UNIVERSIDADE FERDERAL DE SÃO CARLOS
CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAUDE
PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA E RECURSOS NATURAIS

GIORDANO CIOCHETI

Spatial and temporal influences of road duplication on wildlife road kill using habitat suitability models

São Carlos, 2014

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Tese de Doutorado apresentada ao Programa de Pós-Graduação em Ecologia e Recursos Naturais do Centro de Ciências Biológicas e Saúde da Universidade Federal de São Carlos como parte dos requisitos para obtenção do título de Doutor em Ciências (Ciências Biológicas), área de concentração Ecologia e Recursos Naturais.

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Dedicatória

Ao meu pai, Ermetes Ciocheti.

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Resumo

O crescimento urbano e o aumento populacional levaram a construção de uma gigantesca malha rodoviária ao redor do mundo. Essa malha é responsável por diversos impactos causados sobre a fauna, meio físico e flora, tais como: atropelamentos, isolamento de populações, facilitação no estabelecimento de espécies invasoras, assoreamento de rios, entre outros. Entretanto, embora a ecologia de estradas tenha avançado recentemente, ainda existem muitas lacunas sobre como elas afetam a fauna, da mesma forma que pouco se sabe sobre como os efeitos da mudança na estrutura das rodovias podem modificar o atropelamento de animais. Este estudo tem como objetivo avaliar alguns dos impactos das rodovias sobre espécies de mamíferos de médio e grande porte em paisagens fragmentadas e naturalmente heterogêneas. Utilizando uma abordagem de grupos funcionais baseados na sensibilidade à perturbação e na capacidade de deslocamento, me propus a responder três perguntas, sendo cada uma um capítulo: 1) qual a contribuição de diversos índices de paisagem para prever o atropelamento de fauna; 2) a duplicação das rodovias e a implementação de passagens de fauna alteram a taxa de atropelamentos dos animais; 3) a duplicação das rodovias altera a maneira que os atropelamentos de fauna são correlacionados com as métricas da paisagem. Para responder a primeira e terceira perguntas, desenvolvemos métodos inovadores combinando aos dados de atropelamentos, uma abordagem multi-escala de métricas da paisagem envolvendo quantidade e distância de diversos elementos da paisagem, como vegetação natural, cerrado, água, silvicultura e cana-de-açúcar. O método proposto no primeiro capítulo, derivado do modelo de adequabilidade de habitat, se mostrou bastante promissor para estimar a probabilidade de atropelamentos. Cada grupo funcional de espécies respondeu de forma diferente aos elementos da paisagem. Distância e quantidade de vegetação foram mais importantes para prever o atropelamento de mamíferos mais sensíveis, mas quantidade de cana de açúcar também contribuiu para os resultados. O método proposto apresenta alta replicabilidade e pode ser utilizado facilmente em outras regiões e para outros táxons. A segunda pergunta foi abordada de forma mais analítica, com uma abordagem de teste de hipótese convencional. Verificamos que, de modo geral, não houve diferença significativa entre os atropelamentos antes e depois da duplicação da estrada. Entretanto, ao se considerar os grupos funcionais, e mesmo as espécies, algumas alterações foram significativas tanto para o aumento e redução de atropelamentos, conforme o foco da análise. Ainda neste capítulo verificamos que a proximidade das passagens de fauna aos atropelamentos não reduziu a taxa de atropelamento, indicando que tais medidas de mitigação podem não estar sendo apropriadas para reduzir a mortalidade por atropelamentos. Por fim, no terceiro capítulo propusemos uma nova abordagem para estimar as mudanças dos atropelamentos antes e depois da duplicação das rodovias. Neste capítulo registramos um aumento na probabilidade de atropelamento de espécies depois da duplicação para espécies generalistas e com maior mobilidade. O uso dos métodos propostos neste trabalho são de fácil implementação em diversas ações relacionadas a estradas, tanto visando sua melhoria estrutural quanto para torná-las mais sustentáveis para a biodiversidade.

Palavras-chave: Ecologia de Estradas; Maxent; Ecologia de Paisagem; cerrado; mamíferos.

Abstract

Urban growth and population growth led to the construction of a gigantic road network around the world. This network is responsible for several impacts on fauna, flora and the environment, such as road kill, isolation of populations, facilitating the establishment of invasive species, river siltation, among others. However, although road ecology has advanced recently, there are still many gaps on how roads affect fauna, as little is known about how effects of changing the structure of highways can modify animal-vehicle collisions. This study aims to evaluate some of the impacts of roads on species of medium and large mammals in fragmented and naturally heterogeneous landscapes. Using a functional group approach based on animal sensitivity to disturbance and displacement capacity, I set out to answer three questions, one in each chapter: 1) the contribution of various landscape indices to predict wildlife road kill; 2) highway duplication and the implementation of wildlife crossing structures alter animal road kill; 3) duplication of roads change the way fauna road kill is correlated with the landscape metrics. To answer the first and third questions, we have developed innovative methods combining road kill data with a multi-scale approach with landscape metrics involving quantity and distance of various landscape elements, such as natural vegetation, cerrado, water, forestry and sugar cane. This method proposed was derived from habitat suitability model, and proved very promising for estimating the probability of animal road kill. Each functional group of species responded differently to landscape elements. Distance and amount of vegetation has been more important to estimate road kill probability of more sensitive mammals, but the amount of sugar cane also contributed to these results. The proposed method is highly replicable and can be easily applied in other regions with other taxa. The second question was addressed in an analytical way, with a conventional hypothesis testing approach. We found that, in general, there was no significant difference between road kill before and after road duplication. However, when considering the functional groups, and even species, some changes were significant for both increasing and reducing road kill. We also found that the proximity of wildlife crossing structures to road kill records did not reduce the frequency of animal-vehicle collision, indicating that such mitigation measures may not have been appropriate to reduce animal road mortality. Finally, in the third chapter we have proposed a new approach to estimate the changes in animal road kill probability before and after duplication of highways. In this chapter we recorded an increase in the probability of road kill after duplication for generalist species with high mobility. The methods proposed here are easy to implement in several actions related to roads, both for seeking their structural improvement and for making them more sustainable for biodiversity.

Keywords: Road Ecology; Maxent; landscape ecology; cerrado; mammal.

Sumário

Introdução

Ecologia de estradas é uma das ciências mais importantes quando pensamos em áreas dominadas pelo homem. Estradas estão por todas as partes e a necessidade de transportar pessoas e bens criou uma rede densa e dominante de duas principais modalidades de transporte terrestres: Rodovias e Ferrovias. Para alcançarmos a meta de um desenvolvimento sustentável temos de estudar as relações entre estes meios de transporte e as paisagens que permeiam. A ecologia de estradas surgiu há pouco tempo em terras brasileiras, e visa à manutenção de diversas espécies: de invertebrados a vertebrados, de musgos a angiospermas. Estradas são verdadeiras cicatrizes na paisagem, alteram seu entorno e sua configuração. As espécies reagem de forma diferente às estradas. Enquanto algumas são beneficiadas, na sua maioria espécies invasoras e generalistas, outras são altamente prejudicadas. A partir deste ponto de vista, tentamos entender como esses meios de ligação alteram e impactam mamíferos de médio e grande porte. Com base em dados reais de atropelamentos, utilizamos modelos matemáticos para entender quais características da paisagem ao redor dos atropelamentos poderiam estar relacionadas com a preferência de esses animais cruzarem as estradas no ponto da fatalidade. Estudamos uma estrada relativamente homogênea em termos de sua estrutura física (curvas, relevo, fluxo de veículos, etc.) e nos concentramos no entorno dos atropelamentos, respeitando as diferenças comportamentais de cada grupo de espécies. Regiões com maior uso antrópico apresentam altas densidades de estradas, isolando ou impactando espécies que necessitam de áreas amplas para buscar recursos alimentares e reprodutivos. Outras espécies, acostumadas com áreas abertas utilizam as estradas como semi-habitats, o que as torna mais vulneráveis a atropelamentos por veículos. Ainda são necessários estudos que indiquem o comportamento destas espécies que inevitavelmente encontram estradas em seu caminho diário para que a perda de indivíduos de diferentes populações possa ser evitada e não gere impactos irreparáveis na biota local e regional.

Capítulo 1: A novel method for predicting road kill hotspots: integrating habitat suitability modeling and landscape ecology

Capítulo a ser submetido para a revista *Landscape Ecology*.

Predicting road traffic threat to wildlife using an innovative model that combines habitat suitability and landscape variables

A novel method for predicting road kill hotspots: integrating habitat suitability modeling and landscape ecology

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running head

Habitat suitability modeling for road kill prediction

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Abstract

The urbanization process leads to a rapid growth of the highway system. Parallel, fragmentation forces wildlife to move between the few remnants of natural vegetation in search of resources such as reproductive partners, food, shelter, etc. This demand for movement in fragmented environments increases the probability of individuals crossing roads and therefore of wildlife-vehicle collisions. The study of the relationship between species and the environment where they live has always been a central point within ecology and is the core of potential distribution modeling. The association between environmental variables and species occurrence has recently been widely used to predict spatial suitability for biodiversity through habitat suitability models. Besides the importance of choosing which variables to use in these models, the scale of these relationships must also be considered. Scales change according to organisms since different species have different environmental demands. Here we propose an innovative model to predict wildlife road kill probabilities integrating habitat suitability model with landscape variables. In Road Ecology, there are no studies that use this type of model (e.g., Maxent) based on habitat suitability and landscape parameters in the immediate surroundings of highways to predict areas where chances of wildlife-vehicle collision are higher. Species interact differently with natural and altered environments; therefore, models should receive input of biological data from different groups. Since species respond differently to environmental variables to generate the models, we separated the species into three functional groups: i) generalist species with high mobility; ii) generalist species with low mobility; and iii) sensitive species with low mobility. These models were compared with each other, to verify the difference in the contribution of variables to the models that predict road kill probability for the three functional groups. Distance from vegetation and percentage of vegetation were the variables that best explained the model with species sensitive to anthropogenic alterations and with low mobility, but the amount of sugar cane was also an important variable for this group. The method developed in this study can be applied in any landscape, since the variables used are adaptable to different focal species and landscapes.

1. Introduction

Intense land use by human activities is the principal agent in the reduction of biodiversity. Agriculture is the most extensive form of land use, while urbanization, due to population increase, is the more intense and damaging form of land use for biodiversity (Lin & Fuller 2013). These processes lead to a drastic reduction and fragmentation of natural areas, as in the Brazilian Atlantic Rainforest Biome where most of its remnants are small (80% are smaller than 50 ha) and isolated (average distance of 1440 m between fragments) (Ribeiro et al., 2009).

The roads, responsible for connecting urban areas and for transporting materials from agriculture, are among the principal modifying agents of landscapes, having a direct correlation with political, social, economic and cultural parameters (Coffin 2007). The increase of the urban population, as well as the emergence of new urban centers, lead to a significant increase in the number of highways, and road densities (Huijser & Clevenger, 2006), reaching values of 3.31 km of road for each km^2 in the Netherlands, 5.04 km per km^2 in Belgium and 0.67 km per km^2 in the United States (The World Bank, 2011).

The presence of roads is related to the type, extent and intensity of land use, and in different contexts, roads generate impacts on landscape. These impacts affect abiotic components such as water quality (Montgomery 1994, Stoeckeler 1965), the process of soil erosion and the contamination of soil and air (Coffin 2007). Roads can also affect biotic components such as vegetation composition (Benet, 1991; Zwaenepoel, 1997) and wildlife diversity, either directly through traffic mortality (Andrews 1990, Forman & Alexander 1998, Trombulak & Frissel 2000, Seiler 2001) or through the avoidance behavior and consequent isolation of populations (Reijnen et al., 1995; Reijnen et al., 1996; Andrews, 1990).

Wildlife-vehicle collisions are considered a major cause of mortality of large vertebrates, directly causing more deaths than hunting (Forman & Alexander 1998). Estimates indicate high annual rates of road kill: 159 thousand mammals and 653 thousand birds in the Netherlands, 7 million birds in Bulgaria, 5 million amphibians and reptiles in Australia and 1 million vertebrates in the United States (van der Zande et al., 1980; Forman, 1995). These fatal accidents can affect directly animal populations, such as in Florida, USA, where the puma (*Puma concolor*) presented a mortality of 10% of its population per year (Harris & Scheck, 1991 apud Saunders and Hobbs, 1991).

Road Ecology arises from de necessity of understanding the effects of the various impacts of roads on the environment and biodiversity in order to propose strategies of functional mitigation and compensation of such impacts (Forman & Deblinger, 2000; Malo et al., 2004; Ramp et al., 2005; Corlatti et al., 2009). Thus, since the 1980s, Road Ecology has been solidifying as an important tool for biodiversity conservation and planning, leading up to mitigation actions, such as underpasses and forested bridges that have already been implemented on roads with high levels of animal-vehicle collisions (Ng et al., 2004; Corlatti et al., 2009).

1.1. Habitat suitability models and biodiversity conservation

The study of relationship between species and environment has always been a central point in ecology, and quantification of this relationship is at the core of potential species distribution modeling (Guisan & Zimmermann, 2000). The association between environmental variables and species occurrence can be applied in Habitat Suitability Models to understand the species niche requirements and spatial predictions, indicating the suitability of location for a target species, community or biodiversity (Hirzel et al 2006). These models have been used in different areas of conservation biology and applied ecology, such as studies on the effect of climate change, habitat loss, invasive species and conservation planning (Guisan & Thuiller, 2005; Peterson, 2006). The ability to predict the spatial distribution of species, to identify the most appropriate regions for species to occupy, as well as their changes, is extremely important for environmental planning (e.g. implementation of wildlife passages in areas with higher environmental value) involving the landscape as a whole (Buckland & Elston, 1993).

Advances in geographic information systems, statistical analyses, as well as the large amount of metadata available nowadays increase the complexity of the relation between environmental variables and species and, consequently, the range of model algorithms and the factors that should be revealed in these models (Elith & Graham, 2009).

One of these factors is the choice of scale, which must be related to the studied species, since the perception of the landscape by the target species must be considered for modeling (Guisan & Thuiller, 2005). According to Vos et al. (2001) the extent of the study area and the radius of influence (e.g. influence of landscape on the displacement of individuals and on the probability of the individual crossing the road at a given point) should consider biological and ecological aspects of the species or ecological processes of interest. In this sense, Boscolo & Metzger (2009), studying understory birds in the Brazilian Atlantic forest, noted that three species responded to radiuses ranging from 600 to 1000 m from the centroid of remnants. Lyra-Jorge et al. (2010), studying carnivorous mammals in a cerrado region within the state of São Paulo, Brazil, observed that the most sensitive species, *Leopardus pardalis*, responded better to a more restrict scale (radius of 250 m) for the amount of cerrado forest, while species with greater mobility and less habitat specificity (*Chrysocyon brachyurus* and *Puma concolor*) were better explained by the amount of native vegetation edge on a broader scale (radius of 2000 m).

In the case of conservation planning, one of the main decisions relies on the choice and delimitation of regions for biodiversity protection. However, there is still great uncertainty for conservation in general because of the lack of biological information, which can lead to less effective mitigation and conservation strategies (Guisan & Thuiller, 2005). The correlation between species occurrence and environmental variables has been used predominantly to identify sites of importance for conservation and of high quality for the species. Despite all the advances in modeling studies, it is worth emphasizing that modeling methods still have not been widely used to predict the locations of species at risk, such as locations with a high risk of road kill. In this respect, one of the challenges for research on species distribution modeling is the use of higher levels of ecological complexity, such as assemblies, functional groups and niche properties, among others (Guisan & Thuiller, 2005).

1.2. Functional groups: why to use them?

Due to the variety of behaviors and environmental requirements of the species, the effects of fragmentation, isolation and habitat loss affect each organism differently (Lyra-Jorge *et al.* 2008). Species with high mobility are affected by different variables than those which affect less mobile species (Garmendia *et al.*2013, Decout *et al.* 2012). In view of this, planners and researchers, who use models to support actions or ideas, should take into consideration that the association between environmental variables and organisms is unique to those species or group of species with similar characteristics, depending on the conservation target (Taylor and Goldingay, 2011). When generating habitat suitability models using landscape ecology concepts, we have to think of scales and variables (e.g. matrix permeability, functional and structural connectivity) that are relevant to the behavior and the species perception of the landscape (Wu, 2013). For this reason, working with modeling involving diverse groups requires separating each species in functional groups according to their perception of the landscape.

The main objective of this study was to develop an innovative method for predicting road kill probabilities of wildlife, integrating landscape ecology and habitat suitability modeling for different functional groups. Data included road kill records provided by the highway concessionaire, and environmental variables relevant to three functional groups, created according to the displacement capacity of each organism and species sensitivity to land use. We also highlight that this method can be replicated in other landscapes, and used as an important tool for planning either new roads or in the upgrading of existing ones.

2. Material and methods

2.1. Study area

The study area refers to landscapes surrounding the highway SP 225 (Rodovia Engenheiro Paulo Nilo Romano) from km 75 to km 235, where we monitored road kill of medium and large mammals. The region encompasses the municipalities of Itirapina, Brotas and Jau (Figure 1) in the northwest of the state of São Paulo. The original vegetation contains diverse cerrado physiognomies, semi deciduous forest and riparian forest. This highway is homogeneous in its physical structure (undivided, with one lane per roadway), with few curves and smooth, homogeneous relief. However, different types of monocultures develop in its surroundings, such as sugar cane, orange plantation (citrus), pastures and silviculture. Also, there are two protected areas along the highway.

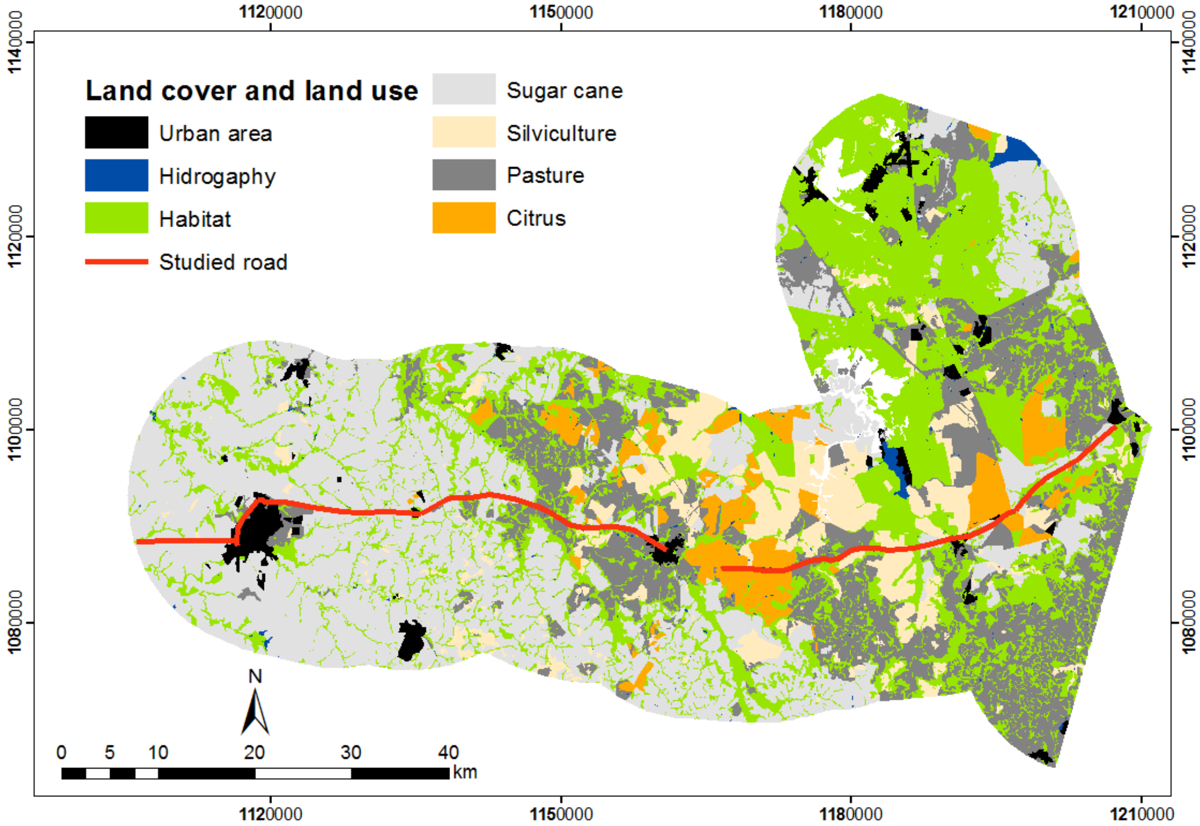

Figure 1- Road stretch of SP 255 between km 75 and 235 in the interior of the state of São Paulo, Brazil, and surrounding landscape classified according to land use and land cover.

