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ORIGINAL ARTICLE

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Assessing road effects on bats: the role of landscape, road features, and bat activity on road-kills

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Abstract Recent studies suggest that roads can significantly impact bat populations. Though bats are one of the most threatened groups of European vertebrates, studies aiming to quantify bat mortality and determine the main factors driving it remain scarce. Between March 16 and October 31 of 2009, we surveyed road-killed bats daily along a 51-km-long transect that incorporates different types of roads in southern Portugal. We found 154 road-killed bats of 11 species. The two most common species in the study area, *Pipistrellus kuhlii* and *P. pygmaeus*, were also the most commonly identified road-kill, representing 72 % of the total specimens collected. About two-thirds of the total mortality occurred between mid July and late September, peaking in the second half of August. We also recorded casualties of threatened and rare species, including *Miniopterus schreibersii*, *Rhinolophus ferrumequinum*, *R. hipposideros*, *Barbastella barbastellus*, and *Nyctalus leisleri*. These species were found mostly in early autumn, corresponding to the mating and swarming periods. Landscape features were the most important variable subset for explaining bat casualties. Road stretches crossing or in the vicinity of high-quality habitats for bats—including dense Mediterranean woodland (''montado'') areas, water courses with riparian gallery, and water reservoirs—yielded a significantly higher number of casualties. Additionally, more road-killed bats were recorded on high-traffic road stretches with viaducts, in areas of higher bat activity and near known roosts.

Keywords Road-kill modeling · Mediterranean landscape · Bat activity · Phenology · Road traffic

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Introduction

Roads are a widespread and an increasingly present feature in modern landscapes (Giulio et al. 2009). They improve human communications and play an important social role in the present-day societies (Trombulak and Frissell 2000; Hawbaker et al. 2004), but they are also a major driving factor of biodiversity loss worldwide (Giulio et al. 2009; Lopez 2009). Overall, the presence of roads is highly correlated with changes in: species composition (Forman and Alexander 1998), population size (Fahrig and Rytwinski 2009; Lopez 2009), reproductive success (Trombulak and Frissell 2000), behavioral responses (Jaeger et al. 2005), and physiological condition of animals (Coffin 2007). Roads can also act as barriers or filters to animal movement during foraging and dispersal, leading to changes in home range size and shape (Clevenger et al. 2003). On a local scale, animal populations may be prevented from accessing a formerly used habitat by roads, which can lead to population declines (Eigenbrod et al. 2008; Clark et al. 2010). These isolation effects may be stronger than expected since even small and recently constructed roads can exert a significant impact upon the population genetic structure of some species (Clark et al. 2010). However, the most immediate and evident effect of roads on wildlife is the mortality of individuals caused by collisions with vehicles, with particular importance for species with large home ranges (Forman and Alexander 1998; Ascensão and Mira 2007).

While much documentation of road-kills has focused on non-flying mammals (Grilo et al. 2009; Barthelmess and Brooks 2010), birds (Gomes et al. 2008), amphibians (Eigenbrod et al. 2008), and reptiles (Woltz et al. 2008), few studies have focused on bats (Bafaluy 2000; Gaisler et al. 2009; Lesiński et al. 2010). In fact, the study of the impacts of roads on bat populations is a relatively new field in conservation, with most information coming from eastern and central Europe (Kiefer et al. 1995; Haensel and Rackow 1996; Limpens et al. 2005; Lesiński 2008; Gaisler et al. 2009; Kerth and Melber 2009; Lesiński et al. 2010; Berthinussen and Altringham 2011). In North America and southern Europe, information remains scarce (Gonzalez-Prieto et al. 1993; Bafaluy 2000). Road mortality may pose additional conservation problems to bats due to their low fecundity and late maturation, which further increases their local extinction risk. Nevertheless, bat species may be differentially affected by road mortality, depending upon species-specific behaviors, in particular, the height that bats fly from the ground and their foraging strategies (Brinkmann et al. 2008). There is mounting evidence that species that usually fly at ground level are more susceptible to road casualties (Haensel and Rackow 1996; Lesiński 2008; Gaisler et al. 2009); while for high-flying species, the risk of collision seems to be negligible, because they fly most of the time above the passing vehicles (Limpens et al. 2005; Zurcher et al. 2010). Other impacts of roads on bats may include habitat fragmentation for bat species that use landscape structures for guidance (Limpens et al. 2005) and for species that show road avoidance while foraging (Zurcher et al. 2010).

Recently, many quantitative models of animal-vehicle collision have been developed (Malo et al. 2004; McDonald and St. Clair 2004; Jaeger et al. 2005; Orlowski and Nowak 2006). However, it is very important that variables describing the space use and population density are included in models of wildlife road mortality (Roger and Ramp 2009), as they greatly improve the models' predictive ability (Baker et al. 2004; Mikusioski et al. 2007; Grilo et al. 2009). Previous studies on bats did not provide much information regarding factors that increase the risk of vehicle-bat collisions (Lesiński 2008), although some authors state that the habitat surrounding the road plays an important role in the frequency of such collisions (Lesiński et al. 2007, 2010; Gaisler et al. 2009). Thus, particularly in Mediterranean landscapes, more information is needed to properly evaluate areas where high bat vehicle-collision risk is predicted, if we aim to implement efficient mitigation measures.

