these relationships were  
5 maintained for variables KAI-N, UNC and COR during other seasons, years and  
6 localities. Thus, a general linear model (GLM) was used separately for each of these  
7 three variables to test the effect of locality (1–8, random effect), season (winter–spring  
8 and summer, fixed effect), and year (2007 and 2008, random effect) on the slope of the  
9 linear regression between the reference method and each index of abundance  
10 (dependent variable). Significant effects by season or year would preclude pooling the  
11 data in the linear regressions. All analyses were performed using STATISTICA 6.0  
12 (StatSoft 2001).

13

## 14 **Results**

### 15 **Density estimates**

16 In summer and in four localities (6, 2, 9 and 8), the low number of sightings precluded  
17 reliable estimation of DEN-N. In locality 6, no rabbits were observed during the three  
18 nights. Consequently, in these localities, rabbit densities were indirectly estimated from  
19 the linear regression between density estimates (dependent variable) and kilometric  
20 abundance index (independent variable, Palomares et al. 2001). The mean  $\pm$  SE rabbit  
21 density considering all localities was then  $0.85 \pm 0.29$  rabbits  $\text{ha}^{-1}$  ( $n = 11$ , range: 0–2.69  
22 rabbits  $\text{ha}^{-1}$ , Fig. 2). To calculate the density estimates at dusk (DEN-D) in localities  
23 with insufficient sightings (2, 6 and 9), we used the linear regression between density  
24 estimates at dusk (dependent variable) and kilometric abundance index at dusk  
25 (independent variable).

## 1 **Comparisons between indices**

2 Of the variables tested, the pellet count indices were the most significantly related to the  
3 reference method (Table 2, Fig. 3). Among these, the COR showed the best fit to the  
4 reference method ( $r^2 = 0.79$ ,  $P \leq 0.001$ ). Nevertheless, we observed a low difference in  
5  $r^2$  among all indices based on pellet counts (see Table 2 and Fig. 3). RAB, based on  
6 rabbit counts during linear transects on foot, was also significantly related to the  
7 reference method ( $r^2 = 0.68$ ,  $P \leq 0.001$ ). KAI-N was also significantly associated with  
8 the reference method ( $r^2 = 0.73$ ,  $P < 0.05$ ), although this was expected because both  
9 KAI-N and DEN-N were derived from the same datasets. KAI-D was also significantly  
10 related to the reference method, although with lower  $r^2$  than the same index at night  
11 (KAI-N). The variables RDI ( $r^2 = 0.5$ ,  $P < 0.05$ ) and WARR ( $r^2 = 0.45$ ,  $P < 0.05$ ), both  
12 based on linear transects on foot, were also significantly related to the reference method.  
13 In contrast, SCR, LATR and DEN-D were not significantly associated with the  
14 reference method ( $P > 0.05$ ).

15 With respect to the model during other seasons, all independent factors except  
16 year ( $P = 0.46$ ) significantly affected the slope between KAI-N and the reference  
17 method (locality,  $F_{7, 17} = 3.30$ ,  $P = 0.02$ , season,  $F_{1, 17} = 5.78$ ,  $P = 0.03$ ). Pooling data  
18 from different years (2007-2009), we found that KAI-N was significantly related to the  
19 reference method both in winter–spring ( $r^2 = 0.63$ ,  $P \leq 0.001$ , Table 3, Fig. 4) and in  
20 summer ( $r^2 = 0.78$ ,  $P \leq 0.001$ , Table 3, Fig. 4). DEN-N estimates were obtained from  
21 these relationships and for each season separately in cases where there were insufficient  
22 observations (see above for details). Because no factor was significant for the UNC and  
23 COR models ( $P > 0.05$ ), we pooled the data among seasons, years and localities for  
24 each variable. Both UNC ( $r^2 = 0.45$ ,  $P \leq 0.001$ ) and COR ( $r^2 = 0.4$ ,  $P \leq 0.001$ ) were  
25 significantly related to DEN-N (Table 3, Fig. 4).

1

2 **Discussion**

3 Using DEN-N as a reference standard, we evaluated the applicability of most rabbits  
4 abundance indices used previously, as described in the literature. We found that most  
5 pellet-count indices may reliably estimate abundances, whereas other indices, such as  
6 scrape and latrine indices and density estimates at dusk were not related to abundance  
7 on a regional scale. Among the pellet-count indices, cleared-plot pellet counts corrected  
8 and uncorrected by persistence were similarly related to the reference method, as  
9 previously demonstrated (Palomares 2001; Wood 1988). Estimating these indices in the  
10 zone of highest relative abundance within a locality may minimize the number of zero  
11 counts in areas of low rabbit density. In yearly monitoring of Mediterranean habitats it  
12 is advisable to obtain pellet-count indices during early summer, at the start of the dry  
13 season, thereby reducing the biases associated with differences in rabbit pellet  
14 persistence due to the effects of rainfall (Fernandez-de-Simon et al. *submitted*; Iborra  
15 and Lumaret 1997). Standing crop counts in summer were also related to density,  
16 resulting in only a slightly lower fit than COR. Therefore, if two visits are not possible  
17 owing to logistical constraints, standing crop counts may be a valid option for  
18 estimating rabbit abundance. Long-term monitoring of rabbit populations may be  
19 accomplished by repeated cleared-plot pellet counts over time, because they were found  
20 to be related to density during both summer and winter–spring periods. Pellet counts,  
21 however, are affected by other factors, such as the non-random distribution of pellets  
22 over the area due to heterogeneity in the environment (that may be corrected  
23 undertaking stratified transects) or the variation in defecation rates between populations  
24 or even between individuals (Putman 1984).