2.2. Area for modeling

The generated models were extrapolated to a region that encompasses the study area and extends to the municipality of Ribeirao Preto, covering a total area of 130 x 200 km. This clipping presents similar characteristics regarding predominance of uses, types of monocultures, relief, climate and physiognomies to those found in the study area (Figure 2).

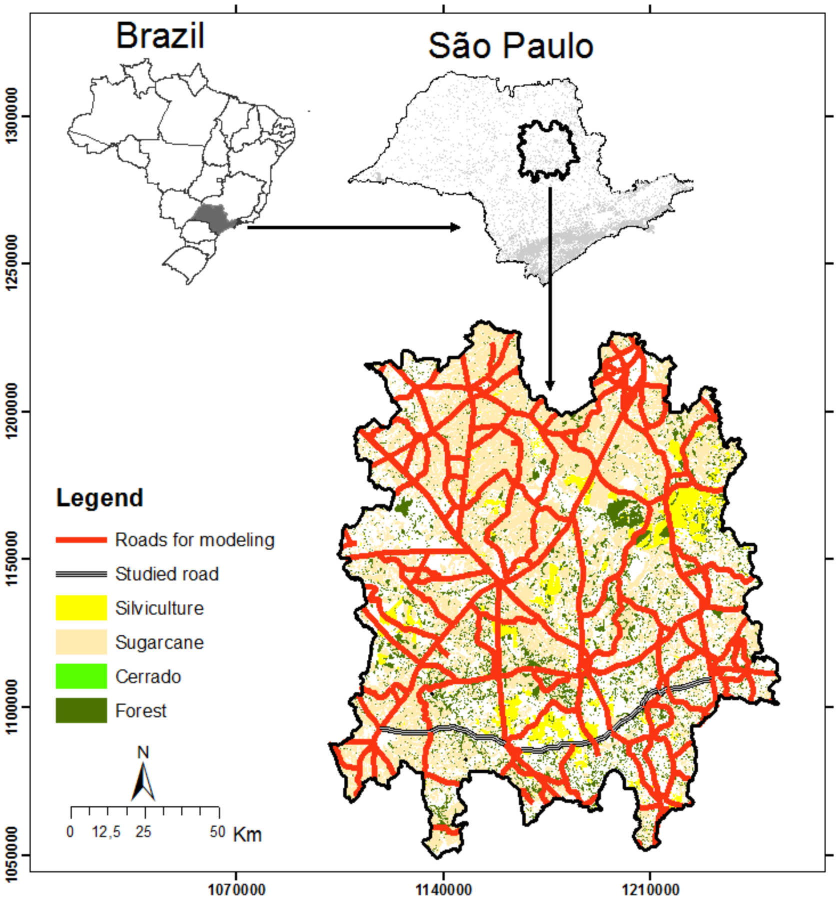

Figure 2- Area for modeling to estimate road kill probabilities for functional groups of medium and large sized mammals using habitat suitability models in the interior of the state of São Paulo, Brazil.

2.3. Biological data and functional groups of medium and large sized mammals

Collection of road kill data was made by the group OHL BRAZIL concessionaire during the years of 2006 and 2007 when the road was still undivided. The Transport Agency of the State of São Paulo (ARTESP), together with Chico Mendes Institute for Biodiversity (ICMBio), requires the monitoring of roads under concession of private companies. The monitoring is done by satellite-monitored car, which travels the road every three hours at a maximum speed of 50 km/h. For each recorded road kill, a photo was taken and the coordinates indicated. The species hit were identified by G. Ciocheti.

The medium and large sized mammals were divided into three functional groups according to displacement capacity and sensitivity to environmental change: a) sensitive animals with low displacement capacity (300 m) (LMS – Low Mobility Sensitive), b) opportunistic animals with low displacement capacity (1500 m) (LMG – Low Mobility Generalist), c) generalist animals with high displacement capacity (3000 m) (HMG – High Mobility Generalist) (Table

1). For the classification of road killed animals in functional groups, we used data from the literature and the knowledge of experts.

Table 1: Separation of medium and large sized mammals in three functional groups, according to sensitivity to land use and estimated displacement capacity.

Functional Group	**Low Mobility Sensitive**	**Low Mobility Generalist**	**High Mobility Generalist**
Acronym	LMS	LMG	HMG
Displacement Capacity	300 m radius	1500 m radius	3000 m radius
Example	*Tamandua tetradactyla* (Southern tamandua) 	*Dasypus sp.* (Armadillo) 	*Mazama gouazoubira* (Gray broket) 

2.4. Selection of landscape variables

The map of land use and land cover in the study area and the area considered for the extrapolation of the model was derived from data from Instituto Florestal (Konkra et al., 2003) and the CANASAT satellite (INPE, 2006). Independent variables were selected to represent landscape heterogeneity and in case they were ecologically related (Lyra-Jorge et al., 2008) to the three defined functional groups. Below we describe each of them:

- Distance from water bodies: shortest distance between road kill and hydrography;
- Percentage of sugar cane: ratio between sugar cane and other classes of land use and land cover;
- Distance from vegetation: shortest distance between road kill and the area of native vegetation;
- Distance from silviculture: shortest distance between road kill and Eucalyptus plantation;
- Distance from sugar cane: shortest distance between road kill and sugar cane plantation;

- Percentage of vegetation: ratio between area of vegetation (native remnants) and other land uses within a search radius from the road kill;
- Percentage of silviculture: ratio between silviculture and other classes of land use and land cover;
- Distance from cerrado: shortest distance between road kill and the area of cerrado;
- Percentage of cerrado: ratio between area of cerrado and other land uses within a search radius from the road kill.

For each of the functional groups, we performed Pearson correlation analysis (Appendix 1) in order to select variables that were not strongly correlated. When two variables presented correlation above 0.7, only one of them was selected.

2.5. Study hypotheses

The working hypotheses are described and represented graphically below:

a. Distance from water bodies: For LMS and HMG, we anticipate a relationship of negative exponential decay. For LMS, the hypothesis motivation would be the best quality the rivers provide to the neighboring fragments and roads occurrence. For HMG, the lowest energy expenditure to move through the riparian forests would indicate this relationship. LMG are not affected by better quality fragments in this area and this variable would not influence the odds of road kill for this group;

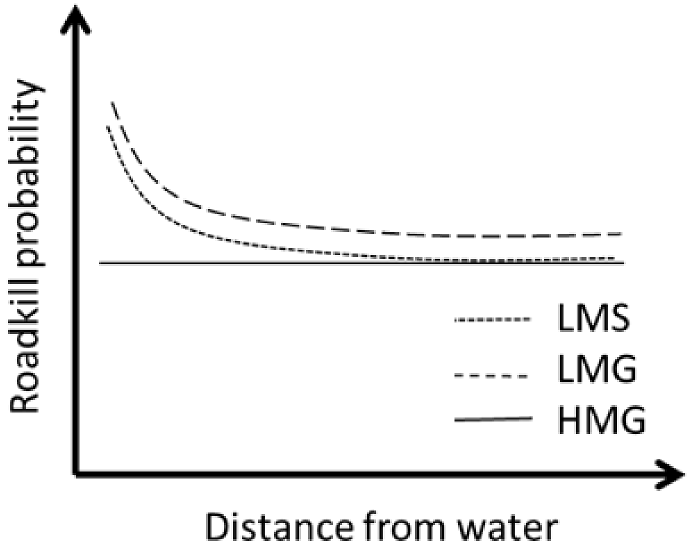

Figure 3- Expected results for road kill probability changes of functional groups relative to increasing distance from water bodies. LMS: low mobility sensitive, LMG: low mobility generalist, HMG: high mobility generalist.

b. Percentage of Sugar cane: For HMG, the percentage of sugar cane would have no influence as it is a very costly matrix for movement and dominates large areas. LMG would also be unaffected by this variable because they adapt to these matrices, and do not change their occurrence in the area. LMS would have a lower probability of being hit with high proportions of sugar cane, which decreases drastically with habitat quality and, consequently, the diversity and density of these species;

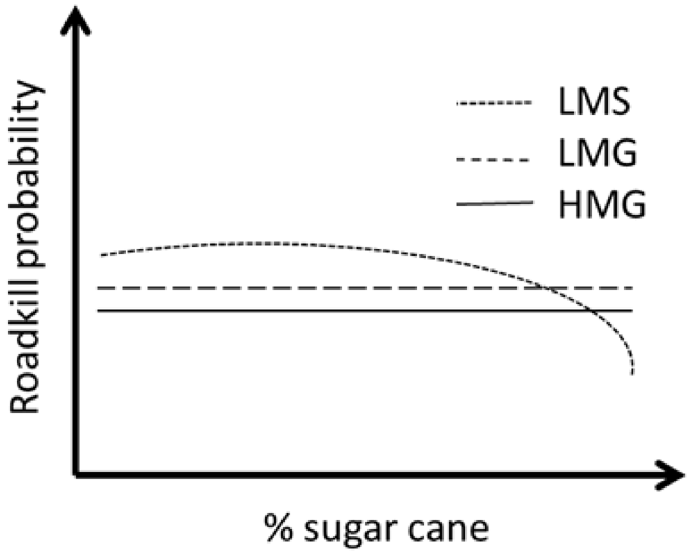

Figure 4- Expected results for road kill probability changes of functional groups relative to increasing proportion of sugar cane. LMS: low mobility sensitive, LMG: low mobility generalist, HMG: high mobility generalist.

c. Distance from vegetation: the three functional groups would have the same kind of response but at different rates, because more sensitive species benefit more from proximity of vegetation;

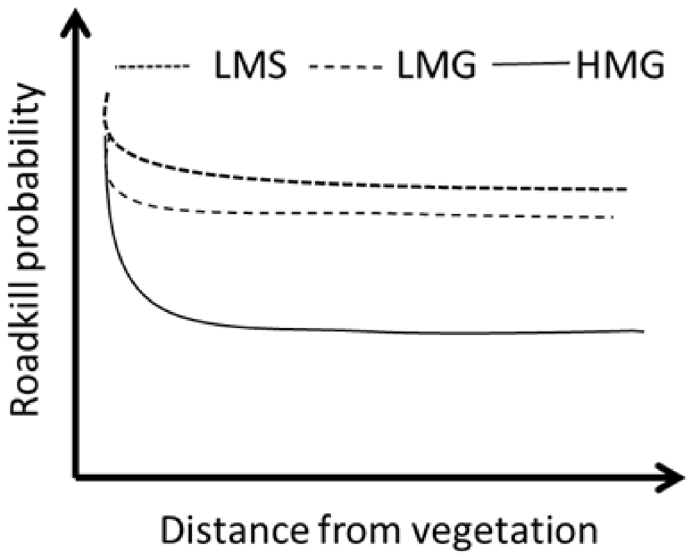

Figure 5- Expected results for road kill probability changes of functional groups relative to increasing distance from vegetation. LMS: low mobility sensitive, LMG: low mobility generalist, HMG: high mobility generalist.

d. Distance from silviculture: for LMS, the curve presents high probability of road kill in the smallest distances from silviculture, with sharp decay until intermediate levels and stabilization at higher levels; the HMG possess medium probability of road kill in areas near silviculture and with a rate of mild stabilization in relation to distance from silviculture. LMG are less affected because the greater permeability of silviculture areas relate weakly with displacement capacity, maintaining average values of road kill;

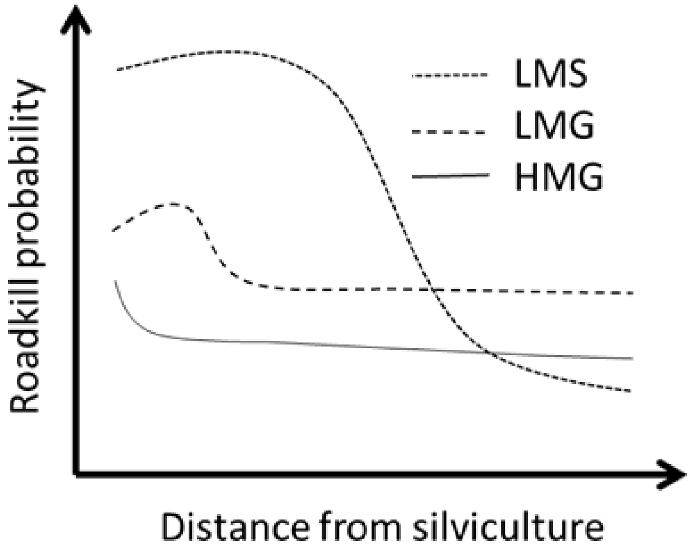

Figure 6- Expected results for road kill probability changes of functional groups relative to increasing distance from silviculture. LMS: low mobility sensitive, LMG: low mobility generalist, HMG: high mobility generalist.

e. Distance from sugar cane: LMS has low probabilities of road kill near sugar cane plantations and a rapid increase of this probability occurs in more distant areas, due to their low sensitivity to this matrix type. The other two groups (HMG and LMG), being opportunistic, are not affected by this distance, maintaining low and medium probability according to their mobility;

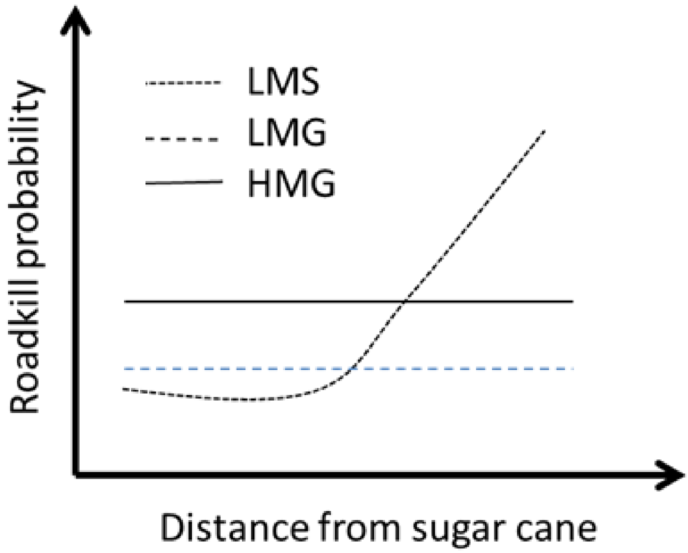

Figure 7- Expected results for road kill probability changes of functional groups relative to increasing distance from sugar cane. LMS: low mobility sensitive, LMG: low mobility generalist, HMG: high mobility generalist.

f. Percentage of vegetation: the three groups have similar responses. Lower probabilities of road kill at smaller proportions of vegetation and a rapid increase in high proportions of vegetation, as biodiversity and densities also increase.

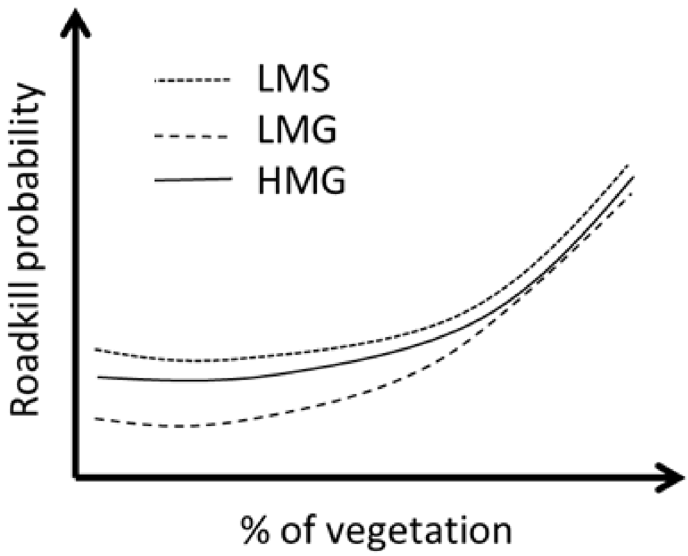

Figure 8- Expected results for road kill probability changes of functional groups relative to increasing proportion of vegetation. LMS: low mobility sensitive, LMG: low mobility generalist, HMG: high mobility generalist.

g. Percentage of silviculture: HMG are not affected by the proportion of silviculture as they move easily within this matrix and others, but maintain high probability of road kill because they use even small areas of eucalyptus when moving. LMG have a high probability of road kill in areas with the highest percentage of silviculture; this probability decreases with the decrease of neighboring silviculture. LMS have high probability of road kill in areas with smaller proportions of silviculture; the probability increases with decrease of neighboring silviculture.

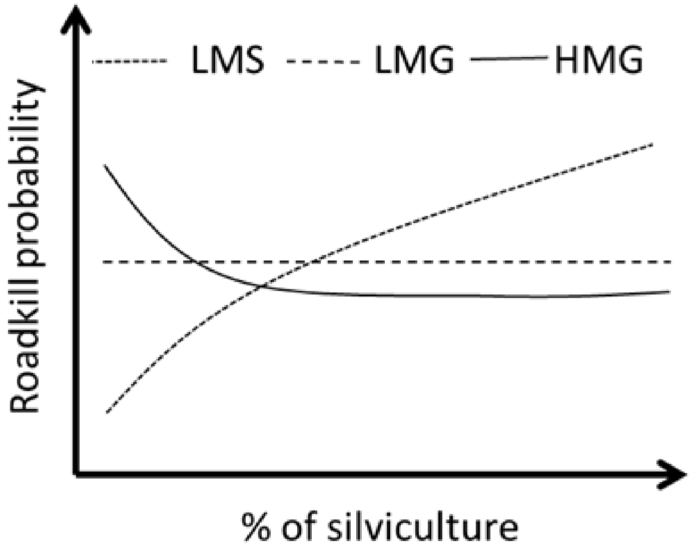

Figure 9- Expected results for road kill probability changes of functional groups relative to increasing proportion of silviculture. LMS: low mobility sensitive, LMG: low mobility generalist, HMG: high mobility generalist.

h. Percentage of cerrado: the three groups have similar responses. Lower probabilities of road kill at smaller proportions of cerrado and a rapid increase in high proportions of cerrado, as biodiversity and densities also increase.