The main goals of our study were: (i) to characterize bat road-kills in a Mediterranean landscape identifying species, gender, and age classes that are more prone to be killed; (ii) to describe the temporal patterns of bat vehicle-collisions; (iii) to identify the most important factors influencing bat casualties and thus provide information for the implementation of efficient mitigation measures.

Methods

Study area

The study area, comprised of 39,400 ha, is located in southern Portugal (38°66′N, 8°07′W), on the main terrestrial transport corridor linking Lisbon and Madrid. The corridor is crossed by different types of roads, including a major motorway (A6), two national roads with medium/high traffic volumes (EN-4 and EN-114), and several other national or municipal paved roads with low traffic volumes (less than 3,000 vehicles/day) (Fig. 1). The climate is Mediterranean, characterized by a dry and hot season (Rivas-Martinez 1999) from June to September, with low rainfall and monthly average temperatures ranging from 20 to 23 °C. Topography is plain, with altitude ranging between 100 and 400 m. The landscape is dominated by a Mediterranean agro-forestry system consisting of tree stands of evergreen cork (*Quercus suber*) and/or holm (*Q. rotundifolia*) oaks intermixed with extensive agricultural areas, "montado". Other less represented land uses include cereal crops, grasslands and meadows, vineyards, olive groves, and small villages. The area partially includes the Natura 2000 site *Serra de Monfurado*.

Bat road-kill survey

From March 16 to October 31, 2009, we surveyed bat road-kills daily along a 51-km road transect including stretches of different types of road (Fig. 1). All surveyed roads generally encompass two paved lanes; EN-114 and EN-4 also have 1 to 2-m-wide paved verges. Surveys were conducted by car, traveling at an average speed of 30 km/h along the verges, whenever possible. The sampling period corresponds to the high bat-flying activity months in the study area.

Bat casualties were sought during the early hours of each day (from 06 h 30 m to 11 h 00 m) to reduce the probability of removal by scavengers (Slater 2002). Indeed, daily searches are the recommended sampling technique for recording small vertebrate road-kills (Slater 2002; Santos et al. 2011), because the mean bat carcass retention time on roads is less than 1 day (Santos et al. 2011). Bat carcasses were removed from the road to avoid double-counting and were identified to species level using morphological identification keys (Palmeirim 1990; Dietz and von Helversen 2004) or genetic analysis. We also recorded each bat carcass sex and age, when possible. Age was determined by the degree of ossification of the carpal joints and the development of nipples and testes (Baagøe 1977).

For genetic identification of carcasses, genomic DNA was extracted from bat corpses using an E.Z.N.A. Tissue DNA kit (Omega Bio-Tek, GA, USA), eluted, and stored in 50 μl of the provided elution buffer. One mitochondrial fragment was amplified: the cytochrome-*b* (cyt *b*). To amplify this fragment, we used the following sets of

