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Research article

Is vertebrate mortality correlated to potential permeability by underpasses along low-traffic roads?

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ABSTRACT

Road permeability to animal movements depends among several factors on structures which, integrated in the road design, operate as safe conducts to mitigate vehicle collision and barrier effects. There is abundant evidence that wildlife makes use of such structures as safe passages to cross roads. We analyzed the spatial relationship between road drainage elements (N = 253; mostly culverts) as potential faunal underpasses, and mortality due to vehicle collisions in two seasons and on four relatively low-traffic roads (< 5000 cars/day) traversing oak rangelands of western Andalusia (S Spain). Focusing on amphibians, reptiles and mammals, we recorded and located casualties (N = 238 individuals, 35 species) along these roads, identifying and characterizing all potential underpasses. Overall frequencies of casualties and spatial distribution were highly variable both within and among these roads. We obtained an estimation of potential permeability for the different roads. We detected, located and described a wide supply and a very variable pattern of drainage culverts and other underpasses, with differences among roads in passage attributes potentially affecting permeability for wildlife, such as spatial arrangement, number, density (frequency or concentration of passages) and dimensions. We used Mantel tests to assess spatial congruence of passages and road-killed animals. We applied generalized linear mixed models fitted by maximum likelihood through Akaike Information Criterion to explain the variation in the distance of the 238 casualties to the nearest underpasses, with road transect and season as random factors, and traffic intensity, speed and vertebrate class as fixed effects. Both road-killed animals and underpass distribution followed aggregated patterns, and casualties were not significantly related to underpasses along any of the 4 roads. There were no differences in distance of casualties to the nearest underpass for the three vertebrate classes. Although existing underpasses were abundant, we could not correlate potential permeability with reduced mortality along these roads, and other factors potentially affecting roadkill aggregations should be evaluated along with permeability assessment. Mitigation of road-caused mortality can still be greatly improved for these roads, through measures of reconditioning and proper management of existing underpasses, aiming to maximize road permeability and reducing major impacts upon animal populations of Andalusian rangelands.

1. Introduction

The road network has been designed by humans to connect previously separated areas, increasing the ability of humans to reach formerly remote ecosystems and the capacity to transform the occupied environments. One of the most immediate effects of roads is ecosystem fragmentation, involving ecosystem area reduction, loss or alteration of key processes, and populations loss (and gains) of species leading to altered composition and dynamics (Forman et al., 2002; Coffin, 2007). Amongst these issues, road permeability for wildlife is key in the management of road fragmentation (Bissonette and Adair, 2008; Loro et al., 2014, 2015; Rytwinski et al., 2015).

The Iberian Peninsula is one of the areas of Europe with greater biodiversity, being included in a global biodiversity hotspot. However, proliferation of transport structures and diffuse urban expansion favored by it are accelerating biodiversity loss at local and regional

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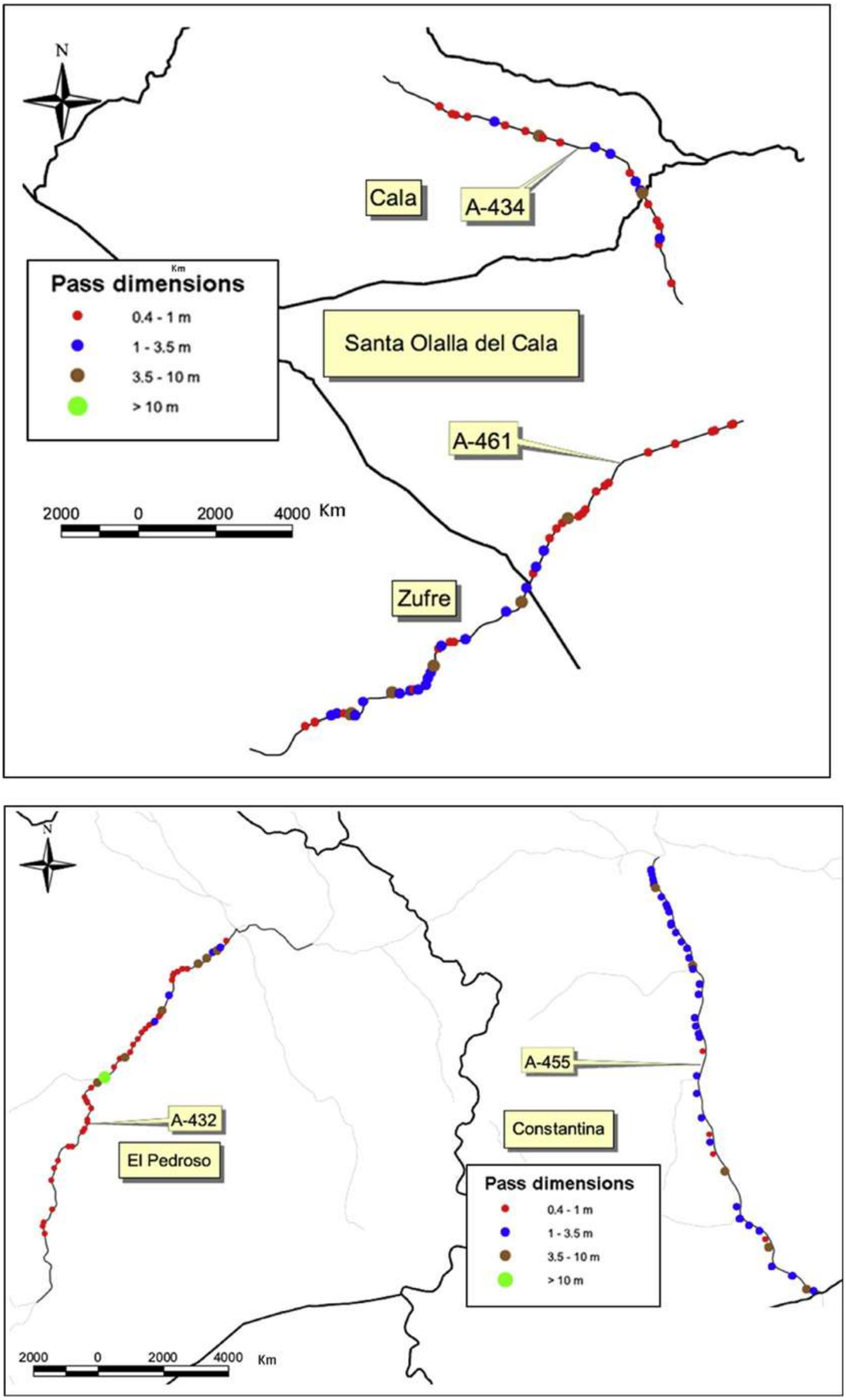

Fig. 1. Location, distribution and attributes of drainage structures (potentially used as faunal underpasses) in the four study roads.

scales. Planning and construction of road corridors actually confronts a saturation of transport networks, especially in central and southern Europe (EuroNatur, 2010). Species with exclusive habitat demands, marked seasonality responses, or contracting range or population size are the most vulnerable (Rodríguez and Delibes, 2004; Grilo et al., 2009; Karlson and Mörtberg, 2015).