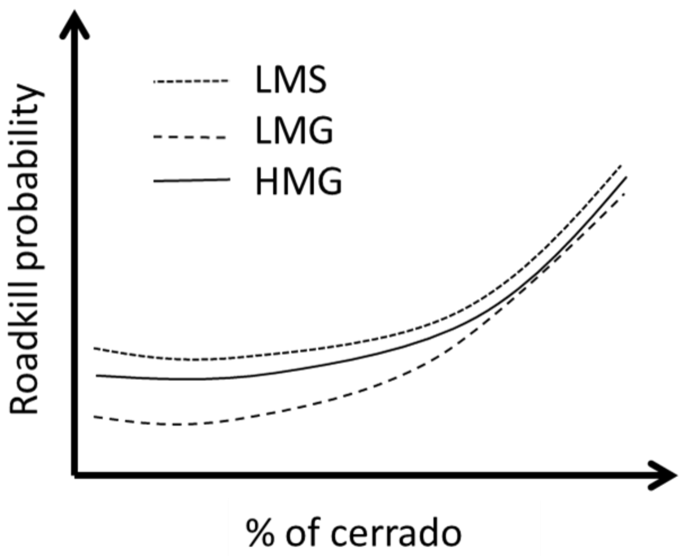

Figure 10- Expected results for road kill probability changes of functional groups relative to increasing proportion of cerrado. LMS: low mobility sensitive, LMG: low mobility generalist, HMG: high mobility generalist.

i. Distance from cerrado: the three functional groups would have the same kind of response but at different rates, because more sensitive species benefit more from proximity of cerrado;

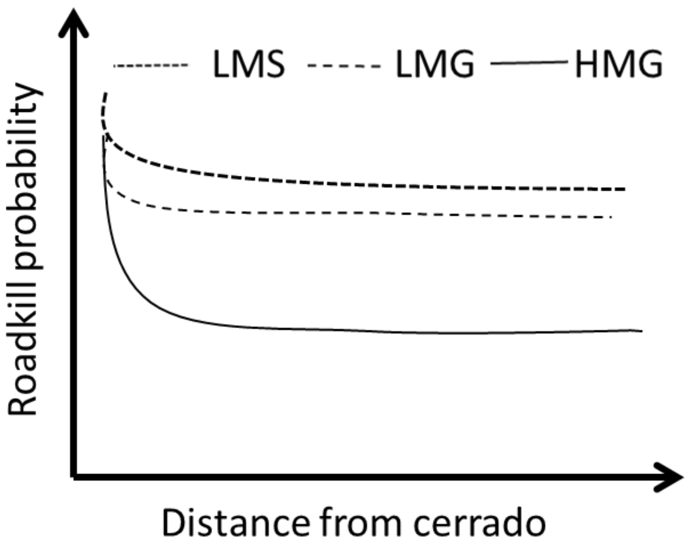

Figure 11- Expected results for road kill probability changes of functional groups relative to increasing distance from cerrado. LMS: low mobility sensitive, LMG: low mobility generalist, HMG: high mobility generalist.

2.6. Modeling procedures

The models to predict probability of road kill were made in *Maxent 3.3.3 software* (Phillips et al., 2004; Phillips et al., 2006). We used as a reference the modeling developed by Ferraz et al. (2012), which considers landscape variables to predict habitat suitability for occurrence of birds. We applied *Jacknife* statistics to quantify the relative contribution of each landscape variable to model the probability of road kill for each of the functional groups. In our case, however, we used road kill occurrences of species organized in functional groups. For modeling, we used 70% of the presence data for training and 30% for the test. We sampled the data using *bootstrap* method, generating 10 replicas of the models, followed by an estimate of the average of these 10 replicas generated through *Maxent*. The models were evaluated by the values of AUC (*Area Under the Curve*) (Fielding & Bell, 1997; Pearson, 2007). Throughout the text, we refer to the above models as real models.

To verify that the proposed models differ from the models created at random, we generated neutral models from random points for each functional group. The number of points to generate neutral models was equal to the number of data obtained for each functional group. This assessment, complementary to the AUC, allowed greater assurance that what guides the estimates are causal relationships (see Figures 3 to 9) and not just the predominance of certain landscape attributes. To make sure that the real models differ from the neutral models, we generated scatterplots and Pearson's correlation analysis. These analyses were conducted independently for each functional group. Correlations between functional groups and respective neutral models were weak (Appendix 2).

2.7. Statistical analyses to compare functional groups

To verify that the resulting maps for each functional group (LMS, LMG and HMG) have differing probabilities of road kill, we used Pearson's correlation, where all models were correlated with each other. In addition to the Pearson's correlations, we also generated scatterplots between the pairs of real models of functional groups. All the statistical analyses that were not made by Maxent were performed in the software R version 2.15 (R Core Team, 2013).

3. Results and discussion

Road kill records obtained for the three functional groups are presented in Table 1 separated by species.

Table1: Species of medium and large mammals, organized in functional groups and the number of road kill records, between 2006 and 2007, used to develop predictive models.

Functional groups and species		Number of recorded road kill events
High Mobility Generalist		
	Crab-eating Fox (*Cerdocyon thous*)	42
	Capybara (*Hydrochoerus hydrochaeris*)	14
	Maned Wolf (*Chrysocyon brachyuros)*	11
	Mountain Lion (*Puma concolor*)	1
	Brocket deer (*Mazama gouazoubira)*	10
Sub-total		**83**
Low Mobility Generalist		
	European Hare (*Lepus europaeus*)	16
	White-eared opossum (*Didelphis albiventris*)	2
	Black-capped Capuchin (*Sapajus apella*)	4
	Coypo (*Myocastor coypus*)	1
	South American Coati (*Nasua Nasua*)	3
	Armadillo (*Dasypus sp.*)	10
Sub-total		**36**
Low Mobility Sensitive		
	Tayra (*Eira barbara*)	1
	Ocelot (*Leopardus pardalis)*	1
	Crab-eating Raccoon (*Procyon cancrivorus)*	7
	Brazilian Porcupine (*Coendou prehensilis*)	3
	Black-pencilled Marmocet (*Callithrix Penicillata*)	1
	Southern Tamandua (*Tamandua tetradactyla*)	8
Sub-Total		**21**
Total		**140**

3.1. Quality of the models and contribution of landscape variables

The AUC values estimated for the road kill models for three functional groups were high, ranging from 0.89 to 0.91, indicating good quality of the models generated by *Maxent*. Through the *Jacknife* statistics we identified five key variables: percentage of sugarcane (HMG, LMS and LMG), distance from cerrado (LMS and LMG), distance from sugarcane (LMG and HMG), percentage of silviculture (HMG) and distance from hydrography (LMG). Table 2 shows the results of *Jacknife* for the contributions of landscape variables per functional group, as well as the AUC values.

Table 2- Relative contribution of variables estimated by *Jacknife* and AUC of the models (±standard deviation) resulting from the process of modeling the prediction of road kill of medium and large mammals for three functional groups within the State of São Paulo, Brazil. LMS: Low Mobility Sensitive, LMG: Low Mobility Generalist and HMG: High Mobility Generalist.

Variables	LMS	LMG	HMG
Distance from cerrado (m)	**43.5**	**21.3**	9.5
Distance from vegetation (m)	2.2	3.7	0.6
Percentage of cerrado	2.3	7.2	6.3
Percentage of vegetation	4	2.6	3.4
Distance from water bodies (m)	4.8	**16.1**	4.5
Percentage of water bodies	0	4.1	0.9
Distance from silviculture (m)	1.5	3.1	2
Percentage of silviculture	7.1	3.9	**23.6**
Distance from sugar cane (m)	5.7	**24.6**	**12.7**
Percentage of sugar cane	**28.9**	**13.6**	**36.6**
AUC ± 1 standard deviation	0.906 ± 0.045	0.911 ± 0.022	0.891 ±0.016

According to the hypothesis (Figure 4), the influence of the variable percentage of sugarcane would be significant only for the model HMG (36.6%). However, the results of the *Jacknife* analyses indicated that the variable was also important in the models of LMS (28.9%) and LMG (13.6%).

The second most important variable for the models was distance from cerrado. Our hypotheses predicted that all groups would be similarly influenced by the distance from cerrado, however with higher contribution for sensitive species. This was corroborated by a significant value for the functional group LMS (43.5), followed by HMG (9.5).

In relation to the distance from sugarcane, the hypothesis would be that the variable would not influence generalist species of high mobility (Figure 7). However, the results pointed in another direction, indicating that both the distance as well as the proportion significantly influence (36.6 and 12.7, respectively) the estimation of the probabilities of road kill.

Further, we expected that the distance from sugarcane would influence LMG (Figure 7). Again the results differed, as both the distance as well as the ratio significantly influenced LMG (13.6 and 24.6 respectively). That the LMS group should be influenced by the percentage of sugar cane was verified in the results (28.9).

In the analysis of the principal variables that contributed to the estimate of the proportion of road kill in the case study, we observed that the variable distance from cerrado (Figure 11) showed negative correlation to the probability of road kill for LMS. The same was observed for percentage of sugar cane (Figure 4). The variable percentage of sugar cane showed a negative correlation with the probability of road kill for the functional group LMG, which also presented negative relationship with distance from cerrado and distance from water bodies. In relation to the functional group HMG, the probability of road kill showed a positive relationship with distance from sugar cane, but showed no relationship with the variable distance from silviculture. Figure 12 shows the response curves between probabilities of road kill according to landscape variables that contributed most to the modeling of the three functional groups.

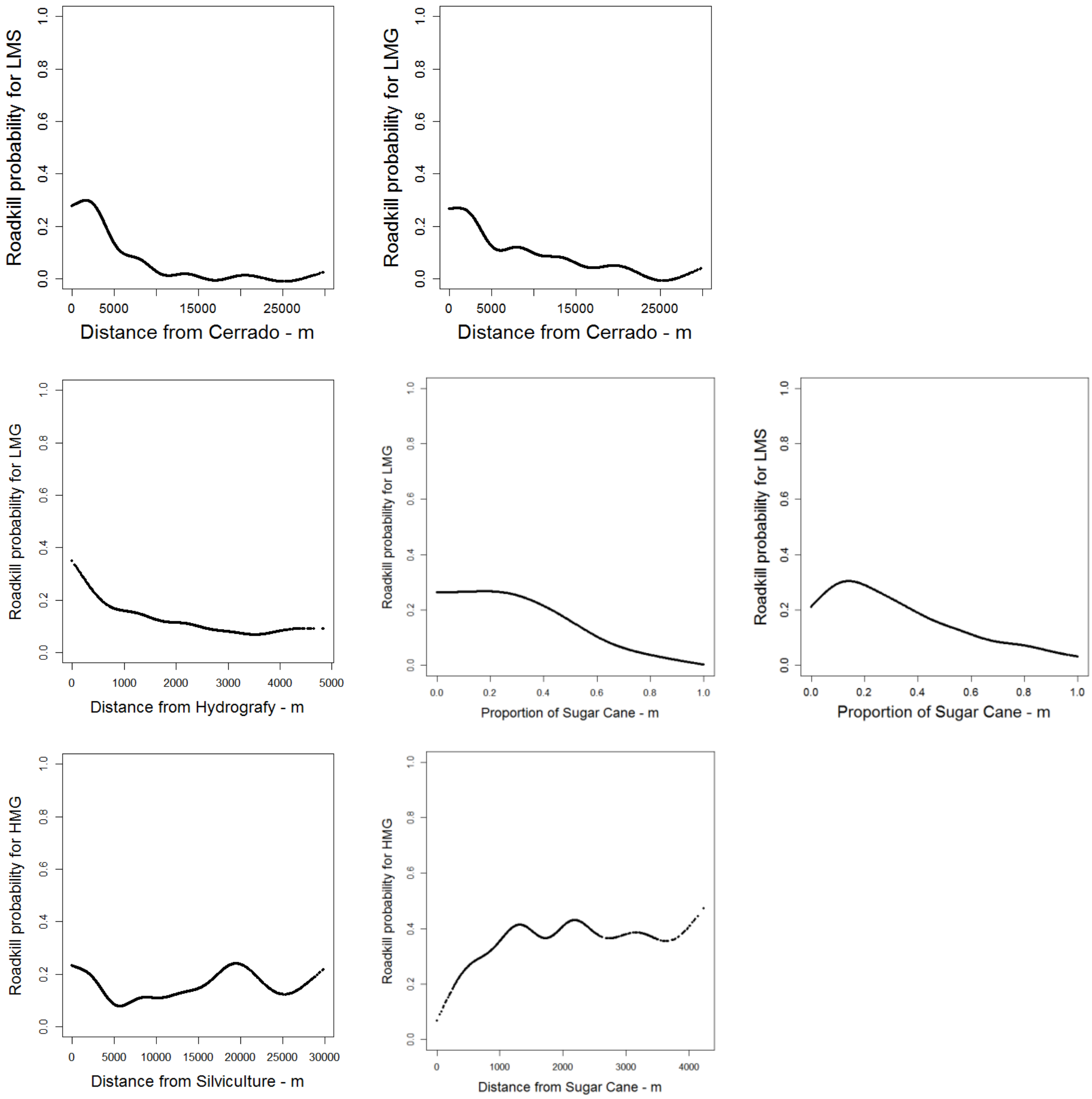

Figure 12. Responses curves between the probability of road kill and landscape variables that contributed most to the three functional groups analyzed (HMG = High mobility generalist, LMS = Low mobility sensitive and LMG = Low mobility generalist), estimated for roads in the interior of the State of São Paulo, Brazil.

3.2. Probability maps of road kill

The maps with the probabilities of road kill for medium and large mammals of the functional groups LMS, LMG and HMG are presented in Figures 13, 14 and 15, respectively. In these, the probability values of road kill range from 0 to 1, with cooler colors (blue) representing lower probability values of road kill and warmer tones (red) indicate higher probabilities. The three maps illustrate that there are few areas of high probability of road kill, with a predominance of probabilities less than 0.1. The details, presented to the right of the figures mentioned above, show great variation in the spatial pattern of response of the road kill probabilities when comparing the three functional groups.

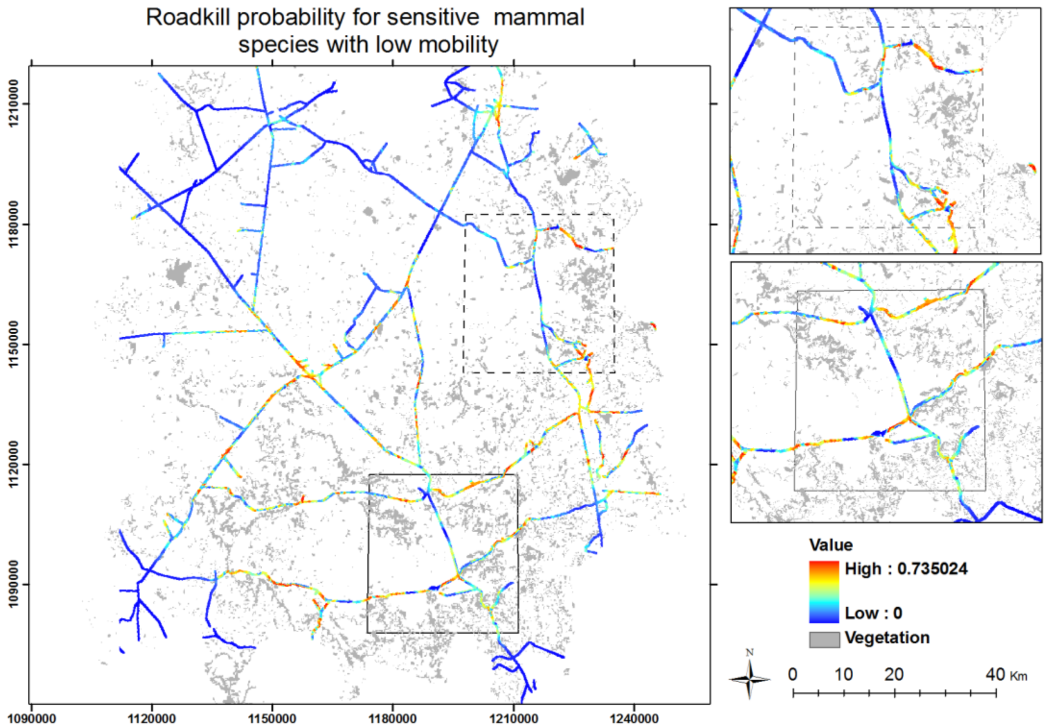

Figure 13. Probabilities of road kill for medium and large mammals for the functional group Low Mobility Sensitive (LMS) for highways in the interior of the State of São Paulo, Brazil. Two details are presented on the right.

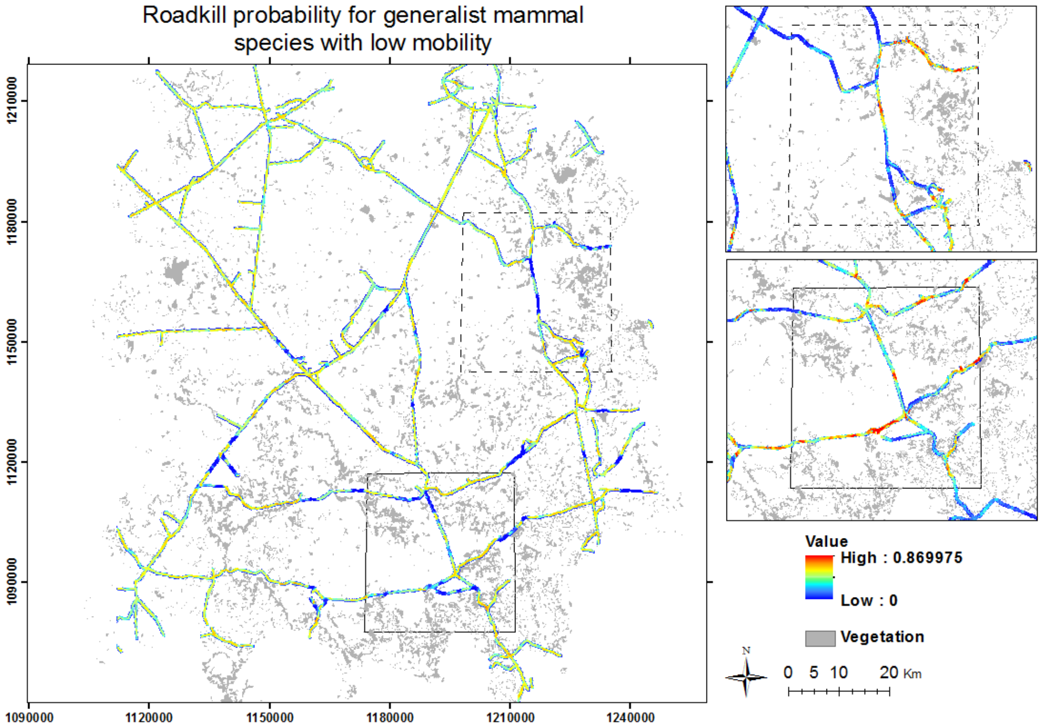

Figure 14. Probabilities of road kill for medium and large mammals for the functional group Low Mobility Generalist (LMG) for highways in the interior of the State of São Paulo, Brazil. Two details are presented on the right.