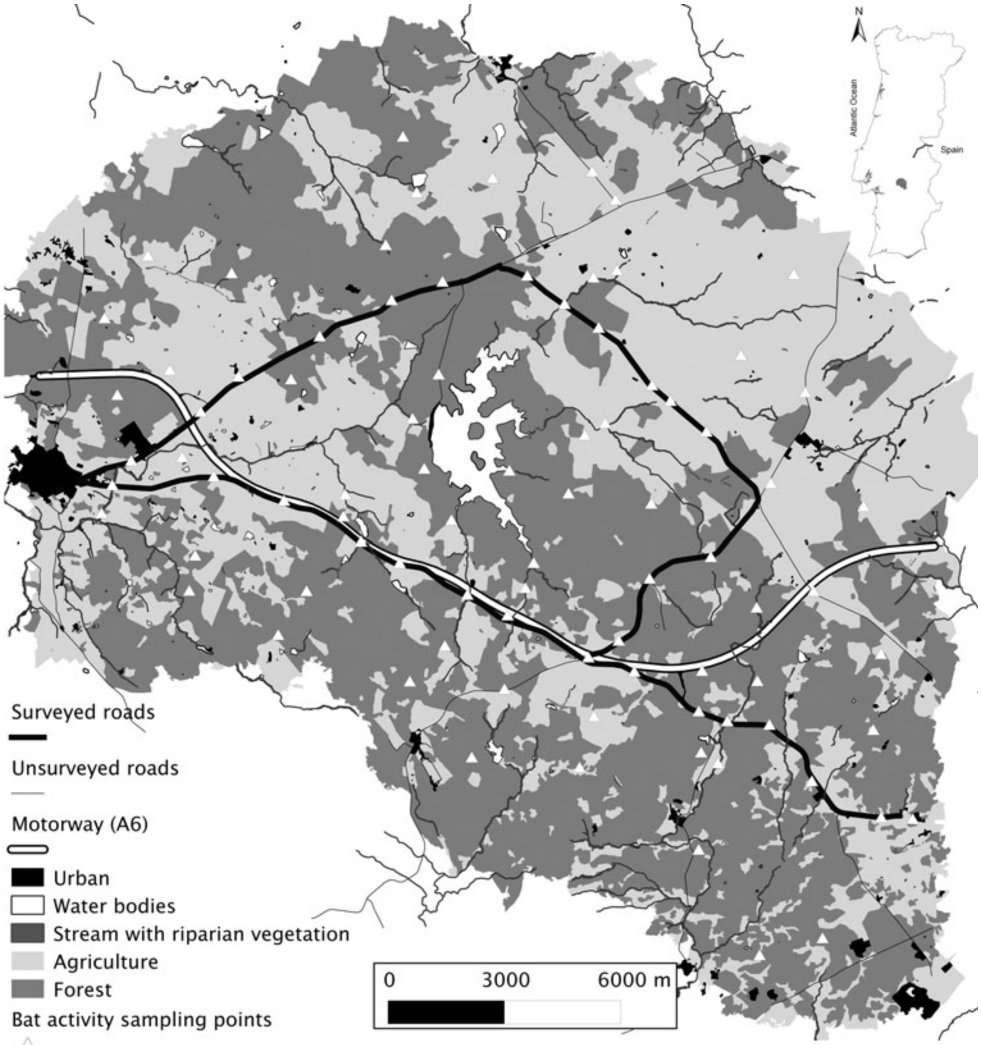

Fig. 1 Location of the study area, surveyed roads, and bat activity sampling points

primers: MVZ16 (Smith and Patton 1993) and Molcit-F (Ibáñez et al. 2006). Polymerase chain reactions (PCR) contained 5 μl of Qiagen's MasterMix (Qiagen Multiplex PCR Kit, Qiagen, CA, USA), 0.4 of each primer at 10 pmol, 3.2 μl of H2O and 1 μl of DNA extraction. The amplification consisted of an initial denaturation at 95 °C for 15 min, followed by 40 cycles at 95 °C for 30 s, 50 °C for 45 s, and 72 °C for 60 s, with a final elongation step at 72 °C for 10 min. The amplified fragments of cyt *b* were sequenced on an automated sequencer (ABI 310; Applied Biosystems) in both directions, using the same primers. All sequences are available at GenBank (accession numbers JX566924-JX566948).

Data analyses

Explanatory variables

In order to identify the main factors influencing bat casualties, we used 22 explanatory variables categorized into three subsets: land use and landscape metrics (LAND); road features (ROAD); and bat ecology variables (BAT) (Table 1). For analysis purposes, we subdivided the surveyed roads into 500-m segments (n = 101), each designated as a sampling unit (SU). Most LAND explanatory variables were extracted from a digital land-use map, using a 500-m buffer around each road segment. The main land uses were obtained through the interpretation of aerial photos (year 2005), complemented by ground validation and including detailed cartography of both built and natural linear structures (e.g., roads, rivers, streams with and without riparian gallery). Areas with structural resemblance were grouped and land uses were reclassified into five classes: (1) "open agricultural-areas" (OPEN), which include cereal crops, fallow, and "montados" with less than 10 % tree cover, (2) "sparse montado" (SMT), areas with evergreen oak tree cover of between 10 and 30 %, (3) "dense montado" (DMT), "montado" areas with more than 30 % tree cover, (4) "forest areas" (FA), wooded areas other than "montados" with more than

Table 1 Description and summary statistics of explanatory variables of the three subsets: landscape features (LAND), road characteristics (ROAD), and bat ecology (BAT)

