ORIGINAL ARTICLE

Bad moon rising? The influence of the lunar cycle on amphibian roadkills

F. Mestre[1] · H. Lopes[2] · T. Pinto[2] · L. G. Sousa[2] · A. Mira[2] · S. M. Santos[2]

Abstract

Annually, roads, and their associated users, are responsible for millions of roadkills worldwide. Mortality affects multiple taxonomic groups, but amphibians are particularly vulnerable, due to their size and underreporting. In fact, very high mortality frequencies can occur, mostly during short periods of time, when individuals migrate to and from reproduction areas (e.g., ponds). In this study, we assess the influence of the lunar cycle on amphibian roadkills, while accounting for weather conditions. As expected, the main environmental effects explaining roadkill numbers were weather related, with increases in minimum air temperature, average relative air humidity, and cumulative rainfall during the previous 24 h having a positive effect on roadkill numbers for all studied species. However, the lunar cycle also affected roadkills for two of the studied species. Darker nights had higher numbers of roadkills of *Pleurodeles waltl*, while moonlit nights had higher numbers of *Salamandra salamandra*. As such, these moon effects are species specific. Animals that are more active in moonlight may be at an advantage if their visual acuity is better than that of their predators. We hypothesize that differences between species in the response to moonlight may be due to differences perceived in predation risk. This information should be considered when designing mitigation measures. Volunteer actions, for instance, can be planned and coordinated keeping in mind the most appropriate weather conditions for the general amphibian community and specific phases of the lunar cycle for particular species.

Keywords Lunar effects · Migration · Moonlight · Roads · Weather conditions

Introduction

The threat posed to biodiversity by linear infrastructures, including roads and railways, is widely acknowledged in the scientific literature (Seiler and Helldin 2006; Coffin 2007;

✉ F. Mestre
fmestre@uevora.pt

1   Cátedra 'Rui Nabeiro' de Biodiversidade, University of Évora, Casa Cordovil 2° Andar, Rua Dr. Joaquim Henrique da Fonseca, 7000-890 Évora, Portugal

2   ICAAM - Instituto de Ciências Agrárias e Ambientais Mediterrânicas; UBC – Conservation Biology Lab, University of Évora, Mitra, 7002-554 Évora, Portugal

Benítez-López et al. 2010; van der Ree et al. 2015; Richardson et al. 2017; Santos et al. 2017). In particular, road networks have three mainly reported negative impacts (van der Ree et al. 2015): (i) indirect landscape effects (habitat degradation or loss and barrier effect); (ii) direct mortality (e.g., roadkill); and (iii) avoidance behavior, due to the high disturbance caused by the road traffic to its surroundings. By comparison, some positive effects have been reported. In fact, some species find the road margins an attractive and suitable habitat as in the case of several birds (reviewed in Morelli et al. 2014) and small mammals, like the Cabrera's vole (*Microtus cabrerae*) in studies conducted in Portugal (Santos et al. 2007a) and the common vole (*Microtus arvalis*) and crowned shrew (*Sorex coronatus*) in studies in France (Redon et al. 2015). Moreover, some studies have documented the use of road verges as preferential movement corridors such as for the hazel dormouse (*Muscardinus avellanarius*) in Germany (Encarnação and Becker 2015). However, this attraction to road surroundings may also transform the road into an ecological trap, leading animals to suffer increased mortality (Morelli et al. 2014), with the positive effects of roads resulting in negative consequences.

Mortality caused by roads affects all taxonomic groups, but amphibians are particularly vulnerable (Síllero 2008; Holderegger and Di Giulio 2010; Colino-Rabanal and Lizana 2012; D'Amico et al. 2015; Franch et al. 2015). Due to their small body size and lower conspicuousness, amphibian roadkill is frequently underestimated (Hels and Buchwald 2001; Santos et al. 2011). However, some studies have reported that an important proportion of roadkill species belongs to this group, highlighting the relevance of the threat to amphibians (Ascensão and Mira 2005; Glista et al. 2007; Carvalho and Mira 2011; Matos et al. 2012; Beebee 2013). In a study conducted in Portugal, Carvalho and Mira (2011) reported that amphibian mortality can reach values of up to 70% of total vertebrate mortality during the wet season and amphibians are one of the taxa most affected by roadkills, along with passerine birds (which may reach 60–70% in certain spring months). In another study in the same region (Ascensão and Mira 2005), amphibians represented around 56% of the total mortality, while a study conducted in the USA (Glista et al. 2007) determined that amphibians represent around 95% of total roadkills. Several factors impact amphibian mortality numbers, with the more relevant being (i) movement ability, with species with higher vagility being more affected by roads (Carr and Fahrig 2001; Colino-Rabanal and Lizana 2012; Meek 2012); (ii) immobility in the face of an approaching vehicle (Mazerolle et al. 2005; Lima et al. 2015); (iii) traffic intensity (Hels and Buchwald 2001; Mazerolle 2004); (iv) surrounding habitat (Síllero 2008; Matos et al. 2012; Heigl et al. 2017); and (v) proximity to reproduction sites, such as ponds and streams (Ascensão and Mira 2005; Santos et al. 2007b).

High mortality numbers of amphibians are often aggregated in time, particularly when individuals migrate to and from reproduction areas (e.g., ponds) (Síllero 2008; Brzeziński et al. 2012). In fact, the complex reproduction cycles of most amphibian species, which include both terrestrial and aquatic phases (for breeding), imply seasonal movements of large numbers of individuals between different habitats which are performed mostly at night (Ferrand de Almeida et al. 2001; Hels and Buchwald 2001; Loureiro et al. 2008; Langen et al. 2009; Colino-Rabanal and Lizana 2012). For most species residing in Mediterranean climates, the onset of movement toward the aquatic breeding sites is determined by the first autumn rainfall (September–November), while monthly average temperatures are still mild (> 10 °C; Díaz-Paniagua 1992; IPMA 2014) and ends in late spring (May) (Ferrand de Almeida et al. 2001; Richter-Boix et al. 2006; Loureiro et al. 2008). Following the breeding period, adults of most species return to their terrestrial habitats with juveniles dispersing to these habitats later in the season (Rothermel 2004; Cushman 2006; Sinsch 1997). Activity may decrease during the winter if temperatures are too low, but the summer is generally the most limiting period as many reproduction sites dry out, and the high temperatures force amphibians to remain hidden or to aestivate (Speybroeck et al. 2016; Sinsch et al. 2007). This partially explains why so many amphibians are susceptible to roadkill during their peak activity periods (Hels and Buchwald 2001; Puky 2005).

While season has a significant influence on species activity, there are several examples in the literature acknowledging the effects of moonlight on different species behaviors (reviewed in Kronfeld-Schor et al. 2013). Many species, marine and terrestrial, show some kind of adjustment in their activity levels and movement patterns to the lunar cycle (Kronfeld-Schor et al. 2013; Chakraborty 2018). The various moon phases and their influence on tides and geomagnetic fields are perceived by animals and affect their behavior (Kronfeld-Schor et al. 2013). For example, the reproductive activity of fiddler crabs (genus *Uca*) reflects the 14-day semilunar cycle in the Atlantic Coast of USA, while deer mice (*Peromyscus maniculatus*) foraged less during a full moon when compared to the new moon in a study conducted in Santa Barbara Island, USA, to decrease predation risk by barn owls (*Tyto alba*) (Chakraborty 2018), and maned wolves (*Canis brachyurus*) in Brazil responded to temporally reduced prey availability by reducing their distance traveled on full moon nights when compared to other nights (Chakraborty 2018). Geomagnetic fields are also influenced by the lunar cycle, with peaks during the third quarter moon and decreases at the first quarter (Stolov 1965). These fields provide navigational cues for animals (Phillips 1986; Fischer et al. 2001; Grant et al. 2009; Mestre et al. 2014) as birds are well known to use geomagnetic fields for orientation when migrating long distances (e.g., Ossenkopp and Barbeito 1978).

