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ARE ROAD-KILL HOTSPOTS COINCIDENT AMONG DIFFERENT VERTEBRATE GROUPS?

Fernanda Z. Teixeira[1], Igor P. Coelho[1], Isadora B. Esperandio[1], Nicole Rosa Oliveira[2], Flávia Porto Peter[3], Sidnei S. Dornelles[4], Natália Rosa Delazeri[5], Maurício Tavares[6], Márcio Borges Martins[7]& Andreas Kindel[1]

[1]Federal University of Rio Grande do Sul (UFRGS), Bioscience Institute, Post Graduate Program in Ecology, Av. Bento Gonçalves, 9500, setor IV, prédio 434222, sala 207, Caixa Postal 15007, CEP: 91501-970, Porto Alegre, RS, Brasil.
[2]Federal University of Rio Grande do Sul (UFRGS), Bioscience Institute, Ecology Department, Av. Bento Gonçalves, 9500, setor IV, prédio 434222, sala 207, Caixa Postal 15007, CEP: 91501-970, Porto Alegre, RS, Brasil.
[3]Departamento de Biología de la Conservación, Estación Biológica de Doñana (EBD-CSIC), Calle Américo Vespucio s/n, E-41092 Sevilla, España.
[4]University of Joinville Region (UNIVILLE), Biology Departament, Laboratory of Zoology, Rua Paulo Malschitzki, 10, Campus Universitário - Zona Industrial, Caixa Postal: 246, CEP: 89219-710, Joinville, SC, Brasil.
[5]Department of Environment of Rio Grande do Sul (DEFAP/SEMA). Rua Tiradentes, 587, CEP: 95900-000, Lajeado, RS, Brasil.
[6]Federal University of Rio Grande do Sul (UFRGS), Bioscience Institute, CECLIMAR, Av. Tramandaí 976, Imbé, RS, Brasil, CEP: 95625-000.
[7]Federal University of Rio Grande do Sul - UFRGS, Bioscience Institute, Post Graduate Program in Animal Biology, Av. Bento Gonçalves, 9500, setor IV, prédio 434351, sala 103, CEP: 91501-970, Porto Alegre, RS, Brasil.
E-mails: fernandazteixeira@gmail.com, borges.martins@ufrgs.br, djakare@gmail.com,isadora_e@yahoo.com.br, nicole.rosa.bio@gmail.com, flavia_peter@yahoo.com.br,sdornelles@univille.br, natalia-delazeri@sema.rs.gov.br, mauricio.gemars@gmail.com,andreas.kindel@ufrgs.br

ABSTRACT

The evaluation of road-kill spatial patterns is an important tool to identify the priority of locations for mitigation measures aiming to reduce wildlife mortality on roads. Single-target or multi-species approaches are usually adopted on the implementation of such measures, although their success must be assessed. We aim to test if road-kill hotspots are coincident among different vertebrate groups. If this proves to be right, data on accidents from one group could be used to plan measures applicable to other groups. We identified hotspots using five different grouping criteria: vertebrate Classes (reptiles, birds or mammals), body size (large or small), species commonness (common or rare), type of locomotion (flying or non-flying), and time of activity (nocturnal/crepuscular or diurnal). We analyzed data from road-kill surveys on four roads in southern Brazil, each with at least one year of monitoring. We performed a modified Ripley's K-statistic to recognize scales of road-kill aggregation, and we carried out a hotspot analyses to identify the location of road-kill aggregations for each group described above on each road. To test for similarity in hotspot location among different groups we performed an association test using correlation as the resemblance measure. Hotspot analyses and association tests were done using different spatial scales to evaluate the effect of scales on similarities. Correlation results between groups presented low values at small scales although they had a tendency to increase with raising scales. Our results show that road-kill hotspots are different among groups, especially when analyzed on small scales. We suggest that, for a successful biodiversity approach to mitigation, one should first select general hotspots on large scales and then identify specific hotspots on small scales to implement specific measures. These findings are relevant in a context of existing road networks, where mitigation measures are being planned to reduce impact on wildlife.
Keywords: Road mortality; animal-vehicle collision; spatial pattern; mitigation; scale effect.