Variable (unit)	Description	Mean ± SE	Range
LAND			
PDMT (%)	Proportion of dense montado (> 30 % tree cover)	34.4 ± 0.1	0–100.0
PSMT (%)	Proportion of sparse montado (10–30 % tree cover)	18.2 ± 0.1	0–99.5
PAA (%)	Proportion of agricultural areas (arable fields and pasturelands with < 10 % tree cover)	64.5 ± 0.1	0–100.0
PFA (%)	Proportion of forest (> 30 % tree cover) (including eucalyptus, pines, and oaks)	35.5 ± 0.1	0–100.0
PURB (%)	Proportion of urban area	11.2 ± 0.1	0–41.6
PWB (%)	Proportion of water bodies	0.5 ± 0.1	0–15.0
POLIV (%)	Proportion of olive groves	12.5 ± 0.1	0–35.0
PVIN (%)	Proportion of vineyards	2.2 ± 0.1	0–46.7
PTE (%)	Proportion of pine or eucalyptus plantations	1.1 ± 0.1	0–34.1
ED_FA (m/ha)	Forest edge density	8.2 ± 2.7	1.2–238.9
MPS_FA (ha)	Mean patch size of forest patches	16.7 ± 1.2	4.5–50.0
AWMSI (m^2)	Area weighted mean shape index	1.5 ± 0.1	1.2–2.2
D_SWG (m)	Minimum distance to stream with riparian gallery	716.4 ± 48.7	3.2–2,127.4
D_SNG (m)	Minimum distance to stream without riparian gallery	965.0 ± 55.6	64.3–2,633.5
D_WB (m)	Minimum distance to water bodies	554.7 ± 29.3	22.1–1,471.1
D_URB (m)	Minimum distance to urban areas (> 10 ha)	3820.1 ± 156.4	14.6–7,187.0
D_A6 (m)	Minimum distance to motorway–A6	2,693.0 ± 254.9	38.7–7,932.1
ROAD			
TRAF (categoric)	Traffic intensity estimated (number of vehicles per night: TRAF1 < 150; TRAF2 150–1200; TRAF3 > 1200)	–	1; 2; 3
N_Culv (categoric)	Number of culverts in road segment (CULV 0; CULV 1; CULV 2; CULV3)	–	0; 1; 2; 3
PA_Viad (binary)	Presence (PRES1) or absence (PRES0) of Viaducts or Bridges in the road segment	–	0; 1
BAT			
ACT	Mean bat activity on segments measured from inverse distance Weighted interpolation	7.7 ± 0.4	1.4–22.7
D_Roost (km)	Nearest distance to known roosts	4.4 ± 0.2	0.1–8.0

30 % tree cover of any type, excluding riparian galleries, and (5) “urban areas” (URB), represented by cities, small villages, and isolated farmhouses. All landscape metric descriptors were computed for each SU. Specifically for wooded areas, edge density (ED) and mean patch size (MPS) were computed considering “sparse montado”, “dense montado”, and forest areas all together (SMT + DMT + FA). Minimum distances to other roads, streams, villages, and known bat roosts were measured from the central point of each SU.

Bat activity at each SU was estimated from a bat activity map for the whole study area. This map was generated using the inverse distance-weighted interpolation (Supplementary material) of the mean number of bat passes for 87 sampling points, located on the representative land uses (Soeller 2004), and at different distances from roads (Fig. 1). At each sampling point, bat activity was monitored for 15 min using a D240x bat detector (Pettersson Elektronik AB) and a digital recorder (Archos AV 500 mobile digital video recorder) to record sound samples of each bat pass. Acoustic sampling was repeated three times at each sampling point between April and September in the same year of the road-kill surveys, with a 2-month interval between visits, excluding windy or rainy nights.

The road features were also mapped for each SU. These included number of culverts, presence of viaducts, and the location and length of fences on each side of the road. Traffic data was obtained from direct counts of vehicles on 15-min periods. They were carried out at different times of the night and on different occasions along the study period (EN-4 = 14 nights, EN-114 = 24 nights, and EN-370/M-529 = 25 nights). These data were classified into three nocturnal traffic categories, complying with the classification of the Portuguese Road Institute: high/moderate traffic (EN-114; ≈1210 vehicles/night), moderate traffic (EN-4; ≈277 vehicles/night), and low traffic (EN-370/M-529 < 100 vehicles/night). All the information was included in an ArcGis 9.3 GIS project to perform the spatial analyses (ESRI 2008).

Statistical analyses

Prior to statistical analysis, we used Moran’s I to evaluate the presence of spatial autocorrelation in bat fatality data, because ignoring spatial autocorrelation in model building can lead to the selection of predictors that do not present statistically significant relationships (Legendre and Legendre 1998). The distances between bat mortality locations that occurred in different SUs were measured along the road “network”, using Crimestat III software (Levine 2004).

We used Chi-square analysis to evaluate significant differences in casualties by sex, road type, and SU (Sokal and Rohlf 1995). These computations were performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA) (SPSS 2008).