With regard to amphibians, the effect of the moon as a cue to some phases of their phenological cycle has been discussed in the literature (Grant et al. 2009, 2012; Vignoli and Luiselli 2013; Underhill and Höbel 2018), raising the possibility that the movement intensity of amphibians might be somewhat affected by the moon, with indirect effects in roadkill numbers, although this is species specific (Grant et al. 2012). For example, Bufonidae, Ranidae, Salamandridae, and Hylidae are influenced more by lunar cycles than other families (Grant et al. 2012), and while the adaptive significance for amphibians of lunar-mediated behaviors is rare, predator avoidance, reproductive synchronization, visual signaling, foraging, and navigation have all been observed (Grant et al. 2012). The activity of several individuals of the genus *Litoria* was significantly greater close to full moon nights, possibly as an adaptation to predation by snakes (Brown and Shine 2002; Grant et al. 2012). By comparison, large arrival, and amplexus and spawning events in *B. spinosus* are more frequent around the full moon (Grant et al. 2009). Phillips (1986), for example, suggests that the eastern red-spotted newt (*Notophtalmus viridiscens*) can detect and respond to small geomagnetic changes in navigation and orientation. We assumed that roadkill events result from both breeding and non-breeding movements, and that many individuals use the road for foraging (T. Pinto and L.G. Sousa, personal observations).

To our knowledge, very few works have investigated the effect of the moon on roadkill intensity and amphibians (e.g., Mizuta 2014). In the present study, we aim to assess the influence of the lunar cycle on the roadkill of six amphibian species in
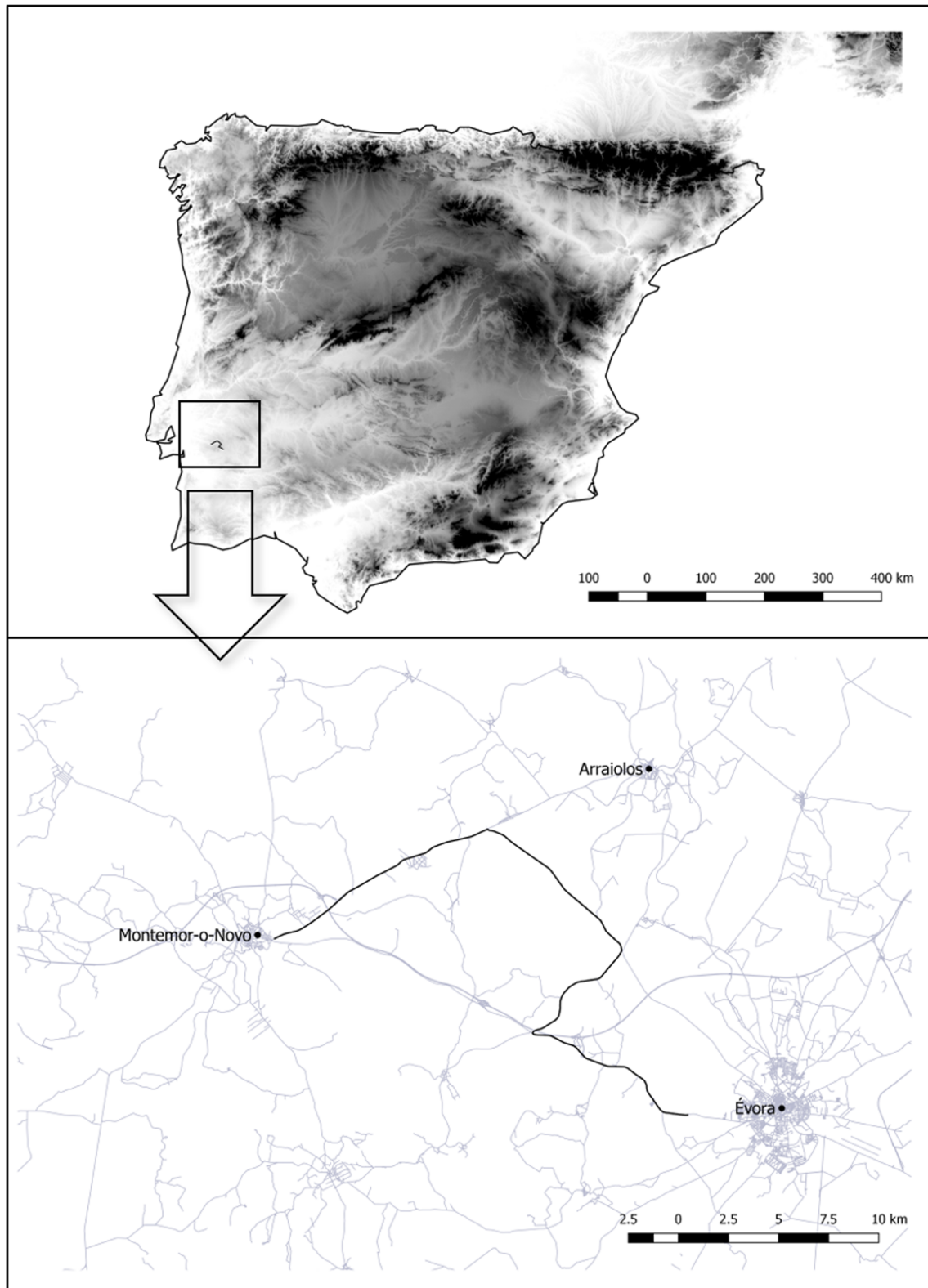

Fig. 1 Study site, near Évora (Portugal) and the surveyed road (37 km) from March 2010 and May 2012

Southern Portugal and on the total number of amphibian roadkill recorded during roadkill surveys, while accounting for weather conditions. We predict that, at least to some of the studied species, the moon cycle–related variables affect roadkill numbers given the influence of the moon cycle in the nighttime luminosity and geomagnetism. We demonstrate the relevance of the lunar cycle on the mortality of amphibian species, despite the expected influence of weather conditions, particularly temperature and humidity. Studying the influence of weather and the moon cycle on roadkill numbers of amphibians may help predict peak mortality events and plan conservation efforts, such as road circulating restrictions near amphibian breeding areas.

Material and methods

We conducted this study in southern Portugal (38° 32′ 24″ to 38° 47′ 33″ N; − 08° 13′ 33″ to − 07° 55′ 45″ W) (Fig. 1). The study area is dominated by a mixture of *montado* woodlands (Pinto-Correia et al. 2011) with varying tree density (*Quercus suber* and/or *Q. rotundifolia*) and agricultural areas, while topography is generally undulating and under 400 m a.s.l. (for details, see Santos et al. 2011). The climate is Mediterranean, with mild winters and hot and dry summers. Mean monthly temperature averages 15.8 °C (9.3–23.3 °C) and mean monthly rainfall averages 50.8 mm (6.6–102.7 mm; Évora 1971–2000; IPMA 2014).

Roadkills were undertaken between 16 Mar 2010 and 31 May 2012, in order to have two complete breeding cycles and thus cover possible interannual variability in weather conditions. Four road segments (N4 = 12.3, M529 = 9.1, N370 = 6.4, and N114 = 9.2 km, respectively) were surveyed, totaling 37 km: N4 and N114 are national main roads (ca. 4000 to 10,000 vehicles/day), and M529 and N370 are secondary roads with lower traffic volume (< 4000 vehicles/day; EP 2005). These roads were selected as they represent the most frequent and abundant type of roads in the country, and they cross important areas for biodiversity of southern Portugal (e.g., *montado* open forest). All roads are two-lane wide (9.25 m in average), and central traffic dividers are present in only two road crossings (Fig. 1).

Road surveys were performed daily and started within 2 h after sunrise (see Santos et al. 2011). This sampling effort equally covered all moon phases. One observer, driving a car at 20–40 km/h, checked both sides of the road (lanes and verges) for all amphibian carcasses. The surveys were always carried out by experienced observers (> 100 roadkill surveys), and most (84%) were carried out by the same four observers, thereby assuring consistency of procedures and minimizing errors associated with variation in observer skills (Collinson et al. 2014). The car speed was as low as possible in order to maximize detection probabilities of small carcasses (Collinson et al. 2014; Santos et al. 2016b). However, due to

driver safety reasons, a constant speed was not always possible. The standard road sampling width corresponded to both lanes and paved shoulders (see Santos et al. 2013, 2016a). For each amphibian roadkill detected, identification was in situ or later in the laboratory to the lowest possible taxonomic level, and its GPS position was recorded (Santos et al. 2011). After species identification, the carcasses were removed from the road to avoid recounts (Guinard et al. 2012).