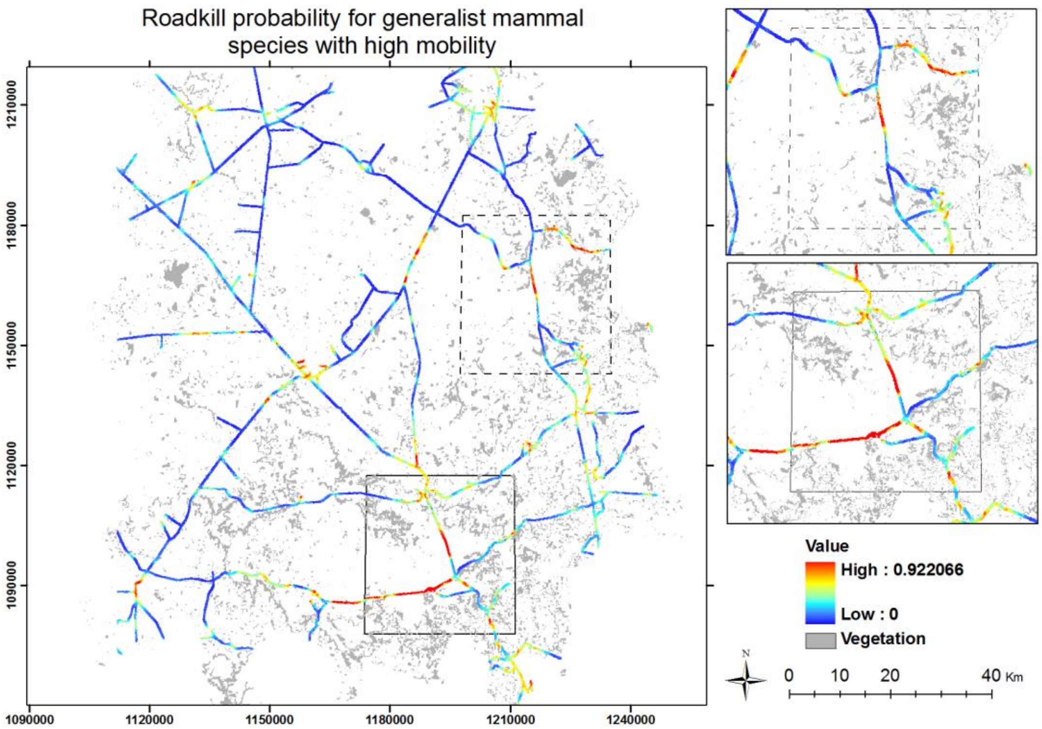

Figure 15. Probabilities of road kill for medium and large mammals for the functional group High Mobility Generalist (HMG) for highways in the interior of the State of São Paulo, Brazil. Two details are presented on the right.

The analyses of the maps presented in Figures 13 to 15 indicate that the vast majority of highway positions refer to low probabilities of road kill provides the opportunity to propose strategies that might be more suitable than others. Among them include local strategies for road kill mitigation, such as speed reducers, radars and wildlife corridors, which should be well constructed and consider the particularities of each functional group (Clevenger & Waltho, 2005, Lesbarrères & Fahrig, 2012). This also suggests that the strategy of fencing the entire length of the roads could not work, because it would impede transit of the animals, as well as increase monetary and conservationist cost (Pearce & Ferrier, 2000, Ng *et al.*, 2004).

3.3. Frequency of road kill probabilities

Corroborating what was observed in Figures 13 to 15 the vast majority of positions on the roads present less than 10% probability of road kill. This result recurred in all three analyzed functional groups (Figure 16).

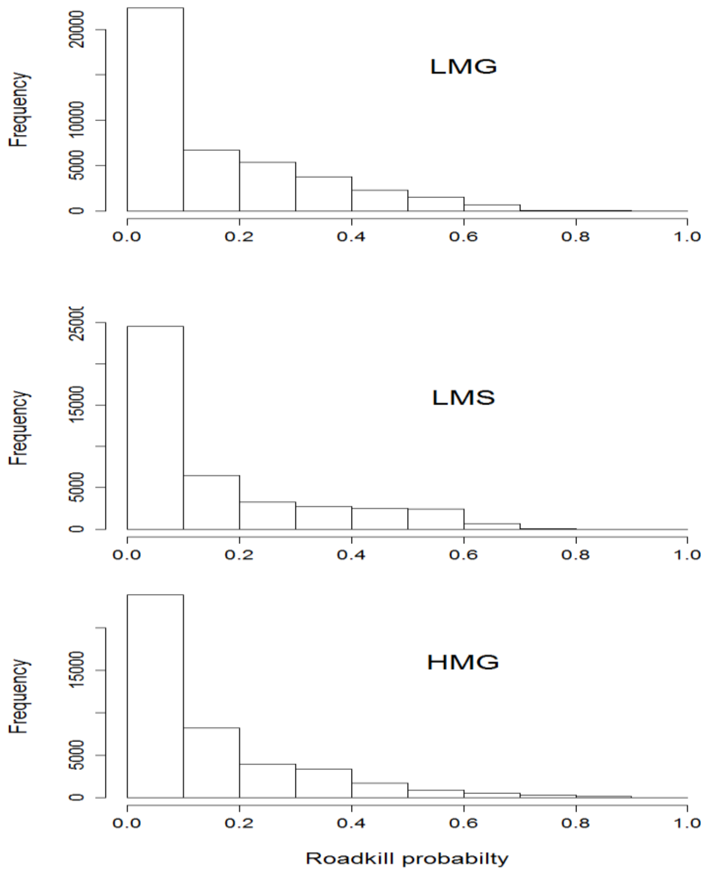

Figure 16: Histogram of probabilities of road kill for medium and large mammals on roads in the interior of the State of São Paulo, Brazil. LMS: High mobility sensitive; LMG: Low mobility generalist; HMG: High mobility generalist.

Again, such results suggest that mitigation actions should be intensely localized in certain stretches of roads.

To increase the efficiency of road kill mitigation strategies, the differences between the functional groups require consideration (Clevenger *et al.*, 2003, Ng *et al.*, 2004, Sandra *et al.*, 2004, Kusak *et al.*, 2009). For example, many wildlife corridors, whether pre-existing or planned, are not functional because they were not specifically developed for the most at-risk species in the region where they were implemented (Soanes *et al*, 2013).

3.4. Correlations between functional groups

To assess whether the functional groups present spatially distinct patterns in the modeled region, we conducted Pearson's correlation coefficient (r) analyses. The following values were estimated: LMS x LMG = 0.75; LMG x HMG = 0.56 and LMS x HMG = 0.3. From the road kill probability maps for the functional groups, we generated scatterplots between the probabilities for paired functional groups, as well as trend analyses using Generalized Additive Models (GAM) (Figure 17). As shown in the values of the above correlations, models LMS and LMG have high correlation (r = 0.75), indicating similar probabilities in the models generated for the two functional groups. For groups HMG and LMG, a medium value of correlation exists, indicating that the probabilities are partially similar; however, there is much variability among them, stressing the importance of maintaining these separate groups.

Finally, the analysis of functional groups LMS and HMG shows very low correlation value (r = 0.3) and a tendency to demonstrate significant differences between the spatial patterns of road kill probability for the two models. This result is consistent with the different behavior of the species that make up the two groups in relation to the environment: generalist species of high displacement capacity (HMG) are very distinct from sensitive species of low displacement capacity (LMS). These species have differences in relation to environmental variables that facilitate or hinder their movement through the landscape. As such, it is essential to consider the different patterns observed for different functional groups to determine appropriate strategies and actions to reduce or mitigate the effects of the highways and the landscape on wildlife (Nally, 2001).

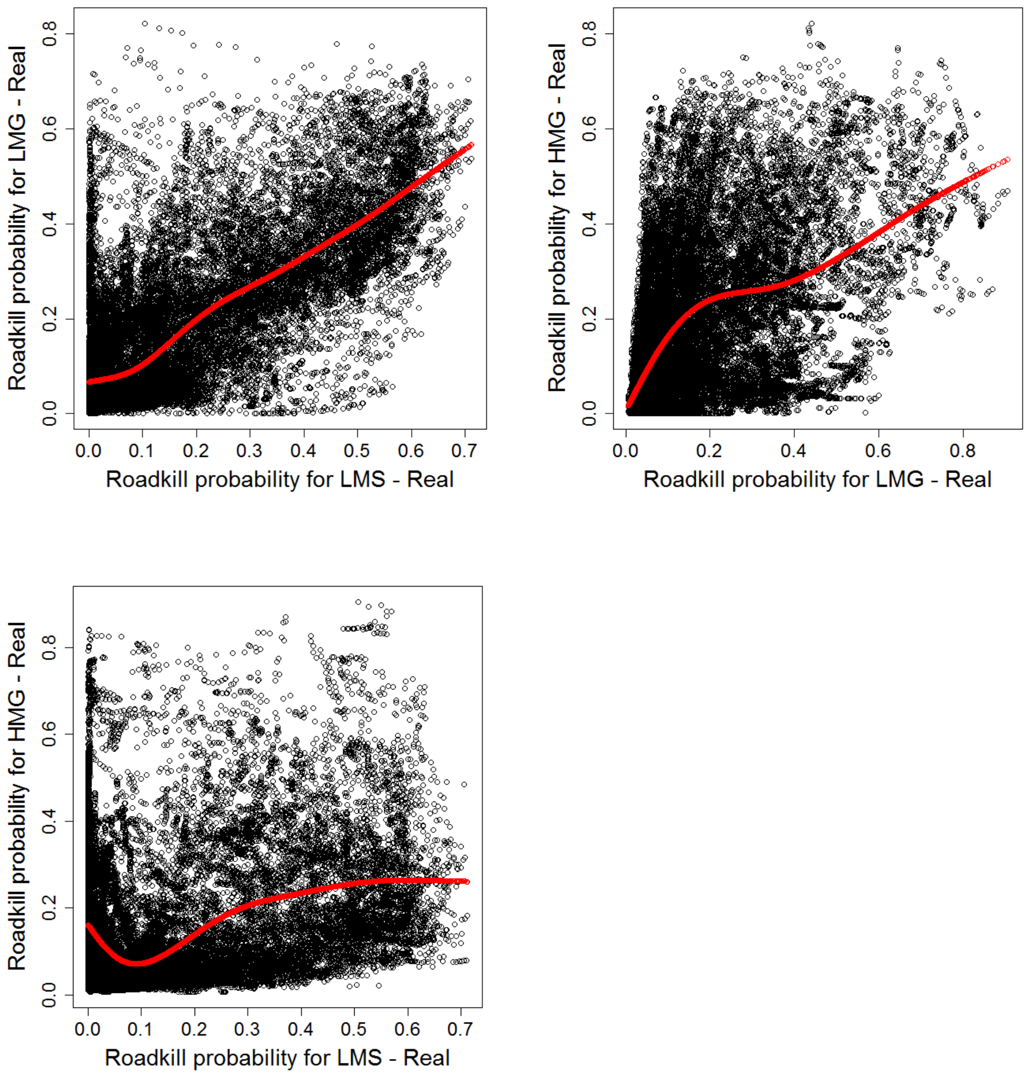

Figure 17: Dispersion between probabilities of road kill of medium and large mammals on roads in the State of São Paulo, Brazil, relating models for pairs of functional groups LMS = Low Movement Sensitive, LMG = Low Movement Generalist and HMG = High Movement Generalist. The red lines indicate the trend generated by the generalized additive model (GAM) in the program R.

4. Final considerations and new perspectives

4.1. Habitat suitability models and prediction of road kill

There are several types of prediction models for species distribution, environmental and habitat suitability models being examples of approaches. In the case of Road Ecology, we need to work with finer scales, analyzing the classes of land use and land cover in proximity to places of road kill (Roger & Ramp, 2009), assuming individuals select locations to cross the road according to landscape and environmental characteristics (Heglund, 2002).

Most road ecology studies focus only on *hotspots* and rates of road kill (Forman & Alexander 1998). In general, they don't allow proper identification and quantification of the effects of landscape or local features on road kill events, and the extrapolation of data to predict road kill hotspots for other regions lacking information is limited. The method proposed here provides a significant advance in this sense, as it allows: a) evaluation of the relative contribution of the various landscape attributes on the probability of wildlife being hit by vehicles, b) separate or combine evaluation of species with distinct ecological characteristics, and c) extrapolation of road kill probability from one region to a similar one, provided that it is pertinent; d) hypothesis testing with robust approaches, statistically, ecologically, and of the modeling itself. Besides consisting of a transparent and simple method, it can potentially be used in any region worldwide. Adaptations are viable for analysis of other species or groups, and even other response variables (e.g. use of wildlife corridors, effectiveness of crossing structures, effects of infrastructure such as guide fences or Jersey barrier).

4.2. Functional groups, landscape and road kill

All species that have similar characteristics and relationships with the environment (i.e. obtaining food resources, sensitivity to anthropogenic changes, displacement capacity through matrices and landscape perception) will probably have similar behavior. This study highlights the importance of grouping species by environmental requirement to identify differences in the contribution of each element of anthropogenic landscapes in wildlife-vehicle collisions for functional groups (Ferrier *et al.*, 2002; Cain *et al,* 2003) and consequently for establishing relevant road kill mitigation strategies. This importance was most evident when we analyzed the correlation between ecologically distinct functional groups (LMS and HMG) and their road kill probabilities.

The vast majority of the highways in areas of high population density lie in landscapes altered by humans. Consequently, for what remains of the wildlife found in altered environments, anthropogenic matrices may exhibit significant influences on the various ecological processes. This was evident as we determined that the variables percentage of sugarcane and distance from cerrado were of great contribution in models to estimate probability of road kill (Roger & Ramp, 2009). Since there are few studies about how animals move through and use the landscape, we consider the use of different scales among functional groups (Boscolo & Metzger 2009) in the analyses as an important strategy in the process. Thus, we were able to quantify the effect of the landscape on wildlife-vehicle collisions for organisms with a wide range of characteristics, from sensitive animals of small living area such as Brazilian Porcupine (*Coendou prehensilis*) to large carnivores such as the puma (*Puma concolor*). The use of different scales seems imperative to portray the influence of the landscape on the displacement of these species.

The majority of areas with dense road network are in landscapes with anthropogenic matrices (e.g. eucalyptus, pastures, plantations) and to obtain resources, wildlife must adapt to use these areas (Lesbarréres & Fahrig, 2012). As the landscapes differ both in their degree of conservation and in the geography and habitat types, the models must be transparent (comprehensible methodology) and replicable (Nally *et al*, 1999, Malo *et al.*, 2004, Kociolek & Clevenger, 2007) so that they can be applied to other types of landscapes. The capacity for extrapolation (analyze a small area to understand a large similar area) is of extreme importance to the practical effectiveness of the model (Ramp *et al.*, 2005).

4.3. Future prospects

We believe that the method proposed here, although very promising, can be perfected and have its approach broadened. As a starting point, we present a list of study foci or refinements identified so far:

- Evaluate the effects of landscape on road kill of organisms belonging to other groups, such as birds, amphibians, snakes, invertebrates and small mammals;
- Compare within the same functional group the relative contributions of each variable, if multiple scales were used simultaneously;
- Validate the model for regions where data were not used, to see the level of consistency of the estimates in relation to other similar regions;
- Combine modeling for habitat suitability with genetic information to verify if road kill predictions can explain genetic structuring or reduced gene flow of species;
- Reconcile the approach presented with movement simulation models for individuals, to verify if there are emergent patterns related to the ecology of movement of the species;

- Assess whether aspects of infrastructure (Ng *et al.*, 2001) implemented in other regions may alter road kill predictions, particularly with the comparison of databases before and after implementation;
- Test the present approach in distinct regions, which include landscapes along gradients of conservation, fragmentation, natural and anthropogenic heterogeneity, natural aspects such as relief, slope and other parameters of geomorphometry;
- Assess whether highways with distinct characteristics (freeways, double lanes, single lane asphalt, dirt roads and access roads) result in differences in the relative contribution of the landscape parameters on road kill;
- Integrate the present approach with radio telemetry or GPS data, to identify conditions for highways or landscapes that allow, prevent or are indifferent to the probability of highway crossing by wildlife.

The method presented here opens a new avenue for the ecology of roads, highway management, definitions of strategies for impact mitigation, and even for the most appropriate action planning in support of biodiversity conservation and landscape restoration. While we seek to advance the science, we are aware that society requires quick and reliable responses for how best to plan or design its actions. Methods that present low-cost data acquisition, user-friendly techniques, high power of extrapolation and ability to generate efficient communication material (e.g. maps) extends the return potential of academia for practical issues in the different spheres of government, managers, decision makers and society.

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Appendix 1

Correlations between the five variables that contributed most in the models generated for the interior of the State of São Paulo, Brazil, for each group (LMS: Low mobility sensitive, LMG: Low mobility generalist e HMG: High mobility generalist).