1           Of the driving transect indices, KAIs were related to the reference method for  
2 the seasons tested, although the relationships with DEN-N differed between seasons.  
3 Caution must be taken with KAIs because they do not control for habitat variation in  
4 rabbit detectability (Marchandeaude et al. 2006). Furthermore, sight counts may  
5 underestimate rabbit abundance (Poole et al. 2003; Twigg et al. 1998), since the  
6 proportion of rabbits seen in a population are inversely related to rabbit density due to  
7 social interactions (Twigg et al. 1998). In the Iberian Peninsula, however, the densities  
8 reached are much lower than those of Australia (Barrio 2010), and at such densities the  
9 activity levels are relatively high during the spotlight counts (Twigg et al. 1998). These  
10 factors probably affects the slope of the regressions and make relationships obtained for  
11 each locality and season more reliable. KAI-D was related to the reference method,  
12 albeit with a lower fit than KAI-N, as found by Barrio et al. (2010a). DEN-D was not  
13 related to the reference method, although the transects were identical and performed on  
14 the same days. This may be related to an avoidance of dusk-time hours to prevent  
15 higher predation risk (hunting and diurnal raptors, Fernández de Simón et al. 2009) or to  
16 an avoidance of dirt tracks during the day to prevent human disturbance (similarly as the  
17 roe deer *Capreolus capreolus*; Ward et al. 2004). Driving transect counts may be  
18 affected by environmental factors such as visibility, wind speed and rainfall, which may  
19 affect to rabbit activity (Martins et al. 2003; Twigg et al. 1998; Villafuerte et al. 1993).  
20 Similarly, numbers of predators and hunting pressure should be considered when  
21 conducting the se surveys as rabbits may shift their activity to reduce the predation risk  
22 (Fernández de Simón et al. 2009). All these factors make advisable to conduct replicates  
23 and to complement these estimates with alternative indices to increase the results  
24 reliability.

1           The lack of significant relationships of LATR and SCR with the reference  
2 method may be due to the strong behavioral component of latrine (Monclús and de  
3 Miguel 2003) and scrape (Burggraaf-van Nierop and van der Meijden 1984) abundance,  
4 which may not be linked to rabbit abundance. Biases concerning latrine counts may also  
5 derive from subjective criteria for latrines (Delibes-Mateos et al. 2008b). Although  
6 these indices may locally result in reproducible variations in densities, we do not  
7 recommend their use in regional monitoring programs if they are not complemented by  
8 additional rabbit data (e.g., RDI). Despite LATR being recently adopted as a  
9 standardized methodology for monitoring rabbits in Portugal (Ferreira and Delibes-  
10 Mateos *in press*), our findings indicate this method is not reliable for monitoring  
11 abundance on large spatial and temporal scales, being preferable other pellet-count  
12 indices.

13

#### 14 **Recommendations**

15 Overall, DEN-N may be used to provide reliable estimate of rabbit abundance when  
16 sufficient resources are available and when terrain, habitat and weather conditions  
17 permit. In other circumstances (wide-scale monitoring programs, many localities  
18 surveyed, long-term monitoring), pellet-count indices (except LATR) may be the most  
19 reliable for obtaining estimates of abundance when rabbit numbers are low to moderate.  
20 In summer and depending on the scale, the best indices for estimating rabbit abundance  
21 on small-, intermediate- and large-scales are the cleared-plot pellet counts corrected by  
22 persistence, the standing crop pellet index collected along a transect and the standing  
23 crop index at stratified transects, respectively. However, other indices obtained from  
24 linear transects on foot (rabbit, relative density and warren indices) may also be  
25 appropriate. Using an abundance index without knowing its relationship to estimated

1 densities throughout the range of abundances likely to be encountered may compromise  
2 the reliability of the results. It would also make it difficult to compare these results with  
3 those of other studies. Therefore, standardization of abundance indices allows the most  
4 appropriate method to be selected, depending on the characteristics and objectives of the  
5 study. This can be performed using the methods described in this study, though the  
6 standardization with techniques such as density estimates from night counts may not  
7 always be possible. In this situation, equations provided here may be useful for  
8 monitoring studies and projects carried out in the same study area. Further research is  
9 also encouraged to standardize the abundance and density indices in other study areas or  
10 with other reference methods (e.g., live-trapping). Here, we concluded that COR may be  
11 optimal for monitoring rabbits on a large-scale and may be implemented as standardized  
12 methods for rabbit conservation programs, as well as for management issues (e.g.,  
13 hunting, agriculture, etc.). Administrators, researchers and other personnel monitoring  
14 rabbits should promote the use of these methods for uniform monitoring and should  
15 abandon less reliable methods such as LATR. Effective monitoring to manage and  
16 conserve rabbit populations and their endangered predators is only possible when  
17 standardized and comparable monitoring methods are used.

