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Dinâmica de Atropelamento de Fauna Silvestre no Entorno de Unidades de Conservação do Distrito Federal

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“Escolhe um trabalho de que gostes e não terás que trabalhar nem um dia na tua vida”

Confúcio

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Apresentação

Os estudos sobre o impacto das estradas na biodiversidade tem crescido exponencialmente nos últimos anos, principalmente com enfoque no atropelamento de fauna. Há uma busca incessante dos pesquisadores pelo conhecimento dos principais fatores na causa desses atropelamentos, bem como pela adequação das metodologias utilizadas para estudá-los e definição de medidas mitigadoras. Dentro desse escopo a presente tese foi elaborada com o intuito de responder algumas lacunas ainda existentes na temática. Um dos objetivos desse estudo é auxiliar no processo de licenciamento ambiental de rodovias, indicando e sugerindo aos tomadores de decisões ferramentas de manejo para preservação da biodiversidade.

Segundo o Departamento Nacional de Infraestruturas e Transportes (DNIT), o Brasil possui uma malha viária de pouco mais de 1,7 milhão de quilômetros de estradas, dos quais 80% (mais de 1,3 milhão de quilômetros) não são pavimentados. Apenas 12% das estradas são pavimentadas (pistas simples e duplicadas), e o restante são vias planejadas para pavimentação, segundo relatório publicado pelo órgão em 2014. Diante desse panorama de constante aumento da malha viária pavimentada no país, o foco dessa tese foi no aprimoramento das de estimativas de abundância e distribuição de animais atropelados, assim como das ferramentas de análise e processamento de informações advindas do impacto das estradas sobre a fauna. O trabalho desenvolvido é fruto de uma amostragem intensiva e sistemática, onde cada capítulo é complementar aos demais, de modo que sua análise conjunta convença o leitor da tese central do estudo: fornecer mecanismos para um adequado manejo da biodiversidade e mitigação dos impactos das estradas sobre a fauna.

A presente tese está dividia em três capítulos: Capitulo I - Carcass persistence and detectability: reducing the uncertainty surrounding wildlife-vehicle collision surveys; Capítulo II - Assessing the reliability of patterns of hotspots and hot-moments of wildlife road mortality over time; Capítulo III - Predicting the roadkill risk using occupancy models. Os três capítulos estão redigidos em inglês pois foram submetidos à publicação. Como cada capítulo foi escrito para uma revista diferente, a formatação textual varia ao longo da tese. Os capítulos estão precedidos pela introdução geral, cujo objetivo é fornecer ao leitor o arcabouço teórico para a melhor compreensão do trabalho.

O objetivo principal do primeiro capítulo foi avaliar a influência da paisagem, das condições climáticas e da estrutura viária na remoção das carcaças nas rodovias em uma região de Cerrado do Brasil Central. Além disso, a proposta foi mensurar a eficiência do observador na coleta de dados e estimar a mortalidade de animais atropelados com os dados corrigidos pelo tempo de remoção e detectabilidade. Já no segundo capitulo o objetivo foi investigar se os padrões de atropelamento, tanto espaciais (*hotspots*) quanto temporais (*hot-moments*) se mantém ao longo dos anos sob diferentes escalas espaciais e temporais. A proposta foi avaliar se os mesmos locais de agregação de atropelamento na estrada vão permanecer com o passar do tempo na mesma secção de estrada, e se os períodos de maior atropelamento serão na mesma época ano. Por fim, o objetivo do terceiro capítulo foi avaliar a influência de diferentes fatores ambientais (como a paisagem do entorno da estrada e as características da rodovia) na dinâmica de atropelamento de seis espécies, por meio de modelos de ocupação. A proposta foi elaborar um modelo preditivo de potenciais locais de colisões entre veículos e animais.