The daily moon phase at the geographical location of the study site was obtained from the Naval Oceanography Portal services (http://www.usno.navy.mil/USNO/astronomical-applications/data-services/rs-one-day-world) and was expressed as the fraction of moon disk illuminated that was converted into radians (π). One lunar cycle thus corresponded to a gradual change from 0 to 2 π radians (0 and 2 π radians corresponded to the full moon, and π radians corresponded to the new moon). The transformations cos(π) and sin(π) were used in the statistical analyses as explanatory variables to investigate possible lunar effects on amphibian roadkills. High values of "cos(π)" represent full moon (versus new moon for low values), while high values of "sin(π)" represent last quarter moon (versus first quarter moon; see deBruyn and Meeuwig 2001; Penteriani et al. 2011). Hourly weather conditions were obtained from the Centro de Geofísica de Évora (University of Évora; Mitra's Weather Station) and added to the dataset. Weather-related variables included minimum and maximum air temperatures during the previous 24 h (°C), average relative air humidity during the previous 24 h (%), cumulative rainfall during the previous 24 h (mm), and average wind speed during the previous 8 h (Suppl. Material, Table S1). We excluded the period of June to August (3 months) after preliminary data exploration, as those months corresponded to minimum values of amphibian roadkills and to the period with the lowest amphibian activity in Mediterranean areas (e.g., Jakob et al. 2003; D'Amico et al. 2015). The season was based on average meteorological conditions and defined as a three-category variable: autumn (September to November), winter (December to February), and spring (March to May; Suppl. Material, Table S1). Species with roadkill frequency higher than 3% were selected for analyses.

The data analysis was a two-step process: a previous screening of the candidate variables followed by an evaluation of the significance of the explanatory variables in explaining the roadkill numbers of each species and of the total number of amphibians. First, a pairwise Spearman correlation was computed among candidate explanatory variables and, to each pair of highly correlated variables ($|rs| > 0.7$), only the one with higher biological relevance for the species was retained for further analysis (Tabachnick and Fidell 1996). Where appropriate, explanatory variables were transformed to reduce the influence of outliers (Zuur et al. 2009). This was followed by generalized linear mixed model for count data (Poisson) in

order to evaluate the importance of the lunar cycle and weather variables in each species and for the total amount of amphibian roadkills. Season was modeled as a random effect to account for time dependencies (Zuur et al. 2009). A zero-inflation component was added to models in which species counts showed excess of zeros (> 70%). Thus, each species was modeled with the zero-inflation component while the overall data was modeled without it. Moreover, since some of the observed mortality "zeros" can be due to measurement error (Santos et al. 2011), the zero-inflation component was applied (Zuur et al. 2009). Models were fitted with a negative binomial distribution if overdispersion was too high (>> 1; Zuur et al. 2009). Model selection was not performed, since we opted to test the effect of all explanatory variables. Model diagnostics were based on plots of Pearson residuals versus the fitted values.

The statistical analysis was carried out within the R environment (R Core Team 2016), using the "glmmADMB" package (Fournier et al. 2012; Skaug et al. 2016).

Results

Over nearly a 26-month period, a total of 4276 amphibian roadkills were recorded (two orders, 11 genera, and 14 species), of which 94% were identified to species level during a total of 807 surveys. The species with higher roadkill percentages (> 3% of

all amphibian carcasses) in the study area comprised two urodeles and four anurans: sharp-ribbed salamander (*Pleurodeles waltl*), fire salamander (*Salamandra salamandra*), Iberian painted frog (*Discoglossus galganoi*), western spadefoot toad (*Pelobates cultripes*), spiny common toad (*Bufo spinosus*), and natterjack toad (*Epidalea calamita*). The species with the highest roadkill frequencies (> 0.02 km/day) were *P. cultripes* (*n* = 934), *P. waltl* (*n* = 897), and *S. salamandra* (*n* = 864), all with 0.03 roadkills/km/day. Together, the six species comprised 91.5% (*n* = 3911) of all carcasses found (Table 1; Suppl. Material).

Weather effects on roadkills

Considering the potential effect of cloud cover in the results, for species showing higher mortality during higher luminosity nights, we re-fitted models with a reduced dataset (excluding the observations of full moon nights with potential interference of high cloud cover: high luminosity (ncos > 0.5), highest rainfall (p_24 > 10 mm), and maximum humidity values (hr_24 > 80%). The new estimated coefficient for "ncos" remained positive and significant (estimate = 0.32, *p* = 0.019) and very similar to the values obtained with the full dataset. Therefore, we conclude that cloud cover is not masking any moon luminosity effect in our study.

For the *D. galganoi* (overdispersion = 0.84) Poisson model and for all the negative binomial models (other species and the global dataset which had overdispersion > 2), the inspection of residual plots revealed an adequate fit to data. In all models,

Table 1 Number, number of roadkills per km per day, and percentage of roadkills by order and species. On bold, the total carcass numbers, overall and per order

Order	Scientific name	Common name	Carcass number	No/km/day	%
Caudata	*Pleurodeles waltl**	Sharp-ribbed salamander	897	0.0300	20.98
	*Salamandra salamandra**	Fire salamander	864	0.0289	20.21
	Triturus pygmaeus	Southern marbled newt	31	0.001	0.72
	Lissotriton boscai	Bosca's newt	8	0.0003	0.19
	Total Caudata		**1800**	0.0603	**42.10**
Anura	*Pelobates cultripes**	Western spadefoot toad	934	0.0313	21.84
	*Epidalea calamita**	Natterjack toad	543	0.0181	12.70
	*Bufo spinosus**	Spiny common toad	540	0.0181	12.63
	*Discoglossus galganoi**	Iberian painted frog	133	0.0044	3.11
	Hyla meridionalis	Mediterranean tree frog	27	0.0009	0.63
	Pelophylax perezi	Perez's frog	25	0.0008	0.58
	Pelodytes ibericus	Iberian parsley frog	10	0.0003	0.23
	Hyla molleri	Iberian tree frog	5	0.0002	0.12
	Alytes cisternasii	Iberian midwife toad	1	0.00003	0.02
	Non-identified anurans		258	0.0086	6.03
	Total Anura		**2476**	0.0829	**57.90**
Total			**4276**		

*Marked species were considered in this study (those with > 3% of the roadkills)

Table 2 Roadkill models for the species of the order Caudata (*Salamandra salamandra* and *Pleurodeles waltl*)

	Salamandra salamandra					*Pleurodeles waltl*				
	Estimate	Std. error	*z* value	Sig.		Estimate	Std. error	*z* value	Sig.	
(Intercept)	− 5.350	0.659	− 8.12	0.000	*	− 5.848	0.825	− 7.09	0.000	*
ncos	0.331	0.136	2.43	0.015	*	− 0.493	0.142	− 3.48	0.000	*
nsin	0.173	0.139	1.25	0.212		0.060	0.145	0.42	0.676	
mt24	0.004	0.024	0.16	0.872		0.118	0.029	4.05	0.000	*
cp24 (log + 1)	1.084	0.133	8.16	0.000	*	0.629	0.132	4.76	0.000	*
ahr24	0.0659	0.008	6.90	0.000	*	0.060	0.009	6.35	0.000	*
mws8	0.081	0.289	0.87	0.384		0.001	0.097	0.01	0.993	
ncos:nsin	− 0.058	0.289	− 0.20	0.841		− 0.001	0.281	− 0.01	0.996	

*$p < 0.05$

Variables: *ncos*, cosine of the fraction of moon disk illuminated (in radians); *nsin*, sine of the fraction of moon disk illuminated (in radians); *mt24*, minimum air temperature (°C) in the last 24 h; *cp24*, cumulative rainfall (mm) in the last 24 h; *arh24*, average relative air humidity (%) in the last 24 h; *mws8*, average wind speed in the last 8 h; additional parameters (*S. salamandra*: random effects: variance = 0.0000; SD = 0.0003; negative binomial dispersion parameter = 0.3514; zero-inflation parameter = 0.0000; *P. waltl*: random effects: variance = 0.5024; SD = 0.7088; negative binomial dispersion parameter = 0.3377; zero-inflation parameter = 0.0000)

weather variables were the most relevant in explaining roadkills, with average relative air humidity in the previous 24 h being always significant; cumulative rainfall during the previous 24 h being significant to all models, except for the *E. calamita*; and minimum air temperature in the previous 24 h being significant to all models except for *S. salamandra* (Tables 2, 3, and 4).