A: Functional group with 300 m diameter influence of the landscape

	Sug_dist	%Sug300m	Silv_dist	%Silv300m	Cerra_dist	%Cerr300m	%For300m	Veg_Dist	%Veg300m
%Sug300m	-0.63								
Silv_dist	-0.41	0.44							
%Silv300m	0.69	-0.32	-0.48						
Cerra_dist	-0.25	0.60	0.35	-0.20					
%Cerr300m	-0.03	-0.18	-0.11	-0.03	-0.22				
%For300m	-0.20	0.01	-0.19	-0.10	-0.20	-0.04			
Veg_diSt	0.27	0.35	0.12	0.18	0.43	-0.22	-0.34		
%Veg300m	-0.26	-0.12	-0.08	-0.12	-0.32	0.45	0.72	-0.59	
For(diSt)	-0.14	0.11	0.12	-0.12	-0.14	-0.04	0.47	0.07	0.27

B: **Functional group with 600 m diameter influence of the landscape**

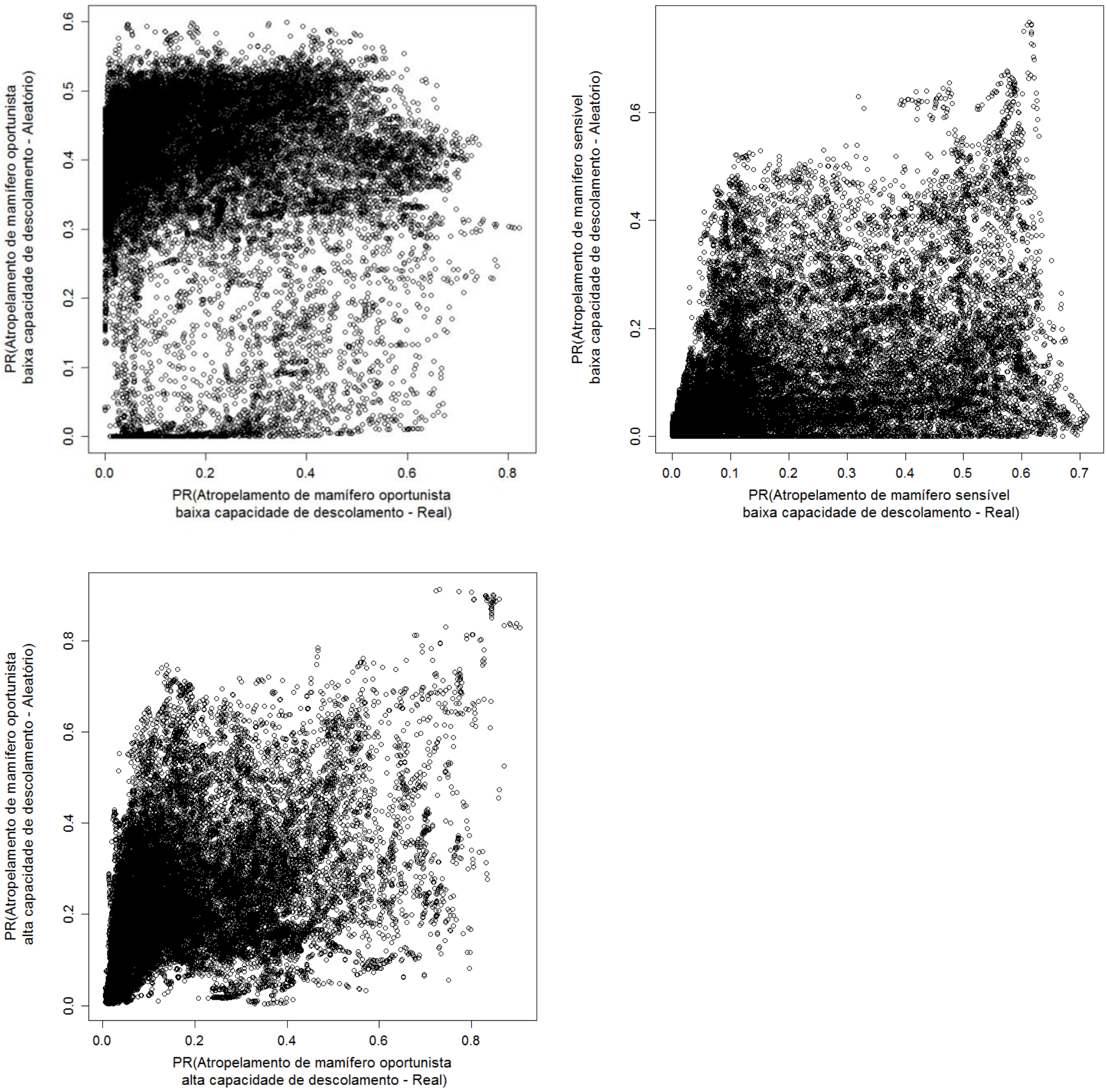
	Sug_dist	Silv_euc	Cerra_dist	Veg_euc	For_euc	Cerra_600m	Silv_600m	Sug_600m	For_600m
Silv_euc	-0.41								
Cerra_dist	-0.25	0.35							
Veg_euc	0.27	0.12	0.43						
For_euc	-0.14	0.12	-0.14	0.07					
Cerra_600m	0.06	-0.16	-0.28	-0.21	-0.09				
Silv_600m	0.70	-0.51	-0.21	0.19	-0.12	0.03			
Sug_600m	-0.66	0.45	0.56	0.38	0.19	-0.28	-0.38		
For_600m	-0.25	-0.12	-0.27	-0.47	0.32	-0.03	-0.22	-0.04	
Veg_600m	-0.27	-0.09	-0.40	-0.66	0.11	0.34	-0.24	-0.19	0.82

C: **Functional group with 1000 m diameter influence of the landscape**

	Sug_dist	Silv_euc	Cerra_dist	Veg_euc	For_euc	Cerra_1000m	For_1000m	Veg_1000m	Sug_1000m
Silv_euc	-0.41								
Cerra_dist	-0.25	0.35							
Veg_euc	0.27	0.12	0.43						
For_euc	-0.14	0.12	-0.14	0.07					
Cerra_1000m	0.05	-0.35	-0.38	-0.24	-0.13				
For_1000m	-0.30	0.28	-0.27	-0.57	0.12	-0.18			
Veg_1000m	-0.31	0.17	-0.43	-0.71	0.01	0.19	0.90		
Sug_1000m	-0.55	0.50	0.62	0.48	0.20	-0.27	-0.23	-0.33	
Silv_1000m	0.70	-0.61	-0.27	0.20	-0.10	0.10	-0.36	-0.34	-0.44

Appendix 2

Dispersion between road kill probabilities for medium and large mammals on roads of the interior of the State of São Paulo, Brazil, relating real and neutral models. LMS = Low Mobility Sensitive, LMG = Low Mobility Generalist e HMG = High Mobility Generalist.



CAPÍTULO 2 - Highway duplication and underpass interference on vertebrate road mortality

Capítulo a ser submetido à revista *Biotropica (Commentary, up to 2000 words*).

Highway duplication and underpass interference on vertebrate road mortality

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Abstract

Wildlife road kill may be altered by road duplication and the presence of crossing structures. We used road kill records of large and medium sized mammals obtained in a 150 km before and after road duplication to evaluate changes. Only the group of animals with low mobility and generalists showed a significant difference with an increase in road kill records after road duplication. Road kill records of functional groups did not vary according to distance to underpasses.

1. Introduction

Road upgrading through duplication and widening has become a trend in a globalized world with increasing demands for production flow and human mobility. Often road upgrading involves adding lanes to undivided highways and transforming undivided into divided highways with more than two lanes per roadway. Environmental consequences of such changes are not accounted for in advance and not many efforts have targeted duplication effects on biodiversity (e.g. Taylor and Goldingay, 2014).

Mitigation measures to minimize road effects on animal populations include wildlife warning signs, traffic volume and speed reduction tools, wildlife fences and crossing structures, such as culverts, underpasses and overpasses (Iuell et al., 2003; Glista et al., 2009). Also modification of road design using viaducts, bridges and changes in road-verge management (van der Grift et al., 2013) may prevent road mortality and the barrier effect. Among several mitigation measures, modified road design and wildlife crossing structures must be planned together with the road construction. However, lack of information about the effectiveness of such measures has being used to disregard this type of investment (van der Grift et al., 2013).

Although road duplication may cause changes in road mortality, these influences rarely has been investigated. Taylor and Goldingay (2014) detected a decline in the usage of underpasses by bandicoots after road duplication in Australia. Furthermore, understanding how upgrading roads impact animal populations and mobility through landscape is of utmost importance to ensure the adoption of appropriate mitigation measures. Frequently, road structures for water runoff have been adapted as crossing structures, but their efficiency in reducing road mortality has not been tested.

Here we analyzed whether road kill records changed after road duplication in comparison with before road mortality. We also investigated if the proximity to underpass implemented with road duplication reduced animal mortality. In both questions we analyzed medium and large sized mammals species classified into three functional groups, according to species mobility and sensitivity to habitat disturbance.

2. Material and method

2.1. Study site and data collection

Road kill records were collected two years before (2006 and 2007) and two years after (2008 and 2009) duplication along 150 km of state road SP 225 (Rodovia Engenheiro Paulo Nilo Romano), between km 75 and 235. Before duplication, the road was undivided with only 2 lanes and after the duplication process it was divided with at least 2 lanes each roadway (total of 4 lanes with median). This road has a relatively homogeneous structure with few curves and predominantly flat relief. Land use within the region is dominated by sugarcane, but also presents orange plantation (citrus), pasture and eucalyptus and pinus plantations. The original vegetation of the study site is cerrado, semi deciduous forest and riparian forest, all still present in some remaining fragments.

Animal road kill was systematically recorded by OHL BRASIL concessionaire, responsible for SP 225 road. Carcass detection and registration was made from a car at less than 50 km/h, according to São Paulo Transportation Agency (ARTESP) and Instituto Chico Mendes de Biodiversidade (ICMBio) normative. The road was checked every three hours. GPS location and photos of killed animals were registered for posterior identification. In this study only records of medium and large-sized mammals were analyzed.

2.2. Before and after road duplication road mortality

Species were classified into three functional groups, according their mobility and sensitivity to habitat disturbance: Low Mobility Sensitivity species (LMS), for species that are more sensitive to habitat disturbance, but with restricted (<300 meters) mobility into the matrix; Low Mobility Generalist (LMG), for species more adapted to habitat disturbance, but with mobility into the matrix up to 1,500 meters; High Mobility Generalist (HMG), for species with high adaptability to disturbed areas, and with highly capacity of move into the matrix (3,000 meters). The number of animal mortality before and after road duplication was accounted for each species and functional group. χ^2 test in R allowed us to estimate if there was significant increase or decreased on these road kills.

2.3. Distance to underpass and changes on road kills

We used two approaches to assess the relationship between road kill and distance from underpass. First we used road kill records position before and after road duplication and estimated the distance of each record to the nearest underpass. Then we grouped the distances from the nearest underpasses in equally spaced classes of 250 meters. After that, we did a χ^2 test in R in order to assess if there was a significant difference between the frequency of road kill records after road duplication between classes of distance from underpasses, when compared to the reference (before road duplication).

3. Results

Of 293 road killed medium and large size mammals of 19 species, half (N=148) was made in the period monitored before road duplication (2006 and 2007) and half (N=145) after duplication (2008 and 2009) – Table 1. Roughly, more animals with low mobility generalist (LMG) were killed in the road after duplication (χ^2 =2.84, p=0.09), especially European hares and armadillos. The amount of animals with high mobility generalist (HMG) killed after road duplication decreased, but this difference was not significant (χ^2 =1.35, p=0.25). Animals with low mobility and sensitive (LMS) also presented less road kills in a smaller magnitude than HMG, and the difference was not significant as well (χ^2 =0.44, p=0.50).

Species present some variation in number of road killed animals before and after duplication. However, χ^2 significant differences were detected only for Giant anteater (*Myrmecophaga tridactyla*), Crab eating fox (*Cerdocyon thous*), Brown capuchin (*Sapajus apella*) and European hare (*Lepus europaeus*) – See Figure 1.

Table 1: Number of road kill records of medium and large-sized mammals before (2006 and 2007) and after (2008 and 2009) road duplication within highly fragmented and heterogeneous landscapes of São Paulo state, Brazil. Species are classified according to their mobility capacity and sensitivity to habitat disturbance.

High Mobility Generalist (HMG)	**Before**	**After**
Cerdocyon thous (Crab eating fox)	51	31
Hydrochoerus hydrochaeris (Capibara)	24	14
Mazama gouazoubira (Gray broket)	10	8
Myrmecophaga tridactyla (Giant anteater)	0	7
Chysocyon brachyurus (Maned wolf)	4	4
Puma concolor (Mountain lion)	2	2
Subtotal	***91***	***76***
Low Mobile Generalist (LMG)		
Lepus europaeus (European hare)	16	32
Dasypus sp. (Armadillo)	10	11
Didelphis albiventris (White-eared opossum)	2	3
Myocastor coypus (Coypu)	1	3
Nasua nasua (South American coati)	3	3
Sapajus apella (Brown capuchin)	4	0
Callithrix jacchus (White-tufted-ear marmoset)	1	1
Subtotal	***37***	***53***
Low Mobility Sensitive (LMS)		
Tamandua tetradactyla (Southern tamandua)	8	5
Coendou prehensilis (Brazilian porcupine)	3	4
Procyon cancrivorus (Crab-eating raccoon)	7	3
Leopardus pardalis (Ocellot)	1	3
Cuniculos paca (Spotted paca)	0	1
Eira barbara (Tayra)	1	0
Subtotal	***20***	***16***
TOTAL	148	145

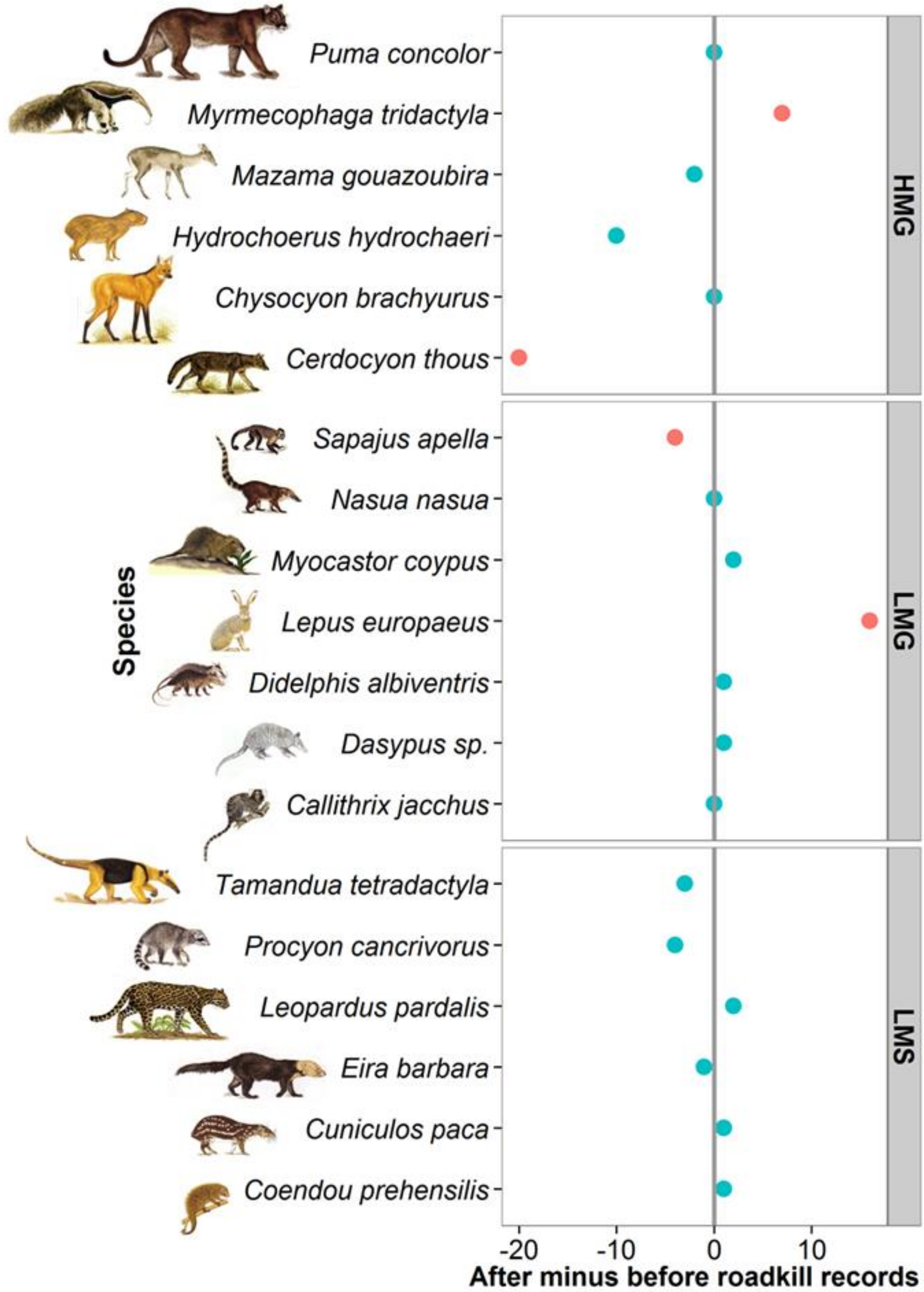

Figure 1 – Differences on road kill records before (2006 and 2007) and after (2008 and 2009) road duplication per species, organized according to functional group. HMG: High mobility generalist; LMG: Low mobility generalist; LMS: Low mobility sensitive. Positive values indicate increase on road kills, negative values the contrary. Species with red dots presented significant changes according to χ^2 test (α=0.05).

Although we expected a reduction in animal road kills nearby underpasses implemented after duplication, according to χ^2 test there was no significant changes on the frequency of killed fauna along road. This was similar for all the three functional groups of mammals: χ^2 for HMG=9.95 (p=0.44); χ^2 for LMG=12.84 (p=0.17); χ^2 for LMS=3.02 (p=0.81). Figure 2 presents the density of road kills before and after road duplication, for the three functional groups.

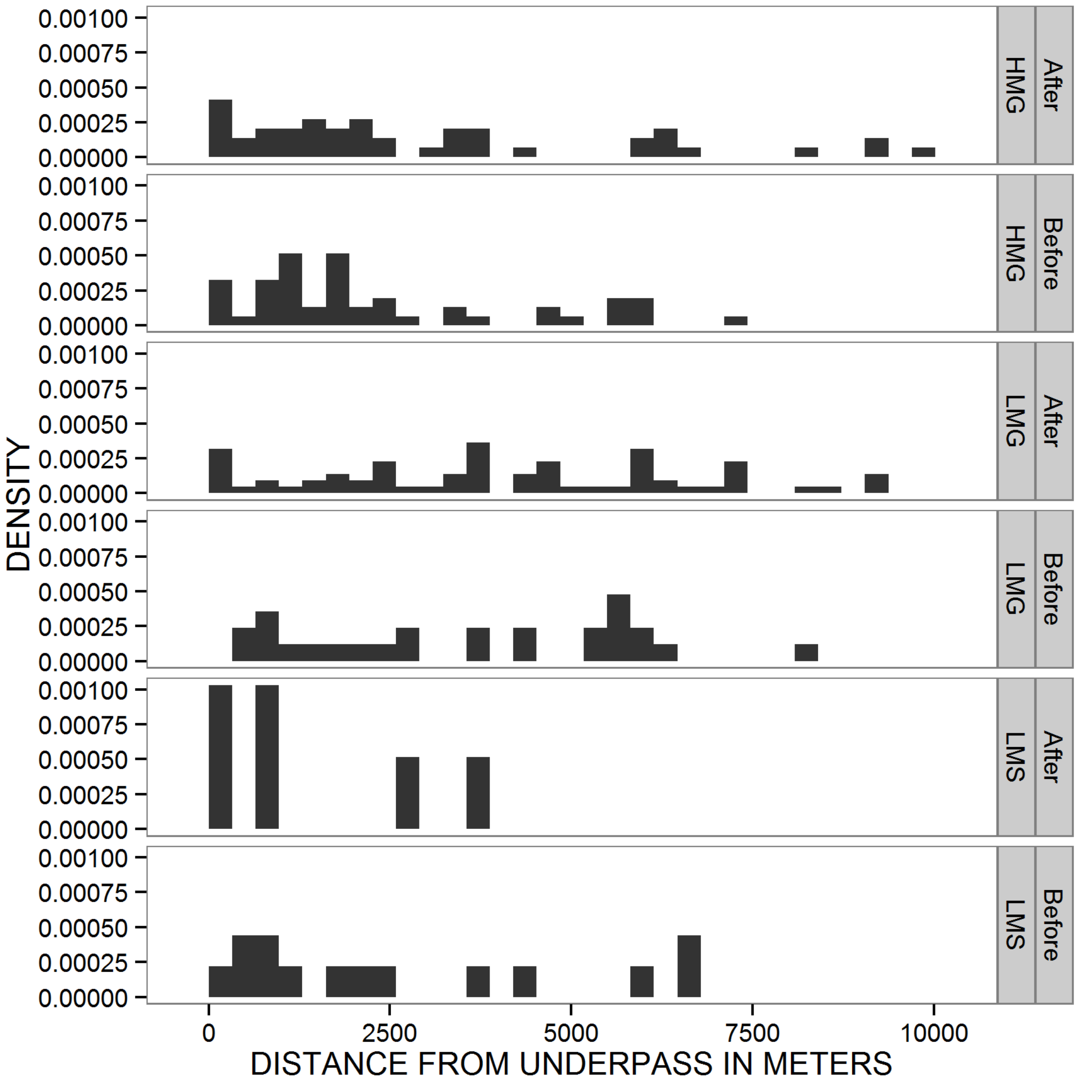

Figure 2 – Spatial distribution of road kill records before and after road duplication according to distance to closest underpass in meters. Information related to functional groups. HMG: High mobility generalist; LMG: Low mobility generalist; LMS: Low mobility sensitive.