18

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8

## 9 **References**

- 10 Angulo E, Villafuerte R (2003) Modelling hunting strategies for the conservation of  
11 wild rabbit populations. *Biol Conserv* 115: 291-301
- 12 Ballinger A, Morgan DG (2002) Validating two methods for monitoring population size  
13 of the European rabbit (*Oryctolagus cuniculus*). *Wildl Res* 29: 431-437
- 14 Barrio IC (2010) The rabbit as an agricultural pest in its native distribution range.  
15 Dissertation, Universidad de Castilla-La Mancha
- 16 Barrio IC, Acevedo P, Tortosa FS (2010a) Assessment of methods for estimating wild  
17 rabbit abundance in agricultural landscapes. *Eur J Wildl Res* 56: 335-340
- 18 Barrio IC, Bueno GC, Tortosa FS (2010b) Alternative food and rabbit damage in  
19 vineyards of southern Spain. *Agr Ecosyst Environ* 138: 51-54
- 20 Beltrán JF (1991) Temporal abundance pattern of the wild rabbit in Doñana, SW Spain.  
21 *Mammalia* 55: 591-599
- 22 Blondel J, Aronson J (1999) *Biology and wildlife of the Mediterranean region*. Oxford  
23 University Press, Oxford
- 24 Buckland ST, Anderson DR, Burnham KP, Laake JL (1993) *Distance sampling:*  
25 *estimating abundance of biological populations*. Chapman and Hall, London



- 1 Buckland ST, Anderson DR, Burnham KP, Laake JL, Borchers DL, Thomas L (2001)  
2 Introduction to distance sampling: estimating abundance of biological populations.  
3 Oxford University Press, Oxford
- 4 Burggraaf-van Nierop YD, van der Meijden E (1984) The influence of rabbit scrapes on  
5 dune vegetation. *Biol Conserv* 30: 133-146
- 6 Burnham KP, Anderson DR, Laake JL (1980) Estimation of density from line transect  
7 sampling of biological populations. *Wildl Monogr* 72: 1-202
- 8 Cabezas S, Moreno S (2007) An experimental study of translocation success and habitat  
9 improvement in wild rabbits. *Anim Conserv* 10: 340-348
- 10 Calvete C, Pelayo E, Sampietro J (2006) Habitat factors related to wild rabbit  
11 population trends after the initial impact of rabbit haemorrhagic disease. *Wildl Res*  
12 33: 467-474
- 13 Catalán I, Rodríguez-Hidalgo P, Tortosa FS (2008) Is habitat management an effective  
14 tool for wild rabbit (*Oryctolagus cuniculus*) population reinforcement? *Eur J*  
15 *Wildl Res* 54: 449-453
- 16 Delibes M, Hiraldo F (1981) The rabbit as prey in the Iberian Mediterranean ecosystem.  
17 In Myers K, MacInnes CD (eds) *Proceedings of the World Lagomorph*  
18 *Conference*. University of Guelph, Ontario, pp 614-622
- 19 Delibes-Mateos M, Delibes M, Ferreras P, Villafuerte R (2008a) Key role of European  
20 rabbits in the conservation of the western Mediterranean basin hotspot. *Conserv*  
21 *Biol* 22: 1106-1117
- 22 Delibes-Mateos M, Farfán MA, Olivero J, Vargas JM (2010) Land use changes as a  
23 critical factor for long-term wild rabbit conservation in the Iberian Peninsula.  
24 *Environ Conserv* 37: 169-176

- 1 Delibes-Mateos M, Ferreras P, Villafuerte R (2008b) Rabbit populations and game  
2 management: the situation after 15 years of rabbit haemorrhagic disease in central-  
3 southern Spain. *Biodivers Conserv* 17: 559-574
- 4 Delibes-Mateos M, Ferreras P, Villafuerte R (2009) European rabbit population trends  
5 and associated factors: a review of the situation in the Iberian Peninsula. *Mammal*  
6 *Rev* 39: 124-140
- 7 Delibes-Mateos M, Redpath SM, Angulo E, Ferreras P, Villafuerte R (2007) Rabbits as  
8 keystone species in southern Europe. *Biol Conserv* 137: 149-156
- 9 Díez C, Pérez JA, Prieto R, Alonso ME, Olmedo JA (2005) Activity patterns of wild  
10 rabbit (*Oryctolagus cuniculus*, L. 1758), under semi-freedom conditions, during  
11 autumn and winter. *Wildl Biol Pract* 1: 41-46
- 12 Fernández de Simón J, Díaz-Ruiz F, Cirilli F, Sánchez F, Villafuerte F, Ferreras P.  
13 (2009) Relación entre la presión de depredación y los ritmos de actividad del  
14 conejo de monte: aplicación a las estimas de densidad. In VIII Jornadas de la  
15 Sociedad Española para la Conservación y Estudio de los Mamíferos. Sociedad  
16 Española para la Conservación y Estudio de los Mamíferos, Málaga, pp 72
- 17 Ferreira C, Delibes-Mateos M (*in press*) Wild rabbit management in the Iberian  
18 Peninsula: state of the art and future perspectives for Iberian lynx conservation.  
19 *Wildl Biol Pract Lynx* special issue. Link:  
20 <http://socpvs.org/journals/index.php/wbp/article/view/146/212>
- 21 Gálvez L, Belliure J, Rebollo S (2009) European rabbits as ecosystem engineers:  
22 warrens increase lizard density and diversity. *Biodivers Conserv* 18: 869-885
- 23 Iborra OL, Lumaret JP (1997) Validity limits of the pellet group counts in wild rabbit  
24 (*Oryctolagus cuniculus*). *Mammalia* 61: 205-218

