Amphibia-Reptilia 29 (2008): 337-347

Amphibian mortality levels on Spanish country roads: descriptive and spatial analysis

Neftalí Sillero

Abstract. Road-kills are the greatest source of direct human-induced wildlife mortality, especially in amphibians. Country roads could act as the most important source of mortality when main roads act as strong barriers hampering the migration movements of some species. Mortality patterns of amphibians on country roads (1380 km) were studied in Salamanca (Spain) in order to quantify the mortality levels, to test the effects of sex and age factors on road-kills, to determine the spatial distribution patterns of road-kills, and to identify routes of migration through a friction map and hotspots of road-kills. From a total of 819 records of amphibians, 38.1% were road-killed and 61.9% were live. Fourteen amphibian species were recorded during the surveys (10 anurans and four urodeles). The species more affected by road-kills were the anurans *Bufo calamita*, *Pelobates cultripes* and *B. bufo* (38.5, 23.4 and 11.9%, respectively). Females had higher incidence of road-kills than males, due to the differential activity patterns of both sexes during the reproductive period. Adults were the most common age period and also the most road-killed. The spatial distribution patterns of live and road-killed records were clustered. On the sampled roads, there were 0.23 road-kills per kilometre and 52 hotspots of road-kills. The friction map showed that most of the road-killed and live specimens were located on migration routes crossing suitable habitats. Conservation measures should be implemented in these areas, as these mortality patterns may be causing significant negative impacts at the population level.

Keywords: Amphibians, road-kills, country roads, Spain, friction map, GIS.

Introduction

Roads have various effects on wildlife (Forman and Alexander, 1998; Ruediger, 1998; Spellerberg, 1998; Trombulak and Frissell, 2000): (1) mortality from road construction and vehicle collisions; (2) habitat loss and fragmentation by alteration of the physical environment; (3) subdivision of populations into smaller and more vulnerable units; (4) interference with animal behaviour, changing home ranges, movement, reproductive success, escape response, and physiological state; and (5) spread of exotic species promoted by habitat alteration, stressing native species, and providing movement corridors. All these effects can extend from 100 m to 1 km around the road itself (Forman and Deblinger, 2000). However, roads affect species populations and ecosystems in different ways depending on several factors (Trombulak and Frissel, 2000; Gibbs and Shriver, 2002; Jaeger et al., 2005), namely road avoidance behaviour of the animals (i.e. noise, road surface, and car avoidance), population sensitivity to road effects, road size, and traffic volume.

Roads are currently modifying the environment. In the United States, for example, 15 to 20% of the land surface is ecologically affected by roads (Forman and Alexander, 1998). In the United States and Europe, road-kills are the greatest source of direct human-induced terrestrial wildlife mortality, with significant impacts at the population level (Clarke et al., 1998; Kline and Swann, 1998; Hauer et al., 2002). Generally, road density decreases diversity and abundance of species (Bury et al., 1977; Reijnen et al., 1996; Rudolph et al., 1998; Haskell, 2000). Moreover, the effects of roads on species diversity could remain undetectable for decades after the road construction; eight years in the case of wetland birds, amphibians and reptiles (Findlay and Bourdages, 2000).

Amphibians in particular, due to their activity patterns, population structure, and preferred habitats, are strongly affected by traffic inten-

Centro de Investigação em Ciências Geo-Espaciais (CICGE), Universidade do Porto, Departamento de Matemática Aplicada, Rua do Campo Alegre, 687, 4169-007 Porto, Portugal
e-mail: neftali.pablos@fc.up.pt

sity and road density (Carr and Fahrig, 2001; Hels and Buchwald, 2001). Recent worldwide increases in traffic volumes probably contribute to the decline of amphibian populations, especially in highly populated areas (Fahrig et al., 1995). In fact, amphibians are the group more affected by road-kills (González-Prieto et al., 1993; Ashley and Robinson, 1996; Kline and Swann, 1998; Carretero and Rosell, 2000). However, almost all these studies focused only on the effects of highways (González-Prieto et al., 1993; Vogel and Puky, 1995; Ashley and Robinson, 1996; Carretero and Rosell, 2000; Forman and Deblinger, 2000; Kenneth et al., 2004), and only a few have focused on roads of lower categories, frequently treating them together with highways (Vos and Chardon, 1998; Orlowski, 2007; Santos et al., in press). Therefore, there are few data available on country road effects on amphibians.

Country roads could be the main source of wildlife mortality when main roads act as strong barriers hampering the migration movements of some species (Clarke et al., 1998; Rondonini and Doncaster, 2002). For amphibians, this fact could be especially important, as their small size often makes it impossible to cross the road verges (Marsh et al., 2005). Also, country roads could be biasing sex and age ratios in amphibians, if road effects act on a specific sex or age group, as described previously (Steen and Gibbs, 2004; Aresco, 2005; Gibbs and Steen, 2005). In such cases, population viability could be at risk. Conservation measures for mitigating these problems are restricted to the limited available knowledge on country road mortality patterns. However, any application of conservation measures relies not only on data resulting from descriptive analysis but also from spatial analysis. The distribution of road-kills is usually not random, because animals (and amphibians in particular) frequently cross roads using the same routes (Malo et al., 2004; Ramp et al., 2005; Seiler, 2005; Ramp et al., 2006). Thus, it is possible to analyse the distribution of road-kills, to identify the main migration routes, and then to define road-kill hotspots where conservation measures should be implemented.