Mitigation measures for road fragmentation include the construction of wildlife-adapted faunal conducts to provide animals with alternative routes which a priori may prevent or reduce the risk of collision with vehicles. In road schemes of most countries, proper wildlife under or/and overpasses have not been introduced in the planning phase of the projects until very recently. However, from the design desk, most roads incorporate transverse drainage elements (i.e. culverts) designed to serve brooks and rivers and to provide evacuation for surface runoff. Such structures are opportunistically used by many animals mostly as conducts (amphibians, reptiles, cursorial birds and small to large mammals), although they were not originally designed for animal crossing purposes (Yanes et al., 1995; Rodríguez et al., 1996; van der Ree et al., 2015).

Animal movements across the road barrier is partly verified through crossing structures designed or not to enhance connectivity (Alexander et al., 2005; see Berthinussen and Altringham, 2012 for bats). Most transversal road perforations with value as wildlife passes are water drainage works and easements or rights-of-way for vehicles and cattle (Clevenger and Waltho, 2000; Forman et al., 2002). Drainage conducts serving hydrographic basins are in fact used by wildlife, and their design can be enhanced to maximize efficiency as faunal passes, thus optimizing road permeability (Clevenger and Waltho, 2005; EuroNatur, 2010; Ministry of Agriculture, Foodand the Environment, 2016).

Both explicitly-devised, integrated wildlife passes and runoff culverts unplanned for wildlife are used by animals to cross roads. Most of the existing road-perforating elements are in fact under-rather than overpasses (i.e. ecoducts), since the latter are more expensive and difficult to build. Underpasses of different dimensions (diameter, length) and design (i.e. section, faunal adaptions) can contribute with contrasting efficiency to the road "permeabilization" process for animals (Forman et al., 2002; Puig et al., 2012). It is generally assumed that the longer and narrower the underpass, the harder to cross for most species. However, there is little empirical evidence for such extreme. The frequency of use and crossing efficiency of underpasses and overpasses would depend on animal location in the landscape, on frequency of road perforations, entrance and conduct dimensions and naturalness, shape, and particular adaptation design features (i.e. dry ledges, ramps), among other factors.

Has the arrangement and frequency of underpasses any effect on the distribution of wildlife casualties? It would be expected, for a given set of road underpasses, and assuming that these are operational and effectively mitigate the barrier effect, that the frequency of road-kills should be lower in those sectors of the road with more underpasses. Does wildlife mortality concentrate at random around road underpasses or is there a pattern of concentration of casualties far from underpasses? In other words, is animal mortality along roads dependent to some degree on the availability of safe conducts below the asphalt level? Vehicle-caused mortality could be substantially reduced in those road stretches with a higher potential permeability provided by underpasses. In addition, improvements in road permeability and minimizing of damage to populations could be attained through spatially explicit evaluation of the congruence of use of underpass elements for vertebrate fauna.

Hence, we assessed the relation between underpass frequency and their spatial distribution along roads, and the occurrence of road-killed vertebrates in different road segments for a globally relevant agroforestry ecosystem, the southern Spanish holm-cork oak rangeland in the Seville and Huelva provinces, western Andalusia. Our general steering question was how animal mortality arranges along roads as a function of distance to underpasses. To our knowledge, a thorough examination of the spatial congruence between roadkill events and road permeability for a set of several roads in sensible, high biodiversity habitats, has barely been approached. We aimed to relate spatial distribution of wildlife casualties to underpass spatial distribution pattern and dimensions, along four road segments differing in length, and in the number, frequency and longitudinal disposition of drainage culverts and other underpass types installed during the construction phase of road projects. We controlled also the variation due to seasonality and pertinence to faunal higher taxa, and attempted to cope with local variability of road configurations and moderate traffic intensities.

2. Materials and methods

We performed surveys of vertebrate casualties along four segments of double-lane rural asphalt roads in the provinces of Seville and Huelva (Sierra Morena range, W Andalusia) (Fig. 1). The traversed ecosystems were mainly Andalusian rangelands (agro-sylvo-pastoral systems called "dehesas" in Spanish). Dominant vegetation is formed by a disperse oak (*Quercus suber, Q. ilex* and *Q. rotundifolia*) woodland and an understory layer of annual grasses and shrubs (Marañón, 1988). Agroforestry, livestock and hunting are main exploitative activities hold by this ecosystem. Oak rangelands are a dominant landscape feature in the hilly parts of SW Iberian Peninsula with more than one million hectares only in Andalusia.

Average altitude of the study roads was ∼400 m a.s.l. (159–583 m a.s.l.). Average road width was 9.5 m (± 3.66 standard deviation, SE). Main road and traffic features, including traffic intensity, vehicle type (heavy and light traffic), and speed, were obtained from the local road authorities (Servicio de Conservación y Dominio Público Viario, Sevilla, 2009) (see Table 1 for details).

Each of the 4 roads was surveyed twice for a total of 30 days of fieldwork distributed as follows: end of autumn-early winter: 09/10/2009-22/01/2010; and spring-summer: 20/04/2010-20/07/2010. Overall length of the combined 4 surveyed roads was 53 km. We walked all the complete road length along both sides (total length walked for the survey was 106 km) on each season. 1–4 trained observers walking at 1–2 km/h detected and identified to the finest taxonomic level possible all vertebrate casualties. Birds were excluded from the analysis in this paper due to the lower use of underpasses by this group. There is certainty of use of road underpasses by birds, but this is especially true for large structures with ample habitat space under the road (e.g. viaducts and bridges; Foster and Humphrey, 1995). In our study area, perhaps partridges (*Alectoris rufa*) could make moderate use of such underpasses, and red-rumped swallows (*Cecropis daurica*) certainly use passages for nesting. However, only few bird species will frequently use underpasses as safe passage in this area, especially concerning narrow culverts, which are the dominant underpass type in our study area.

We decided to perform foot censuses because they allow a higher efficiency in casualty detection off the asphalt surface than vehicle samplings. Carcass detectability from a moving car has been found to be very low (10% on average) compared to foot surveys (Lima et al., 2016). In addition, amphibian and other small vertebrate casualties can result severely underestimated when surveyed by car instead of on foot (Teixeira et al., 2013). All casualties found on the asphalt right-of-way, verges and ditches to c. 5 m on each roadside were noted and georeferenced with a Garmin GPS (model GPS 60) set in UTM mode. To avoid repeating observations of casualties, we removed carcasses from the asphalt or, when this was not feasible, we used color marks.