RESUMO

OS HOTSPOSTS DE ATROPELAMENTOS NAS ESTRADAS SÃO COINCIDENTES ENTRE DIFERENTES GRUPOS DE VERTEBRADOS? A avaliação de padrões espaciais de atropelamento em estradas é uma ferramenta importante para identificar locais prioritários para medidas de mitigação voltadas à redução da mortalidade da vida silvestre nas estradas. Abordagens voltadas a uma ou várias espécies são geralmente adotadas para a implantação de tais medidas, apesar de seu sucesso dever ser avaliado. Nosso

objetivo foi testar se os *hotspots* de atropelamento em estradas coincidem entre os diferentes grupos de vertebrados. Se isto for confirmado, dados sobre acidentes com um grupo podem ser usados para planejarem-se medidas aplicáveis aos outros grupos. Nós identificamos os *hotspots* usando cinco critérios de agrupamento diferentes: Classes de vertebrados (répteis, aves ou mamíferos), tamanho corporal (pequeno ou grande), densidade da espécie (comum ou rara), tipo de locomoção (voadora ou não voadora), e horário de atividade (noturna/crepuscular ou diurna). Nós analisamos dados de registros de atropelamentos em quatro estradas no sul do Brasil, cada uma com pelo menos um ano de monitoramento. Realizamos um teste estatístico K de Ripley modificado para reconhecer as escalas de agregação dos atropelamentos, e realizamos análises de *hotspots* para identificar os locais de agrupamento dos atropelamentos para cada grupo indicado previamente em cada uma das estradas. Para testar a proximidade na localização dos *hotspots* entre os diferentes grupos, realizamos um teste de associação usando a correlação como uma medida de semelhança. A análise de *hotspots* e os testes de associação foram realizados usando-se diferentes escalas espaciais para avaliar o efeito da escala nas semelhanças. Os resultados das correlações entre os grupos apresentaram baixos valores em pequenas escalas, apesar de apresentarem uma tendência a aumentar com o aumento das escalas. Nossos resultados mostram que os *hotspots* de atropelamento em estradas são diferentes entre os grupos, especialmente quando analisados em escalas menores. Nós sugerimos que, para uma abordagem de sucesso para a mitigação dos impactos sobre a biodiversidade, deve-se primeiro escolher *hotspots* gerais em grandes escalas, para então identificar *hotspots* específicos em escalas menores, onde serão efetivadas medidas específicas. Estes resultados são relevantes em um contexto de redes de estradas já implantadas, onde as medidas de mitigação estão sendo planejadas para reduzir o impacto sobre a vida silvestre.
Palavras-chave: Mortalidade nas estradas; colisão veículo-animal; padrão espacial; mitigação; efeito de escala.

RESUMEN

¿LOS PUNTOS CRÍTICOS DE MORTALIDAD EN CARRETERA COINCIDEN ENTRE DIFERENTES GRUPOS DE VERTEBRADOS? La evaluación de patrones espaciales de mortalidad de la vida salvaje en carreteras es una herramienta importante para priorizar localidades para implementar medidas de mitigación, destinadas a minimizarla. Abordajes enfocados en un solo grupo o en varias especies son adoptados en la implementación de estas medidas, aunque su éxito debe ser evaluado. Buscamos comprobar si los puntos críticos (*hotspots*) de mortalidad en carreteras coinciden entre diferentes grupos de vertebrados. Si esto fuera cierto, los datos de atropellamiento de un grupo podrían ser usados para planear medidas aplicables a otros grupos. Identificamos *hostpots* usando cinco criterios de agrupamiento: clase de vertebrados (reptiles, aves o mamíferos), tamaño del cuerpo (grande o pequeño), rareza de la especie (común o rara), tipo de locomoción (volador o no), y hora de actividad (nocturno/crepuscular o diurno). Analizamos datos de mortalidad en cuatro carreteras en el sur de Brasil, cada una con al menos un año de monitoreo. Calculamos un estadístico K de Ripley modificado, para reconocer escalas de agregación de las muertes, y llevamos a cabo un análisis de *hotspot* para identificar la agregación de las muertes en carretera para cada grupo en cada carretera. Para testar la semejanza de la ubicación de los *hotspots* entre grupos usamos un test de asociación con correlación como medida de similitud. Los análisis de *hotspot* y tests de asociación fueron realizados usando diferentes escalas espaciales para evaluar el efecto de la escala en las semejanzas. La correlación entre grupos presentó valores bajos a escalas pequeñas y una tendencia a aumentar con la escala. Nuestros resultados muestran que los *hotspots* de mortalidad en carretera son diferentes entre grupos, especialmente cuando son analizados a pequeña escala. Sugerimos que, para tener éxito en la mitigación, se deben seleccionar *hotspots* generales a gran escala y después identificar *hotspots* específicos a menor escala, para implementar medidas específicas. Estos resultados son relevantes en el contexto de la red vial existente, donde se están planeando medidas de mitigación para reducir el impacto sobre la vida silvestre.
Palabras clave: Mortalidad en carreteras; colisiones con animales; patrones espaciales; mitigación; efecto de escala.