The objective of this work was to clarify the mortality patterns of amphibians on country roads in the province of Salamanca (Spain). Firstly, mortality levels of amphibians were quantified by species, and the effects of sex and age on these patterns of mortality were evaluated. Secondly, the spatial distribution patterns of road-kills and respective hotspots were identified. Finally, the relationship between road-kills spatial distribution patterns and landscape connectivity around roads was determined using a friction map.

Materials and methods

Study area

The study was conducted in the province of Salamanca, Spain (fig. 1). Salamanca is essentially a plain (12 500 km^2) with an average altitude of 700 to 900 m a.s.l., disrupted to the south by the Central Mountain Range (2450 m) and to the north-west by the Duero River canyon (112 m). The climate is typically Mediterranean, with low average precipitation (400 mm), summer drought and cold temperatures in winter. These general characteristics of the climate are modified perceptibly by the relief to the south and north-west.

Species data

Road-kill species data was collected during 2000-2002 within the scope of other projects (Pleguezuelos et al., 2002; Sillero et al., 2005). The aim of these projects was to collect and analyse chorological data for amphibians and reptiles in the Iberian Peninsula. The priority was to sample the territory of Salamanca as inclusively as possible, and such roads were sampled only once. Records were collected for the northern, southern and eastern part of Salamanca (fig. 1).

Amphibians were surveyed from sunset to sunrise during 17 rainy nights in spring by car at a very low speed (20-30 km/h), while amphibian activity was detected. Amphibian's activity reduces drastically with temperatures lower than 6-8°C (Garcia-Paris et al., 2004). Rainy nights were considered as nights with rain, or nights with a very high air humidity (90% or more) and a temperature higher than 6-8°C after a day with rain. This method was selected because it allows for a large study area to be sampled, as it cannot be sampled by foot, and the method has been extensively used in previous studies (Fahrig et al., 1995; Kline and Swann, 1998; Carretero and Rosell, 2000; Llorente et al., 2005; Ramp et al., 2006; Orlowski, 2007; Santos et al., in press). In 2000, 4 transects were performed; 10 in 2001; and 3 in 2002. Salamanca is a region with low precipita-

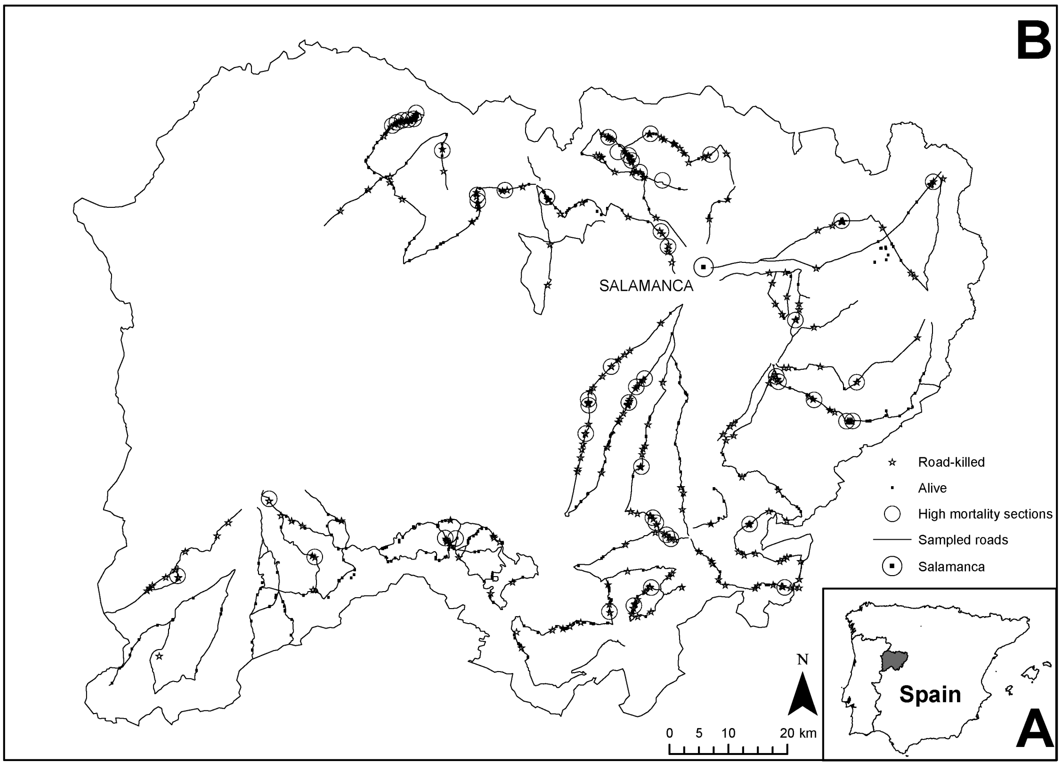

Figure 1. Map A: Location of the province of Salamanca, Spain (in grey colour). Map B: Locations of road-killed and live records (312 and 507, respectively) of amphibians species on the sampled roads. The 52 road sections of 1 km with the hotspots of road-kills are also showed.

tion (see Study Area section) and nights with the appropriate conditions for amphibians are not frequent. Because of this, surveys were performed on all possible rainy nights. The differences in the number of surveys per year were due to different weather conditions on the three years: 2001, for example, was an exceptionally rainy year with a higher number of rainy nights.

All surveys were carried out on roads classified as country roads with similar characteristics of road width (4-6 m) and substrate. There is no information about traffic intensity for these country roads in Salamanca (Martín-Almeida and González-Arias, 2007). Each individual found on the road was recorded as one data point. When specimens were collected within a range smaller than 1 m, they were pooled into one single data point given that this range is inferior to the GPS resolution. Geographic coordinates were collected by a Corvallis Microtechnology MARCH-II GPS. For each data point, the species, sex, age and state (road-killed or alive) of the specimen was recorded. Data points were exported to ArcGIS 9.2 GIS (ESRI, 2000) in shapefile format. All points were recorded using the UTM coordinate system and the European Datum of 1950 for Spain and Portugal.