Table 3 Roadkill models for the species of the order Anura (*Discoglossus galganoi, Bufo spinosus, Epidalea calamita, Pelobates cultripes*)

	Bufo spinosus					*Epidalea calamita*				
	Estimate	Std. error	*z* value	Sig.		Estimate	Std. error	*z* value	Sig.	
(Intercept)	− 3.272	0.617	− 5.30	0.000	*	− 4.522	0.680	− 6.65	0.000	*
ncos	0.064	0.125	0.51	0.611		0.094	0.136	0.69	0.489	
nsin	− 0.113	0.133	− 0.85	0.393		0.011	0.147	0.08	0.939	
mt24	0.103	0.023	4.48	0.000	*	0.061	0.031	1.97	0.049	*
cp24 (log + 1)	0.965	0.134	7.19	0.000	*	0.888	0.146	6.07	0.000	
ahr24	0.019	0.008	2.41	0.016	*	0.048	0.009	5.39	0.000	*
mws8	0.061	0.091	0.67	0.506		− 0.199	0.110	− 1.81	0.070	
ncos:nsin	− 0.119	0.269	− 0.44	0.658		− 0.281	0.301	− 0.93	0.352	
	Pelobates cultripes					*Discoglossus galganoi*				
	Estimate	Std. error	*z* value	Sig.		Estimate	Std. error	*z* value	Sig.	
(Intercept)	− 4.811	0.650	− 7.40	0.000	*	− 3.464	0.903	− 3.84	0.000	*
ncos	− 0.237	0.130	− 1.82	0.068		− 0.205	0.155	− 1.32	0.186	
nsin	0.220	0.131	1.68	0.092		− 0.138	0.172	− 0.80	0.421	
mt24	0.183	0.024	7.73	0.000	*	0.070	0.031	2.26	0.024	*
cp24 (log + 1)	0.830	0.133	6.25	0.000	*	0.616	0.144	4.27	0.000	*
ahr24	0.035	0.008	4.36	0.000	*	0.023	0.011	2.06	0.040	*
mws8	0.057	0.088	0.66	0.512		0.053	0.104	0.51	0.613	
ncos:nsin	0.265	0.264	1.01	0.314		− 0.212	0.368	− 0.58	0.565	

$p < 0.05$

Variables: *ncos*, cosine of the fraction of moon disk illuminated (in radians); *nsin*, sine of the fraction of moon disk illuminated (in radians); *mt24*, minimum air temperature (°C) in the last 24 h; *cp24*, cumulative rainfall (mm) in the last 24 h; *arh24*, average relative air humidity (%) in the last 24 h; *mws8*, average wind speed in the last 8 h,; additional parameters (*B. spinosus*: random effects: variance = 0.0000; SD = 0.0004; negative binomial dispersion parameter = 0.4022; zero-inflation parameter = 0.0000; *E. calamita*: random effects: variance = 0.0591; SD = 0.2430; negative binomial dispersion parameter = 0.3259; zero-inflation parameter = 0.0000; *P. cultripes*: random effects: variance = 0.0000; SD = 0.0003; negative binomial dispersion parameter = 0.3804; zero-inflation parameter = 0.0000; *D. galganoi*: random effects: variance = 0.0000; SD = 0.0009; zero-inflation parameter = 0.6817)

Table 4   Roadkill models for all the species

	Estimate	Std. error	z value	Sig.	
(Intercept)	−2.181	0.493	− 4.43	0.000	*
ncos	− 0.095	0.101	− 0.94	0.350	
nsin	− 0.164	0.101	− 1.63	0.100	
mt24	0.092	0.020	4.71	0.000	*
cp24 (log + 1)	0.909	0.113	8.04	0.000	*
ahr24	0.037	0.006	5.87	0.000	*
mws8	− 0.062	0.072	− 0.87	0.390	
ncos:nsin	0.161	0.203	0.79	0.430	

*$p < 0.05$

Variables: *ncos*, cosine of the fraction of moon disk illuminated (in radians); *nsin*, sine of the fraction of moon disk illuminated (in radians); *mt24*, minimum air temperature (°C) in the last 24 h; *cp24*, cumulative rainfall (mm) in the last 24 h; *arh24*, average relative air humidity (%) in the last 24 h; *mws8*, average wind speed in the last 8 h; additional parameters (random effects: variance = 0.1037; SD = 0.3221; negative binomial dispersion parameter = 0.4127)

Overall, the increases in minimum air temperature, average relative air humidity, and cumulative rainfall in the previous 24 h to road surveys increase the probability of higher roadkill numbers for all studied species and total number of amphibians (Tables 2, 3, and 4).

Moon cycle effects on roadkills

Two species were significantly influenced by moon-related variables: *S. salamandra* and *P. waltl*. The former had a positive coefficient (0.33, $p = 0.015$, Table 2) for "ncos," demonstrating significantly higher mortality during the full moon. The latter depicted a negative coefficient (− 0.49, $p = 0.0005$, Table 2) which demonstrates significantly higher mortality during the new moon. The response of *P. cultripes*, which was just above the significance threshold ($0.05 < p < 0.10$), also had a negative coefficient in ncos (− 0.24, $p = 0.068$, Table 3) and a positive coefficient in nsin (0.22, $p = 0.092$, Table 3).

Discussion

As predicted, our results demonstrate that temperature and humidity are the main drivers for amphibian species mobility, explaining most of the roadkills. However, the most relevant result of this work is the association between roadkill amphibian numbers, in the order Caudata, and the lunar cycle.

Previous research has already revealed amphibians' life history might respond to lunar cycle–related cues (Vignoli and Luiselli 2013; Grant et al. 2009, 2012). Additionally, the influence of the lunar cycle has also been related with roadkill numbers in other taxa, namely ungulates (Gundersen and Andreassen 1998; Colino-Rabanal et al. 2018), kangaroos

(Coulson 1982), or birds (Mizuta 2014). Colino-Rabanal et al. (2018) found higher roadkill during the full moon in three out of the four ungulate species studied. Although no clear conclusion is reached in what concerns the causal factors, the authors hypothesize that either driver behavior or (most probably) the species life cycles are behind these patterns. Mizuta (2014), studying a bird species (Amami woodcock *Scolopax mira*), found that roadkill was more frequent in the first half of the synodic month, with a waxing moon, and from dusk to midnight. To our knowledge, this is the first approach relating amphibian roadkill data with the lunar cycle.

All studied species are characterized by a crepuscular/nocturnal activity, and all rely on water bodies for reproduction, having a terrestrial phase outside the breeding season (Ferrand de Almeida et al. 2001; Loureiro et al. 2008). All, except *B. spinosus*, have decreasing population trends across their distribution areas, and *P. waltl* and *P. cultripes* are classified as Near Threatened by IUCN (Agasyan et al. 2009; Beja et al. 2009a, b, c; Bosch et al. 2009; Kuzmin et al. 2009). The characteristics of waterbodies used for breeding differ among species. *Bufo spinosus* breeds mostly in permanent waterbodies (Agasyan et al. 2009), *P. waltl*, *S. salamandra*, and *D. galganoi* breed in permanent or temporary ponds and small streams (Beja et al. 2009a; Bosch et al. 2009; Kuzmin et al. 2009), *P. cultripes* breeds mostly in temporary ponds (Beja et al. 2009b), and *E. calamita* prefers shallow water like puddles (Beja et al. 2009c). Furthermore, *B. spinosus* and *E. calamita* are also known to show philopatric migratory activity (Reading et al. 1991; Miaud et al. 2000). In years of autumn drought, the start of breeding may be delayed until reproduction sites have enough water for species requirements, particularly the early breeders, such as *P. waltl*, *S. salamandra*, and *P. cultripes* (Díaz-Paniagua 1992; Segev and Blaustein 2007).