We identified, characterized and georeferenced all underpass-like structures potentially serving as passages for wildlife below the four roads. Such structures were surveyed regardless they were or not explicitly designed as faunal passages. Our searches along road verges were focused but not restricted to elements such as drainage culverts, of any type and dimensions, and bridges. For every accessible structure we measured passage length, and width (diameter in case tubular drainages), height, and cross-sectional structure (apart from 3 bridges,

Table 1
Descriptive attributes of the studied roads. *Source: Servicio de Conservación y Dominio Público Viario, Sevilla, 2009. MDI: Mean daily intensity of traffic. Underpass ("units") dimensions (width and height) are given as mean and standard deviations (SD). Cumulative width of underpass units is the sum of underpass widths along the complete length of the road segment.** = average values for the 4 roads, in bold (otherwise, sums, also in bold).

Road	1. Lora del Río-Constantina (A-455)	2. Cantillana-El Pedroso (A-432)	3. Cala-Santa Olalla del Cala (A-434)	4. Santa Olalla del Cala-Zufre (A-461)	Overall
Road basic traits					
Province	Sevilla	Sevilla	Huelva	Huelva	
Road length (km)	16.00	14.70	14.10	8.50	**53.30**
Elevation (m a.s.l.)	390.61 ± 51.97 (328–523)	360.29 ± 48.84 (159–404)	530.94 ± 21.82 (494–583)	421.36 ± 87.67 (268–529)	
Width (m) (± 1 SD) (min.-max.)	9.92 ± 4.25 (7.82–37)	9.59 ± 2.89 (7.65–21.2)	7.92 ± 0.13 (7.75–8.4)	8.54 ± 2.96 (7.6–25)	
Traffic intensity class (vehicles/day)*	High (2000–5000)	High (2000–5000)	Low (1000–2000)	Low (1000–2000)	
MDI (vehicles/day)*	2910	2141	1419	1236	
Average speed (km/h)*	91–100	71–80	71–80	81–90	
Permeability parameters					
Nº underpasses	75	66	90	22	**253**
Nº cattle passages	19	4	4	0	**27**
Nº passages/km	4.69	4.49	6.38	2.59	**4.54****
Mean distance between passages (m)	213.33	222.73	156.67	386.36	**244.77****
Cumulative width of passages (m/km of road)	146.30	131.95	137.73	37.16	**113.29****
Mean width of passages (m)	2.00 (1.46)	2.00 (4.70)	1.55 (1.24)	1.69 (1.83)	**1.81****
Mean height of passages (m)	3.27 (1.21)	1.28 (1.09)	1.36 (1.07)	1.51 (1.06)	**1.89****
Tubular culverts	60	48	87	17	**212**
Arc culverts	0	17	3	5	**25**
Box culverts	13	0	0	0	**13**
Bridges	2	1	0	0	**3**
Passes with obstacles:	11	22	3	5	**41**
Ditch decantation box	7	5	0	3	**15**
Artificially blocked (for cattle)	4	5	0	1	**10**
Clogged by sediment	0	12	3	1	**16**

mainly tubular, arc and box or square culverts) at one of the passage mouth. Due to accessibility constraints, the number of passage for which we could report dimensions could be slightly smaller than the actual passage number perforating the roads. As proxies for overall degree of road permeability we also calculated the number of passes per km of road, the mean distance between passes for the same road, and the cumulative width of passes in meters/km of road. We also counted the number of cattle underpasses per road, and took note of the structures representing obstacles for wildlife movements (such as decantation pits). Permeability data are summarized in Table 1. Underpass distribution and dimensions are shown in Fig. 1.

2.1. Statistical analysis

To study the spatial congruence or mismatch between the pattern of road kills and road underpass location along roads, we used a Mantel test (Mantel, 1967). We divided the surveyed roads into standardized spatial units, using 1-km squares positioned along the roads (N = 60, for the 4 roads, see Fig. 2). All roadkills and road underpasses were plotted using ArcGIS 10.0 (ESRI, 2012). Then, we quantified the number of roadkills and road underpasses in each road unit, to estimate the frequency of occurrences by overlapping the layers. The Mantel statistic called r_M is a measure of the correlation between the two matrices and results from the cross-product of the matrix elements after standardization (Legendre and Fortin, 2010). The statistic r_M is bounded between −1 and + 1 and behaves like a correlation coefficient (Fortin and Payette, 2002). The Mantel test evaluates the similarity between two matrices measuring ecological or environmental distance (difference in values among sites, as for example in frequency of roadkill values vs. density of road underpasses) calculated as a geometric distance matrix (Legendre and Legendre, 2012). When spatial autocorrelation exists, then the closer the plots are in geometric space, the more similar the pattern of values between matrices should be. Thus, the Mantel test measures the correlation between the Euclidean plot-to-plot dissimilarity matrices for testing for plot-level associations. We used Monte Carlo permutations with 9999

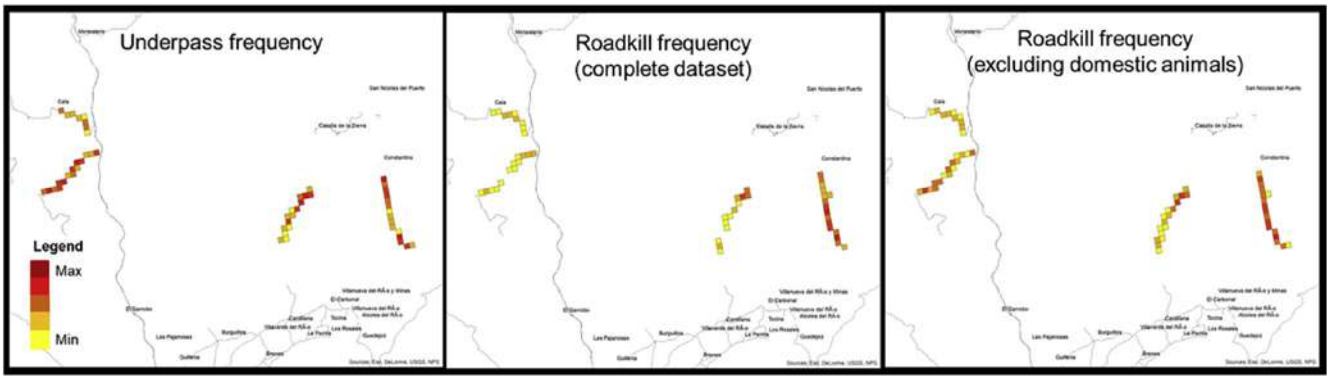

Fig. 2. Mantel configuration for density of faunal underpasses and road kills along the four study roads. Left panel: underpass frequency; central panel: roadkill frequency including domestic animals in the database; right panel: roadkill frequency excluding domestic animals (only wildlife).

randomizations to test for significance (Manly, 2006; Oksanen, 2014).