Spatial distribution of species records

The Nearest Neighbour Index (hereafter NNI; Clark and Evans, 1954) was used to determine if species records on roads were clustered or not. The NNI is expressed as the ratio of the observed distance between points and the expected distance, i.e. the average distance between neighbours in a hypothetical random distribution. If the index is less than 1, records are clustered; if the index is greater than 1, records are dispersed. The Z score value measures the statistical significance of the NNI. The null hypothesis states that the points are randomly distributed. The NNI was calculated separately for road-killed and live specimens. This allowed examining whether the spatial distribution of both groups qualitatively differed or not. Both groups can have a different distribution pattern when road-kills are determined by some factors such as traffic intensity. In that case, points with road-kills could be separated spatially from points with live specimens.

If the NNI showed clustering for both groups, then road sections with a high number of road-kills (hotspots) could be defined following the method described in Malo et al. (2004), which detects spatial clusters of road-kill records. Species records were grouped in 1380 road sections of 1 km, using the ArcGIS command "Divide". The number of GPS points belonging to a particular road section was counted with the command "Count points" using Hawth's Analysis Tools (Beyer, 2004). The spatial pattern of road-kills was compared with a random model where the likelihood of road-kills for each road section follows a Poisson distrib-

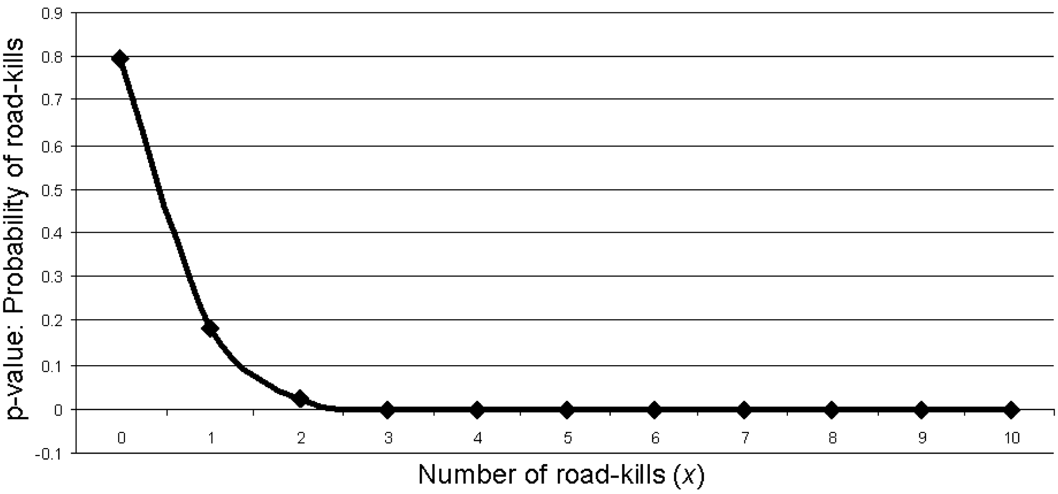

Figure 2. Graph showing the probability of any road section having x number of road-kills versus the number of road-kills (x). The sections situated in the zone of the graph curve that reaches the asymptote were considered as hotspots of road-kills. For more details, see Methods section and Malo et al. (2004).

ution. Using this hypothesis and given the mean number of road-kills per road section (λ), the probability of any road section having x number of road-kills, is:

[1] $$p(x) = \lambda^x/(x!e^\lambda).$$

In a random distribution of road-killed specimens, the hotspots of road-kills should be distributed at random across the roads, and their aggregation would be extremely unlikely. The clusters of hotspots of road-kills were identified using a graph with the p-values versus the number of road-kills (x). These sections were located in the graph zone were the curve reaches the asymptote (fig. 2).

Spatial analysis with friction maps

If the spatial distribution of amphibians on roads (live or road-killed) is clustered, then they probably use specific routes to cross roads. These crossing routes are determined by many factors, namely, habitat composition or structure and road texture, among others. Small and linear habitats can enhance or limit dispersal movements, depending if they act as corridors or barriers (Ray et al., 2002). Amphibians usually move through routes with the lowest energy costs for dispersal movements. Then, crossing routes can be identified using a permeability matrix or friction map, where permeability is defined as the energy cost of moving from one place to another (Ray et al., 2002). Therefore, the friction map is composed by a matrix of suitable and unsuitable habitat patches, where each cell is weighted by a value that represents its permeability.

In this study, the permeability of each cell was defined using the land use and the distance to water surfaces (rivers and ponds) as well as urban settlements. These distances were calculated with three buffers of 100, 300 and 500 m to water surfaces, and four buffers of 100, 300, 500 and 1000 m to urban settlements. A permeability weight (from −400 to 400) was assigned to each distance or land cover type (table 1), following published literature (Garcia-Paris et al., 2004) and fieldwork experience. The highest permeability was assigned to the most favourable variable value for the species movements. The final friction map was the addition of all variables. Pixel size was set to 100 m, as this was the minimal spatial resolution available for the variables used in the friction map calculations. The correspondence between each friction map value and each point of road-kills, live specimens and road-kill hotspots was determined by overlapping using the ArcGIS tool "Extract values to points". Some points of road-killed (9) and live specimens (21) were not included in the friction map analysis because they were recorded on roads for which there was no digital data.