Model building

We used generalized linear models (GLM) with a Poisson error structure to model the number of bat road-kills per road segment (i.e., sampling unit [SU]). Logarithmic and angular transformations were applied to continuous variables and proportions, respectively, in order to achieve normality and reduce the effects of extreme values (Zuur et al. 2007). To avoid collinearity, prior to model building we performed pair-wise Pearson rank correlations between all non-categorical explanatory variables. From pairs of variables showing correlation values higher than 0.7, we only retained the most biologically meaningful variable for further analysis (Tabachnick and Fidell 2001). We also did a preliminary screening of explanatory variables using univariate analysis to reduce the variables available for the model building phase and detect patterns of variation of road-kills with each explanatory variable. The variable reduction was important because our sample size (i.e., number of road segments) was $n = 101$ and according to Harrell et al. (1996), the number of predictor variables should not exceed $n/10$. Only significant and nearly significant predictors ($p < 0.1$) were used in multiple regression model building. The nearly significant predictors were considered to reduce the influence of type II errors and avoid rejecting ecologically relevant effects at an early analysis stage (Underwood 1997). Multiple Poisson regression models were performed for each variable-subset (LAND, ROAD, and BAT). For each subset, models with all possible combinations of remaining variables (after univariate screening) were developed and compared with Akaike’s information criterion (Burnham and Anderson 2002). At this stage, the best models were selected strictly on the basis of AIC. However, the significance levels of the variables were presented and discussed because, despite Burnham and Anderson (2002) do not recommend mixing information-theoretic model comparison (ITMC) and null-hypothesis theory, it is possible and helpful to use the later to enhance the confidence in the coefficients of the final models (Stephens et al. 2005; Whittingham et al. 2006). Whenever overdispersion was detected, standard errors of the regression coefficients and their significance levels were rescaled by the square root of $D/(n - p)$ where D is the model deviance and $n - p$ the degrees of freedom (Berk and MacDonald 2008; Zuur et al. 2009). To evaluate the differential effects of each subset of predictors on bat road-kills, we used a variance partition approach (Borcard et al. 1992) and extended this method to the three subsets of explanatory variables (Heikkinen et al. 2004). All modeling was done using the R 2.10.1 statistical package with library MASS (R 2009).

Results

Species, sex, and age composition of road-kills

We recorded 154 road-killed bats belonging to 11 species (Table 2). Three common bat species represented 86 % of the total casualties: *Pipistrellus kuhlii* ($n = 67$), *P. pygmaeus* ($n = 45$) and, *P. pipistrellus* ($n = 21$). However, we also recorded species that are threatened or data deficient: *Rhinolophus hipposideros* ($n = 7$); *Rhinolophus ferrumequinum* ($n = 1$); *Barbastella barbastellus* ($n = 3$); *Miniopterus schreibersii* ($n = 1$); *Nyctalus leisleri* ($n = 1$); and *Myotis escalerai* ($n = 1$).

The majority of the collected bat carcasses were adults (Table 2). Juveniles represented 15 % of the total specimens aged and were only recorded from June onwards. Males outnumbered females, except in July, but the overall difference was not significant ($\chi^2 = 1.29$, $p = 0.26$).

Temporal patterns of road-kills

The number of casualties varied considerably over the survey period (Fig. 2), being higher from late July to mid-September, during which time 66 % of the casualties were concentrated. Over the last 2 weeks of August, we detected a mortality peak corresponding to about 27 % of all bat casualties. Additionally, the number of road-killed bat species also varied throughout the survey. A greater number of species, many of them threatened, was found during the mating season and the migration of bats to swarming roosts. Indeed, *Rhinolophus* spp., *Nyctalus leisleri*, and *Miniopterus schreibersii* fatalities were recorded mostly in September and October.

Factors influencing bat road-kills

On average, roughly three bats were road-killed per kilometer during the survey period but, there were different contributions of the three road stretches. Bat

Table 2 Number of bats road-killed, by species, sex, and age

Species	Sex: Male	Female	Ud	Age: Adult	Juv	Ud	Total
Rhinolophus ferrumequinum		1		1			1
Rhinolophus hipposideros	3		4	3	1	3	7
Myotis daubentonii	2	1		3			3
Myotis escalerai	1			1			1
Nyctalus leisleri		1		1			1
Eptesicus serotinus	1		3	4			4
Pipistrellus kuhlii	19	2	46	44	7	16	67
Pipistrellus pipistrellus	3	4	14	14		7	21
Pipistrellus pygmaeus	14	10	21	26	9	10	45
Miniopterus schreibersii	1					1	1
Barbastella barbastellus		1	2	2		1	3

Ud undetermined sex or age category, *Juv* bats younger than 6 months old

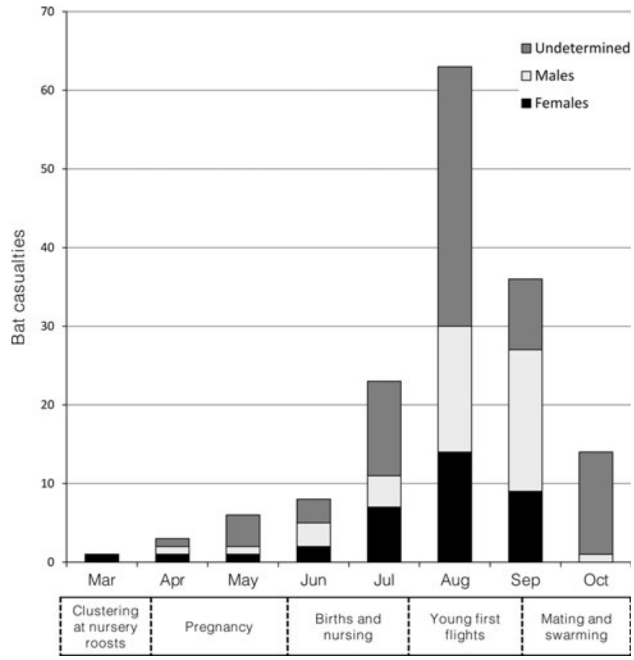

Fig. 2 Total number of bat casualties per month found during the survey period. Bat annual cycle phases are shown beneath the *X* axis