INTRODUCTION

Roads are a source of important impacts on wildlife, especially through mortality caused by animal-vehicle collisions and by isolation of populations due to barrier effects (Forman & Alexander 1998, Trombulak & Frissell 2000, Jaeger *et al.* 2006). Since road mortality has detrimental effects on populations' persistence (Jackson & Fahrig 2011), many measures have been designed and implemented on roads to mitigate this impact, such as wildlife crossing structures, fences, speed reducers, and wildlife warning signs (Glista *et al.* 2009). A major factor defining the effectiveness of these spatially restricted measures is their accurate placement (Glista *et al.* 2009). Road mortality patterns are usually not random and concentrate at some locations, according to the spatial distribution of explanatory factors related to vehicle collisions (Malo *et al.* 2004, Seiler 2005, Ramp *et al.* 2006, Gunson *et al*. 2011, Coelho *et al*. 2012). Factors such as traffic flow and speed, road design, presence of landscape corridors, and habitat availability may influence road-kills at different spatial scales. Thus, evaluating road-kill spatial distribution and identifying road-kill hotspots is an important step to implement successful mortality mitigation on existing roads (Clevenger *et al.* 2003).

After identifying the locations with high numbers of animal-vehicle collisions, options for mitigation can be planned and implemented. Either single-target or multi-species measures may be adopted. Recorded rates of use of wildlife passages suggest that different species preferentially use different types of passages and that some species require particular features in the passage (Foresman 2003, Gordon & Anderson 2003, Lesbarrères *et al.* 2004, Clevenger & Waltho 2005). Even recognizing that each passage may have a different effectiveness for each species, some types of passages may facilitate connectivity for a wide range of species. Also, it is not financially viable to build mitigation measures with optimum efficacy for all species. Lesbarrères & Fahrig (2012) show that, when studies on wildlife passages are synthesized, the conclusion that a variety of passage types for different species is required is not supported. They also point out that one passage design that works for most species is the 'extended stream crossing': "an elongated, open-span structure over a natural stream, including wide banks on both sides" (p. 377). These elongated crossing structures can be built over streams, but also at locations without water bodies, as an elongated underpass. Since these long bridges are one of the most expensive mitigation measures of road impacts (Huijser *et al.* 2008), it is important to build them where there is a road-kill hotspot for a high number of species. On the other side, if some location is a mortality hotspot only for a small group of species, it may be more effective to implement a specific mitigation measure for the target group of species.

Road-kill surveys often address mortality spatial patterns for specific taxonomic groups. Large mammals are the best documented group, probably due to their size and to the interest in their demography (Trombulak & Frissel 2000, Glista *et al.* 2008), while others are often neglected. A review of studies on road impacts by Taylor & Goldingay (2010) found a taxonomic bias towards mammals, with only a few studies concerning birds, amphibians and reptiles, and less than one fourth of the studies including multiple taxonomic groups. Spatial analysis carried out by grouping species is common, and taxonomy is the grouping factor most often used. Consequently, it is elementary to know if the road-kill spatial pattern of one taxonomic group can be a surrogate for the spatial patterns of others. If mortality hotspots are similar for different taxonomic groups, the spatial pattern of one group might be used to plan mitigation measures for all groups. On the other hand, if this does not prove to be right, surveys should focus on all the vertebrate taxa of the study area and analyze them separately.

The accuracy of monitoring data can be influenced by observer's detection ability, removal of carcasses by scavengers, and carcass persistence on the road, which varies according to animal body size, among other factors (Slater 2002, Gerow *et al*. 2010, Santos *et al*. 2011, Guinard *et al*. 2012, Teixeira *et al*. 2013). It is not known if these groups that have different detection probability and removal rates also have different mortality spatial patterns. Thus, if spatial patterns are similar between small- and large-sized species, road-kill assessments could consider only large carcasses of easy detection to plan mitigation measures that could reach both groups. In the same way, common species are usually more abundant in road-kill data, although conservation goals are more often focused on rare species. So, if mortality hotspots of common and rare species are spatially coincident, mitigation measures could be potentially addressed for rare species, using the whole data set, even if most road-kill events in the dataset represent common species.