Results

Amphibian mortality levels on country roads

A total of 819 amphibians were collected during the three years (year 2000: 134; 2001: 534; and 2002: 151; table 1 and fig. 1). From these, 312 (38.1%) were road-killed and 507 (61.9%) were live. Analysing each year separately, the number of road-kills was always lower than the number of live specimens: year 2000, 32 road kills (102 live); 2001, 216 (318); and 2002, 64 (87). The number of road-kills was higher in only six transects (table 2). From the 1380 road sections, 219 presented only road-killed specimens, 299

Table 1. Variables used in the friction map calculation. Each value was weighted depending on the assumed permeability. For each variable, permeability values were estimated from literature and fieldwork experience, varying from −400 to 400. The highest permeability value was attributed to the variable most favourable for the movements of the species. The pixel size of the friction map was 100 m. ESRI: Environmental Systems Research Institute, Inc. INE: National Institute of Statistics.

Variable	Value	Weight	Origin
Land use	Artificial	−400	CORINE 2005
	Arable lands	−300	
	Pastures	−200	
	Pines	−100	
	Natural agriculture	100	
	Agro-forestry areas	200	
	Shrubs	300	
	Woods	400	
Distance to ponds Distance to rivers	0-100 m	300	Fieldwork and ESRI data
	100-300 m	200	
	300-500 m	100	
	>500 m	0	
Distance to human settlements	0-100 m	−400	INE
	100-300 m	−300	
	300-500 m	−200	
	500-1000 m	−100	
	>1000 m	0	

only live specimens, and 85 both road-killed and live specimens. From the 1380 road sections, there were 433 road sections with data (31.2%) and 947 without data (68.6%).

Fourteen amphibian species were collected during the surveys (10 anurans and four urodeles: table 3). The species with the highest number of road-kills were the anurans *Bufo calamita*, *Pelobates cultripes* and *B. bufo* (38.5, 23.4, 11.9%, respectively; table 3). The sex of the amphibians could be determined in 561 specimens (table 2): 154 (27.5%) were males and 407 (72.5%) were females (χ^2 with Yates' correction = 9.464, *df* = 1, $P = 0.002$). In males, 56 (36.4%) were road-killed and 98 (63.6%) were live, while in females, 136 (33.4%) were road-killed and 271 (66.7%), live. The age of the amphibians could be determined in all specimens: 117 (14.3%) were juveniles, 68 (8.3%) sub-adults and 634 (77.4%) adults ($\chi^2 = 28.221$, *df* = 2, $P < 0.001$). In juveniles, 28 (23.9%) were road-killed and 89 (76.1%) were live. In sub-adults, 12 (17.6%) were road-killed and 56 (82.4%), live. In adults,

Table 2. Number of road-killed and live specimens for each night transect in the province of Salamanca (Spain), separated by age and sex. The length of each transect is indicated in kilometres. The transects 5, 6, 8, 10, 15 and 16 (in bold letters) had a larger amount of road-killed specimens than live ones.

	Road-kills						Live								
Survey	Total	Ad	Sub	Juv	Males	Females	Total	Ad	Sub	Juv	Males	Females	Total	Length (km)	Year
1	11	11	0	0	3	5	15	15	0	0		3	26	115.2	2000
2	3	3	0	0		2	28	26	2	0	9	18	31	90.4	2000
3	6	5	1	0	2	4	41	33	8	0	7	29	47	93.7	2000
4	12	12	0	0	3	2	18	16	1	1	4	4	30	60.1	2000
5	41	41	0	0	3	15	26	26	0	0	6	18	67	87.9	2001
6	24	23	1	0		12	22	22	0	0	6	15	46	86.6	2001
7	19	19	0	0	4	7	22	22	0	0	5	15	41	82.7	2001
8	45	45	0	0	16	25	35	31	3	1	14	18	80	91.9	2001
9	17	17	0	0	9	6	18	16	1	1	8	9	35	104.3	2001
10	11	10	1	0	2	7	8	8	0	0	3	5	19	65.1	2001
11	27	4	4	19		1	73	11	11	51	2	17	100	29.1	2001
12	16	10	1	5	3	7	46	26	2	18	10	18	62	91.9	2001
13	14	13	0	1	3	5	29	27	0	2	7	20	43	96.7	2001
14	2	2	0	0	1		39	23	14	2	2	26	41	122.0	2001
15	34	34	0	0	5	18	29	25	3	1	8	17	63	129.4	2002
16	20	20	0	0	1	15	17	15	0	2	5	10	37	71.4	2002
17	10	3	4	3	1	5	41	20	11	10	2	29	51	74.0	2002
Total	**312**	**272**	**12**	**28**	**56**	**136**	**507**	**362**	**56**	**89**	**98**	**271**	**819**	**1492.4**	

Table 3. Number of road-killed and live specimens for each amphibian species recorded in the province of Salamanca, Spain. The species with a larger amount of road-killed specimens than live ones are indicated in bold letters. The names of species follow the last taxonomical review for the Spanish herpetofauna (Comisión de Taxonomía de la AHE 2005).

Species	Road-killed	Live	Total
Pleurodeles waltl	27	11	38
Salamandra salamandra	5	10	15
Lissotriton boscai	0	1	1
Triturus marmoratus	9	20	29
Alytes cisternasii	0	2	2
Alytes obstetricans	5	5	10
Discoglossus galganoi	1	2	3
Pelobates cultripes	73	166	239
Bufo bufo	37	83	120
Bufo calamita	120	156	276
Hyla arborea	16	11	27
Hyla meridionalis	2	0	2
Rana iberica	0	2	2
Rana perezi	17	38	55
Total	**312**	**517**	**819**

272 (42.9%) were road-killed and 362 (57.1%), live.