4. Discussion

4.1. Road duplication interference on road mortality

We weren't able to identify significant differences between road kill before and after duplication for all three functional groups. Only LMG presented significant difference with a higher number of road kills after duplication. We still don't have enough information to state whether animal behavior is the driving force for such results, but one plausible explanation is the way animals respond to structural changes and traffic increase. Some generalist species with broader home ranges seem to be more affected by road duplication than those that are more sensitive to disturbances. In this sense, for species with low movement capacity, the barrier effect may cause a reduction on road kill but it can also increase population isolation.

Collection of road kill data should be mandatory and standardized to provide further information on road impact for wildlife. Comparison between periods before and after duplication is as important as studies carried out during and after road construction. Concessionaires and road agencies are responsible for road impacts and road kill must be avoided in order to maintain landscape permeability as much as possible, reducing the chance of isolating populations by roads.

4.2. Underpass versus road kill: Beyond use, towards efficiency

In order to move beyond assessing the use of crossing structures, researchers and road agencies must work together to evaluate mitigation strategies effectiveness, such as underpasses. All groups of interest (e.g. road ecologists, road concessionaires, road government agencies should be involved in the process of designing evaluation and monitoring programs for mitigation. This includes collecting information at previous stages (before road construction or duplication), adequate replication and appropriate spatial and temporal scales for evaluation (van der Grift et al., 2013).

Although underpasses were implemented in this case, we observed that the proximity to underpass did not diminish road kill. This is critical, because worldwide underpasses are suggested as an effective way of mitigating road mortality. For the three functional groups analyzed, road kill before and after road duplication remained unaffected by proximity to underpasses. We suggest that the type of structure used as underpass must be a focus of research.

Traffic control and location of mitigation measures may be used to promote road permeability and therefore population persistence (Jaeger et al., 2005). Crossing structures that already exist must be thoroughly monitored to provide information about their efficiency in order to support the selection and location of appropriate mitigation measures. Results as those produced by Malo et al. (2004) optimize the location of mitigation structures in Spain at detailed and larger scales with focus on three species (roe deer, wild boar and red deer).

Similarly to what Taylor and Goldingay (2014) identified in their study in Australia, we also verified that underpasses became much longer after duplication. Demonstrating how animals are affected by this change remains a challenge, because other attributes of crossing structures and landscape surrounding them may also be of importance. Additionally, Taylor and Goldingay (2014) verified a reduction in underpass use by bandicoots after duplication. Individuals' behavior and perception of underpasses must be considered in the mitigation planning process to cover a variety of species.

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CAPÍTULO 3 - Highway duplication changes landscape influence on vertebrates road kill

Capítulo a ser submetido à revista *Biological Conservation*.

Highway duplication changes landscape influence on vertebrates road kill

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Abstract

Predicting road kill is a challenge worldwide, but understanding how recurring changes on road structure (such as road duplication) influence animal road mortality has no methods to properly addresses the influences of local and landscape variables. In this study we proposed a novel method to analyze the effects of road duplication on road kill and investigated how road duplication changed the influence of landscape structure on road kill of three functional groups of vertebrates. The novel method proposed to analyze changes in road kill after road duplication, tailors habitat suitability models to combine species characteristics and landscape attributes at various scales. The method has proved to bring important contribution to the field of road ecology. The transparence and reliability of the method consist of very favorable aspects for its replicability. Also, by considering ecologically relevant functional groups as target for the analysis we increased the generalization of results for a great variety of regions worldwide. Particularly for the study region, large amounts of sugar cane increased road kill probabilities after road duplication, and large amounts of forest decreased road kill probabilities. Each functional group responded differently to road duplication, land use and landscape structure. We believe opening a new venue combining techniques largely used is helpful to provide answers to ecologically relevant questions faced in road ecology. Biological conservation field will benefit worldwide if road ecologists provide information about how fauna interacts with both landscape features and road structures. In this sense, transportation planning will be able to incorporate effective mitigation for road effects considering both aspects.

1. Introduction

Road networks and traffic volume will continue expanding in most countries throughout the world, particularly in Eastern Europe, China, India and Latin America (Taylor e Goldingay, 2010; van der Ree et al, 2011). Additionally, upgrading existing roads has become a common strategy to overcome congestion and increase mobility (White, 2007; Rhodes et al, 2014). Roads interference on ecological processes is well studied worldwide. Nonetheless, species response to road effects vary and little is known on its pervasiveness and predictability across taxa (Fahrig and Rytwinski, 2009; Taylor and Goldingay, 2010). Road upgrading includes highway duplication and traffic intensification. The effects of these modifications may change the way animals respond to landscape and road elements combined. However, duplication has not been addressed from the perspective of changes and predictability of animal road kill along road segments (i.e. 10 km or more) taking animal responses and landscape context into account.

Most studies in road ecology have been developed in North America, Europe and Australia, with very few being held in tropical environments (Taylor and Goldingay, 2010). Among well explored local road impacts are habitat loss and fragmentation, reduction of habitat quality and spread of invasive species. Consequences to wildlife population processes comprise vehicle-induced mortality of individuals and the effect of filter or barrier to animal movement (Jaeger et al, 2005; Taylor and Goldingay, 2010). Road mortality is of great concern once it causes loss of individuals to a degree which may interfere in the genetic viability of populations (Epps et al, 2005; Holderegger and Di Giulio, 2010; Laporte et al, 2013). In addition, when roads act as filters or barriers, animals develop a behavioral response, most commonly avoidance (Trombulak and Frissell, 2000; Jaeger et al, 2005, Brehme et al, 2013), which may be less harmful to population once it minimizes road mortality, but it also promotes subdivision and isolation of populations (Trocmé et al, 2003; Jaeger et al, 2005; Reding et al, 2013).

Although road density and traffic volume can have substantial impacts on road mortality, it seems that increasing road density has higher effects on mortality rates than increasing traffic volume on existing roads (Rhodes et al, 2014). However, road duplication implies wider road surface, increased traffic volume and, consequently amplified barriers to animal movement (Taylor and Goldingay, 2010; Rhodes et al, 2014). In this case, animal behavior of road avoidance can be more harmful to populations than that of road mortality because subdivision into smaller populations also increases extinction risk (Jaeger and Fahrig, 2004; Jaeger et al, 2005).

Road impacts on populations are determined by interactions between population density, movement behavior, landscape configuration and habitat distribution (Seiler, 2002; Malo et al, 2004; Rhodes et al, 2014). Animal activities may cluster near roadsides when resources and wetlands are concentrated along them. Also, road kill hotspots can occur as a consequence of movement funneled by topographic features (Litvaits and Tash, 2008) and landscape attributes (Forman and Alexander, 1998). Many factors are recognized to contribute to the spatial distribution of wildlife-vehicle collisions, namely traffic volume, vehicle speed, road width, roadside vegetation, driver awareness and animal behavior (Seiler, 2002; Litvaitis and Tash, 2008). Road mortality rates may be related to population density, though the overall population impact of large, high-volume roads is recognized as alarming (Taylor and Goldingay, 2010).

Animals use and avoid types of roads differently (Brehme et al, 2013), nevertheless road related movement behavior studies commonly focus on a single species or on a specific road type. Even so, few data about animal avoidance to road and traffic is available. Spatial location of roads and proximity to habitat or other available resources are determinant to infer animal behavior as they can result in animal attraction to roadsides and consequently increasing road mortality depending on species mobility and sensitivity (Ramp et al, 2005; Rhodes et al, 2014).

Brehme et al (2013) analyzed movement behavior of small mammals and lizards towards different road types. They found small mammals seem to avoid paved surfaces, yet this behavior is not generalizable to all species. They also verified lizards can be attracted to dirt and secondary roads with relative low traffic volume, but tend to avoid heavy traffic (Brehme et al, 2013).

Litvaits and Tash (2008) reviewed three approaches to investigate wildlife-vehicle collisions. The first is the identification of road kill hotspots based in the comparison of local features present at sites where road kills occur and where no collisions were recorded. The second refers to road-density thresholds that consider road abundance and animal distribution to indicate densities that may limit population expansion at broader scales. The third approach deals with wildlife-vehicle collision models which may be more adequate for species-specific or road specific purposes once it incorporates more detailed information to estimate road kill probability per crossing (Litvaits and Tash, 2008).

In their study, Rhodes et al (2014) developed a simple model using animal movement and road mortality data. They suggest that wildlife may be less harmed when an existing network is enhanced rather than when new roads are built as a strategy to expand traffic. Jaeger et al, 2005 considered three types of road avoidance behavior (surface, noise and cars) in their model for predicting the risk caused by roads upon animal populations. They analyzed how animal responses varied according to different road types and traffic through simulations. Results allowed general insights, perhaps the most important being the generation of directions for field studies designs to access empirical data about populations to further investigate road avoidance behavior (Jaeger et al, 2005).

Models have been designed to detect road kill hotspots (Nielsen et al, 2003; Ramp et al, 2005; Teixeira et al, 2013) and identify areas more prone to road casualties (Malo et al, 2005; Ramp et al, 2005; Hobday and Minstrell, 2008). This type of modeling usually considers road kill information and, in some cases, traffic volume, vehicles speed and landscape features (Malo et al, 2005; Litvaits and Tash, 2008). For Ramp and Roger (2008), the identification of road kill hotspots is of utmost importance for management and the identification of species specific hotspots or functional tendencies could improve mitigation efforts (Ramp and Roger, 2008).

Nielsen et al (2003) used the hotspot approach to investigate deer-vehicle accidents. They selected landscape variables associated with deer-related accidents to guide habitat management to minimize collision risk. Malo et al (2004) developed models associating environmental variables with road kill information from a database to make assumptions at local and landscape scales. Results allowed inference about probable collision locations and identification of relevant variables that favor and hinder animal movement. For instance, presence of forest and high habitat diversity were associated with road kill clustering. Whereas, apparently, animals preferred to cross roads far from the presence of humans, in areas with low crop cover and low presence of buildings, without high embankments at roadsides (Malo et al, 2004).

Teixeira et al (2013) analyzed spatial patterns of road kill hotspots for groups of vertebrate species using SIRIEMA v1.1 software (www.ufrgs.br/biociencias/siriema). They claim using a single species approach constrains results for less common species with high conservation interest because only common species have high numbers of road kill records. They didn’t find a clear correlation among groups and concluded taxonomic groups and other grouping criteria (body size, commonness, locomotion type and time of activity) present low similarities of hotspots location.

The quality and precision of input data used to develop predictive models is paramount to the reliability and application of results, especially in cases that combine local and landscape variables (Taylor and Goldingay, 2010). Ciocheti et al (in prep, Chapter 1) proposed an application of software Maxent 3.3.3 (Phillips et al, 2006) to estimate road kill probability combining road kill records and landscape attributes instead of its common use to identify habitat suitability for species of interest. They used only road kill records of large and medium size vertebrates because of high carcass detectability and assertion in identification. They also used functional groups based on mobility and sensitivity to optimize inferences and empower statistical approach.

Considering that 1) no meaningful changes have occurred in land use close to the road, 2) the main alteration in this landscape was road duplication, 3) studies that investigate road mortality before and after road improvement are scarce, 4) we have already developed a model to access road mortality probability and, 5) there is available data on road kill for the period before and after duplication, we investigated road kill probability changes in face of road duplication for three functional groups of vertebrates. We also explored how road duplication influences landscape variables effect on road kill probability.

2. Material and Methods

2.1. Study area and road

The study region is located within highly fragmented and heterogeneous landscapes of interior of São Paulo state, in the municipalities of Jau, Brotas and Itirapina (Figure 1). The main road of the region is a segment of SP 255 (Rodovia Engenheiro Paulo Nilo Romano), between km 75 and 235. The original vegetation is cerrado (Brazilian savannah), semi deciduous forest and riparian forest. At the period of data collection, SP 255 road was an undivided highway with relatively homogeneous structure, few curves and predominantly flat relief. Land use within the region is dominated by sugar cane, citrus (orange plantation), pasture and eucalyptus plantation.

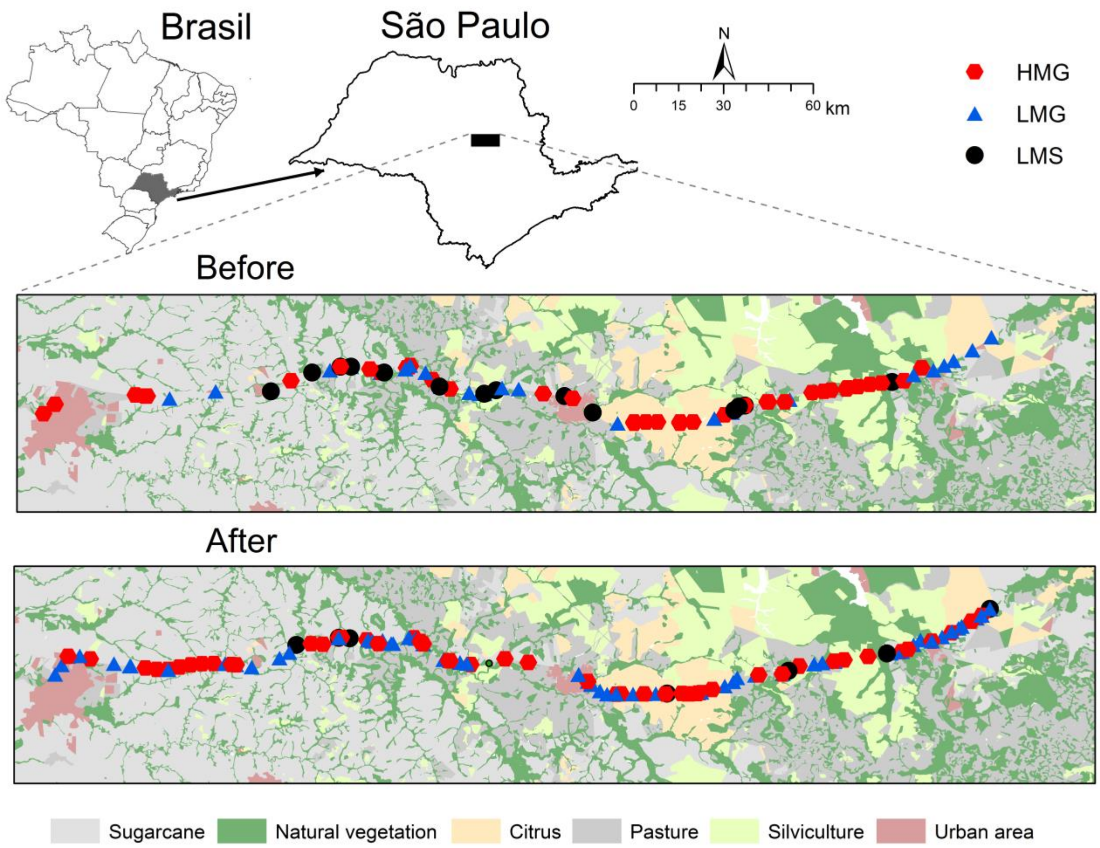


Figure 1 - Road stretch of SP 255 between km 75 and 235 in the interior of the state of São Paulo, Brazil, before and after road duplication. Road kill records are separated by functional groups: High Mobility Generalist (HMG), Low Mobility Generalist (LMG), Low Mobility Sensitive (LMS). Land use and land cover classes are presented according to legend.

2.2. Road kill data and functional mammal groups

The road kill records were obtained from OHL BRASIL concessionaire, which administrates the SP 255 road. Data were collected two years before (2006 and 2007) and two years after (2008 and 2009) road duplication following São Paulo Transportation Agency (ARTESP) and Instituto Chico Mendes de Biodiversidade (ICMBio) normative. Monitoring was made by car, with speed up to 50 km per hour. The road was checked every three hours. All animals found killed in the road were recorded, and pictures were taken to facilitate species identification by experts. All records had their geographic coordinates taken using a Garmin GPS. In this study, only medium and large-sized mammal records were analyzed.

Species were classified into three functional groups, according their mobility capacity and sensitivity to habitat disturbance: a) Low Mobility Sensitive species (LMS), for species that are more sensitive to habitat disturbance, but with restricted mobility into the matrix (<300 m); b) Low Mobility Generalist

(LMG), for species more adapted to habitat disturbance, but with mobility into the matrix up to 1500 m; c) High Mobility Generalist (HMG), for species with high adaptability to disturbed areas, and with high capacity of moving into the matrix (3000 m). Table 1 presents the number of road kill records before and after road duplication for species and functional groups.

Table 1: Number of road kill records of medium and large-sized mammals before (years 2006 and 2007) and after (years 2008 and 2009) road duplication within highly fragmented and heterogeneous landscapes of Sao Paulo state, Brazil. Species are classified according to their mobility capacity and sensitivity to habitat disturbance.

High Mobility Generalist (HMG)	**Before**	**After**
Cerdocyon thous (Crab eating fox)	51	31
Hydrochoerus hydrochaeris (Capibara)	24	14
Mazama gouazoubira (Gray broket)	10	8
Myrmecophaga tridactyla (Giant anteater)	0	7
Chysocyon brachyurus (Maned wolf)	4	4
Puma concolor (Mountain lion)	2	2
Subtotal	***91***	***76***
Low Mobile Generalist (LMG)		
Lepus europaeus (European hare)	16	32
Dasypus sp. (Armadillo)	10	11
Didelphis albiventris (White-eared opossum)	2	3
Myocastor coypus (Coypu)	1	3
Nasua nasua (South American coati)	3	3
Sapajus apella (Brown capuchin)	4	0
Callithrix jacchus (White-tufted-ear marmoset)	1	1
Subtotal	***37***	***53***
Low Mobility Sensitive (LMS)		
Tamandua tetradactyla (Southern tamandua)	8	5
Coendou prehensilis (Brazilian porcupine)	3	4
Procyon cancrivorus (Crab-eating raccoon)	7	3
Leopardus pardalis (Ocellot)	1	3
Cuniculos paca (Spotted paca)	0	1
Eira barbara (Tayra)	1	0
Subtotal	***20***	***16***
TOTAL	148	145

2.3. Modeling road kill probability before and after road duplication

Following Ciocheti et al (in prep), we used habitat suitability modeling to predict road kill probability for each functional group using landscape metrics as input variables. All road kill records within a functional group and for a period (before and after) were considered as road kill occurrence.