Road-kill of flying animals such as bats and some birds can sometimes be mitigated by the same measures installed for terrestrial animals, for example large underpasses (Jacobson 2005, Berthinussen & Altringham 2012). Therefore, data on road-killed flying animals should be preferentially used to define the placement of common mitigation if road-kill hotpots of flying and non-flying species are coincident. If they are not coincident, mitigation should be planned separately for flying and non-flying species, with the implementation of mitigation appropriate and more effective for non-flying and flying species independently. Also, some mitigation measures can be managed in time, such as temporary road closures at night (Huijser & McGowen 2010), temporary educational campaigns (Sullivan *et al.* 2004), vehicle speed limiter and night traffic calming. The implementation of such temporary mitigation only makes sense if road-kill is concentrated in time and if groups of species with different activity times (diurnal or nocturnal) have spatially dissimilar hotspots of mortality.

Here we use data from road-kill surveys on four roads in southern Brazil to test if road-kill hotspots are coincident among vertebrates grouped using five criteria: taxonomic Class (reptiles, birds or mammals), body size (small or large), commonness (common or rare), type of locomotion (flying or non-flying), and time of activity (nocturnal/crepuscular or diurnal). For each group we identified hotspot locations and tested for spatial correlation between groups within each grouping criteria. Additionally, we evaluated how scale affects the resulting pattern. If correlation levels are high, spatial mortality patterns from one group could be potentially used as surrogates for planning measures that could also benefit other groups.

METHODS

STUDY AREA

We surveyed a total of 494.8 km on four roads (BR-101OT, BR-101TP, ERS-389, and ERS-486) located in southern Brazil, in the Atlantic Forest Biosphere Reserve. The roads lie between the Atlantic Ocean and the Serra Geral highlands, where important remnants of the Atlantic Forest still exist. The regional climate is characterized as warm temperate humid with hot summers (Kottek *et al.* 2006). These roads pass through and near protected areas and cross different geomorphological regions (lowland, hillside and highland), differing on human impact on vegetation cover and landscape structure (Brack 2009). The regional landscape is composed of patches of Atlantic Forest, including forest with *Araucaria angustifolia* in a mosaic with grasslands in highlands, and *restinga* forest in lowlands. Lowland areas are much more fragmented than highlands and hillsides (Ribeiro *et al.* 2009), with high density of rural settlements and small villages and predominance of agriculture. Despite urban occupation, the patches of Atlantic Forest in this region are important for biodiversity conservation of this biome (MMA/SBF 2000), and regional roads may act as barriers or filters to wildlife movements.

The BR-101 road was divided into two segments for this study and treated as two different roads as different surveys were carried out. BR-101OT, from Osório to Torres (29º 53’ 08” S, 50º 16’ 16” W/ 29º 19’ 23” S, 49º 46’ 38” W), was a 95-kilometer section of a two-lane road at the time of the road-kill survey (since 2010 it has been a four-lane road). This road borders the slopes of the Serra Geral and passes by coastal lagoons. Vehicle flow is heavy and relatively homogeneous throughout the year (although higher in summer), with heavy nighttime traffic. In 2001, mean traffic was 6,884 vehicles per day, varying from 5,831 in June to 8,895 in February (DNIT 2001). Additionally, BR-101TP, between Torres and Palhoça (27º 39’ 58” S, 48º 40’ 27” W/ 29º 19’ 12” S, 49º 46’ 33” W), is still being widened. It has 245.8 kilometers, with a traffic of more than 10,000 vehicles per day throughout the year (DNER 1999). The speed limit is 80km/h in the two-lane stretches and 100-110km/h in the four-lane stretches on BR-101.

ERS-389 (29º 20’ 01” S, 49º 45’ 46” W/ 29º 54’ 44” S, 50º 15’ 53” W) is a two-lane road of 88 kilometers located near the coast, with restricted traffic of heavy vehicles such as trucks and buses and a speed limit of 80km/h. The daily mean traffic was 4,881 vehicles in 2002, ranging from 2,671 in August to 9,028 in February (DAER-RS 2002). ERS-486 is a 66-kilometer section of RSC-453/ERS-486 (29º 15’

58" S, 50º 19' 12" W/ 29º 36' 59" S, 49º 57' 29" W), a two-lane road with a mean daily traffic of 3,108 vehicles (DAER-RS 2009).