Spatial distribution patterns of road-kills on country roads

The NNI for the road-killed group was 0.37 ($Z = -21.56$; $P < 0.01$) and for the live group, 0.29 ($Z = -30.72$; $P < 0.01$). In both cases, the NNI showed a clustered distribution of records. The number of road-kills per road section (λ) was 0.23 ± 0.71 (range: 0-10). Consequently, 98% of road sections should have zero or one collisions (fig. 2), meaning that the accumulation of two or more collisions per section cannot be attributed to coincidence. Therefore, sections with two or more collisions were considered as road-kill hotspots, which resulted in the detection of 52 such sections (fig. 1). The abundance kilometre index (IKA) for road specimens (0.23) was equal to λ.

Friction map spatial analysis

As the distribution of road-killed and live specimens were clustered, it was possible to identify crossing routes on roads using a friction map. Permeability values of the friction map were distributed between -800 and 1300. Most of the road-killed (205 from 308; 66.6%) and live (356 from 486; 73.3%) specimens were located in cells with a permeability values higher than 0 (suitable habitats), corresponding to 57.2% of the friction map total surface (fig. 3). On the other hand, 86 road-killed (27.9%) and 114 live (23.5%) specimens were located in cells with permeability values between -800 and -100 (unsuitable habitats: 37.8% of the total surface). There were 17 (5.5%) and 16 (3.3%) road-killed and live specimens, respectively, in habitats without effect on dispersal capacity (cells with a permeability value equal to 0: 5% of the total surface). Finally, 34 (from 52; 65.4%) road-kill hotspots were located on cells with a permeability value higher than 0 (fig. 3).

Discussion

Amphibian mortality levels on country roads

All amphibian species present in Salamanca (14 species: see Merchán et al., 2004; Sillero et al., 2005) were also represented in the road surveys (14 species). Thus, all amphibian species were liable to be road-killed although in different numbers. The three species with the highest number of collisions were *Bufo calamita* (120), *Pelobates cultripes* (73) and *B. bufo* (37) (table 3). Others like *Discoglossus galganoi* (1), *Hyla meridionalis* (2), *Salamandra salamandra* (5), *Triturus marmoratus* (9), and *Alytes obstetricans* (5), had a very low number of collisions but also a low number of live records (table 3). Similarly, road-kills of *Rana iberica* and *Lissotriton boscai* were not recorded. These differences could probably be due to species density and behaviour (González-Prieto et al., 1993), and also to the fast disappearance of the carcasses because of the skin structure (Hels and Buchwald, 2001; Llorente et al., 2005). Sampling by car allows detecting a larger number of carcasses in less time than alternative methods due to a higher survey speed. Therefore, the

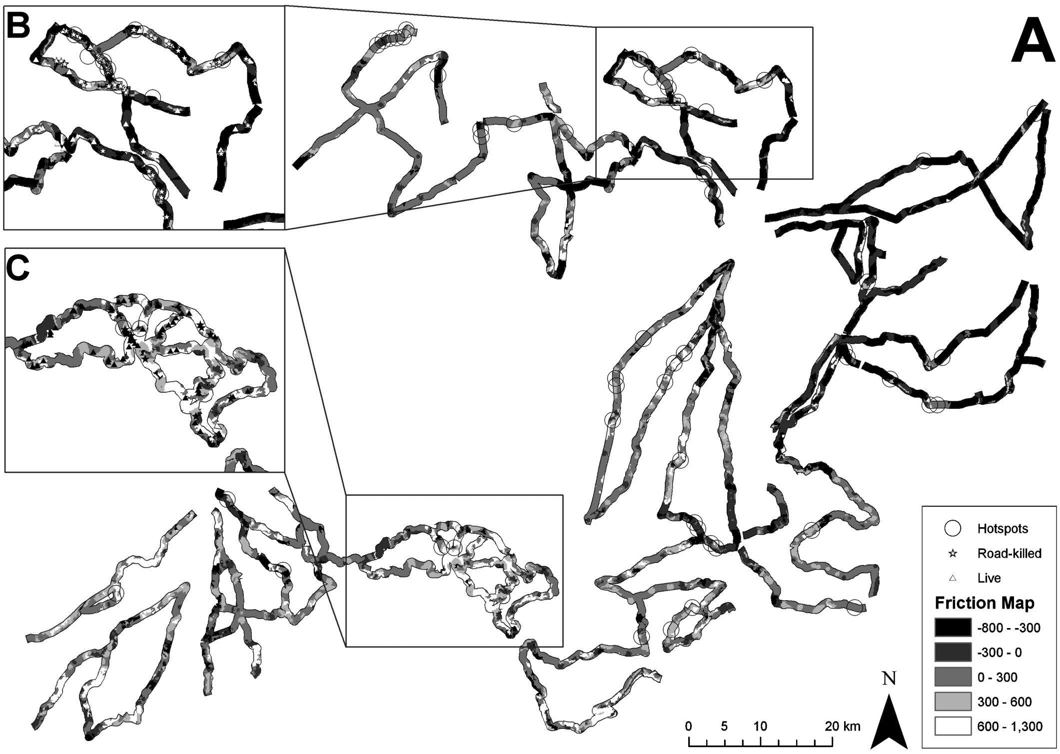

Figure 3. Friction map for analysing the permeability of habitats around country roads (map A). Zones of low and high permeability are shown in more detail in maps B and C, respectively. The points with road-killed (stars) and live specimens (triangles) are represented in maps B and C in white and black colours. The hotspots of road-kills are represented in the three maps with an empty circle.

time for removing the carcasses from roads is lower, because all road-kills are detected a short time after killing. Other specimens disappear so quickly that they are not detected, but such cases cannot be recorded by any alternative sampling method.