- 1 King DR Wheeler SH (1985) The European rabbit in south-western Australia I. Study  
2 sites and population dynamics. *Aust Wildl Res* 12: 183-196
- 3 Lees AC, Bell DJ (2008) A conservation paradox for the 21st century: the European  
4 wild rabbit *Oryctolagus cuniculus*, an invasive alien and an endangered native  
5 species. *Mammal Rev* 38: 304-320
- 6 Marchandeu S, Aubineau J, Berger F, Gaudin JC, Roobrouck A, Corda E, Reitz F.  
7 (2006) Abundance indices: reliability testing is crucial - a field case of wild rabbit  
8 *Oryctolagus cuniculus*. *Wildl Biol* 12: 19-26
- 9 Martins H, Barbosa H, Hodgson M, Borralho R, Rego F. (2003) Effect of vegetation  
10 type and environmental factors on European wild rabbit *Oryctolagus cuniculus*  
11 counts in a southern Portuguese montado. *Acta Theriol* 48: 385-398
- 12 Massei G, Bacon P, Genov PV (1998) Fallow deer and wild boar pellet group  
13 disappearance in a Mediterranean area. *J Wildl Mgmt* 62: 1086-1094
- 14 Monclús R, de Miguel FJ (2003). Distribución espacial de las letrinas de conejo  
15 (*Oryctolagus cuniculus*) en el monte de Valdelatas (Madrid). *Galemys* 15: 157-  
16 165
- 17 Moreno S, Beltrán JF, Cotilla I, Kuffner B, Laffite R, Jordán G, Ayala J, Quintero C,  
18 Jiménez A, Castro F, Cabezas S, Villafuerte R (2007) Long-term decline of the  
19 European wild rabbit (*Oryctolagus cuniculus*) in south-western Spain. *Wildl Res*  
20 34: 652-658
- 21 Moreno S, Villafuerte R (1995) Traditional management of scrubland for the  
22 conservation of rabbits *Oryctolagus cuniculus* and their predators in Doñana  
23 National Park, Spain. *Biol Conserv* 73: 81-85
- 24 Muñoz G (1960) Anverso y reverso de la mixomatosis. Dirección General de Montes,  
25 Caza y Pesca Fluvial, Madrid

- 1 Murray DL, Roth JD, Ellsworth E, Wirsing AJ, Steury, TD (2002) Estimating low-  
2 density snowshoe hare populations using fecal pellet counts. *Can J Zool* 80: 771-  
3 781.
- 4 Myers K, Parker BS, Dunsmore JD (1975) Changes in numbers of rabbits and their  
5 burrows in a subalpine environment in south-eastern New South Wales. *Aust*  
6 *Wildl Res* 2: 121-133
- 7 Newey S, Bell M, Enthoven S, Thirgood S. (2003) Can distance sampling and dung  
8 plots be used to assess the density of mountain hares *Lepus timidus*? *Wildl Biol* 9:  
9 185-192
- 10 Palomares F (2001) Comparison of 3 methods to estimate rabbit abundance in a  
11 Mediterranean environment. *Wildl Soc B* 29: 578-585
- 12 Palomares F, Delibes M, Revilla E, Calzada J, Fedriani JM (2001) Spatial ecology of  
13 Iberian lynx and abundance of European rabbits in southwestern Spain. *Wildl*  
14 *Monogr* 148: 1-36
- 15 Poole DW, Cowan DP, Smith GC (2003) Developing a census method based on sight  
16 counts to estimate rabbit (*Oryctolagus cuniculus*) numbers. *Wildl Res* 30: 487-493
- 17 Putman RJ (1984) Facts from faeces. *Mammal Rev* 14: 79-97
- 18 Ríos CA (2010) Los Planes Técnicos de Caza de Castilla-La Mancha y su aplicación en  
19 la gestión y conservación de las especies cinegéticas. Dissertation, Universidad de  
20 Castilla-La Mancha
- 21 StatSoft, Inc. (2001) STATISTICA (data analysis software system), version 6.  
22 [www.statsoft.com](http://www.statsoft.com)
- 23 Thomas L, Buckland ST, Rextad EA, Laake, JL, Strindberg SS, Hedley SL, Bishop  
24 JRB, Marques TA, Burnham KP (2010) Distance software: design and analysis of  
25 distance sampling surveys for estimating population size. *J Appl Ecol* 47: 5-14

- 1 Thompson HV, King CM (1994) The European rabbit. The history and biology of a  
2 successful colonizer. Oxford University Press, Oxford
- 3 Twigg LE, Lowe TJ, Gray GS, Martin GR, Wheeler AG, Barker W (1998) Spotlight  
4 counts, site fidelity and migration of European rabbit (*Oryctolagus cuniculus*).  
5 Wildl Res 25: 113-122
- 6 Twigg LE, Gray GS, Massam MC, Lowe TJ, Kirkpatrick W, Bendotti G, Chester DR  
7 (2001) Evaluation of bait stations for control of urban rabbits. Wildl Res 28: 299-  
8 310
- 9 Valverde JA (1967) Estructura de una comunidad Mediterránea de vertebrados  
10 terrestres. Monografías de la Estación Biológica de Doñana 1. Consejo Superior  
11 de Investigaciones Científicas, Madrid
- 12 Villafuerte R, Calvete C, Blanco JC, Lucientes J (1995) Incidence of viral hemorrhagic  
13 disease in wild rabbit populations in Spain. Mammalia 59: 651-659
- 14 Villafuerte R, Kufner MB, Delibes M, Moreno S (1993) Environmental factors  
15 influencing the seasonal daily activity of the European rabbit (*Oryctolagus*  
16 *cuniculus*) in a Mediterranean area. Mammalia 57: 341-347
- 17 Villafuerte R, Viñuela J, Blanco JC (1998) Extensive predator persecution caused by  
18 population crash in a game species: the case of red kites and rabbits in Spain. Biol  
19 Conserv 84: 181-188
- 20 Ward AL, White PCL, Critchley CH (2004) Roe deer *Capreolus capreolus* behaviour  
21 affects density estimates from distance sampling surveys. Mammal Rev. 34: 315-  
22 319
- 23 Williams D, Acevedo P, Gortázar C, Escudero MA, Labarta JL, Marco J, Villafuerte R  
24 (2007) Hunting for answers: Rabbit (*Oryctolagus cuniculus*) population trends in  
25 northeastern Spain. Eur J Wildl Res 53: 19-28