DATA COLLECTION

We surveyed the mortality of native vertebrate species on the four road segments from a moving vehicle travelling at speeds between 40-60km/h, and we stopped at every carcass recorded to confirm taxa identifications. All vertebrate road-kills found were identified to the most accurate taxonomic category possible, and their locations determined with a GPS receiver. BR-101TP and BR-101OT were monitored seasonally (four times per year, once each season) between 2005 and 2008, the latter also being monitored monthly between January 2003 and January 2004. ERS-389 was monitored monthly between January 2003 and January 2004, and again between December 2009 and November 2010. ERS-486 was monitored during four consecutive days per month between July 2009 and July 2010.

ROAD-KILL CLASSIFICATION

We classified all road-kill records according to taxonomic Class, body size, commonness, type of locomotion, and period of activity. Each classification was carried out using the criteria that follow. (a) Taxonomic Class – reptiles, birds or mammals. We did not include amphibians since road-kill surveys were carried out by car. (b) Body size – snakes and worm lizards (*Amphisbaena* sp.) less than 1m in length, birds less than 30cm in length, mammals and other reptiles less than 1kg were classified as small animals, whereas all remaining species were classified as large. (c) Commonness – species were divided in common and rare. This classification was carried out following the rarity classification of Rabinowitz (1981), in which each species is classified in regard to distribution (small or large), habitat specificity (small or large) and population size (small or large), and is considered 'rare' when in one of these criteria the species is considered 'small'. Species locally threatened with extinction (Fontana *et al.* 2003, CONSEMA 2011) were considered rare. (d) Type of locomotion – species were divided in two groups, based on how the species usually crosses roads – flying or on the ground (flying and non-flying species). (e) Time of activity – species were classified as preferably nocturnal or preferably diurnal. When a species did not have a preference in regard to the period of greatest activity, its road-kill records were included in both groups (n= 10). Table 1 presents the number of carcasses recorded along each road for each group. Carcasses identified at the species level (or genus in some cases) were classified for all criteria, but carcasses identified at higher taxonomic levels were only used in the analysis based on taxonomic classification.

DATA ANALYSIS

Ripley's K-statistic is used to evaluate dispersion of events on different spatial scales (Ripley 1981, Levine 2000, Clevenger *et al.* 2003). In order to determine the scales on which road-kills were significantly aggregated in space, we used a modified Ripley's K (Coelho *et al.* 2008) provided by the SIRIEMA v1.1 software (www.ufrgs.br/biociencias/siriema). To define the different scales evaluated, we used an initial radius of 100m and increments of 400m for each step. This initial radius size was chosen because we considered that it corresponds to a scale on which most common mitigation measures, like underpasses and speed reducers, can be effective. To evaluate the significance of possible aggregations, we subtracted the observed K-values from the mean obtained from 1000 Monte Carlo simulations of random road-kill distributions for each scale (named L function, Levine 2000). Values above the confidence limits (95%) obtained from the simulations indicate scales with significant aggregations.

To identify the location of road-kill hotspots for the same groups used in the previous analyses, we carried out a 2D HotSpot Identification analysis (Coelho *et al.* 2012) using the SIRIEMA v1.1 software. In this analysis, the road was divided into segments of the same length (we used segments of 200m each). A circle with radius *r* was centered on the first segment, and all road-kill events inside the circle area were summed. This sum was multiplied by a correction factor that considers the length of the road inside the circle in this position. Then, the circle was centered on the next segment and the sum was again computed

Table 1. Number of carcasses recorded on each road for each group.

		Roads			
Grouping criteria	**Groups**	**BR-101OT 95 km 26 surveys**	**BR-101TP 245.8 km 13 surveys**	**ERS-389 88 km 24 surveys**	**ERS-486 66 km 49 surveys**
Total		**1142**	**828**	**477**	**373**
Taxonomy	Reptiles	130	79	154	75
	Birds	238	381	124	149
	Mammals	774	368	199	149
Body size	Small	174	259	139	127
	Large	931	523	302	193
Commonness	Common	973	593	289	236
	Rare	90	143	149	65
Type of locomotion	Flying	216	372	94	109
	Non-flying	903	436	368	222
Time of activity	Nocturnal	773	444	238	159
	Diurnal	348	370	243	214

and multiplied by the correction factor. This procedure was repeated for all segments of the road, resulting in an aggregation intensity value for each road segment. Hotspot analyses were performed using different radius sizes (100m, 300m, 500m, 1000m, 2000m, 3000m and 5000m) to evaluate scale dependence in correlation patterns. However, if the results obtained using Ripley's K analysis indicated that one of these scales did not have significant road-kill aggregations, we did not perform the HotSpot analysis for that radius size. To evaluate the significance of the aggregation intensity for each road segment, we subtracted the intensity value from the mean value of 1000 Monte Carlo simulations of random distribution of the road-kill events. Values for aggregation intensity above the upper confidence limit (95%) indicate significant road-kill hotspots.