Discoglossus galganoi and *H. meridionalis* are rare in the province of Salamanca (Merchán et al., 2004; Sillero et al., 2005). *A. cysternasii* and *A. obstetricans* are small species with a secretive life: migrations using roads could be less frequent in these species. *B. calamita*, *P. cultripes* and *B. bufo*, together with *R. perezi*, are the most abundant species in Salamanca (Sillero et al., 2005). However, *R. perezi* was a species with a relatively low number of road-killed specimens (17 records), probably because it lives mainly in ponds and rivers and it does not migrate frequently to other locations (García-París et al., 2004). The low number of *L. boscai*'s records (both road-killed and live) could be due to its low mobility but also to its small size and discrete colouration. Both of these species could hibernate in their home river and migrate only through the hidrographic network, thus avoiding roads. On the other hand, *B. calamita*, *P. cultripes* and *B. bufo* display a more terrestrial behaviour, thus using roads more frequently. Moreover, *B. calamita* and *P. cultripes* reproduce in temporal ponds (García-París et al., 2004), like road ditches, which are often flooded in rainy nights (pers. obs.). In general, anurans showed a higher number of road-killed specimens than urodeles, which are more sedentary and less terrestrial active (Herrmann et al., 2005). In Catalonia (Spain), the species most road-killed were the most terrestrial amphibians (*S. salamandra*, *B. bufo* and *B. calamita*; Llorente et al., 2005).

Female amphibians were the most frequently recorded sex on Salamanca roads, either live or road-killed, probably due to different activity patterns between both sexes during the reproductive period. However, the proportion of road-killed/live specimens within each sex was similar (males: 37.6% road-killed, 62.4% live; females: 33.6% road-killed, 66.4% live). Males move to breeding locations earlier than females during the first rainy nights, and remain there for an extended period (García-París et al., 2004). On the contrary, females travel during rainy nights to those same breeding locations and back, resulting in a higher presence of females on roads and consequently in a higher risk of being road-killed. In fact, females cover larger distances than males during the breeding season (up to 2600 m/day for females, and 1100 m for males: Salvador and García-París, 2001).

Adults were the most recorded age group in Salamanca roads and also the most road-killed. However, if the transects are performed in the end of the metamorphic season (late summer-early autumn in Salamanca), juveniles moving from hatching to feeding locations may be recorded in higher number than adults. This was the case for transect 11 (table 2), which had the highest number of amphibians (165) on the lowest transect distance (29.1 km), due to a massive early juvenile migration being recorded. A similar situation was found in the Lake Erie, where nearly 90% of 32 482 road-killed specimens were new-born Leopard Frogs (*Rana pipiens*; Ashley and Robinson, 1996).

Spatial distribution patterns of road-kills on country roads

In total, 38% of amphibians were road-killed, corresponding to a mean of 0.23 road-kills/km. Distribution patterns of live and road-killed records were not uniform, being significantly spatially clustered as shown by the Nearest Neighbour Index (NNI). Therefore, 52 sections were identified in Salamanca as road-kill hotspots, defined as clusters of two or more road-kills. These points are distributed mainly on roads connecting the main villages to the province capital city, Salamanca (fig. 1). The north-eastern and south-western parts of the province had a low number of road-kill hotspots, probably because they have a lower human population density (Fahrig et al., 1995). An exception is transect 11 (fig. 1), due to the high number of juveniles recorded (table 2). Thus, road-kill hotspots are probably located in roads with high traffic intensity as suggested in previous works (Fahrig et al., 1995; Reijnen et al., 1996; Clarke et al., 1998; Jaeger et al., 2005).

Globally, the friction map identified two different areas: the north-eastern part essentially characterized by unsuitable habitats, and the remaining areas characterized by suitable habitats. The former area is composed mainly by crops, while the latter is composed by broad-leaved forests (and their vegetation series of substitution) and agro-forestry systems. Most of the points with road-killed and live specimens, and in particular the road-kill hotspots, were located in cells with a high value of permeability, i.e. in suitable habitat patches. Through these high quality habitat areas, amphibians establish specific migratory routes used for travelling from the feeding to the breeding locations, and vice versa (Santos et al., in press). This fact is an important conservation problem, because suitable habitats do not guarantee a good protection for amphibians, as they are road-killed mainly there. It is wrong to assume that a suitable habitat is enough protection for amphibians. Therefore, conservation measures to avoid road-kills in country roads should be applied preferentially in the most suitable habitats. Moreover, if amphibians always use the same migration routes, it could be possible to model the spatial distribution patterns of road-kills (Malo et al., 2004; Ramp et al., 2005; Seiler, 2005; Ramp et al., 2006). Modelling techniques could improve the application of conservation measures.

Comparing the number of road-kills and road-kill hotspots between different studies is very difficult due to differences in study areas, species communities, road types, traffic intensity and species surveyed. In Catalonia, for example, country roads showed 1.3 road-kills/km of *B. bufo* (Santos et al., in press), while a much higher value was described in Poland, with 25.9 road-kills/km of the same species (Orlowski, 2007). The results may also vary if massive migrations of juveniles are detected. However, it is very rare to find such situations in Salamanca, as autumn in the region is usually very short. Therefore, the results of this paper should be considered only for adults.