casualties were higher on road EN-114 (3.99 ± 0.83/km), representing about 60 % of all mortality, followed by road EN-4 (3.60 ± 0.89/km), and roads EN-370/M-529 (1.00 ± 0.30/km). Accordingly, the number of casualties differed significantly by road type ($\chi^2 = 331.87$, $p < 0.001$), as well as between different SUs ($\chi^2 = 244.58$, $p < 0.001$). Despite the concentration of bat mortality on road EN-114, there was no apparent spatial autocorrelation in our data (Moran's $I = -0.007$, $Z = -0.0005$, *ns*).

Results of the multiple regression GLM models for each of the three variable subsets analyzed and the full model are shown in Table 3. Landscape and land use features (LAND), are the most important variable subset, explaining 26.6 % of the total variance in bat road-kills (Fig. 3). According to this model, casualties occurred mainly on segments crossing high-quality habitats, including dense "montado" areas and water courses with riparian vegetation, and in proximity to water reservoirs. Road features (ROAD) explained 20.9 % of the variance, showing that bat road-kills occurred mostly on segments with higher traffic volume and with viaducts. The bat ecology subset (BAT) of variables accounted for 20.6 % of the variance in fatalities, suggesting that road-kills occur mostly in areas of high bat activity and in the vicinity of known bat roosts. Specifically, the LAND partial model had the strongest pure effect (11.5 %), whereas the pure effect of the ROAD and BAT partial models explained 4.5 and 4.2 % of the variance, respectively. On the whole, the full model accounted for 42.8 % of the variation in bat fatality data, and the shared effects among the three partial models was 22.4 %, suggesting strong interactions between them.

Table 3 Partial models for the landscape features (LAND), road characteristics (ROAD), and bat ecology (BAT) subsets, and the full model used for variance partitioning

Variables	Partial models		Full model	
	β	p value	β	p value
LAND				
PDMT	0.528	0.008	0.825	0.002
PSMT	−0.782	0.148	−0.355	0.522
PTE	2.574	0.206	23.013	0.304
PWB	12.742	< 0.001	−15.617	0.733
D_SWG	−0.544	0.022	−0.147	0.536
Intercept	1.690	0.008		
ROAD				
TRAF1				Indicator
TRAF2	1.301	0.003	10.376	0.005
TRAF3	1.293	0.001	0.706	0.087
N_Viad	0.953	0.002	0.934	0.008
Intercept	−0.759	0.041		
BAT				
ACT	1.629	0.005	0.534	0.380
D_Roost	−0.751	0.002	−0.949	0.016
Intercept	1.460	0.220		

Response variable of the models is number of bat casualties per sampling unit (i.e., 500-m road segments)

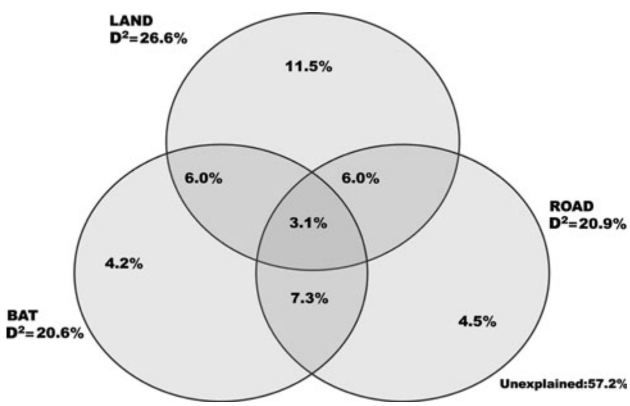

Fig. 3 Results of variance decomposition of bat road-kill models, shown as fraction of total variance explained. Pure and joint effects variance are presented for the three subsets of explanatory variables: landscape features (*LAND*), road characteristics (*ROAD*), and bat ecology (*BAT*). *Unexplained* is the percentage of unexplained variation

Discussion

Species, sex, and age composition of road-kills

We recorded a high bat mortality rate, about 3 bats/km/year, even though the surveys were conducted by car. Nevertheless, other authors have reported higher numbers of bat kills along road stretches located in the vicinity of important roosts (Russell et al. 2009) or when sampling by foot (Gonzalez-Prieto et al. 1993). Moreover, for small sized species (< 150 g), under-detection is inherent in road surveys because small carcasses remain on paved road for less time than larger ones (Slater 2002; Santos et al. 2011). In fact, when smashed, carcasses of small species are easily destroyed by cars or removed by scavengers, or may be thrown into roadside vegetation where their detection is unlikely (Prosser et al. 2008). Therefore, the real road-kill rate is certainly higher than the one detected in our study. Despite the problems associated with carcass detection and differences in sampled areas and methodologies, which turns difficult strict comparisons, the number of killed bats reported in our study is at least of the same order of the magnitude of the one often reported for wind farms. Bat mortality on these farms is repeatedly pointed out as a major issue of conservation concern (Arnett et al. 2008; Rydell et al. 2010). Nevertheless, taking into account the high fatality rate recorded on roads and the widespread road network, we believe that bat road-kills should be also an issue of high concern for long-term bat population viability.