We performed two Mantel tests: 1) on the complete dataset; 2) on the dataset after removing all cases of domestic animals (namely dogs, cats, pigs and goats). We removed these domestic species because these may follow an idiosyncratic pattern of space use and dispersion, regarding roads and traffic, which would difficult finding patterns for the wildlife in which we are focusing the study.

To investigate which class of vertebrate (amphibians, reptiles or mammals) is more associated to the occurrence of road kills in relation to the distance to nearest underpass we used generalized linear mixed models (GLMM) (Bolker et al., 2009). Roadkill distance to nearest underpass was log transformed after having explored the distribution of variable using the package 'MASS' (Venables and Ripley, 2002) and then used as response variable in the model, while "animal class", "type of animal" (domestic or not), "traffic intensity" and "speed limit" were added as fixed effects.

To avoid spatial autocorrelation problems, the focal road (n = 4 studied roads) where sample points (casualties and passages) were collected, was added as random factor. The interaction between "site" (road's name) and "season" (two study seasons) was added as random factor.

Full model incorporating all above mentioned variables was fitted by maximum likelihood using the package 'lme4' (Bates et al., 2014), and Akaike Information Criterion (AIC) was applied on determination of 'best' model (Burnham and Anderson, 2002), where best model is characterized by the smallest AIC (Mazerolle, 2016). The confidence intervals for the significant variables selected in the best model were calculated by the Wald method (Rao and Scott, 1984). Tests were performed with R software (R Development Core Team, 2017).

3. Results

We found a total of 238 vertebrates belonging to 35 species (amphibians, reptiles, and mammals) killed along the four roads and the two field seasons (Table 1, detailed in Appendix). Mammals were the most abundant casualties detected (108 cases, 45.4% of casualties), followed by amphibians (31.5%) and reptiles (23.1%) (Table 1, Appendix). Each of the four studied roads presented its own idiosyncratic distribution pattern of road kills. Kilometric indices, used to establish comparable measures of killing frequency among roads and vertebrate classes, were highest for mammals along all roads except for the A-455, where amphibians were the prevalent group (Table 2). The A-455 road also presented the highest road kill rates in absolute terms (overall number of casualties and relative intensity through kilometric indices in all animal groups), and impacted on a higher number of species (Table 2). On the other hand, A-461 was the road with a lower overall road kill impact in these same terms.

Also, the four roads varied highly in their attributes of potential permeability conferred by the distribution and density of faunal underpasses (Fig. 1). We found, measured and georeferenced a total of 253 underpass structures for the four roads (Table 1). The number of passages seemed not to depend strictly on road length (Table 1). For example, the A-434 was the second shortest road stretch, but the one with most passage structures (n = 90 underpasses) (Table 1). The road A-434 was also the segment with a higher mean pass density (~6.4/km) and consequently with a smaller average distance between passes (~156.7 m). Additionally, the road A-434 was also perforated by the second largest cumulative width of potentially usable faunal passages (~137.7 m). On the other hand, regarding pass dimensions, the average width of individual passages of the A-434 was the smallest of the 4 roads (~1.6 m).

In addition, there were a small proportion (16.2% for all the four roads) of underpasses presenting obstacles to animal transit due to presence of runoff decantation boxes at pass mouths (5.93% of all passes), concealing of underpass entrances to prevent cattle and livestock escaping (3.95%), and various degrees of clogging by sediment and plant debris in culvert-type passages (6.3%) (Table 1). A total of 27 structures, especially the larger ones (including 3 bridges) were used as cattle and livestock trails (Table 1, Fig. 1).

The occurrence of roadkills was independent from the number of underpasses along the road (rM = 0.109, n = 60 units, nº permutations = 9999, p = 0.09, Fig. 2). However, when filtering and removing all cases of domestic animals from the dataset (i.e. only wildlife included in the analysis), we found a significant spatial congruence between number of roadkills and number of underpasses along the road (rM = 0.526, n = 60, permutations = 9999, p = 1e-04, Fig. 2).

We did not find statistically significant differences in the distance from the casualty points to the nearest road underpasses, in any of the three vertebrate groups (Fig. 3). Also the distance from roadkill occurrence to the nearest road underpass was similar among these vertebrate classes (Fig. 4). Casualties in all the three vertebrate groups tended to be found at c.100 m (median value) from an underpass, within a range of 50–200 m of nearest underpass (Fig. 4).

The results of the mixed model showed that the roadkill distance to nearest underpass was independent from the animal class, traffic density and speed limit on the road, but was positively associated to the occurrence of wildlife species (i.e. non-domestic animals) (Table 3). Based on the 238 sampled casualty points along the 4 roads, the full model obtained through GLMM with fixed-effect parameters, accounting for variation in casualty distance to nearest road underpass, rendered no significant effects of vertebrate class, traffic intensity or speed. On the other hand, "random" factors such as sampling season, road, traffic attributes or the particular vertebrate class under focus did not determine the pattern of occurrence of road-kills along these four roads.

Table 2
Wildlife casualty data. Roadkill kilometric indices were calculated for the whole length of every focal road. Overall sumes of casualty data are in bold.

Road	1. Lora del Río- Constantina (A-455)	2. Cantillana- El Pedroso (A-432)	3. Cala-Santa Olalla del Cala (A-434)	4. Santa Olalla del Cala-Zufre (A-461)	Overall
Wildlife casualty data					
Total nº species killed	24	19	12	14	**35**
Total nº individuals killed	140	52	21	25	**238**
Amphibians	52	9	6	8	**75**
Reptiles	42	12	0	1	**55**
Mammals	46	31	15	16	**108**
Roadkill kilometric indices					
Amphibians	3.25	0.61	0.71	0.57	
Reptiles	2.63	0.82	0.00	0.07	
Mammals	2.88	2.11	1.76	1.13	
Average nº individuals killed/km	8.75	3.54	2.47	1.77	
Average nº species killed/km	1.50	1.29	1.41	0.99	

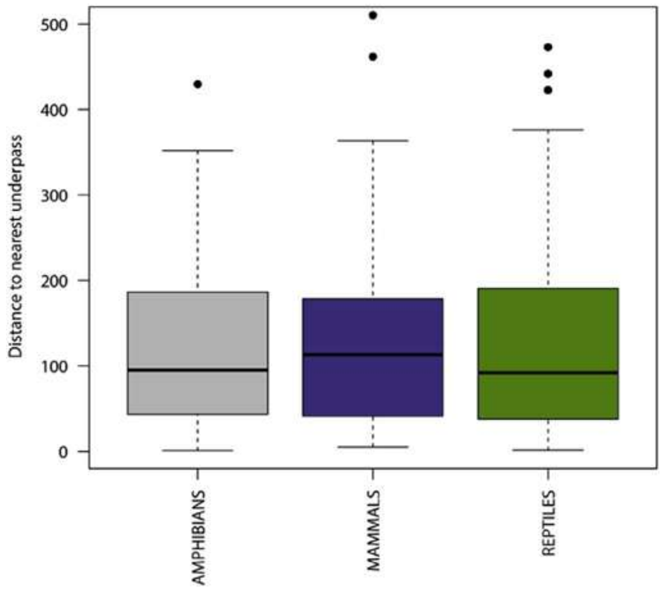

Fig. 3. Distance of roadkill points to the nearest underpass for three vertebrate classes (excluding domestic animals). The y-axis represents the estimated distance variable, in meters. The boxplots show the median (horizontal black bar within box), upper and lower quartiles and extreme values.