- 1 Williams CJ, Parer I, Coman BJ, Burley J, Braysher ML (1995) Managing Vertebrate
- 2 Pests: Rabbits. Bureau of Rural Resources/CSIRO Wildlife & Ecology, Australian
- 3 Government Printing Service, Canberra
- 4 Wood DH (1988) Estimating rabbit density by counting dung pellets. Aust Wildl Res
- 5 15: 665-671
- 6 Zar JH (1984) Biostatistical analysis. Prentice-Hall, New Jersey
- 7

1 **Table 1** Variables obtained with the different methods. The reference method (DEN-N)  
 2 is highlighted in italics

Driving transect counts	
<i>DEN-N</i>	<i>Density estimates at night (rabbits ha<sup>-1</sup>)</i>
KAI-N	Kilometric abundance index at night (rabbits km <sup>-1</sup> )
DEN-D	Density estimates at dusk (rabbits ha <sup>-1</sup> )
KAI-D	Kilometric abundance index at dusk (rabbits km <sup>-1</sup> )
Linear transects on foot	
LATR	Latrine index (latrines km <sup>-1</sup> )
WARR	Warren index (warren entrances km <sup>-1</sup> )
RAB	Rabbits seen index (rabbits km <sup>-1</sup> )
SCR	Scrapes index (scrapes km <sup>-1</sup> )
PEL	Standing crop pellet index (pellets m <sup>-2</sup> ) collected along the transect
RDI	Relative density index (PCA from LATR, WARR, RAB and SCR)
Local pellet counts in permanent plots	
COR	Cleared-plot pellet counts corrected by persistence (pellets m <sup>-2</sup> day <sup>-1</sup> )
UNC	Uncorrected cleared-plot pellet counts (pellets m <sup>-2</sup> day <sup>-1</sup> )
STA	Standing crop counts (pellets m <sup>-2</sup> ) in the high-density area
Stratified pellet counts	
STR	Standing crop counts (pellets m <sup>-2</sup> ) at stratified transects

3

4

1 **Table 2** Significant linear regressions ( $P < 0.05$ ) between the reference method of rabbit  
2 density estimated at night from driving transect spotlight counts (DEN-N, dependent  
3 variable), and several rabbit abundance indices in decreasing order of coefficient of  
4 determination ( $r^2$ ). Data were collected during summer surveys of 11 localities in  
5 central-southern Spain, and are shown graphically in Fig. 3. Codes of variables are  
6 shown in Table 1

Independent variable	$r^2$	Degrees of freedom	$F$	$P$	Equation
COR	0.79	1,10	38.27	$\leq 0.001$	$y=0.708x$
PEL	0.77	1,9	30.87	$\leq 0.001$	$y=0.018x$
UNC	0.73	1,10	27.5	$\leq 0.001$	$y=0.759x$
KAI-N	0.73	1,7	18.64	0.003	$y=0.154x$
STR	0.71	1,9	22.28	$\leq 0.001$	$y=0.026x$
STA	0.7	1,10	23.29	$\leq 0.001$	$y=0.004x$
RAB	0.68	1,10	21.55	$\leq 0.001$	$y=0.153x$
KAI-D	0.5	1,9	9.04	0.02	$y=0.115x$
RDI	0.5	1,10	9.82	0.01	$y=0.798x$
WARR	0.45	1,10	8.11	0.02	$y=0.025x$

7

8



1 **Table 3** Significant linear regressions ( $P < 0.05$ ) between the reference method of rabbit  
2 density estimated at night from driving transect spotlight counts (DEN-N, dependent  
3 variable), and several rabbit abundance indices in decreasing order of coefficient of  
4 determination ( $r^2$ ). Data were collected during surveys in different seasons (winter–  
5 spring and summer) at 11 localities in central-southern Spain, and are shown graphically  
6 in Fig. 4. Significant regressions pooling the data from different seasons (pooled) are  
7 also exhibited. See Table 1 for codes of variables

Season	Independent variable	$r^2$	Degrees of freedom	$F$	$P$	Equation
Winter-spring	KAI-N	0.63	1,17	28.5	$\leq 0.001$	$y=0.103x$
Summer	KAI-N	0.78	1,14	49.11	$\leq 0.001$	$y=0.161x$
Pooled	UNC	0.45	1,41	33.85	$\leq 0.001$	$y=0.625x$
	COR	0.4	1,40	26.67	$\leq 0.001$	$y=0.515x$