To test if hotspots for different groups overlapped, we transformed aggregation intensity data into a binary variable representing road-kill hotspot presence. With these binary data, we performed an association test using Pearson's correlation as the resemblance measure between variables. Correlation values above 0.7 were considered high. Significance values ($\alpha = 0.05$) were obtained using 1000 randomizations (Manly 1997). The road stretches were considered sampling units, and the presence/absence of road-kill hotspots on road stretches for each group was considered the variable. We tested the correlation between hotspots using the different scales (described above in HotSpot analysis) to verify if overlapping is a scale-dependent pattern. Association tests were performed in MULTIV 2.4 (Pillar 2006).

RESULTS

Results of Ripley's K-analyses indicated that all groups considered have significant aggregation on some scales. Road-kill aggregations were not significant for small species on the 100-meter scale on the ERS-389 road, for rare species on scales of 100, 300 and 500m on ERS-486 and on the 100-meter scale on BR-101TP. For that reason, we did not carry out HotSpot analyses and association tests for the body size and commonness criteria on these scales on these roads. An example of intermediary results for each group for a single criteria and road (time of activity and ERS-389) is provided on Appendix 1 and 2, respectively.

On small scales (up to 1000m), mean correlation between groups was lower than 0.3 for all grouping criteria. However, there is a general pattern of increase of correlations of road-kill hotspots with raising scales, although with a higher variance on larger scales (Figure 1). On the largest scale analyzed (5000m) mean correlation values reached around 0.6 for most groups, whereas in the case of the comparison between mammals and reptiles even on the largest scale analyzed (5000m) the mean correlation value was 0.13 (Figure 1a).