4. Discussion

We inventoried a large supply of potential passage structures (N = 253) along 4 rangeland roads in southern Spain. However, the road-killed fauna was distributed following a mostly independent spatial pattern regarding underpass proximity. A similar spatial pattern of occurrence of roadkills in relation to distance to the nearest underpass was found for amphibians, reptiles and mammals. These conducts seemed to be widely available along these roads, and they could be potentially used by animals attempting to cross these roads, thus reducing mortality rates. Despite this, the highest mortality frequency was detected near rather than far from underpasses. Nevertheless, attributing causality to underpass location can be misleading. It can be tempting to infer that underpasses such as culverts cause reductions in road mortality or, on the contrary (and somewhat paradoxically)

Table 3
Results of fixed-effect parameters in a GLMM, accounting for variation in roadkill distance to the nearest road underpass in relation to class of killed animals, type of animals (domestic species included and excluded), traffic intensity and speed limit recorded in the section of the road where data on roadkills were collected. The full model is based on 236 sampled sites. Random effect included in the GLMM procedure: interaction between site and season (groups = 8). Significant variables are showed in bold in the table. Abbreviations: CI, confidence interval; SE, standard error.

Fixed effects	Estimate	CI (lower/ higher)	SE	t	P
Intercept	**3.642**	**(2.871, 4.414)**	**0.394**	**9.253**	**< 2e-16**
Class: Mammals	0.150	(-0.402, 0.703)	0.282	0.533	0.594
Class: Reptiles	−0.022	(-0.580, 0.517)	0.281	−0.121	0.904
Traffic intensity: Low	−0.112	(-0.925, 0.701)	0.415	−0.271	0.787
Speed limit: 81-90	−0.256	(-1.166, 0.654)	0.464	−0.552	0.581
Speed limit: 91-100	0.196	(-0.430, 0.612)	0.266	0.342	0.733
Type of animal: Wildlife species (Non domestic)	**0.754**	**(0.148, 1.361)**	**0.309**	**2.438**	**0.015**

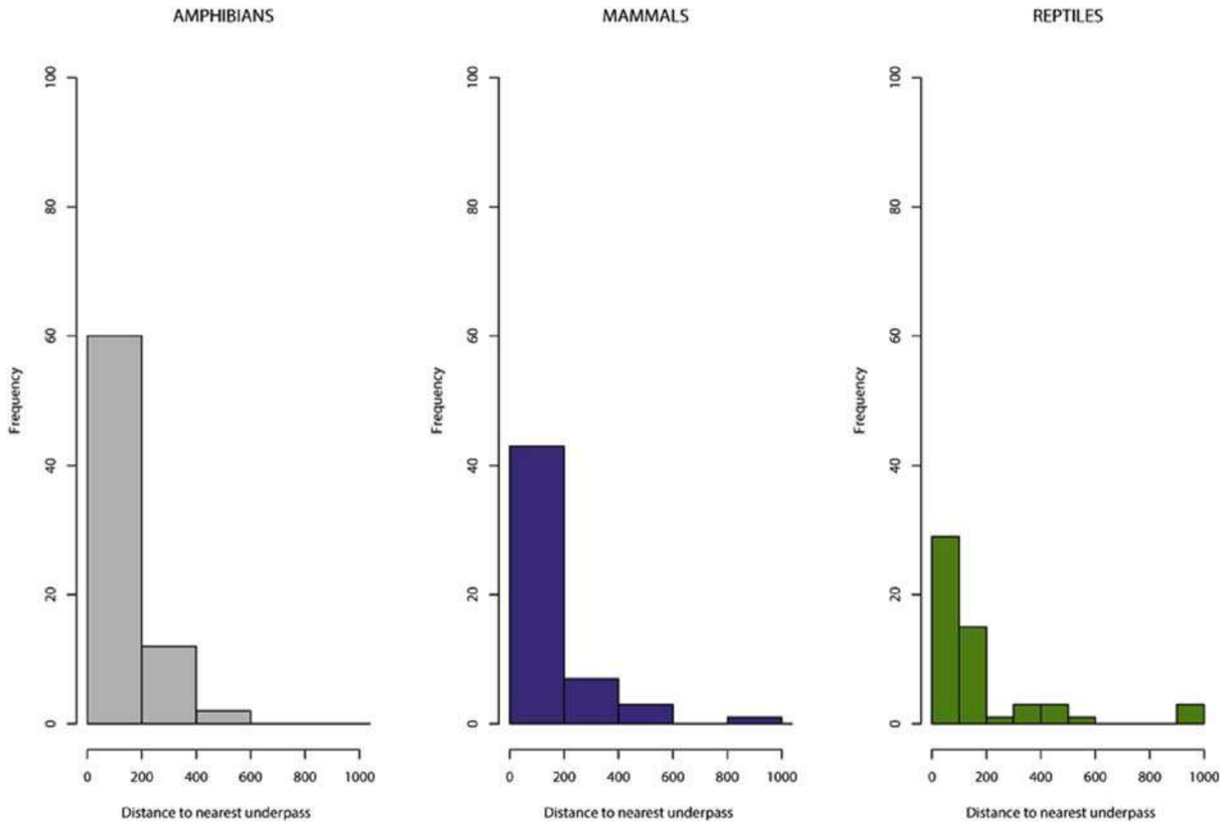

Fig. 4. Distribution of frequency of the distance of roadkill events to nearest road underpass among the three vertebrate classes.

mortality increase due to passage location in sensible spots for wildlife (i.e. water with dense vegetated strips). The roads surveyed in this study are profusely perforated with such structures, so it seems inherently likely to find casualties near than far underpasses. Then, such effect could also be interpreted as an artifact if other interacting factors are not evaluated with adequate inference. Our analysis did not reveal significant effects of factors of transect location and season, and road killing appeared to be produced regardless the existence of "safe" passages, road attributes like traffic intensity and speed for each road. It can be then hypothesized that there must be additional interacting factors affecting distribution patterns of roadkill aggregations in these rangeland roads.