Weather conditions

Rain and temperature are often referred to as major determinants of amphibian movements (Mazerolle 2001; Vasconcelos and Calhoun 2004; Todd and Winne 2006) and our results confirm the relation of these environmental cues with amphibian mortality, with more amphibians being killed on roads during rainy and warmer nights. Although these relations have also been found for reptiles, birds, and mammals (D'Amico et al. 2015; Garriga et al. 2017), numerous studies dealing with amphibian roadkills confirm that mortality peaks with these weather conditions (Síllero 2008; Carvalho and Mira 2011; Beebee 2013; D'Amico et al. 2015; Garriga et al. 2017). This can be explained by the amphibians' highly permeable skin, which can rapidly desiccate (Todd and Winne 2006) and to the lower metabolic cost of movements during warmer nights (Paladino 1985). The combination of these

characteristics could explain the amphibian movement peaks during humid and warmer nights for our study.

Lunar cycle

Moonlight avoidance by animals has been related to an increased risk of predation by visually oriented predators (Lima and Dill 1990; Vignoli and Luisielli 2013) and thus may translate into an anti-predatory behavior. By contrast, animals active on moonlight nights may be at an advantage if their visual acuity is better than that of their predators or if, somehow, they are protected from predators (with toxins, for example). This might be the case of *S. salamandra*, that is preyed on by mammals (like hedgehogs, otters, badgers, and wild boars (Carretero and Rosell 1999; Salvador and García-París 2001; Morales et al. 2004)), reptiles (such as snakes (Bas López et al. 1979; Braña 1998)), and owls (e.g., tawny owl; Otero et al. 1978). Most mammals and reptiles rely on vision, but also on olfactory or infrared senses, respectively, to detect their prey (Newman and Hartline 1982; Nummela et al. 2013). Owls have an accurate vision and, although not exclusively, use it to detect their prey (Martin 1982). Thus, despite predator's abundance, *S. salamandra* might afford to move in full moon nights (higher luminosity) considering its aposematic coloration, which has a dissuading effect on most predators. It is known that predators associate these as warning signals for venom/toxicity or unappealing prey (Ham et al. 2006; Martínez-Freiría et al. 2017). On the other hand, being a highly terrestrial species, it is possible that visual interspecific communication is facilitated during moonlight nights as it happens with other taxa (Penteriani et al. 2010). By comparison, *P. waltl* spends longer periods of time in water and moves less frequently on land than *S. salamandra* (Salvador 2015). *P. waltl* shares common predators with *S. salamandra* (Máñez-Rodríguez 1983; Díaz-Paniagua et al. 2007), meaning this species may have advantages in moving on darker nights to avoid visually oriented predators (e.g., owls), since they lack the aposematic coloration and moving on darker nights may bring advantages in avoiding being detected by predators. However, the information on the biology of these urodeles might be too scarce to allow a consistent discussion on the adaptive function of high activity under full moon/new moon nights.

Lunar cycles influence changes not only in light intensity, but also in the Earth's magnetic field (Grant et al. 2012). The response of *P. cultripes* to the lunar cycle was less clear than that of the urodeles, suggesting that mortality peaked during the new moon (when compared with full moon) and during the last quarter moon (when compared with the first quarter moon). Geomagnetism is regulated by lunar cycles (Lohmann and Willows 1987) with minimum values just before the full moon (first quarter) and peaking at the third quarter (Stolov 1965). Grant and collaborators (Grant et al. 2009) studied the

influence of the lunar cycle on breeding biology of amphibians and found a correlation between the arrival events of newts (urodeles) to breeding areas and geomagnetic changes around moon quarters. *P. cultripes* may be responding to both luminosity and geomagnetic signals, although the results are not clear. Although not focused on roadkills, some studies found that amphibian responses to the lunar cycle are highly species specific and are explained mostly by each species' behavior and specific ecological traits (Grant et al. 2012; Onorati and Vignoli 2017). This supports our results since the two salamander species showed opposite responses to the moon.

Potential limitations

Several factors might induce underestimates of roadkill numbers, namely carcass persistence and detectability (Santos et al. 2011; Teixeira et al. 2013; Barrientos et al. 2018). After an amphibian-vehicle collision, the carcasses do not remain on the road for a long time and are removed by scavengers, by decomposition or destroyed by vehicle wheels (carcass persistence; Santos et al. 2011). The carcass detectability refers to the observer error that fails to register all carcasses present at the time of the survey (Teixeira et al. 2013). Both error types are mostly influenced by carcass size (Santos et al. 2011; Barrientos et al. 2018). We believe that persistence biases were minimized in the present study by performing road surveys with a daily frequency and as close as possible to the moment of roadkill (starting at sunrise; Santos et al. 2011, Collinson et al. 2014). The detectability biases were also minimized by using experienced observers and by driving the car at a very low speed (Collinson et al. 2014; Santos et al. 2016b). In addition, most target species of our study have similar body sizes, varying between 18 g (*S. salamandra*) and 30 g (*P. cultripes*), having thus very similar persistence and detectability probabilities. Only *B. spinosus* has a higher body mass (125 g), with expected higher persistence time and higher detectability by observers. We believe that these size differences are not problematic considering that we related each species independently with the environmental variables. Furthermore, our analyses concerned the sum of roadkill events for each night, thus lowering the effect of particular road sections with lower persistence or detection. Moreover, the visibility conditions during surveys were optimal most of the days (enough sunlight and low traffic volumes), and rarely was there strong persistent rain during the surveys that could interfere with carcass visibility. Thus, we are confident that the roadkill numbers used in analyses are an accurate sample of amphibian mortality in our study area.

A further potential limitation with our study is that we did not include cloud cover as an explanatory variable. It is known that nights with high and continuous cloud cover may reduce the amount of moonlight reaching the soil surface (Onorati

and Vignoli 2017). However, a recent study showed that the moonlight effects on anuran reproductive behavior were not influenced by cloud cover (Underhill and Höbel 2018). Furthermore, as occurred in similar studies (Colino-Rabanal and Lizana 2012), cloud cover varied locally, and differently from the coarser weather data available. However, we addressed this potential bias, which would be most felt on species moving in higher luminosity conditions, in our case *S. salamandra*, by resorting to the approach described in the "Material and methods" section, concluding that the cloud cover is not masking the moon luminosity effect.

Conservation implications

Our study showed that mortality numbers were higher during specific weather conditions (rainy/humid and warmer nights) for almost all studied species: *P. waltl*, *D. galganoi*, *P. cultripes*, *B. spinosus*, and *E. calamita*. Mortality of *S. salamandra* was higher during rainy and humid conditions. Moreover, the mortality of *P. waltl* and *S. salamandra* was also higher during specific lunar phases. These are crucial results for the application of temporal mitigation measures, such as temporarily closing the road to traffic, setting temporary drift fences, or organizing volunteer groups to carry amphibians across roads to save them from death (Schmidt and Zumbach 2008; Beebee 2013). In our study area, these actions should be planned and coordinated in rainy and warmer nights for the general amphibian community. For actions targeting *S. salamandra*, actions should concentrate on humid and rainy nights with a full moon with translocation of individuals from the road surface to safer habitats, for instance. On the other hand, road sections with high roadkill frequency of *P. waltl* could be similarly targeted on rainy/humid and warmer nights with a new moon, as mortality numbers are increased in these periods. Thus, our results contribute to the optimization of resources and temporary mitigation measures (e.g., volunteer campaigns, temporary road signs, or temporary drift fences) by specifying optimal periods for particular species mitigation, which can improve the cost–benefit of some actions. For this reason, our findings have important implications for the road agencies and conservation of amphibians which face a global decline (Houlahan et al. 2000).