8

9

1 **Figure captions**

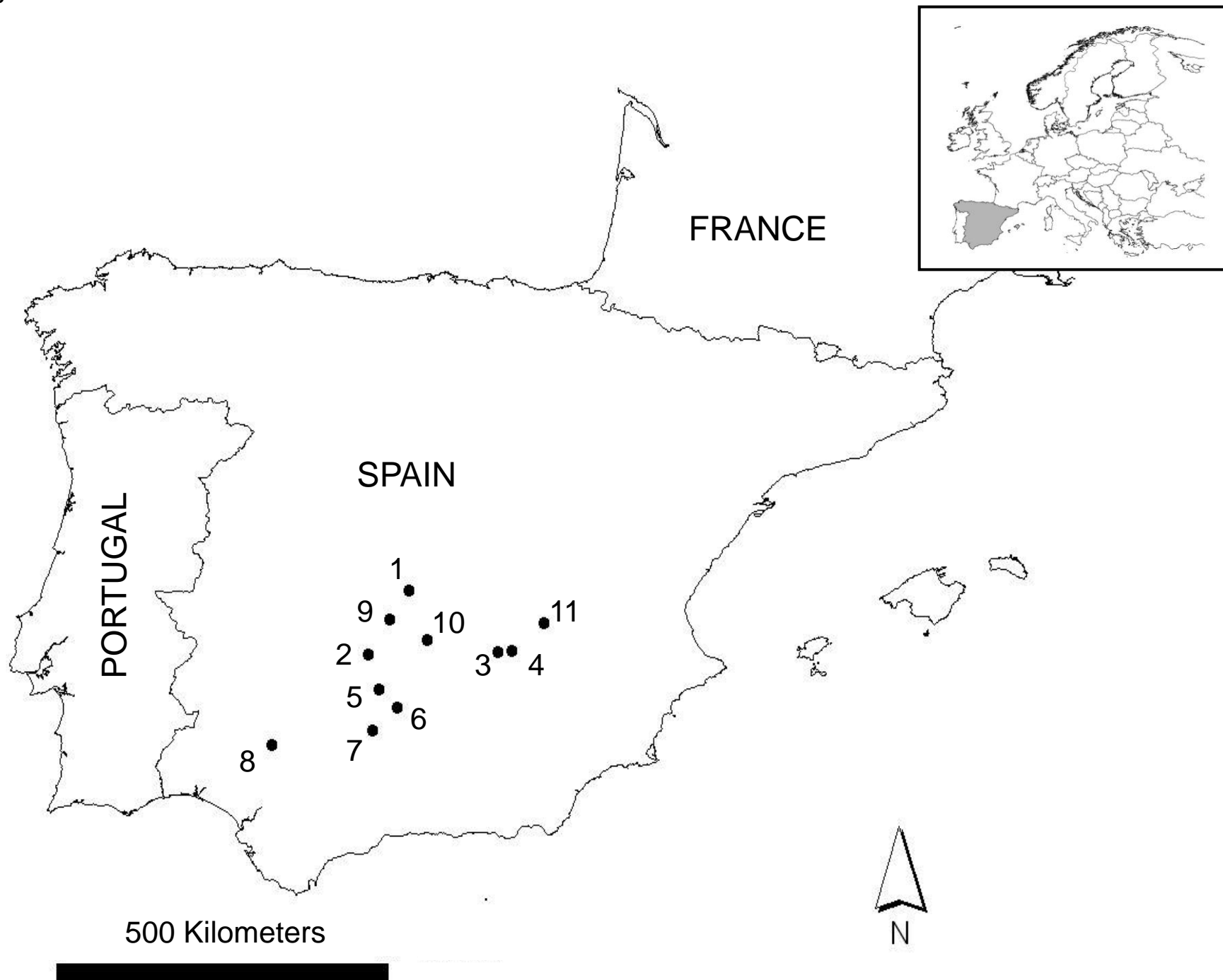
2 **Fig. 1** Location of study areas in the Iberian Peninsula

3 **Fig. 2** Rabbit density at each locality estimated from driving transects using the  
4 Distance Sampling method at night (DEN-N). Error bars represent the standard errors.  
5 Numbers correspond to the localities codes shown in Fig. 1