Apart from the presence, availability, design and spatial arrangement of underpasses, other road attributes have been shown to contribute shaping road kill patterns (Puig et al., 2012; Barthelmess, 2014; D'Amico et al., 2015). Despite lack of significance of our GLLM results, there was a certain tendency for a higher mortality rate on those roads with higher speed and traffic intensity, albeit circulation intensity on all four roads evaluated here was moderate to low compared with that of highways. In this sense, it has been shown in some studies that higher mortalities occur on roads with a high average traffic speed and with high traffic intensity, compared to rural roads and secondary roads (Forman et al., 2002).

Notwithstanding, road size is not a precise predictor of its capacity to cause heavy roadkill rates at a large landscape scale, since minor roads can cause large and even higher damages than major roads in certain contexts (van Langevelde et al., 2009). Traffic intensity and maximum vehicle speed were lower for roads A-434 and A-461. Interestingly, these two roads displayed the smaller numbers of animal casualties, both in absolute and relative (kilometric indices) terms. One of these roads, A-434, in addition, had the highest availability (N = 90 underpasses), passage density and second largest cumulative width of passages (a gross proxy for total road permeability), and the smaller distance between passages (Table 1).

It is likely, in view of the killing aggregations, that not all these passages are used by wildlife, or that they are used with different frequency or intensity by different species. The incidence of wildlife casualties could not only be explained by the distribution and frequency of passages, and other factors related to the characteristics of the roads and traversed habitats could be also influential (Mata et al., 2008; Rytwinski et al., 2015). This could be due to differences in the structure of the underpass itself, but also due to seasonality in selection of structures and intensity of use, as shown by other studies in Spain (Bond and Jones, 2008; Mata et al., 2009), its internal conservation and degree of openness, as well as the habitats it connects (Iglesias et al., 2012; Puig et al., 2012; Villalva et al., 2013). In fact, it has been shown that culverts, the dominant underpass type in our study roads, had the lowest frequency of use by vertebrates, while the type and width of structure were the most influential factors in their selection by crossing vertebrates (Mata et al., 2008). In this sense, underpasses do not ensure total prevention of road killing in their vicinity, although for the widest passage types (e.g. viaducts, bridges) a more thorough protection from road killing has been suggested (Puig et al., 2012).

Moreover, most road-killed species in our roads were amphibians and reptiles (which together made up to 130 cases or 54.62% of casualties). Amphibians and reptiles selectively use non-adapted passages based on passage dimensions and may find it difficult to enter and pass the longest and narrowest culverts (Woltz et al., 2008), and amphibians seemed to avoid crossing narrow culverts in a study in central Spain (Rodríguez et al., 1996). Opening diameter and length, but also type of coating substrate, and sunlight availability are the most influential structural variables for passage crossing in some amphibians and reptiles (Rodríguez et al., 1996; Woltz et al., 2008). Some small mammals, however, are likely to prefer narrow passages due to lower predation risk (Rodríguez et al. 1996). This could be considered as another reason for the lack of correlation between casualties and underpasses, but neither association was found for mammals.

These underpasses did not present, in general, concrete adaptations to promote their use by vertebrates, in particular those of small size and limited dispersal abilities, and thus can be considered non-wildlife passages. This aspect in particular is critical for many amphibians and reptiles, which depending on the taxa, may refuse to penetrate too long and poorly illuminated conduits (Woltz et al., 2008; however, see Delgado and Gómez, 2016), or in case of passages completely flooded for small vertebrates (Niemi et al., 2014). Hence, actual or effective permeability of these 4 roadways can be greatly different (probably smaller) than that potentially provided by the underpasses reported here, as other studies also suggest (Glista et al., 2009; Mata et al., 2008, 2009).

Human disturbance has been postulated as a cause in deterring animals from crossing through such structures (Rodríguez et al., 1996). Disturbance due to passage frequentation is an almost negligible factor for most underpasses in this rangeland landscape. Despite this, along with structural design, another influential factor affecting real underpass effectiveness is maintenance, which determines functionality (Clevenger and Waltho, 2000). In this regard, a common practice in these Spanish rangelands is to block access to some culverts to prevent escape of livestock. This could be an impediment also for certain wildlife species. Blocked passage mouths or interior space can be important obstacles even for some reptiles and amphibians, and also for small and medium-sized mammals (dominant mammal casualties in our study), becoming a true barrier for medium to large ungulates and carnivores (Puig et al., 2012).

Drainage culverts represent the vast majority of passages, and vegetation is highly differentiated along water courses served by such drainages, attracting more wildlife and channeling wildlife to these openings. On the other hand, underpass availability was probably related to the orographic complexity of the terrain on which the structure is inserted, and on the number and width of the watercourses to be drawn, since these underpasses are placed during the construction phase of the road project.

The mere existence and abundance of conducts under roads, as those found in this work, does not necessarily guarantee an increased permeability for wildlife, especially if such passes are not specifically designed and/or adapted to fauna and placed in the adequate landscape spots (Yanes et al., 1995; Rodríguez et al., 1996). Thus, a possible future strategy would be to adapt and selectively enlarge those passages more likely to reduce mortality through favoring their use as a good alternative to moving animals.

It is desirable that road authorities and stakeholders maximize road permeability to ecological fluxes. This can be accomplished by perforating the road barrier with enough but at the same time adequate, ample conducts such as modified culverts and group-specific or/and multifunctional underpasses. Aside from enlarging existing underpasses, overpasses (i.e. ecoducts, multifunctional overpasses) would be also a good option. A practical mitigation alternative is to perform correction measures on selected preexistent drainage elements, such as selectively adapting the underpasses to the requirements of the local vertebrate fauna. Rather than build many narrow underpasses, which may provide a limited connectivity service for wildlife, selective enhancement/placement of a discrete number of specifically designed passes may prove a better solution on the long term, without conflicts with agroforestry and livestock uses in these rangelands (Mata et al., 2008).

Ideally, these transversal elements should be spaced along the transport corridor considering the presence of trouble segments of higher mortality due to traffic. Such are the zones of influence of brooks, rivers, and both temporary and permanent water courses with differentiated vegetation, which act as faunal attractors and channeling elements for fauna across the landscape.

Distance among consecutive underpasses should encompass ranging areas of those species with higher space requirements or/and most vulnerable taxa (traits that are often coincident). The precise relations between animal landscape requirements (i.e., area needs, landscape type, food accessibility and road-response behaviors, see Jaeger et al., 2005; Beckmann et al., 2010) and road permeability attributes should be included as variables in the location and design of faunal passes. Wide conducts for larger taxa would benefit also smaller species, but, in general, patterns of conduct use in relation to passage shape and dimensions also deserve further research.

In addition, maintenance and inspection of preexistent underpasses should be mandatory to ensure their functionality (not only for drainage but also specifically for animal movements). A number of conducts, i.e. those placed in sites of frequent clogging by sediments or debris, or by artificial blockage, could be relocated or remodeled to improve and extend its lifespan.