References

Agasyan A, Avisi A, Tuniyev B, Isailovic JC, Lymberakis P, Andrén C, Cogalniceanu D, Wilkinson J, Ananjeva N, Üzüm N, Orlov N, Podloucky R, Tuniyev S, Kaya U (2009) *Bufo bufo*. The IUCN red list of threatened species 2009: e.T54596A11159939. https://www.iucnredlist.org/species/54596/11159939. Accessed 07 Feb 2018

Ascensão F, Mira A (2005) Spatial patterns of road kills: a case study in southern Portugal. ICOET 2005 proc, 641–646

Barrientos R, Martins RC, Ascensão F, D'Amico M, Moreira F, Borda-de-Água L (2018) A review of searcher efficiency and carcass persistence in infrastructure-driven mortality assessment studies. Biol Conserv 222:146–153. https://doi.org/10.1016/j.biocon.2018.04.014

Bas López S, Guitián Rivera J, de Castro Lorenzo A, Sánchez Canals JL (1979) Datos sobre la alimentación de la salamandra (*Salamandra salamandra* L) en Galicia. Bol Est Cent Ecol 8(16):73–78

Beebee TJC (2013) Effects of road mortality and mitigation measures on amphibian populations. Conserv Biol 27:657–668. https://doi.org/10.1111/cobi.12063

Beja P, Bosch J, Tejedo M, Edgar P, Donaire-Barroso D, Lizana M, Martínez-Solano I, Salvador A, García-París M, Recuero Gil E, Slimani T, El Mouden EH, Geniez P, Slimani T (2009a) Pleurodeles waltl. The IUCN red list of threatened species 2009: e.T59463A11926338. https://doi.org/10.2305/IUCN.UK.2009.RLTS.T59463A11926338.en. Accessed on 07 Feb 2018

Beja P, Bosch J, Tejedo M, Lizana M, Martinez Solano I, Salvador A, García París M, Recuero Gil E, Pérez Mellado V, Díaz-Paniagua C, Cheylan M, Márquez R, Geniez P (2009b) *Pelobates cultripes* (errata version published in 2016). The IUCN red list of threatened species 2009: e.T58052A86242868. https://doi.org/10.2305/IUCN.UK.2009.RLTS.T58052A11722636.en. Accessed on 07 Feb 2018

Beja P, Kuzmin S, Beebee T, Denoel M, Schmidt B, Tarkhnishvili D, Ananjeva NB, Orlov NL, Nyström P, Ogrodowczyk A, Ogielska M, Bosch J, Miaud C, Tejedo M, Lizana M, Martinez Solano I (2009c) *Epidalea calamita* (errata version published in 2016). The IUCN red list of threatened species 2009: e.T54598A86640094. https://doi.org/10.2305/IUCN.UK.2009.RLTS.T54598A11160828.en. Accessed on 07 Feb 2018

Benítez-López A, Alkemade R, Verweij PA (2010) The impacts of roads and other infrastructure on mammal and bird populations: a meta-analysis. Biol Conserv 143:1307–1316. https://doi.org/10.1016/j.biocon.2010.02.009

Bosch J, Beja P, Tejedo M, Lizana M, Martinez Solano I, Salvador A, García París M, Recuero Gil E, Díaz-Paniagua C, Pérez Mellado V, Márquez R (2009) *Discoglossus galganoi* (errata version published in 2016). The IUCN red list of threatened species 2009: e.T55269A86637387. https://doi.org/10.2305/IUCN.UK.2009.RLTS.T55269A11284123.en. Accessed on 07 Feb 2018

Braña F (1998) *Natrix natrix* Linnaeus, (1758). In: Salvador A, Ramos MA et al (eds) Fauna Ibérica, Reptiles, vol 10. Museo Nacional de Ciencias Naturales-CSIC, Madrid, pp 454–466

Brown GP, Shine R (2002) Influence of weather conditions on activity of tropical snakes. Aust Ecol 27:596–605

Brzeziński M, Eliava G, Żmihorski M (2012) Road mortality of pond-breeding amphibians during spring migrations in the Mazurian Lakeland, NE Poland. Eur J Wildl Res 58(4):685–693. https://doi.org/10.1007/s10344-012-0618-2

Carr LW, Fahrig L (2001) Effect of road traffic on two amphibian species of differing vagility. Conserv Biol 15:107–1078. https://doi.org/10.1046/j.1523-1739.2001.0150041071.x

Carretero MA, Rosell C (1999) Salamandra salamandra (fire salamander). Predation. Herpetoll Rev 30(3):161

Carvalho F, Mira A (2011) Comparing annual vertebrate road kills over two time periods, 9 years apart: a case study in Mediterranean farmland. Eur J Wildl Res 57:157–174. https://doi.org/10.1007/s10344-010-0410-0

Chakraborty U (2018) Effects of different phases of the lunar month on living organisms. Biol Rhythm Res 1:–29. https://doi.org/10.1080/09291016.2018.1526502

Coffin AW (2007) From roadkill to road ecology: a review of the ecological effects of roads. J Transp Geogr 15:396–406. https://doi.org/10.1016/j.jtrangeo.2006.11.006

Colino-Rabanal VJ, Lizana M (2012) Herpetofauna and roads: a review. Basic Appl Herpetol 26:5–31

Colino-Rabanal VJ, Langen TA, Peris SJ, Lizana M (2018) Ungulate: vehicle collision rates are associated with the phase of the moon. Biodivers Conserv 27:681–694. https://doi.org/10.1007/s10531-017-1458-x

Collinson WJ, Parker DM, Bernard RTF, Reilly BK, Davies-Mostert HT (2014) Wildlife road traffic accidents: a standardized protocol for counting flattened fauna. Ecol Evol 4:3060–3071. https://doi.org/10.1002/ece3.1097

Coulson GM (1982) Road-kills of macropods on a section of highway in Central Victoria. Aus Wildl Res 9:21–26

Cushman SA (2006) Effects of habitat loss and fragmentation on amphibians: a review and prospectus. Biol Conserv 128:231–240. https://doi.org/10.1016/j.biocon.2005.09.031

D'Amico M, Román J, de los Reyes L, Revilla E (2015) Vertebrate road-kill patterns in Mediterranean habitats: who, when and where. Biol Conserv 191:234–242. https://doi.org/10.1016/j.biocon.2015.06.010

deBruyn A, Meeuwig JJ (2001) Detecting lunar cycles in marine ecology: periodic regression versus categorical ANOVA. Mar Ecol Prog Ser 214:307–310. https://doi.org/10.3354/meps214307

Díaz-Paniagua C (1992) Variability in timing of larval season in an amphibian community in SW Spain. Ecography 15:267–272. https://doi.org/10.1111/j.1600-0587.1992.tb00034.x

Díaz-Paniagua C, Portheault A, Gómez Rodríguez C (2007) Depredadores de los anfibios adultos de Doñana: Análisis cualitativo. Pp. 148–157. In: Gosá A, Egaña-Callejo A, Rubio X (ed). Herpetologia iberiarraren egoera = Estado actual da Herpetologia Ibérica = Estado actual de la Herpetología Ibérica : Lehen Herpetologia Kongressua Euskal Herrian, IX Congreso Luso-Espanhol, XIII Congreso Español de Herpetología. Munibe. Suplemento, n° 25. 303 pp.