Final remarks

The number of road-kills in Salamanca indicate that country roads may be an important source of amphibian mortality (Orlowski, 2007; Santos et al., in press), especially if main roads act as strong barriers, by hampering access to the road (Mash et al., 2005). In such cases, amphibian populations can suffer important negative impacts (decrease of population size, increase of genetic isolation, sex and age ratio bias), especially because conservation measures are not usually implemented on country roads. This fact cannot be ignored and solutions have to be promoted in these roads. More studies like this are necessary in order to identify road points with high collision rates. Obviously, to define these points and implement solutions, a study of occasional records is not enough. To achieve better results, the same roads should be sampled for several years to eliminate variations between years.

The intention of this paper is raising a warning about road-kills on country roads, as the number of studies on their effects specifically dealing with the herpetofauna is still insufficient. Different road-kill sampling methodologies could lead to different results. Sampling roads by foot allows finding all live and dead specimens crossing the road at that time (González-Prieto et al., 1993; Ashley and Robinson, 1996; Hels and Buchwald, 2001), but this method cannot be applied to regional or national conservation plans. In large study areas, car surveys are the only possible solution (e.g. Llorente et al., 2005). The number of live and road-killed specimens detected by car is probably lower than by foot, but this fact does not invalidate the results. Since the method applies equally well everywhere, and since the number of live and dead amphibians does not need to be and does not pretend to be exhaustive, car sampling is considered adequate. Finally, this type of study needs to become available to public administrations notifying them about the high number of amphibian road-kills and the locations where they occur more often.

Acknowledgements. Carlos, Josué, María, Tomé, Jabato, Isabel, Peludo and Mario help me in night fieldwork. P. Sá-Sousa and F. Ascensão gave useful ideas and information. S. Martín Alfageme and the Servicio Transfronterizo de Información Geográfica from University of Salamanca provided the GPS. A. Kaliontzopoulou improved an early manuscript version. A. Moura, M.A. Carretero and J. Harris checked the English. Thanks to Tove Hels and an anonymous referee for their useful suggestions. I was supported in the two first years by a Doctoral Grant from Junta de Castilla y León (Spain). Currently, I am supported by a Post-Doctoral grant (SFRH/BPD/26666/2006) from Fundação para a Ciência e Tecnologia (Portugal).

References

Aresco, M.J. (2005): The effect of sex-specific terrestrial movements and roads on the sex ratio of freshwater turtles. Biol. Cons. **123**: 37-44.

Ashley, E.P., Robinson, J.T. (1996): Road mortality of amphibians, reptiles and other wildlife on the Long Point Causeway, Lake Erie, Ontario. Can. Field Nat. **110**: 403-412.

Beyer, H. (2004): Hawth's Analysis Tools, 3.4. Edmonton, Spatial Information Systems Consultant.

Bury, R.B., Luckenbach, R.A., Busack, S.D. (1977): Effects of off-road vehicles on vertebrates in the California Desert. Wildlife Research Report (United States Department of the Interior Fish and Wildlife Service): 1-23.

Carr, L.W., Fahrig, L. (2001): Effect of road traffic on two amphibian species of differing vagility. Cons. Biol. **15**: 1071-1078.

Carretero, M.A., Rosell, C. (2000): Incidencia del atropello de anfibios, reptiles y otros vertebrados en un tramo de carretera de construcción reciente. Bol. Assoc. Herp. Esp. **11**: 39-43.

Clark, P.J., Evans, F.C. (1954): Distance to nearest neighbor as a measure of spatial relationships in populations. Ecology **35**: 445-453.

Clarke, G.P., White, P.C.L., Harris, S. (1998): Effects of roads on badger *Meles meles* populations in south-west England. Biol. Cons. **86**: 117-124.

ESRI (2000): ArcGIS-ArcInfo 9. ESRI. Environmental Systems Research Institute, Inc., Redlands, California.

Fahrig, L., Pedlar, J.H., Pope, S.E., Taylor, P.D., Wegner, J.F. (1995): Effect of road traffic on amphibian density. Biol. Cons. **73**: 177-182.

Findlay, C.S., Bourdages, J. (2000): Response time of wetland biodiversity to road construction on adjacent lands. Cons. Biol. **14**: 86-94.

Forman, R.T.T., Alexander, L.E. (1998): Roads and their major ecological effects. Ann. Rev. Ecol. Syst. **29**: 207-231.

Forman, R.T.T., Deblinger, R.D. (2000): The ecological road-effect zone of a Massachusetts (U.S.A.) suburban highway. Cons. Biol. **14**: 36-46.

Garcia-Paris, M., Montori, A., Herrero, P. (2004): Amphibia, Lissamphibia. Fauna Ibérica, Vol. 24., Museo Nacional de Ciencias Naturales and Consejo Superior de Investigaciones Científicas, Madrid.

Gibbs, J.P., Shriver, W.G. (2002): Estimating the effects of road mortality on turtle populations. Cons. Biol. **16**: 1647-1652.

Gibbs, J.P., Steen, D.A. (2005): Trends in sex ratios of turtles in the United States: implications of road mortality. Cons. Biol. **19**: 552-556.

González-Prieto, S., Villarino, A., Frean, M.M. (1993): Mortalidad de vertebrados por atropello en una carretera nacional del NO de España. Ecología **7**: 375-389.

Haskell, D.G. (2000): Effects of forest roads on macroinvertebrate soil fauna of the Southern Appalachian mountains. Cons. Biol. **14**: 57-63.

Hauer, S., Ansorge, H., Zinke, O. (2002): Mortality patterns of otters (*Lutra lutra*) from eastern Germany. J. Zool. **256**: 361-368.

Hels, T., Buchwald, E. (2001): The effect of road kills on amphibian populations. Biol. Cons. **99**: 331-340.

Herrmann, M.J., Babbitt, K.J., Baber, M.J., Congalton, R.G. (2005): Effects of landscape characteristic on amphibian distribution in a forest-dominated landscape. Biol. Cons. **123**: 139-149.