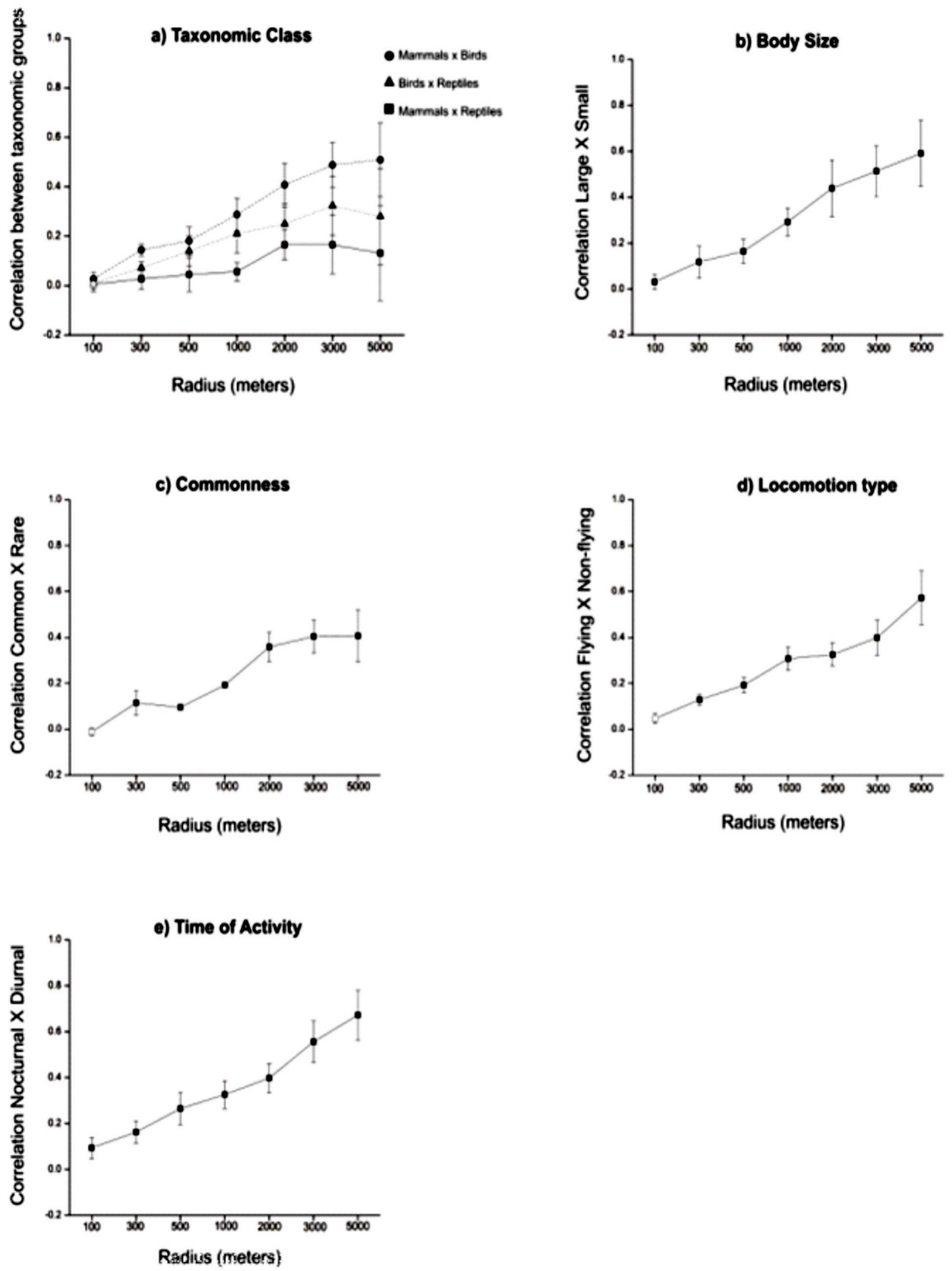

Figure 1. Mean (±standard error) of correlation values between road-kill hotspots of different taxonomic/ecological vertebrate groups obtained for increasing radius size used in the 2D HotSpot Identification analysis. White symbols represent non-significant correlation values (P>0.05) in all roads.

DISCUSSION

Considering the relevance of mitigation to reduce road impacts on wildlife and their frequent high financial costs (Huijser *et al.* 2008), careful planning is needed to adopt the best cost-benefit measures. Due to the high diversity of species affected by vehicle collision, a biodiversity approach in mitigation planning must be pursued, although it may be a challenging strategy. Crossing structures or other mitigation measures are more effective, in a multi-species context, when they satisfy the 'crossing' requirements for as many species as possible (Grilo *et al*. 2010, Taylor & Goldingay 2010). Multi-species mitigation may be achieved by two different ways. The first one is a complementary approach, that is, to build a variety of measures distributed in space to reach different target species or groups. In such approach, different animal groups/species and particular mitigation strategies should be considered. The other way to implement multi-species mitigation is to invest in a single measure that benefits a large group of species, such as an elongated stream crossing (Lesbarrères & Fahrig 2012). The installation of a common measure for different species is a good opportunity for mitigation if road-kill hotspots of different target species or groups are spatially similar.

On the other side, some studies recommend that mitigation should be species-specific to be effective, and the most well-known studies of effective mitigation were developed to mitigate road impacts on individual species (Lesbarrères & Fahrig 2012). Patterns of usage of wildlife passages are influenced by structural, landscape and road-related attributes which may be species-specific (Taylor & Goldingay 2010). However, one important limitation of single-species mitigation is that spatial analysis will only be possible to perform for those species with a high number of road-kill records, which sometimes may be only common species with less conservation interest. An alternative to this sample size problem is to group species in order to analyze road-kill data. This strategy is usually adopted during environmental impact assessments in Brazil and also in research literature (for exemple, Clevenger *et al.* 2003, Ramp *et al.* 2005 and Coelho *et al.* 2008). Due to that issue, in this study we evaluate spatial patterns of groups of species instead of analyzing single-species hotspots.