Encarnação JA, Becker NI (2015) Stealthy at the roadside: connecting role of roadside hedges and copse for silvicolous, small mammal populations. J Nat Conserv 27:37–43

EP (2005) Recenseamento do tráafego. Estradas de Portugal, E.P.E, Evora

Ferrand de Almeida N, Ferrand de Almeida P, Gonçalves H, Sequeira F, Teixeira J, Ferrand de Almeida F (2001) Guias FAPAS Anfíbios e Répteis de Portugal. FAPAS, Câmara Municipal do Porto, Porto

Fischer JH, Freake MJ, Borland SC, Phillips JB (2001) Evidence for the use of magnetic map information by an amphibian. Anim Behav 62:1–10

Fournier DA, Skaug HJ, Ancheta J, Ianelli J, Magnusson A, Maunder MN, Nielsen A, Sibert J (2012) AD model builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. Optim Methods Softw 27:233–249. https://doi.org/10.1080/10556788.2011.597854

Franch M, Silva C, Lopes G, Ribeiro F, Trigueiros P, Seco L, Sillero N (2015) Where to look when identifying roadkilled amphibians? Acta

Herpetol 10(2):103–110. https://doi.org/10.13128/Acta_Herpetol-16441

Garriga N, Franch M, Santos X, Montori A, Llorente GA (2017) Seasonal variation in vertebrate traffic casualties and its implications for mitigations measures. Landsc Urban Plan 157:36–44

Glista DJ, DeVault TL, DeWoody JA (2007) Vertebrate road mortality predominantly impacts amphibians. Herpetol Conserv Biol 3:77–87

Grant RA, Chadwick EA, Halliday T (2009) The lunar cycle: a cue for amphibian reproductive phenology? Anim Behav 78:349–357. https://doi.org/10.1016/j.anbehav.2009.05.007

Grant R, Halliday T, Chadwick E (2012) Amphibians' response to the lunar synodic cycle-a review of current knowledge, recommendations, and implications for conservation. Behav Ecol 24:53–62. https://doi.org/10.1093/beheco/ars135

Guinard E, Julliard R, Barbraud C (2012) Motorways and bird traffic casualties: carcasses surveys and scavenging bias. Biol Conserv 147:40–51

Gundersen H, Andreassen HP (1998) The risk of moose Alces alces collision: a predictive logistic model for moose-train accidents. Wildl Biol 4:103–111

Ham AD, Ihalainen E, Lindström L, Mappes J (2006) Does colour matter? The importance of colour in avoidance learning, memorability and generalisation. Behav Ecol Sociobiol 60(4):482–491. https://doi.org/10.1007/s00265-006-0190-4

Heigl F, Horvath K, Laaha G, Zaller JG (2017) Amphibian and reptile road-kills on tertiary roads in relation to landscape structure: using a citizen science approach with open-access land cover data. BMC Ecol 17:1–11. https://doi.org/10.1186/s12898-017-0134-z

Hels T, Buchwald E (2001) The effect of road kills on amphibian populations. Biol Conserv 99:331–340. https://doi.org/10.1016/S0006-3207(00)00215-9

Holderegger R, Di Giulio M (2010) The genetic effects of roads: a review of empirical evidence. Basic Appl Ecol 11:522–531. https://doi.org/10.1016/j.baae.2010.06.006

Houlahan JE, Findlay CS, Schmidt BR, Meyer AH, Kuzmin SL (2000) Quantitative evidence for global amphibian population declines. Nature 404:752–755. https://doi.org/10.1038/35008052

IPMA (2014) Instituto Português do Mar e da Atmosfera website, IP, Portugal. Available: http://www.ipma.pt (accessed 02.07.14)

Jakob C, Poizat G, Veith M, Seitz A, Crivelli AJ (2003) Breeding phenology and larval distribution of amphibians in a Mediterranean pond network with unpredictable hydrology. Hydrobiologia 499:51–61. https://doi.org/10.1023/A:1026343618150

Kronfeld-Schor N, Dominoni D, de la Iglesia H, Levy O, Herzog ED, Dayan T, Helfrich-Forster (2013) Chronobiology by moonlight. Proc R Soc B 280:20123088. https://doi.org/10.1098/rspb.2012.3088

Kuzmin S, Papenfuss T, Sparreboom M, Ugurtas IH, Anderson S, Beebee T, Denoël M, Andreone F, Anthony B, Schmidt B, Ogrodowczyk A, Ogielska M, Bosch J, Tarkhnishvili D, Ishchenko V (2009) Salamandra salamandra. The IUCN red list of threatened species 2009: e.T59467A11928351. https://doi.org/10.2305/IUCN.UK.2009.RLTS.T59467A11928351.en. Accessed on 07 Feb 2018

Langen TA, Ogden KM, Schwarting LL (2009) Predicting hot spots of Herpetofauna road mortality along highway networks. J Wildl Manag 73(1):104–114. https://doi.org/10.2193/2008-017

Lima SL, Dill LM (1990) Behavioral decisions made under the risk of predation: a review and prospectus. Can J Zool 68:619–640

Lima SL, Blackwell BF, DeVault TL, Fernández-Juricic E (2015) Animal reactions to oncoming vehicles: a conceptual review. Biol Rev 90:60–76. https://doi.org/10.1111/brv.12093

Lohmann KJ, Willows AOD (1987) Lunar-modulated geomagnetic orientation by a marine mollusk. Science 235:331–334. https://doi.org/10.1126/science.3798115

Loureiro A, Ferrand de Almeira N, Carretero MA, Paulo OS (2008) A*tlas dos Anfíbios e Répteis de Portugal*. Instituto da Conservação da Natureza e Biodiversidade, Lisboa. 257 pp.

Máñez-Rodríguez M (1983) Espectro alimentario del mochuelo común (*Athene noctua*) en España. Alytes 1:275–290

Martin GR (1982) An owl's eye: schematic optics and visual performance in Strix aluco L. J Comp Physiol 145:341–349

Martínez-Freiría F, i de Lanuza GP, Pimenta AA, Pinto T, Santos X (2017) Aposematism and crypsis are not enough to explain dorsal polymorphism in the Iberian adder. Acta Oecol 85:165–173. https://doi.org/10.1016/j.actao.2017.11.003

Matos C, Sillero N, Argana E (2012) Spatial analysis of amphibian road mortality levels in northern Portugal country roads. Amphibia-Reptilia 33:469–483. https://doi.org/10.1163/15685381-00002850

Mazerolle MJ (2001) Amphibian activity, movement patterns, and body size in fragmented peat bogs. J Hertetol 35:13–20

Mazerolle MJ (2004) Amphibian road mortality in response to nightly variations in traffic intensity. Herpetologica 60:45–53. https://doi.org/10.1655/02-109

Mazerolle MJ, Huot M, Gravel M (2005) Behavior of amphibians on the road in response to car traffic. Herpetologica 61(4):380–388. https://doi.org/10.1655/04-79.1

Meek R (2012) Patterns of amphibian road-kills in the Vendée region of Western France. Herpetol J 22:51–58

Mestre F, Bragança M, Nunes A, dos Santos ME (2014) Satellite tracking of sea turtles released after prolonged captivity periods. Mar Biol Res 10:996–1006. https://doi.org/10.1080/17451000.2013.872801

Miaud C, Sanuy D, Avrrillier JN (2000) Terrestrial movements of the natterjack toad *Bufo calamita* (Amphibia, Anura) in a semi-arid, agricultural landscape. Amphibia-Reptilia 21:357–369. https://doi.org/10.1163/156853800507426

Mizuta T (2014) Moonlight-related mortality: lunar conditions and road-kill occurrence in the Amami woodcock *Scolopax mira*. Wilson J Ornithol 126:544–552

Morales JJ, Lizana M, Acera F (2004) Ecología trófica de la nutria paleártica *Lutra lutra* en el río Francia (cuenca del Tajo, Salamanca) Galemys 16 (2): 57–77

Morelli F, Beim M, Jerzak L, Jones D, Tryjanowski P (2014) Can roads, railways and related structures have positive effects on birds? - a review. Transport Res D-TR E 30:21–31. https://doi.org/10.1016/j.trd.2014.05.006

Newman EA, Hartline PH (1982) The "infrared" vision of snakes. Sci Am 246:116–127

Nummela S, Pihlström H, Puolamäki K, Fortelius M, Hemilä S, Reuter T (2013) Exploring the mammalian sensory space: co-operations and trade-offs among senses. J Comp Physiol A 199:1077–1092

Onorati M, Vignoli L (2017) The darker the night, the brighter the stars: consequences of nocturnal brightness on amphibian reproduction. Biol J Linn Soc 120:961–976

Ossenkopp KP, Barbeito R (1978) Bird orientation and the geomagnetic field: a review. Neurosci Biobehav R 2:255–270. https://doi.org/10.1016/0149-7634(78)90034-9

Otero C, Castibn E, Senosiain R, Portillo F (1978) Fauna de Cazorla Vertebrados Icona Monogr 19:1–123