5. Conclusions

We found no significant relationship of roadkill aggregations with the distribution pattern of underpasses for these four roads. All three studied vertebrate groups exhibited a similar spatial distribution around these passages. Despite lack of statistical significance of influential factors modelling, most of the casualties tended to be detected in the vicinity of the mouths of the wildlife passages, rather than far from them. Many of the drainage elements surveyed were in potential areas of confluence of animal movements or probable dispersal routes, following bank vegetation alignments, and vegetation strips along small seasonal or permanent streams flowing into the mouths of passages. This, however, could induce to expect a trend to a prevalence of kills concentrated near the underpass mouths by effect of mere proximity. In fact, it could be hypothesized that the tendency to cross through these passes increases the likelihood of being run over because these places concentrate more wildlife, acting as channels and attracting fauna. Our results, however, do not allow us to reject this hypothesis with total confidence, but it would be advisable to adapt and improve existing passages since, although they are abundant, they probably do not confer the desirable permeability attributes for animal movements through these roads with the aim of reducing mortality and conferring true permeability. Hence, it would be desirable to evaluate the actual capacity (functionality) of the underpasses, because it is possible that many are not operational and are totally or partially unusable (eg with decantation boxes, a serious obstacle for most species, closed by farmers, of inadequate dimensions or flooded. This would happen mostly with culverts, whereas under bridges and viaducts contribute diminishing roadkill rates (Puig et al., 2012). Special adaptations are recommendable in particular for smaller vertebrates, and especially for amphibians and reptiles, to mitigate traffic mortality. Finally, our study also suggests that a significant improvement in project appraisal of road permeability to wildlife could be approached by measuring spatial congruence between passage structures and their actual functionality and roadkill patterns.

Authors contributions

JDD designed the study, performed field data collecting and data analysis, and wrote the manuscript; FM performed data analysis and contributed in manuscript writing; NLA performed previous cartographic and GIS analysis and contributed with field work; JDH, ARP, ARR, MVP and JRS contributed to field work and reviewing the paper. All of the authors examined and agreed with the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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Appendix. List of road-killed species per vertebrate class for the four roads. Overall sumes of casualties per road and group are in bold.

Class and species	Cantillana-El Pedroso (A-432)	Santa Olalla del Cala-Cala (A-434)	Santa Olalla del Cala-Zufre (A-461)	Lora del Río-Constantina (A-455)	Total
AMPHIBIANS	**9**	**6**	**8**	**52**	**75**
Anura indet.				4	4
Bufo spinosus	7	3	4	38	52
Bufo calamita	1				1
Hyla meridionalis			1		1
Pelophylax perezi	1	1	3	10	15
Triturus pygmaeus		2			2
MAMMALS	**31**	**15**	**16**	**46**	**108**
Apodemus sylvaticus	2	2		2	6
Canidae indet.	1	1	1		3
Canis familiaris	12	2	6	15	35
Capra hircus			1		1
Carnivora indet.	1			1	2
Barbastella barbastellus				1	1
Chiroptera indet.	1			3	4
Crocidura russula			1		1
Erinaceus europaeus		2		1	3
Felis catus	7	1	1	3	12
Felis cf. *silvestris*			1	1	2
Genetta genetta			1		1

Herpestes ichneumon			1		1
Martes foina	1				1
Meles meles		1		1	2
Microtus duodecimcostatus				2	2
Mustela nivalis				1	1
Mustelidae indet.				1	1
Oryctolagus cuniculus	4	1		13	18
Rhinolophidae indet.			1		1
Sus scrofa cf. *domestica*		1			1
Vulpes vulpes	2	4	2	1	9
REPTILES	**12**		**1**	**42**	**55**
Acanthodactylus erythrurus	1				1
Blanus cinereus	1			1	2
Colubridae indet.	1			1	2
Hemorrhois hippocrepis	3				3
Indet. Snake				3	3
Timon lepidus				4	4
Malpolon monspessulanus	1			6	7
Mauremys leprosa	1		1	6	8
Rhinechis scalaris	4			21	25
Total	**52**	**21**	**25**	**140**	**238**

References

Alexander, S.M., Waters, N.M., Paquet, P.C., 2005. Traffic volume and highway permeability for a mammalian community in the Canadian Rocky Mountains. Can. Geogr. 49, 321–331.

Barthelmess, E.L., 2014. Spatial distribution of road-kills and factors influencing road mortality for mammals in Northern New York State. Biodivers. Conserv. 23, 2491–2514.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2014. lme4: linear mixed-effects models using Eigen and S4-R Package. J. Stat. Softw. 67, 1–48.

Beckmann, J.P., Clevenger, A.P., Huijser, M.P., Hilty, J.A., 2010. In: Safe Passages: Highways, Wildlife and Habitat Connectivity. Island Press, Washington.

Berthinussen, A., Altringham, J., 2012. Do bat gantries and underpasses help bats cross roads safely? PLoS One 7 (6), e38775. http://dx.doi.org/10.1371/journal.pone.0038775.

Bissonette, J.A., Adair, W., 2008. Restoring habitat permeability to roaded landscapes with isometrically-scaled wildlife crossings. Biol. Conserv. 141, 482–488.

Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H., White, J.S.S., 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol. Evol. 24, 127–135.

Bond, A.R., Jones, D.N., 2008. Temporal trends in use of fauna-friendly underpasses and overpasses. Wildl. Res. 35, 103–112.

Burnham, K.P., Anderson, D.R., 2002. Model Selection and Multimodel Inference: a Practical Information-theoretic Approach, second ed. Springer-Verlag, New York, pp. 488.

Clevenger, A.P., Waltho, N., 2000. Factors influencing the effectiveness of wildlife underpasses in Banff National Park, Alberta, Canada. Conserv. Biol. 14, 47–56.

Clevenger, A.P., Waltho, N., 2005. Performance indices to identify attributes of highway crossing structures facilitating movement of large mammals. Biol. Conserv. 121, 453–464.

Coffin, A.W., 2007. From roadkill to road ecology a review of the ecological effects of roads. J. Transp. Geogr. 15, 396–406.

Delgado, J.D., Gómez, M.A., 2016. Evidence of use of road drainage culverts by Timon lepidus (Daudin, 1802) in Western Andalusia. An. Biol. 38, 63–67.

D'Amico, M., Román, J., de los Reyes, L., 2015. Vertebrate road-kill patterns in Mediterranean habitats: who, when and where. Biol. Conserv. 191, 234–242.

ESRI, 2012. ArcGIS Desktop: Release 10.1. Environmental Systems Research Institute. California, Redlands.

EuroNatur, 2010. TEWN Manual. Recommendations for the Reduction of Habitat Fragmentation Caused by Transport Infrastructure Development. EuroNatur Foundation, Radolfzell, pp. 135.