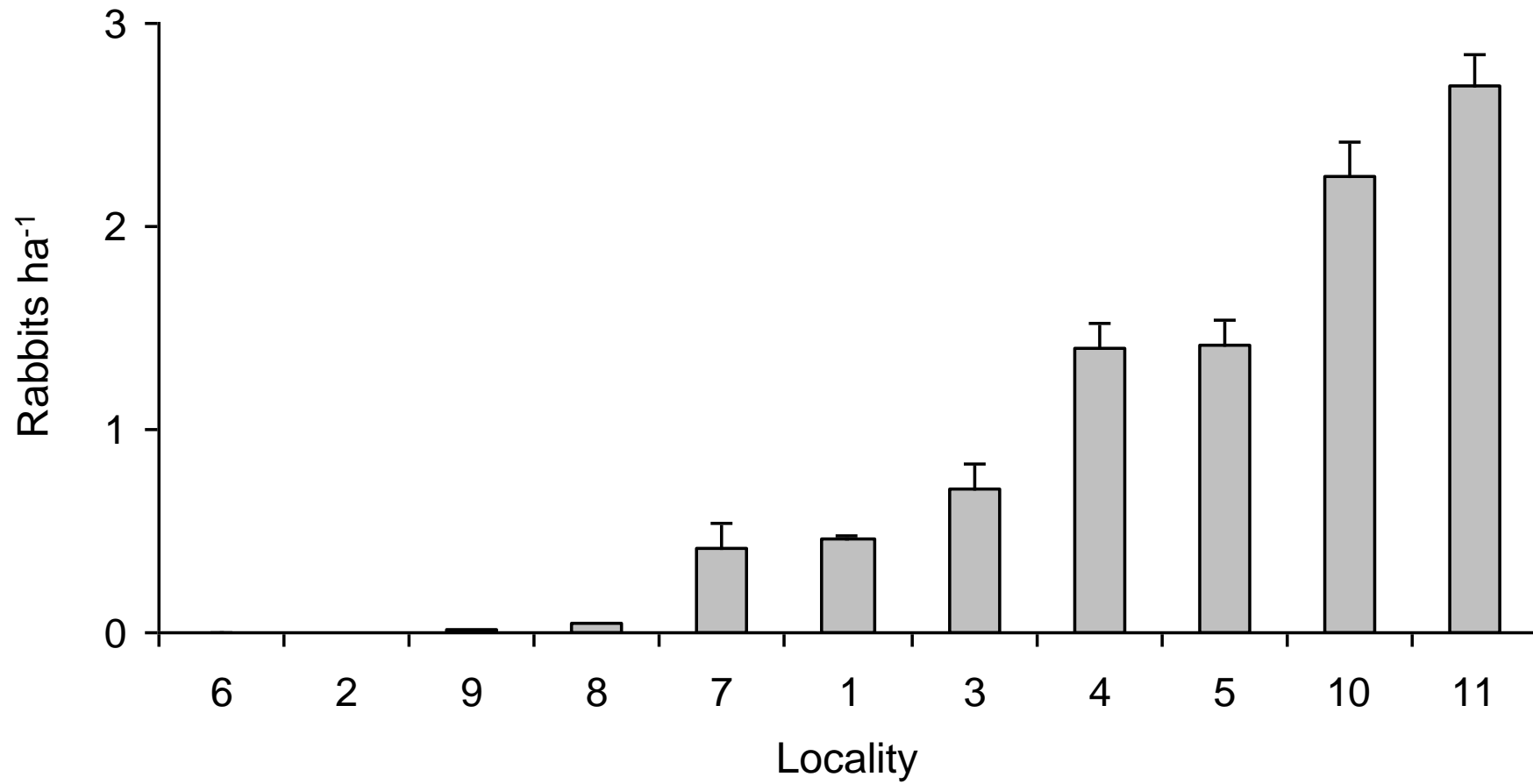
6 **Fig. 3** Relationships and *P*-values between rabbit density estimates at night (DEN-N,  
7 dependent variable and reference method) and the independent variables (a-j) in  
8 summer. The RDI axis lacks units because it was obtained from a principal component  
9 analysis of the latrine (LATR), warren (WARR), rabbits (RAB) and scrapes (SCR)  
10 indices. Codes of variables are shown in Table 1

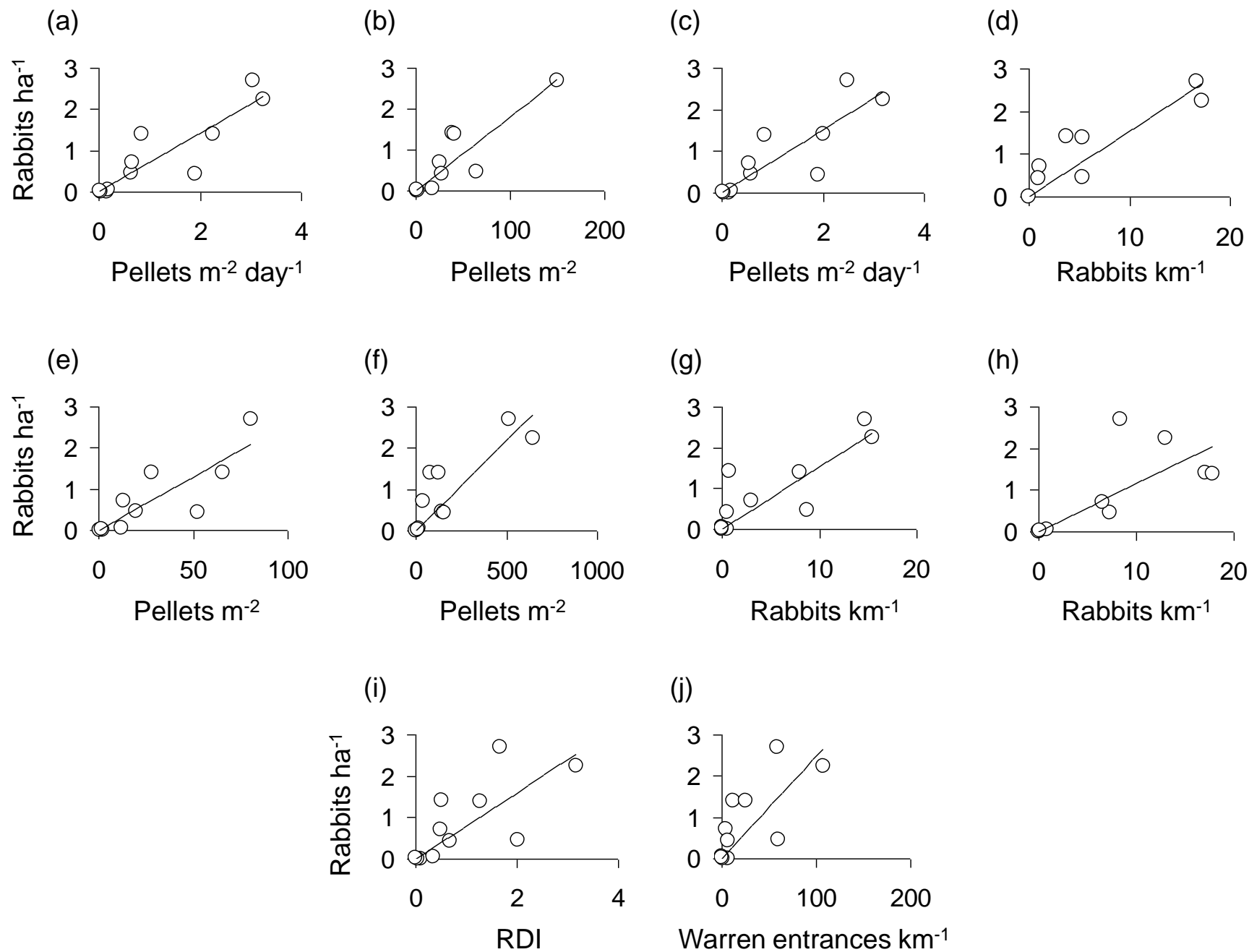
11 **Fig. 4** Relationships and *P*-values between rabbit density estimates at night DEN-N  
12 (dependent variable and reference method) and (a) kilometric abundance index (KAI-N)  
13 at night in winter–spring, (b) KAI-N in summer, (c) cleared-plot pellet counts  
14 uncorrected by persistence (UNC) with pooled dataset from both seasons, and (d)  
15 cleared-plot pellet counts corrected by persistence (COR) with pooled dataset from both  
16 seasons