A large number of bat species described for the study area (about 65 %) (Marques and Rainho 2006) were found road-killed, though the numbers varied greatly between species. Vulnerability of each species to vehicle collision may be related to bat-specific flight and foraging behaviors. In fact, species with a low flight, that forage close to vegetation or ground, and that fly along guiding landscape structures should have a higher risk of collision with vehicles (Limpens et al. 2005; Kerth and Melber 2009). However, in our fatality data, species with these characteristics (i.e., horseshoe and barbastelle bats) are poorly represented, probably because they are rare in the region (Marques and Rainho 2006) and may avoid foraging close to the road (Lesiński et al. 2010; Zurcher et al. 2010; Berthinussen and Altringham 2011).

Pipistrellus kuhlii casualties were twice that of *P. pygmaeus*, despite the fact that the activity level of these two species is similar in the whole study area (Denis Medinas, unpublished data). We believe that our results, besides suggesting differences in foraging behavior (e.g., flying time, number of foraging bouts and distances flown per foraging bout) also may reflect differences in prey selection and flight agility (Goiti et al. 2003; Davidson-Watts and Jones 2006). *P. kuhlii* flies at a lower altitude and with less sinuous routes (Grodzinski et al. 2009), both of which can increase its vulnerability to being road-killed. Conversely, Lesiński et al. (2010) reported that *P. pygmaeus* flies higher and rarely uses forest lanes (e.g., tree lines along roadsides), which may reduce their risk of being struck.

Nevertheless, contrasting patterns of bat road-kills have been described for different parts of Europe. On the Iberian Peninsula and in France, *P. kuhlii* and *P. pipistrellus* are considered the species with the most fatalities (Gonzalez-Prieto et al. 1993; Bafaluy 2000); while in central Europe, *P. nathusii*, *Myotis daubentonii*, and *Plecotus auritus* are the bats most commonly found dead (Lesiński 2008; Dietz et al. 2009; Gaisler et al. 2009).

We recorded a few carcasses of species that forage above tree canopies, such as *N. leisleri* and *E. serotinus*. These species avoid being road killed because they spend most of their active time flying above vehicle height (Norberg and Rayner 1987; Zurcher et al. 2010).

Female mortality is particularly high in early summer, when female casualties were almost twice the number of male road-kills, although the overall result is not statistically significant. This period corresponds to the time of births and lactation, a critical phase in the bat life cycle. This may have strong implications for long-term population viability, because newborns are totally dependent upon their mothers to survive. Perhaps females' higher body mass during this season interferes with their flight performance, making them less maneuverable (Rayner et al. 1989). During late pregnancy and lactation, females also emerge earlier from roosts, which may expose them to the late afternoon and early evening peak traffic. Furthermore, during lactation, females hunt close to the roosts and return several times to suckle their young (Russo et al. 2005). At our study sites, many known roosts are close to the road, thereby increasing female mortality risk.

Several authors have demonstrated that young bats are more vulnerable to traffic than adults (Gaisler et al. 2009). However, in our study, young represented but a small percentage of the total aged bat road-kills, and belonged only to the most common species, *P. kuhlii* and *P. pygmaeus*. This is surprising, because juvenile bats are inexperienced flyers and are expected to be more susceptible to road mortality. However, a few studies have shown that young bats often travel shorter distances (Buchler 1980), which may make them less likely to be hit by a car. Moreover, young bats may show road avoidance behavior, as suggested for other young mammals (Grilo et al. 2009).

Temporal patterns of road-kills

Bat mortality peaked in late summer and early autumn, in accordance with other authors' findings (Bafaluy 2000; Gaisler et al. 2009; Lesiński et al. 2010). This may reflect the greater foraging activity, larger number of flying bats due to the emergence of fledged young, and longer flying times and distances flown by bats during mating and swarming (Davidson-Watts and Jones 2006). The annual bat activity life cycle must be an important driver of bat non-natural mortality, since fatalities on wind turbines across Europe also peak during this period (Rydell et al. 2010).

Fatalities of threatened or data-deficient species, like *R. hipposideros*, *R. ferrumequinum*, *M. schreibersii*, *N. leisleri*, and *B. barbastellus*, are concentrated in autumn, corresponding in the study region to the mating and swarming period. Despite the low numbers of casualties recorded for these species, non-natural mortality from road-kills may exert a significant impact, due to their usually small local populations and rarity countrywide. This result has major implications for the definition of sound bat road-kill monitoring schemes; they should cover an extended time period and include both the mortality peak and the autumn season to identify rare species.