To our knowledge, this is the first time that the coincidence of vertebrate road-kill hotspots has been evaluated considering different grouping criteria. We were not exhaustive in classifying all possible groups, although we selected the criteria that we believe are most relevant to decision making during mitigation planning. Our results show that, in general, road-kill hotspots of groups within each classification criteria are spatially dissimilar and that this pattern is scale-dependent, with increasing similarity at larger scales. However, our analyses have some assumptions that require caution: 1) although some authors have demonstrated that carcass removal is dependent on body size and carcass density (Slater 2002, Teixeira *et al*. 2013) and that detectability varies among taxonomic groups and different body sizes (Teixeira *et al*. 2013), we assumed that spatial pattern is not affected substantially, that is, the effects of removal and detectability are homogeneously or randomly distributed in space; 2) the groups used in analyses have different sample sizes (see Table 1), and we assumed that these sample sizes represent the spatial patterns of each group for the detection of hotspot locations; 3) the monitoring frequency/intensity varied in the four studied roads, and we assumed that the accuracy in spatial patterns of road-killed animals was equivalent along the four roads. These assumptions should be tested in future work.

Our main conclusion is that no multi-species grouping is a surrogate for another in regard to hotspot identification. For a successful multi-species approach to road-kill mitigation, based on the increase in correlation with raising scales, one should first select general hotspot regions on large scales and then identify specific hotspots on small scales to implement species-specific or group-specific measures. These results are very important in the context of mitigating road impact, since recording all species of interest for conservation during monitoring is difficult. We have shown that care must be taken not to use surrogate groups that are not validated as good indicators, and only easy-detection carcasses cannot be used to plan mitigation measures for all species impacted by roads.

Apparently, there is a need for a hybrid mitigation system on multiple scales, considering both one and several species. Species-specific and group-specific mitigation measures must be combined with more general measures for a comprehensive road-kill mitigation program. A few measures can be implemented on large scales, such as the reduction of speed limits (Hobday & Minstrell 2008) and fencing on long road stretches identified as road-kill hotspots for a large group of species. Considering our results of low similarities of hotspots between groups, associated to mitigation in long stretches, local mitigation measures need to be located on smaller scales specifically addressed for each group of interest, such as wildlife crossing structures, speed limiters/controllers, and warning signs.

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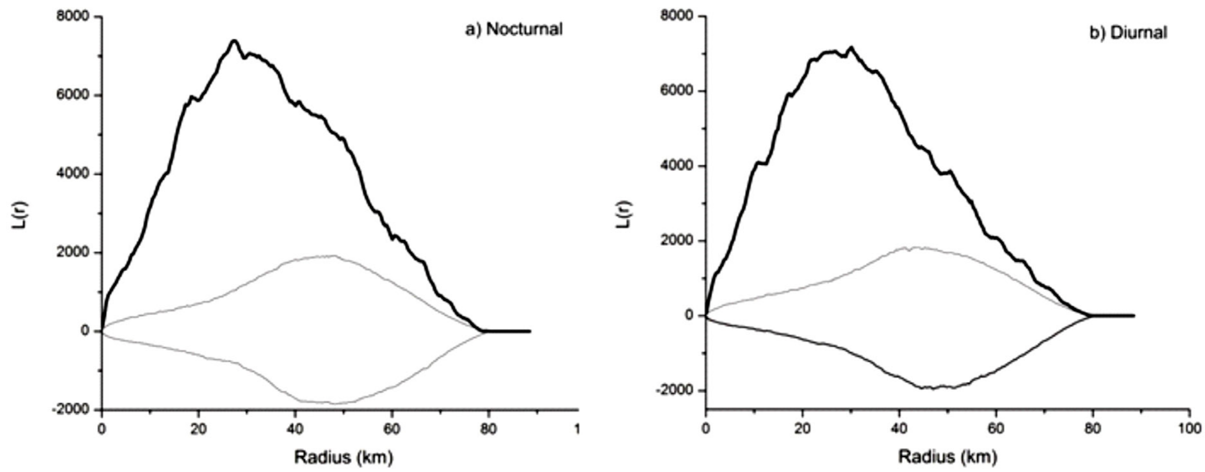
Appendix 1. Example of a result obtained from a Ripley's K-test. The L-statistic is presented as a function of scale distance (radius; black line) and confidence limits of 95% (light-gray lines) for the distribution of (a) nocturnal and (b) diurnal animals on the ERS389 road. L-values above the confidence limits indicate significant aggregation of road-kill events.



Appendix 2. Example of hotspot pattern obtained from the 2D HotSpot Identification analysis. Road-kill intensity of aggregation (black line) and 95% confidence limits (light-gray lines) for (a) nocturnal and (b) diurnal animals on the ERS389 road, for a 2000m scale. Values above the upper confidence limit indicate significant hotspots of mortality. Correlation value between nocturnal and diurnal hotspots for this scale on ERS389 was 0.47.