We used a presence-only modeling method available on Maxent 3.3.3 (Phillips *et al* 2004; Phillips *et al,* 2006) to generate the road kill probability maps. On the modelling process we used 70% of records as training and 30% as test. Maxent sampling method was used as setup as *bootstrap*, with 10 replicates for each functional group and period (before and after road duplication). The models were evaluated using Area Under the Curve (AUC) method (Fielding and Bell, 1997; Pearson, 2007).

Land cover maps with spatial resolution of 30 meters were used to generate the landscape input layers for Maxent. This step used 2006 Landsat imagery, which was classified using visual interpretation within SPRING GIS and intensive field check. Although we used only one cover map, the landscape dynamics is quite low in that region, so we considered that small cover changes do not influence our analysis. Additionally we used two vegetation and land cover official maps as support for the refinement of our map: Instituto Florestal Forest map (Konkra et al, 1993) and CANASAT (INPE, 2006) sugar cane plantation map. Using GRASS 6.4 GIS, a set of eight landscape metric maps were calculated for the region (Table 2). A total of six road kill probability maps were generated, three before (one per functional group) and three after road duplication (one per functional group).

Table 2: Landscape metrics used to model road kill probabilities for medium- and large-sized mammals before (2006 and 2007) and after (2008 and 2009) road duplication, within highly fragmented and heterogeneous landscapes of Sao Paulo state, Brazil.

Landscape metric	**Description**
1. Percentage of native vegetation	Amount of vegetation within a search radius, which varied for each functional group (LMS, LMG and HMG; see dispersability of species above)
2. Percentage of cerrado vegetation	Amount of cerrado vegetation within a search radius, which varied for each functional group
3. Percentage of sugar cane	Amount of sugar cane within a search radius, which varied for each functional group
4. Percentage of silviculture	Amount of eucalyptus plantation within a search radius, which varied for each functional group
5. Distance from vegetation in m	Distance between every road pixels and the nearest native vegetation
6. Distance from water in m	distance between every road pixels and the nearest water body
7. Distance from sugar cane in m	distance between every road pixels and the nearest sugar cane plantation
8. Distance from silviculture in m	distance between every road pixels and the nearest eucalyptus plantation

2.4. Changes on road kill probability and land use

As our focus is on road duplication effect, we calculated the difference between road kill records after duplication minus road kill records before duplication. For each road pixel we had a road kill probability difference for each functional group. Positive differences indicate that road kill probability increased at that position, and negative values refer to reduction of road kill probability.

For each road position (i.e. pixel) we extracted road kill differences for the functional groups, and also identified the predominant land used within a search radius of 100 m. Road kill probability differences for the five main land cover classes were analyzed: forest, citrus, pasture, silviculture and sugar cane plantation. Density analyzed were explored visually in order to identify if road kill probability differences were preferentially negative (i.e. reduction on the probability), positive (increase on the probability), or neutral (equally distributed around zero).

To facilitate the inspection of road kill probability differences along the road and for the different land cover classes, graphics were generated for each functional group.

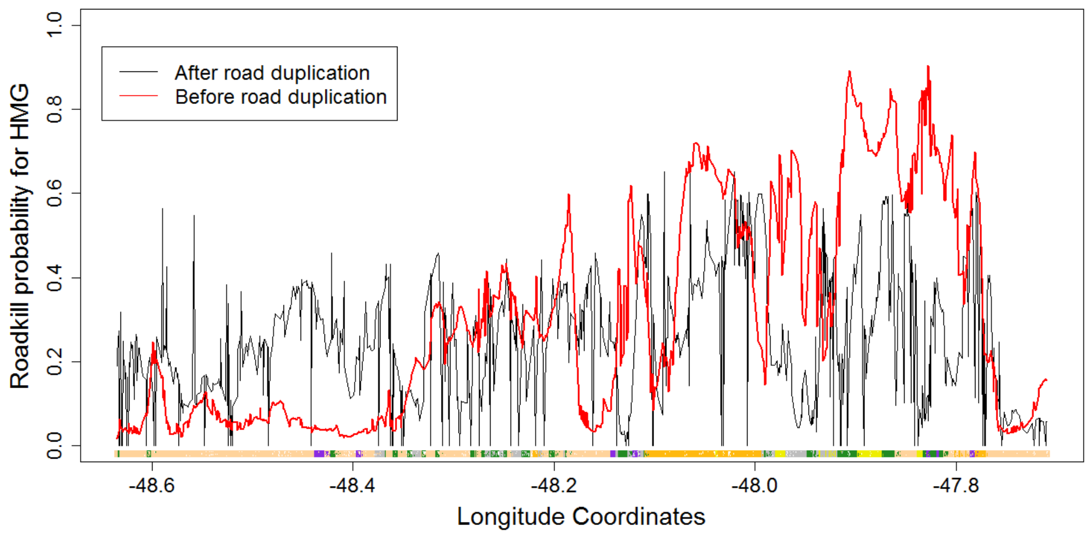
2.5 Relationship between changes on road kill probability and landscape

We used a model selection approach to assess the relative contribution of eight landscape metrics to explain the changes on road kill probabilities before and after road duplication. The difference of after minus before road kill probability for the three functional groups was used as response variable. Generalized Addictive Models (GAM; Zuur et al, 2009), with only one explanatory variable (see Table 2) per model, were fitted to explain road kill probability changes. Akaike Information Criteria corrected for small samples (AICc) were used to compare the models (Burnhan and Anderson, 2002). Graphical representation of response vs explanatory variables were done using ggplot2 (Wickham, 2009) and R 2.15.3 package (R Core Team, 2013).

3. Results

3.1. Land cover and land use influences on road kill changes after highway duplication

Road kill probabilities of high mobility genetalists (HMG) species clearly varied after highway duplication, when compared to before road duplication data (Figure 2A). These chances varied between different land use and land cover types. Road kill probability tends to increase in areas with prevalence of sugar cane (east) and decrease in areas with natural vegetation and silviculture (west) (Figure 2B).



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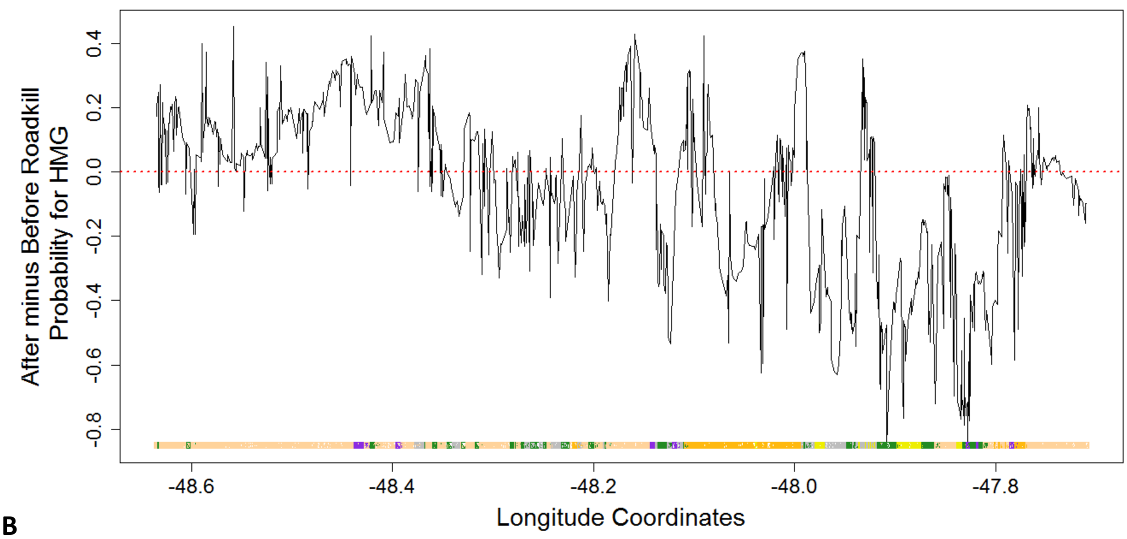

B

Figure 2 – Variation of road kill probability for a functional group of mammals (HMG - high mobility generalist) along studied road. Colored bar along x axis represents predominant land cover or land use within a 100 m radius at that point. A – Road kill probability before and after duplication. B – Difference between after and before road kill probabilities. Positive values mean the probability increased and negative values mean probability decreased. Bottom colors: wheat = sucar cane, dark violet=urban, green = forest, gray = pasture, orange = silviculture and yellow = citrus.

Large amounts of sugar cane poses great influence on medium and large sized mammal because it presents a very dissimilar structure if compared to natural vegetation or silviculture (Figure 3). Our results indicate that sugar cane and silviculture, the dominating land uses in the study area, and forest cover that even in smaller amounts are the highly influential classes on how species use the landscape.

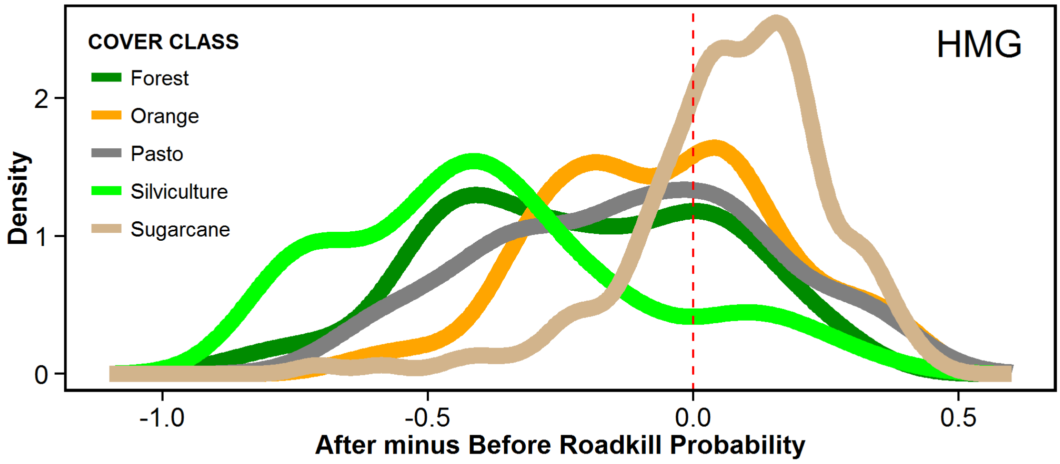

Figure 3 - Relationship between land cover and land use density with road kill probability change after road duplication.

3.2. Quantifying landscape influences on variation of road kill probability

After road duplication forest amount was negatively correlated with road kill probabilities of low mobility mammals (LMS and LMG). For HMG the influence of forest amount on changes in road kill probabilities remains unclear (Figure 4A). Distance to nearest forest patch (Figure 4B) presented a positive relationship with road kills of LMS mammals. On the other side, the further from remaining forests the more negative is the difference between road kill probability after and before duplication for generalist (LMG and HMG) species, particularly after 1000 m from the forests.

In sugar cane dominated areas (~50%), HMG and LMS present an increase in road kill probability after duplication, while the pattern for LMG is indistinct (Figure 4C). With varying distances from sugar cane, the functional groups presented diverging tendencies. LMS had very high road kill probabilities after duplication in areas far from sugar cane, possibly because of the presence of other land cover and use (Figure 4D).

Silviculture amount seems to positively influence HMG road kill probability after road duplication as it decreases drastically with high proportions of this cover type (Figure 4E). Road kill probabilities tend to increase for all functional groups as the distance from water bodies increases (Figure 4F).

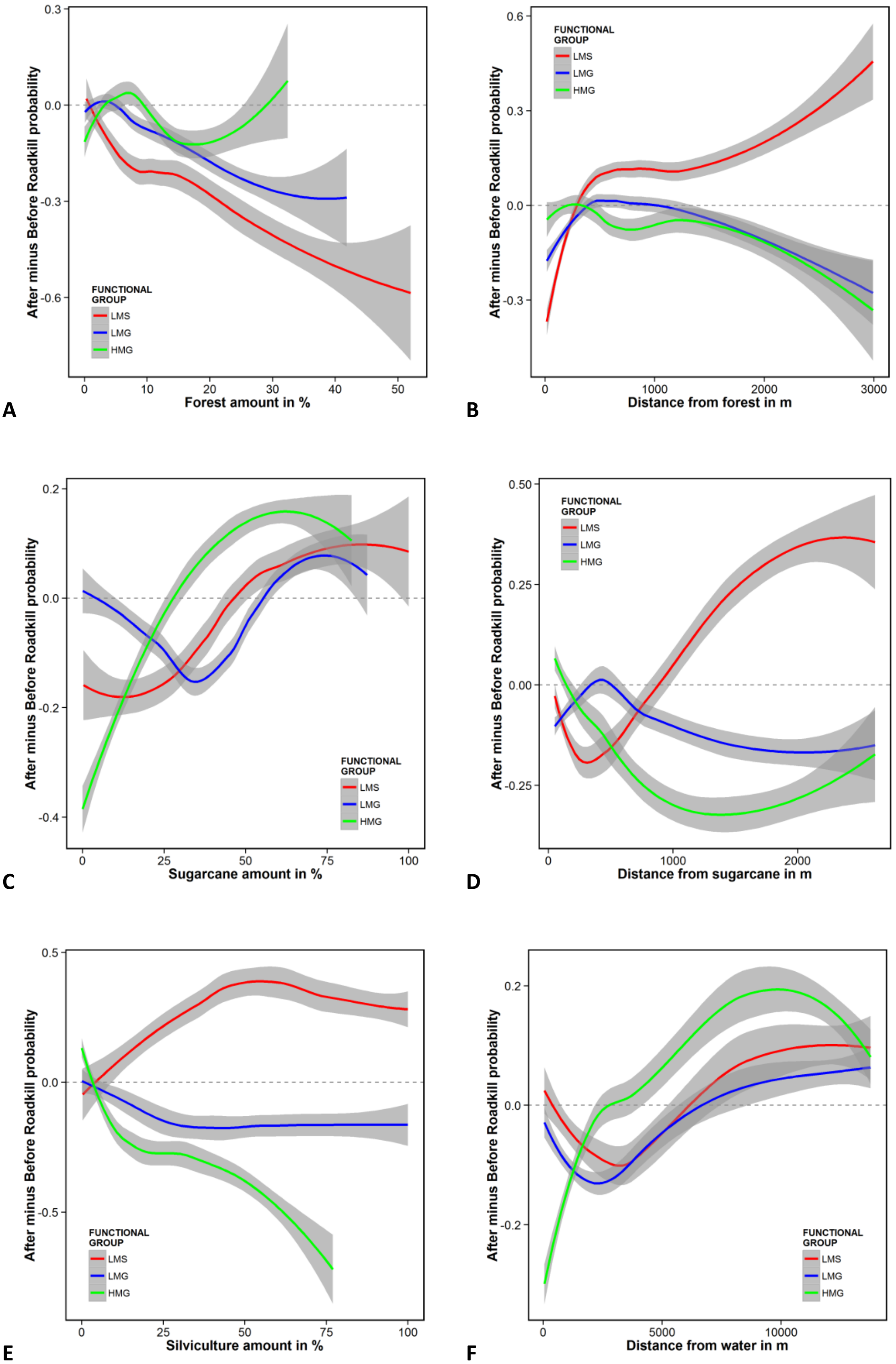

Figure 4 – Variation of road kill probability organized according to gradients of variables for different functional groups of medium and large sized mammals. LMS: low mobility sensitive; LMG: low mobility generalist; HMG: high mobility generalist.

4. Discussion

4.1. Novelty to assess road duplication effects on fauna

The novel method we proposed to analyze changes in road kills after road duplication, tailoring habitat suitability models to combine species characteristics and landscape attributes at various scales, proved to bring important contribution on the field of road ecology. The proposed method is transparent and very adequate to address the influences of road structure changes on wildlife road kill probability. It is also fully and easily replicable. Results reliability encourages its application worldwide. The tools used during the modeling to infer changes in road kill probabilities after duplication allow other researchers to quickly replicate the method, because we use only freely available software to handle the spatial data (GRASS and QGIS), to generate the probability maps (Maxent; but many other free software can also be used) and analyze the data (such as R). The method rely on accessible and affordable data, such as landscape attributes, that can be simply derived from remote sensing data at very low or no costs and road kill records, usually a requirement for road agencies and concessionaires.

A major challenge facing researchers in this field comprises the incorporation of expert knowledge in the development of more reliable models. Therefore, using expert knowledge (Perera et al., 2012) may boost this modeling approach to generate more realistic results. The transparency of this modeling method allows users to verify variables influence and explanatory power separately.

A very important aspect is that, although many studies on road kill focus on species (which many times is difficult for some taxa), we used functional groups which take into consideration the natural history of species. This facilitates both data handling and analysis, diminishes the number of response variables to deal with (as the number of functional groups are hugely small in relation to species number), and also because functional groups allow us to cluster species with ecologically similar characteristics and responses. Road ecology is guided by landscape ecology principles and thus justifies the evaluation of landscape attributes that are ecologically relevant for a particular taxon of interest.

4.2. Road kill and land use relationship before and after highway duplication

For our study case, sugar cane was a key cover type influencing changes in road kill probabilities after road duplication (see Figures 3B and 3C) and the role of silviculture was the second most influential land use. Differently, silviculture is related to a decrease in the road kill probability for HMG and LMS, but increases probabilities for LMG. The east and west parts of the road are contrasting in the results concerning changes in road kill probabilities mainly due to the predominance of different land use. West is dominated by sugar cane and east presents both native vegetation and silviculture. Sugar cane causes animals to move through broader areas, seeking resources, which increases probability of animals being killed apart from being threatened by fire. Forest amount and proximity imply higher habitat quality for all functional groups and this diminishes the need to move through the landscape, causing road kill probabilities to decrease. One possible explanation for these results is because matrix have strong influences on how species are distributed (Anderson et al. 2007), and how they potentially move in the landscape (Baum et al., 2004; Antogiovanni and Metzger, 2005; Castellón and Sieving, 2006).

4.3. Change in landscape effect on mammal functional groups after road duplication

Road kill probabilities for LMS and LMG decrease with higher proportions of forest possibly because animals are not required to cross the road for resources. Otherwise, in areas with less remaining forest road kill probabilities are higher once animals must move across the landscape after resources, which increase road kill probability after duplication. LMG can adapt to certain landscape modifications, but when there is a predominant use of hostile matrix (such as sugar cane), adaptation becomes an issue (Duelli, 1997). We emphasize that evaluating road kill data at two time periods, such as before and after road duplication, even with similar overall records, we observed that results conceal important changes of fauna responses to road conditions. Our results highlight that not only animal road kill must be monitored, but it is also crucial to assess how road structure changes will influence species or functional groups. The ecological aspects of road changes may be much more influential than species road mortality. Alterations on road structure must be evaluated carefully, in the light of ecological processes that maintain ecosystem functions, such as seed dispersal (Bueno et al., 2013; Galetti et al., 2013), natural regeneration modulated by fauna, gene flow and fauna movements (Boscolo et al., 2008; Graves et al., 2012). But unfortunately none or very rare studies combine ecologically scaled questions and road ecology, many of them has explored mainly on estimate road kill rates.