Factors influencing bat road-kills

The location of bat road-kills was not random, being influenced by different features, which include habitat quality, road features, and species ecological traits. To our knowledge, our study is the first attempt to go beyond the description of bat road-kills and model them, taking into account a large number of factors.

The results of variation decomposition analysis show that landscape structure comprises the main set of factors influencing the likelihood of bat collisions with vehicles, followed by road features, and bat ecology predictors, both explaining similar proportions of variation. The variables included in our final model all were indicative of the ecological requirements and habitat preferences of bats. The positive relationships with dense "montados" (forest) and proximity to streams with a riparian gallery suggest that bats have a higher probability of being killed while foraging in their preferred habitats. In fact, "montado" areas and riparian vegetation often are highly suitable habitats for bat communities, because they provide shelter and high availability of insects (Allan et al. 2003; Russo and Jones 2003). Moreover, the structural complexity and diversity of "montados", which can include open spaces intermixed with forested areas with different tree densities, provide foraging grounds for different bat guilds (Morris et al. 2010), including forest-dwelling species like *B. barbastellus* and *M. bechsteinii* (Schofield and Morris 2000) and edge habitat specialists like *P. kuhlii* and *M. escalerai* (Grodzinski et al. 2009). The higher probability of bats being road-killed in the proximity of streams and water reservoirs also may be related to the availability of water, a scarce and unevenly distributed resource in the region (Russo and Jones 2003; Rainho 2007; Rainho and Palmeirim 2011). Moreover, riparian corridors may also be used as bat flyways to cross roads (Abbott et al. 2012), increasing their risk of being hit by a car.

It is widely accepted that large and medium-sized mammals, bats, and birds avoid very high traffic volume roads (Grilo et al. 2009; Lopez 2009; Berthinussen and Altringham 2011), and that higher fatality rates are more likely to occur on roads with moderate to low traffic (Langeveld et al. 2009). Apparently, our data do not conform to this view, as road stretches with higher traffic volumes were those with higher road-kill rates. However, the highest traffic volume road sampled in our study was less than 1,500 vehicles per night, roughly corresponding to medium traffic road stretches in other studies. Moreover, most fatalities were *Pipistrellus* spp., which are considered generalist species, often living in urban environments, being tolerant to noise and artificial lightning, such that they may exhibit limited road avoidance behavior. Some habitat specialists, like *B. barbastellus*, also integrate roads into their foraging areas and regularly cross these infrastructures (Kerth and Melber 2009). However, road avoidance in response to the presence of vehicles has been recently reported in other bat species (Zurcher et al. 2010). In fact, Kerth and Melber (2009) revealed that areas close to roads with high traffic were poor foraging areas, and that roads act as flight boundaries for *M. bechsteinii*. This suggests that road avoidance behavior can be species-specific and may also have a large dependence on road traffic volume.

Bats are referred to regularly use viaducts and underpasses for crossing roads (Kerth and Melber 2009; Abbott et al. 2012); consequently, the likelihood of road-kills close to viaducts should be lower, which also does not conform to our results. This contrasting result may be due to the association of these structures with streams and ponds in our study area, where bat foraging activity is usually enhanced. Furthermore, viaducts often provide roosting places for some species (Keeley and Tuttle 1999), which is the case in our study area, increasing the probability of bats being road-killed when moving to, from, or around their roosts. Further studies are needed to clarify the role of bridges and viaducts in bat road fatalities, since they may be important structures enhancing or reducing the probability of bat road-kills, depending upon the species and landscape context.

The adoption of variables concerning species habitat use within predictive fatality modeling has been done only sporadically. However, Roger and Ramp (2009) reported the importance of incorporating these variables to improve the predictive ability of the models and reduce the number of other possible variables. We found, as expected, a strong positive relationship between locations of bat road-kills and bat activity interpolated for the whole the study area. Thus, according to our data, bat activity may be a simple and useful clue to assess road-kill risk and inform about locations where mitigation may be needed, both for planned and already-constructed roads.

Globally, our results show that high-quality habitats, proximity to roosts, bat activity level, and high traffic volume are the main drivers of road casualties. Moreover, we have shown that bats are more vulnerable during specific life-history periods, such as mating and swarming. Most of the species in the study area are affected by roads, including threatened and data-deficient species, for which the impact of road-kills on long-term population viability may be of particular concern. However, due to sample size constraints, we were able to only model global mortality, without distinguishing between species. Behavioral responses of bats to the presence of roads, and thus their risk of being road-killed, may be species-specific. For this reason, implementing mitigation measures aimed at minimizing fatalities, particularly for species of conservation concern, should be a priority and take into account this possible specificity. As a first step, long-term road-kill monitoring programs with standardized methodologies that include the quantification of the population effects of increased non-natural mortality should be implemented. With these data it will be possible to identify the main species affected and the risks for their long-term population viability at roadside environments, which is the basis for the appropriate solutions to the problems detected.

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