Paladino FV (1985) Temperature effects on locomotion and activity bio-energetics of amphibians, reptiles, and birds. Am Zool 25:965–972. https://doi.org/10.1093/icb/25.4.965

Penteriani V, Delgado MM, Campioni L, Lourenço R (2010) Moonlight makes owls more chatty. PLoS One 5:e8696. https://doi.org/10.1371/journal.pone.0008696

Penteriani V, Kuparinen A, Delgado MM, Lourenço R, Campioni L (2011) Individual status, foraging effort and need for conspicuousness shape behavioural responses of a predator to moon phases. Anim Behav 82:413–420. https://doi.org/10.1016/j.anbehav.2011.05.027

Phillips JB (1986) Magnetic compass orientation in the eastern red-spotted newt (*Notophthalmus viridescens*). J Comp Physiol A 158:103–109

Pinto-Correia T, Ribeiro N, Sá-Sousa P (2011) Introducing the montado, the cork and holm oak agroforestry system of southern Portugal. Agrofor Syst 82:99–104

Puky M (2005) Amphibian road kills: a global perspective. IN: Proceedings of the 2005 International Conference on Ecology and Transportation, Eds. Irwin CL, Garrett P, McDermott KP. Center for Transportation and the Environment, North Carolina State University, Raleigh, NC: pp. 325–338. Retrieved from: http://escholarship.org/uc/item/7j7546qv

R Core Team (2016) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. url: https://www.r-project.org/

Reading CJ, Loman J, Madsen T (1991) Breeding pond fidelity in the common toad, *Bufo bufo*. J Zool (Lond) 225:201–211

Redon L, Viol I, Jiguet F, Machon N, Scher O, Kerbiriou C (2015) Road network in an agrarian landscape: potential habitat, corridor or barrier for small mammals? Acta Oecol 62:58–65

Richardson ML, Wilson BA, Aiuto DAS, Crosby JE, Alonso A, Dallmeier F, Golinski GK (2017) A review of the impact of pipe-lines and power lines on biodiversity and strategies for mitigation. Biodivers Conserv 26:1801–1815. https://doi.org/10.1007/s10531-017-1341-9

Richter-Boix A, Llorente GA, Montori A (2006) Breeding phenology of an amphibian community in a Mediterranean area. Amphibia-Reptilia 27(4):549–559. https://doi.org/10.1163/156853806778877149

Rothermel BB (2004) Migratory success of juveniles: a potential constraint on connectivity for pond-breeding amphibians. Ecol Appl 14(5):1535–1546. https://doi.org/10.1890/03-5206

Salvador A (2015) Gallipato - *Pleurodeles waltl*. Enciclopedia Virtual de los Vertebrados Españoles. Salvador A, Martínez-Solano I. (Eds.). Museo Nacional de Ciencias Naturales, Madrid. http://www.vertebradosibericos.org/. Accessed 1 Sept 2018

Salvador A, García-París M (2001) Anfibios Españoles: Identificación, Historia Natural y Distribución. Esfagnos, Talavera de la Reina

Santos SM, Mathias ML, Mira A, Simões MP (2007a) Vegetation structure and composition of road verge and meadow sites colonized by Cabrera vole (*Microtus cabrerae* THOMAS). Pol J Ecol 55:481–493

Santos X, Llorent GA, Montori A, Carretero MA, Franch M, Garriga N, Richter-Boix A (2007b) Evaluating factors affecting amphibian mortality on roads: the case of the common toad *Bufo bufo*, near a breeding place. Anim Biodivers Conserv 30:97–104

Santos SM, Carvalho F, Mira A (2011) How long do the dead survive on the road? Carcass persistence probability and implications for road-kill monitoring surveys. PLoS One 6(9):e25383. https://doi.org/10.1371/journal.pone.0025383

Santos SM, Lourenço R, Mira A, Beja P (2013) Relative effects of road risk, habitat suitability, and connectivity on wildlife roadkills: the case of tawny owls (Strix aluco). PLoS One 8(11):e79967. https://doi.org/10.1371/journal.pone.0079967

Santos SM, Mira A, Salgueiro PA, Costa P, Medinas D, Beja P (2016a) Avian trait-mediated vulnerability to road traffic collisions. Biol Conserv 200:122–130

Santos RAL, Santos SM, Santos-Reis M, de Figueiredo AP, Bager A, Aguiar LM, Ascensão F (2016b) Carcass persistence and detectability: reducing the uncertainty surrounding wildlife-vehicle collision surveys. PLoS One 11:e0165608. https://doi.org/10.1371/journal.pone.0165608

Santos SM, Carvalho F, Mira A (2017) Current knowledge on wildlife mortality in railways. In: Borda-de-Água L, Barrientos R, Beja P, Pereira HM (eds) Railway ecology. Springer International Publishing, pp 11–22

Schmidt BR, Zumbach S (2008) Chapter 11: amphibian road mortality and how to prevent it: a review. In: Mitchell JC, jung Brown RE, Bartolomew B (eds) Herpetological conservation 3, urban herpetology. Society for the Study of Amphibians and Reptiles

Segev O, Blaustein L (2007) Priority effects of the early breeding fire salamander on the late breeding banded newt. Hydrobiologia 583: 275–283. https://doi.org/10.1007/s10750-006-0565-6

Seiler A, Helldin JO (2006) Mortality in wildlife due to transportation. In: Davenport J, Davenport JL (eds) The ecology of transportation: managing mobility for the environment. Springer, Dordrecht, pp 165–189

Síllero N (2008) Amphibian mortality levels on Spanish country roads: descriptive and spatial analysis. Amphibia-Reptilia 29(3):337–347. https://doi.org/10.1163/156853808785112066

Sinsch U (1997) Postmetamorphic dispersal and recruitment of first breeders in a *Bufo calamita* metapopulation. Oecologia 112(1):42–47. https://doi.org/10.1007/s004420050281

Sinsch U, Oromi N, Sanuy D (2007) Growth marks in natterjack toad (*Bufo calamita*) bones: histological correlates of hibernation and aestivation periods. Herpetol J 17(2):129–137

Skaug H, Fournier D, Bolker B, Magnusson A, Nielsen A (2016) Generalized linear mixed models using "AD Model Builder". R package version 0.8.3.3

Speybroeck J, Beukema W, Bok B, Van der Voort J (2016) Field guide to the amphibians and reptiles of Britain and Europe. British Wildlife Field Guides, Bloomsbury, London

Stolov HL (1965) Further investigations of a variation of geomagnetic activity with lunar phase. J Geophys Res 70:4921–4926. https://doi.org/10.1029/JZ070i019p04921

Tabachnick B, Fidell L (1996) Using multivariate statistics. Pearson Education

Teixeira FZ, Coelho AVP, Esperandio IB, Kindel A (2013) Vertebrate road mortality estimates: effects of sampling methods and carcass removal. Biol Conserv 157:317e323–317e323. https://doi.org/10.1016/j.biocon.2012.09.006

Todd BD, Winne CT (2006) Ontogenetic and interspecific variation in timing of movement and responses to climatic factors during migrations by pond-breeding amphibians. Can J Zool 84:715–722. https://doi.org/10.1139/z06-054

Underhill VA, Höbel G (2018) Moonlighting? - Consequences of lunar cues on anuran reproductive activity. Acta Oecol 87:20–28. https://doi.org/10.1016/j.actao.2018.02.001

van der Ree R, Smith DJ, Grilo C (2015) The ecological effects of linear infrastructure and traffic: challenges and opportunities of rapid global growth. In: van der Ree R, Smith DJ, Grilo C (eds) Handbook of road ecology. Wiley Blackwell, pp 1–9

Vasconcelos D, Calhoun AJK (2004) Movement patterns of adult and juvenile *Rana sylvatica* (LeConte) and *Ambystoma maculatum* (Shaw) in three restored seasonal pools in Maine. J Herpetol 38(4):551–561. https://doi.org/10.1670/157-03A

Vignoli L, Luiselli L (2013) Better in the dark: two Mediterranean amphibians synchronize reproduction with moonlit nights. Web Ecology 13:1–11. https://doi.org/10.5194/we-13-1-2013

Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) Mixed effects models and extensions in ecology with R. Springer, 575pp