Resumo Geral

O tempo de persistência das carcaças nas estradas e a capacidade de detecção (detectabilidade) do observador são as duas principais fontes de incerteza nos estudos de fauna atropelada em rodovias. Considerando o viés amostral produzido por esses dois fatores, a proposta do primeiro capitulo foi mensurar seus efeitos e estimar a real mortalidade nas estradas da área estudada. O principal objetivo desse capítulo foi quantificar o tempo de persistência da carcaça e avaliar como ele é influenciado pelo peso, características da estrada (estradas duplicadas, de único sentido, pavimentadas ou não), condições climáticas, e pela cobertura de vegetação na vizinhança, que foi utilizada como um"proxy" da atividade de carniceiros na rodovia. Além disso, a proposta foi mensurar a taxa de detecção de carcaças ao realizar os levantamentos de animais atropelados por carro e, por fim, estimar o “real” numero de carcaças após corrigir o valor encontrado nas amostragens com os dados de persistência e o viés da detectabilidade. Para estimar o tempo de persistência da carcaça, três observadores incluindo o motorista monitoraram (procurando por animais atropelados) em campanhas de cincos dias consecutivos, durantes 26 meses, 114 quilômetros de estradas. Cada animal encontrado era deixado no mesmo local e o seu tempo de remoção na rodovia era acompanhado nos dias subsequentes. Para estimar a detectabilidade da carcaça, trechos de 500m foram selecionados aleatoriamente para serem monitorados a pé por dois observadores (totalizando 146 km percorridos no período do estudo), enquanto outra equipe percorria todo o trecho de 114 km de veiculo, com três observadores a procura de animais atropelados. Em geral, em cada campanha uma equipe percorria 6 km a pé. Considerando todas as carcaças registradas, o tempo médio de persistência foi de dois dias e a detectabilidade foi baixa (<10%) para todos os grupos analisados. O tamanho do corpo e a alta proporção de cobertura de cerrado típico no entorno da rodovia (como um proxy da presença de carniceiros) foram os principais fatores que influenciam no tempo de persistência da carcaça. Os animais de menor peso corporal e em

áreas com elevada proporção de cerrado típico permaneceram por menos tempo na rodovia. A detectabilidade foi menor para animais com massa corporal menor que 100g. As taxas de mortalidade registradas subestimaram os valores reais de 2 a 10 vezes menos, quando corrigidos pela remoção e detecção. Embora os tempos de persistência fossem semelhantes a outros estudos, as taxas de detectabilidade aqui descritas diferem consideravelmente dos demais estudos com essa abordagem. A detectabilidade é a principal fonte de viés nos estudos de atropelamento de fauna, e portanto, mais do que estimar o tempo de persistência, a detectabilidade deve ser o foco da correção metodológica durante as campanhas de levantamento de fauna atropelada.

No segundo capítulo, o objetivo foi avaliar se os padrões de agregação espacial e temporal de atropelamento de fauna permanecem nos mesmos locais e períodos, ao longo do tempo, e sob diferentes escalas espaciais e temporais. Os padrões de agregação espacial e temporal de atropelamento de fauna são comumente utilizados para informar onde e quando as medidas de mitigação são necessárias. Com o intuito de registrar os animais atropelados foram realizadas campanhas com uma frequência média de duas vezes por semana (n = 484), no período de abril de 2010 a março de 2015, em um trecho de 114 km. Os *hotspots*/*hot-moments* foram definidos com diferentes comprimentos de secção de estrada (500, 1000, 2000m) e períodos de tempo (quinzenal, mensal, bimestral) por meio do método de Malo (calculado por meio de distribuição de Poisson). Os dados foram classificados em períodos anuais, e para cada ano foi calculado o *hotspot*/*hot-moment* e verificado se esses pontos de agregação permaneciam durante os cinco anos de amostragem. Ao longo do período de estudo foram registrados 4422 animais silvestres atropelados e identificado a presença de *hotspots* e *hot-moments* nas diferentes escalas de análise. No entanto, a ocorrência de *hotspots* e *hot-moments* ao longo dos anos foi mais evidente quando consideradas grandes escalas temporais e espaciais. Portanto, recomenda-se a utilização de secções de estrada e períodos de tempo mais longos nas análises de *hotspots*/*hot-moments* de atropelamento. Além disso, o custo/benefício de mitigação ao usar unidades espaciais e temporais maiores é semelhante ao usar escalas menores na identificação de *hotspots*/*hot-moments*.