Figure



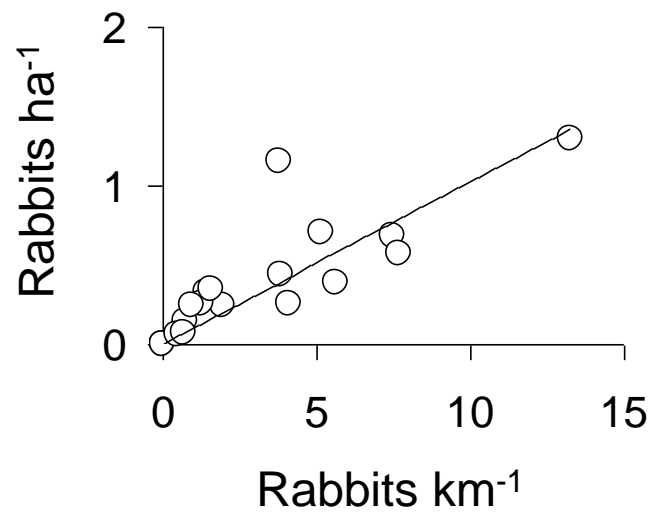
Figure



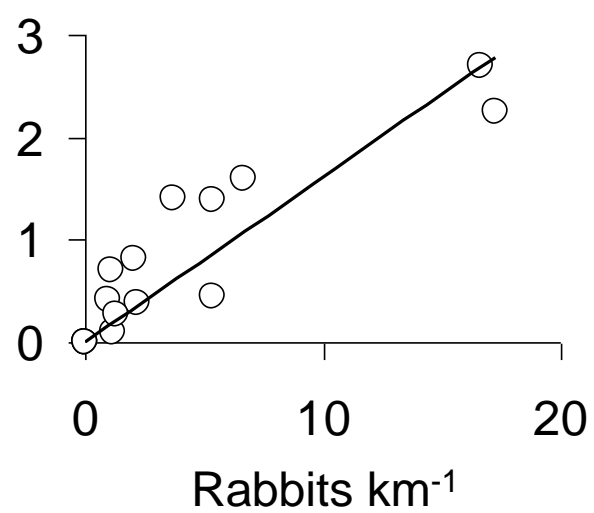
**Figure**

Figure

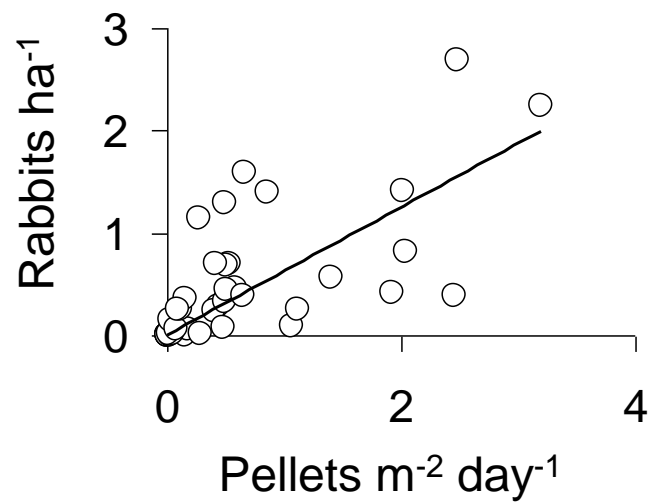
(a)



(b)



(c)



(d)