Jaeger, J.A.G., Bowman, J., Brennan, J., Fahrig, L., Bert, D., Bouchard, J., Charbonneau, N., Frank, K., Gruber, B., von Toschanowitz, K.T. (2005): Predicting when animal populations are at risk from roads: an interactive model of road avoidance behaviour. Ecol. Mod. **185**: 329-348.

Kenneth Dodd, Jr. C., Barichivich, W.J., Smith, L.L. (2004): Effectiveness of a barrier wall and culverts in reducing wildlife mortality on a heavily travelled highway in Florida. Biol. Cons. **118**: 619-631.

Kline, N.C., Swann, D.E. (1998): Quantifying wildlife road mortality Saguaro National Park. ICOWET **9**: 23-318.

Llorente, G.A., Garriga, N., Montori, A., Richter-Boix, A., Santos, X. (2005): Incidencia de les carreteres sobre els amfibis i els reptils de Catalunya; Memoria final. Barcelona, Universitat de Barcelona.

Malo, J.E., Suáez, F., Díez, A. (2004): Can we mitigate animal-vehicle accidents using predictive models? J. App. Ecol. **41**: 701-710.

Marsh, D.M., Milam, G.S., Gorham, N.P., Beckman, N.G. (2005): Forest roads as partial barriers to terrestrial salamander movement. Cons. Biol. **19**: 2004-2008.

Martín-Almeida, J., González-Arias, J. (2007): Plan Regional Sectorial de Carreteras 2002-2007. España, Junta de Castilla y León, Consejería de Fomento, Dirección General de Carreteras e Infraestructuras.

Merchán, T., Sillero, N., Lizana, M., Fontana, F. (2004): Nuevos hallazgos de la ranita meridional (*Hyla meridionalis* Boettger, 1874) en la provincia de Salamanca. Bol. Assoc. Herp. Esp. **15**: 81-85.

Orlowski, G. (2007): Spatial distribution and seasonal pattern in road mortality of the common toad *Bufo bufo* in an agricultural landscape of south-western Poland. Amphibia-Reptilia **28**: 25-31.

Pleguezuelos, J.M., Márquez, R., Lizana, M. (2002): Atlas de distribución y Libro Rojo de los Anfibios y Reptiles de España, 2ª impresión. Madrid, Dirección General de Conservación de la Naturaleza-Asociación Herpetológica Española.

Ramp, D., Caldwell, J., Edwards, K.A., Warton, D., Croft, D.B. (2005): Modelling of wildlife fatality hotspots along the Snowy Mountain Highway in New South Wales, Australia. Biol. Cons. **126**: 474-490.

Ramp, D., Wilson, V.K., Croft, D.B. (2006): Assessing the impacts of roads in peri-urban reserves: road-based fatalities and road usage by wildlife in the Royal National Park, New South Wales, Australia. Biol. Cons. **129**: 348-359.

Ray, N., Lehmann, A., Joly, P. (2002): Modeling spatial distribution of amphibian populations: a GIS approach based on habitat matrix permeability. Biodiv. Cons. **11**: 2143-2164.

Reijnen, R., Foppen, R., Meeuwsen, H. (1996): The effects of traffic on the density of breeding birds in Dutch agricultural grasslands. Biol. Cons. **75**: 255-260.

Rondinini, C., Doncaster, C.P. (2002): Roads as barriers to movement for hedgehogs. Funct. Ecol. **16**: 504-509.

Rudolph, D.C., Burgdorf, S.J., Conner, R.N., Dickson, J.G. (1998): The impact of roads on the Timber Rattlesnakes, (*Crotalus horridus*), in Eastern Texas. ICOWET **9**: 236-240.

Ruediger, B. (1998): Rare carnivores and highways: moving into the 21st century. ICOWET **9**: 10-16.

Salvador, A., Garcia-Paris, M. (2001): Anfibios Españoles. Talavera de la Reina, Canseco Editores, S.L.

Santos, X., Llorente, G.A., Montori, A., Carretero, M.A., Franch, M., Garriga, N., Richter-Boix, A. (in press): Evaluating factors affecting amphibian mortality on roads: the case of the Common Toad, *Bufo bufo* near a breeding place. An. Biodiv. Cons. **30** (1): 97-104.

Seiler, A. (2005): Predicting locations of moose-vehicle collisions in Sweden. J. App. Ecol. **42**: 371-382.

Sillero, N., Celaya, L., Martín-Alfageme, S. (2005): Using GIS to Make An Atlas: A Proposal to Collect, Store, Map and Analyse Chorological Data for Herpetofauna. Revista Española de Herpetología **19**: 87-101.

Spellerberg, I.F. (1998): Ecological effects of roads and traffic: a literature review. Glob. Ecol. Biog. **7**: 317-333.

Steen, D.A., Gibbs, J.P. (2004): Effects of roads on the structure of freshwater turtle populations. Cons. Biol. **18**: 1143-1148.

Trombulak, S.C., Frissell, C.A. (2000): Review of ecological effects of roads on terrestrial and aquatic communities. Cons. Biol. **14**: 18-30.

Vogel, Z., Puky, M. (1995): A fast environmental impact assessment method for the evaluation of road construction effects on amphibian communities. In: Scientia Herpetologica, p. 349-351. Llorente et al., Eds, Barcelona.

Vos, C.C., Chardon, J.P. (1998): Effects of habitat fragmentation and road density on the distribution pattern of the moor frog *Rana arvalis*. J. App. Ecol. **35**: 44-56.

Received: September 6, 2007. Accepted: February 12, 2008.