Forman, R.T.T., Bissonette, J., Clevenger, A., Cutshall, C., Dale, V., Fahrig, L., Goldman, C., Heanue, K., Jones, J., Sperling, D., Swanson, F., Turrentine, T., Winter, T., 2002. Road Ecology: Science and Solutions. Island Press, Washington D.C.

Fortin, M., Payette, S., 2002. How to test the significance of the relation between spatially autocorrelated data at the landscape scale: a case study using fire and forest maps. Ecoscience 9, 213–218.

Foster, M.L., Humphrey, S.R., 1995. Use of highway underpasses by Florida panthers and other wildlife. Wildlife Soc. Bull. 23, 95–100.

Glista, D.J., DeVault, T.L., DeWoody, J.A., 2009. A review of mitigation measures for reducing wildlife mortality on roadways. Landsc. Urban Plan. 91, 1–7.

Grilo, C., Bissonette, J.A., Santos-Reis, M., 2009. Spatial–temporal patterns in Mediterranean carnivore road casualties: consequences for mitigation. Biol. Conserv. 142, 301–313.

Iglesias, C., Mata, C., Malo, J.E., 2012. The influence of traffic noise on vertebrate road crossing through underpasses. Ambio 41, 193–201.

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 behavior. Ecol. Model. 185, 329–348.

Karlson, M., Mörtberg, U., 2015. A spatial ecological assessment of fragmentation and disturbance effects of the Swedish road network. Landsc. Urban Plan. 134, 53–65.

Legendre, P., Fortin, M.-J., 2010. Comparison of the Mantel test and alternative approaches for detecting complex multivariate relationships in the spatial analysis of genetic data. Mol. Ecol. Resour. 10, 831–844.

Legendre, P., Legendre, L., 2012. Numerical Ecology. Elsevier, Amsterdam, The Netherlands, pp. 1006.

Lima, R.A., Santos, S., Santos-Reis, M., de Figueiredo, A., Bager, A., Aguiar, L.M.S., Ascensão, F., 2016. Carcass Persistence and detectability: reducing the uncertainty surrounding wildlife-vehicle collision surveys. PLoS One 11 (11), e0165608.

Loro, M., Arce, R.M., Ortega, E., Martín, B., 2014. Road-corridor planning in the EIA procedure in Spain. A review of case studies. Environ. Impact Assess. Rev. 44, 11–21.

Loro, M., Ortega, E., Arce, R.M., Geneletti, D., 2015. Ecological connectivity analysis to reduce the barrier effect of roads. An innovative graph-theory approach to define wildlife corridors with multiple paths and without bottlenecks. Landsc. Urban Plan. 139, 149–162.

Manly, B.F.J., 2006. Randomization, Bootstrap and Monte Carlo Methods in Biology, third ed. Chapman and Hall/CRC, New York, pp. 480.

Mantel, N., 1967. The detection of disease clustering and a generalized regression approach. Cancer Res. 27, 209–220.

Marañón, T., 1988. Agro-sylvo-pastoral systems in the Iberian Peninsula: dehesas and montados. Rangelands 10, 255–258.

Mata, C., Hervás, I., Herranz, J., Suárez, F., Malo, J.E., 2008. Are motorway wildlife passages worth building? Vertebrate use of road-crossing structures on a Spanish motorway. J. Environ. Manag. 88, 407–415.

Mata, C., Hervás, I., Herranz, J., Malo, J.E., Suárez, F., 2009. Seasonal changes in wildlife use of motorway crossing structures and their implication for monitoring programmes. Transp. Res. Part D Transp. Environ. 14, 447–452.

Mazerolle, M.J., 2016. AICcmodavg: model selection and multimodel inference based on (Q) AIC (c). R package version. http://cran.r-project.org/web/packages/AICcmodavg/index.html.

Ministry of Agriculture, Food and the Environment, 2016. Technical Prescriptions for Wildlife Crossing and Fence Design (Second Edition, Revised and Expanded). Documents for the Mitigation of Habitat Fragmentation Caused by Transport Infrastructure, Number 1. Ministry of Agriculture, Food and the Environment, Madrid, pp. 124.

Niemi, M., Jääskeläinen, N.C., Nummi, P., Mäkelä, T., Norrdahl, K., 2014. Dry paths effectively reduce road mortality of small and medium-sized terrestrial vertebrates. J. Environ. Manag. 144, 51–57.

Oksanen, J., 2014. Multivariate Analysis of Ecological Communities in R: Vegan Tutorial. http://cc.oulu.fi/~jarioksa/opetus/metodi/vegantutor.pdf.

Puig, J., Sanz, L., Serrano, M., Elosegui, J., 2012. Wildlife roadkills and underpass use in Northern Spain. Environ. Eng. Manag. J. 11, 1141–1147.

R Core Team, 2014. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing. The R Foundation for Statistical Computing, Vienna, Austria.

Rao, J.N.K., Scott, A.J., 1984. On Chi-squared Tests for multiway contigency tables with proportions estimated from survey data. Ann. Statistics 12, 46–60.

Rodríguez, A., Crema, G., Delibes, M., 1996. Use of non-wildlife passages across a high speed railway by terrestrial vertebrates. J. Appl. Ecol. 33, 1527–1540.

Rodriguez, A., Delibes, M., 2004. Patterns and causes of non-natural mortality in the Iberian lynx during a 40-year period of range contraction. Biol. Conserv. 118, 151–161.

Rytwinski, T., van der Ree, R., Cunnington, G.M., Fahrig, L., Scott, C., Houlahan, J., Jaeger, J.A.G., Soanes, K., van der Grift, E.A., 2015. Experimental study designs to improve the evaluation of road mitigation measures for wildlife. J. Environ. Manag. 154, 48–64.

Teixeira, F.Z., Coelho, A.V.P., Esperandio, I.B., Kindel, A., 2013. Vertebrate road mortality estimates: effects of sampling methods and carcass removal. Biol. Conserv. 157, 317–323.

van der Ree, R., Smith, D.J., Grilo, C. (Eds.), 2015. Handbook of Road Ecology. John Wiley & Sons, Oxford, pp. 552.

van Langevelde, F., van Dooremalen, C., Jaarsma, C.F., 2009. Traffic mortality and the role of minor roads. J. Environ. Manag. 90, 660–667.

Venables, W.N., Ripley, B.D., 2002. Modern Applied Statistics with S, 4th ed. Springer, Netherlands.

Villalva, P., Reto, D., Santos-Reis, M., Revilla, E., Grilo, C., 2013. Do dry ledges reduce the barrier effect of roads? Ecol. Eng. 57, 143–148.

Woltz, H.W., Gibbs, J.P., Ducey, P.K., 2008. Road crossing structures for amphibians and reptiles: informing design through behavioral analysis. Biol. Conserv. 141, 2745–2750.

Yanes, M., Velasco, J.M., Suárez, F., 1995. Permeability of roads and railways to vertebrates: the importance of culverts. Biol. Conserv. 71, 217–222.