The quality and precision of input data used to develop predictive models is paramount to the reliability and application of results, especially in cases that combine local and landscape variables (Taylor and Goldingay, 2010). And particularly for road ecology, the understanding of how species use landscape features and how they move throughout space is crucial.

More efforts in spatial-movement empirical research can elucidate how movement behavior decisions affect the impacts of roads on wildlife populations (Brehme et al, 2013). Meanwhile, models are the key tool to develop management and mitigation strategies. Despite the fact that extrapolability of models is limited to areas with environmental conditions similar to sampled location (Malo et al, 2004), our method is replicable and may be applied to develop models with local data input elsewhere.

5. Conclusion

Using habitat suitability models to determine the likelihood of road kill of medium and large-sized mammals has proved to be a very suitable tool in cases where the amount of records is not large enough but is spatialized. With this, we can identify variables in different highways that are more evident in changing road kill probabilities according to time and road upgrading, such as duplication. Increasing the width of highways creates an environment that influences behavior of different functional groups, possibly increasing their mortality or increasing isolation of populations. Besides, observing variables influence separately allows researchers to determine what relations these different variables set during time and after road improvement. This study opens a new venue combining techniques that are largely used in the fields of species distribution modeling and landscape ecology to answer ecologically relevant questions that are faced in the road ecology field. More than bringing new techniques to road ecologists, we emphasize that they can benefit from this new utility of road kill records to promote new plausible solutions for transportation planning and management of mitigation measures. These advances on road ecology will contribute to the definition of new regulatory normative which can guide road upgrading towards more robust strategies to encompass transportation planning and conservation of wild populations.

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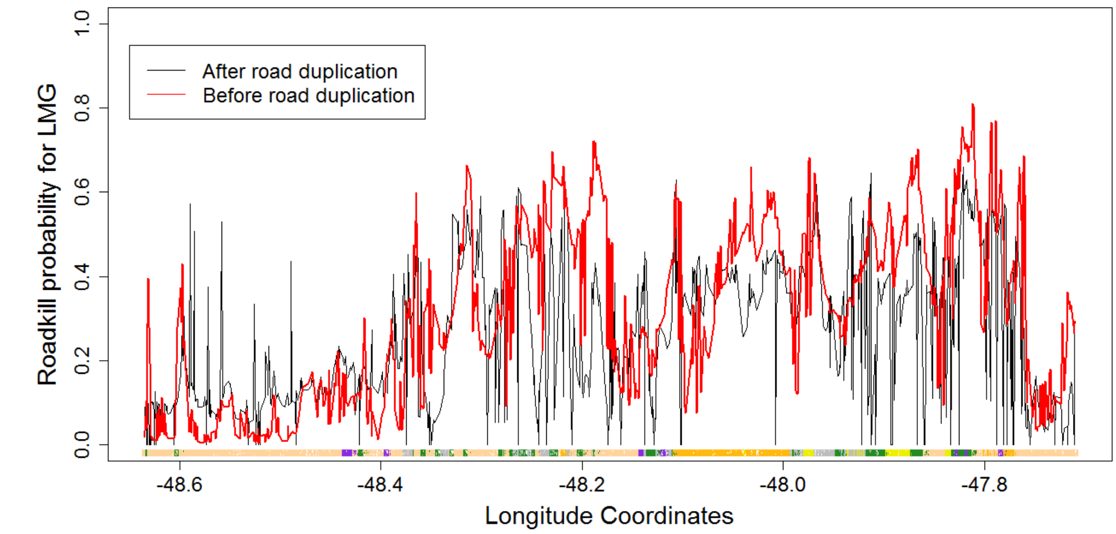
Supplementary Material



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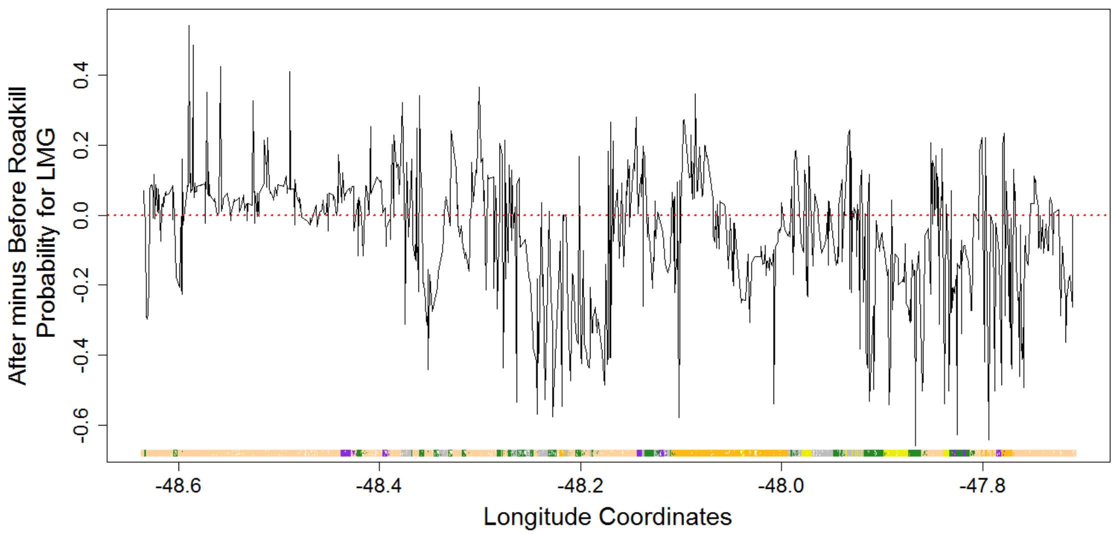
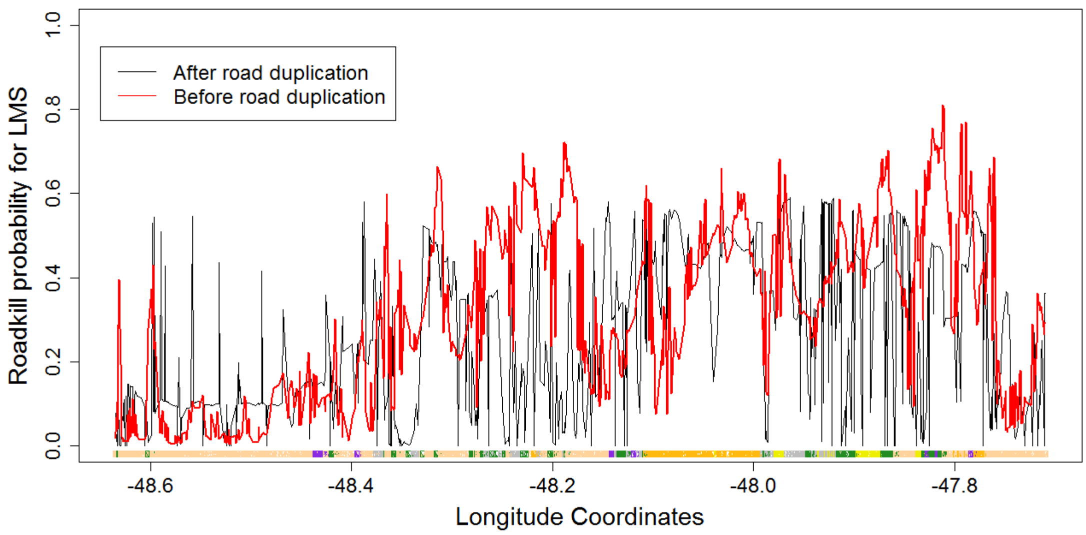


B

Figure S1A – Variation of road kill probability for a functional group of vertebrates along studied road. LMG: low mobility generalist. Colored bar along x axis represents predominant land cover or land use within a 100 m radius at that point. A – Road kill probability before and after duplication. B – Difference between after and before road kill probabilities. Positive values mean the probability increased and negative values mean probability decreased.



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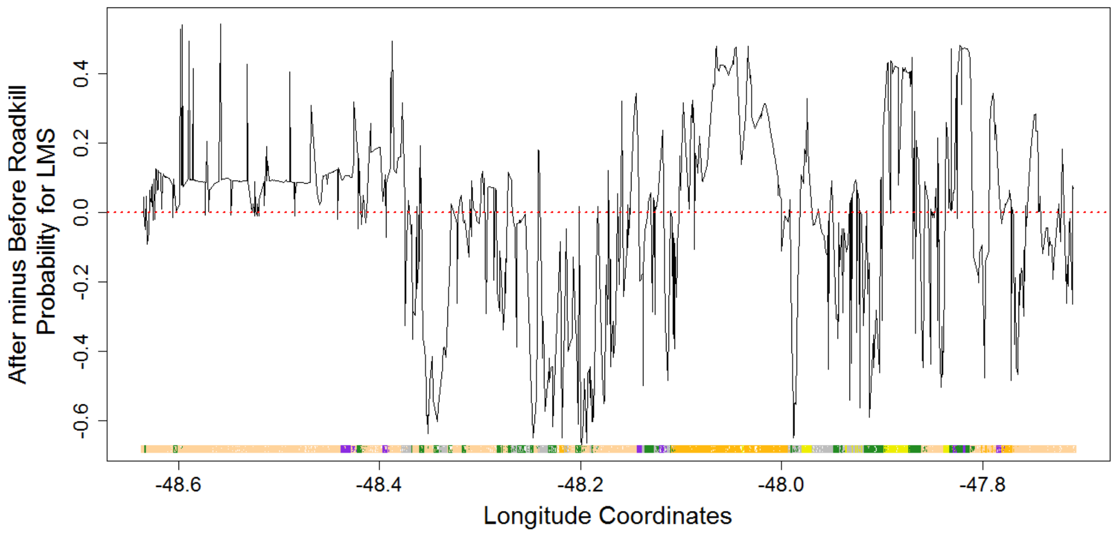

B

Figure S1B – Variation of road kill probability for a functional group of vertebrates along studied road. LMS: low mobility sensitive. Colored bar along x axis represents predominant land cover or land use within a 100 m radius at that point. A – Road kill probability before and after duplication. B – Difference between after and before road kill probabilities. Positive values mean the probability increased and negative values mean probability decreased.

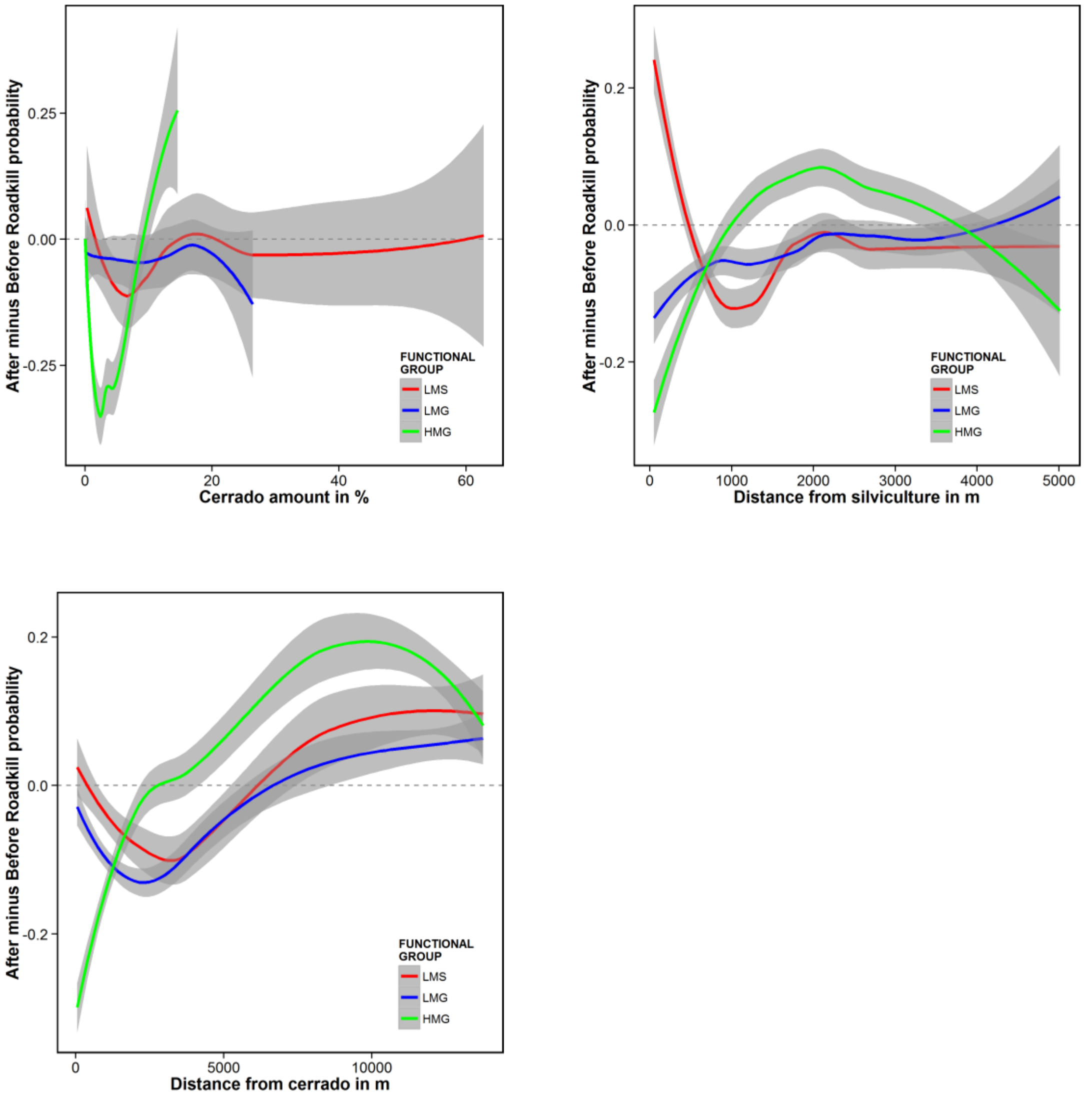

Figure S2– Variation of road kill probability organized according gradients of variable for different functional groups of medium and large vertebrates. LMS: low mobility sensitive; LMG: low mobility generalist; HMG: high mobility generalist.

Ponto de vista de um ecólogo de estradas

Este trabalho demonstrou que a ciência acadêmica tem capacidade funcional de lidar com problemas práticos de demanda social, gerando resultados que possam ser utilizados com facilidade pelos gestores regionais. Estratégias simples e práticas podem ser desenvolvidas para a manutenção da Biodiversidade em ambientes altamente impactados pelo homem. Não é possível aceitar a perda de espécies e/ou populações, nem em regiões extremamente populosas do mundo (não existe conservar uma área em detrimento da outra, a evolução tem de seguir seu rumo na totalidade da superfície terrestre e aquática). O termo desenvolvimento sustentável só é real se os esforços em conservação tratar destas áreas deveras impactadas.

Este trabalho realizado num ambiente com alta densidade de estradas e com um desenvolvimento humano desenfreado, mostrou a possibilidade de gerar metodologias, transparentes, replicáveis e inovadoras para aplicação prática e científica, com resultados que levam à conservação. Usando embasamento científico e com o conhecimento de campo (experiência do pesquisador em entender o reconhecimento de diferentes escalas da paisagem por diferentes espécies) produzimos protocolos passíveis de serem utilizados em qualquer parte do globo. Sem esquecer que "cada caso é um caso", é necessário considerar as principais variáveis independentes (locais e regionais) de interferência sobre as variáveis dependentes (atropelamento e isolamento), assim diminuindo o tempo de monitoramento e podendo embasar leis para garantir uma baixa probabilidade de extinção de espécies. Esta preocupação em determinar quais as variáveis mais adequadas se divide em dois pontos principais: comportamento das espécies/indivíduos e realidade da política pública, de desenvolvimento e de ocupação regional.

O efeito da duplicação de estradas ainda tem que ser melhor avaliado em regiões onde variáveis locais, tais como: fluxo de veículos, relevo, visibilidade etc. possam afetar mais as probabilidades de atropelamento do que variáveis regionais e da paisagem como abordado neste estudo. Mas ficou visivelmente claro que existem diversas alterações na forma como os indivíduos de diferentes espécies reagem com diferentes tipos de uso e cobertura das terras ao se ampliar estradas.

A acessibilidade de estratégias para conservação de espécies que interagem com estradas, deve ser abordada em duas frentes: acadêmica, produzindo informação científica de qualidade para publicação em revistas de alto impacto; e de extensão, visando a divulgação do trabalho acadêmico e suas aplicações por ONGs, instituições públicas (as que gerenciam parques, secretarias de meio ambiente e procuradorias públicas). Os resultados dos trabalhos também devem indicar que cada cidadão, dependendo da sua participação na sociedade (ex. caminhoneiros, turistas ambientais e trabalhadores que utilizam as rodovias como meio de transporte) pode ter uma percepção diferenciada sobre a problemática dos atropelamentos. Esta estratégia deve ser feita com base na ciência de educação ambiental, procurando entender a melhor forma de produzir material que se aplique diretamente e funcionalmente ao grupo estudado.

O que percebo hoje no Brasil é que estratégias com pouco embasamento científico são utilizadas para estabelecer políticas públicas para mitigação dos atropelamentos. Passagens de fauna estão sendo mal concebidas, vídeos poucos animais utilizando as passagens e estimativas de atropelamentos não regionais são utilizados em análises estatísticas fracas e sem padronização, o que pode gerar confusão no entendimento e nas ações direcionadas à sociedade civil, que não tem acesso a informação para discernir sobre o que é divulgado pelos meios de comunicação.

Como o estudo da Ecologia de Estradas ainda está em formação no Brasil, temos que nos precaver com seriedade e responsabilidade sobre os resultados encontrados. Cremos que o próximo passo de importância incontestável será a avaliação dos diferentes tipos de passagens de fauna que, em países em desenvolvimento, ainda são de pouca funcionalidade quando não implementados em complementação a outras medidas de mitigação como cercamento, por exemplo. Utilizar apenas os resultados de passagens já bem avaliadas em climas/regiões diferentes pode ser um grande problema, pois o comportamento das diversas espécies se adéqua a seu habitat e comportamentos.

É impossível, no meu ponto de vista, que estradas possam ter alguma relação positiva na manutenção da biodiversidade. Percebe-se que elas são o fator mais impactante sobre populações e espécies nos dias de hoje. Mesmo que algumas espécies sejam favorecidas, estas são invasoras e até mesmo exóticas, desestabilizando a colonização por espécies nativas.

Em resumo o trabalho demonstrou que existem muitas variáveis envolvidas no atropelamento de fauna, agir rapidamente é necessário, mas agir cientificamente é fundamental, pois se usarmos modelos desenvolvidos no exterior podemos condenar à morte diversos animais, populações e consequentemente espécies. Existe a possibilidade de que sistemas viários e biodiversidade interajam de forma sustentável, tanto economicamente como ambientalmente. Basta mantermos o foco na ciência e que instituições ambientais sigam as recomendações de especialistas que tenham experiência e comprometimento com a causa.