Por fim, no terceiro capítulo, a proposta foi utilizar modelos de ocupação no âmbito dos estudos de ecologia de estradas, visando incorporar a detecção imperfeita nas análises. As colisões entre animais silvestres e veículos representam uma grande ameaça para a vida selvagem e compreender como os padrões espaciais de atropelamento se relacionam com caracteres da paisagem circundante é crucial na decisão de onde implementar medidas de mitigação. No entanto, essas associações entre atropelamento e descritores da paisagem/estrada podem ser tendenciosas, já que muitas carcaças não são detectadas em pesquisas de atropelamento de fauna. Esse fato pode, em última instância, comprometer as ações de mitigação. Para utilização dos modelos de ocupação foi necessário assumir alguns pressupostos: a) a ocupação em nosso estudo representou o risco de uma colisão, no qual o animal usa uma seção de estrada para migrar ou forragear e fica propenso a ser atingido por um veículo; e b) a detectabilidade é a combinação da probabilidade de um indivíduo ser atingido por um veículo e da sua carcaça ser detectável. O objetivo desse estudo foi avaliar o risco de colisões animal-veículo ao longo das estradas e relacioná-lo com as informações da paisagem e da estrada. A coleta de dados foi à mesma já descrita no capitulo dois. Para avaliar padrões espaciais de ocorrência de atropelamento para os seis táxons mais atropelados durante os cinco anos de coleta de dados em campo foi desenvolvido um modelo de ocupação hierárquico bayesiano. Em geral, há um maior risco de atropelamento em trechos de estradas mais próximos às áreas urbanas e os com maior cobertura de habitat campestre. A detectabilidade foi maior para as estradas duplicadas e para a estação chuvosa. Foi constatado que os modelos de ocupação podem ser usados como uma ferramenta útil de manejo para acessar o risco de atropelamento ao longo das estradas, incorporando ainda o problema da detecção imperfeita.

Abstract

Carcass persistence time and detectability are two main sources of uncertainty on road kill surveys. In this study, we evaluate the influence of these uncertainties on roadkill surveys and estimates. The main objective of the first chapter was to quantify carcass persistence time and assess how it is influenced by body mass of carcass, road-related characteristics, weather conditions and cover of (semi-) natural habitat (as a proxy of scavenger activity). In addition, the proposal was to estimate carcass detectability when performing road surveys by car and estimate the proportion of undetected carcasses after correcting for persistence and detectability bias in our studied roads.

To estimate carcass persistence time, three observers (including the driver) surveyed 114 km by car on a monthly basis for two years, searching for wildlife-vehicle collisions (WVC). Each survey consisted of five consecutive days. To estimate carcass detectability, we randomly selected stretches of 500m to be also surveyed on foot by two other observers (total 292 walked stretches, 146 km walked). Overall, we recorded low median persistence times (two days) and low detectability (<10%) for all vertebrates. The results indicate that body size and landscape cover (as a surrogate of scavengers' presence) are the major drivers of carcass persistence. Detectability was lower for animals with body mass less than 100g when compared to carcass with higher body mass. We estimated that our recorded mortality rates underestimated actual values of mortality by 2-10 fold. Although persistence times were similar to previous studies, the detectability rates here described are very different from previous studies. The results suggest that detectability is the main source of bias across WVC studies. Therefore, more than persistence times, studies should carefully account for differing detectability when comparing WVC studies.

In the second chapter, the aim was to assess if spatial and temporal aggregation patterns of Wildlife-Vehicle Collisions (WVC) patterns remain in the same locations and periods over time and at different spatial and temporal scales. Spatial and temporal

aggregation patterns of Wildlife-Vehicle Collisions (WVC) are recurrently used to inform where and when mitigation measures are most needed. We conducted biweekly surveys (n=484) on 114 km of nine roads, searching for WVC (n = 4422). Hotspots/hot-moments were defined using Poisson tests using different lengths of road section (500, 1000, 2000m) and time periods (fortnightly, monthly, bimonthly) to aggregate data. Our results showed that hotspots and hot-moments are present, but at large temporal and spatial scales, except for mammal's hot-moments. We suggest using longer road sections and longer time periods to define hotspots/hot-moments in order to minimize uncertainty. Also, we show that the proportional costs and benefits when using different spatial and temporal units to detect WVA are similar.

Finally, in the third chapter we suggest using occupancy models to overcome imperfect detection issues. Wildlife-vehicle collisions (WVC) represent a major threat for wildlife and understanding how WVC spatial patterns relate to surrounding land cover can provide valuable information for deciding where to implement mitigation measures. However, these relations may be heavily biased as many casualties are undetected in roadkill surveys, e.g. due to scavenger activity, which may ultimately jeopardize conservation actions. Here, we assume that: a) occupancy represents the roadkill risk, i.e. the animal uses a road section for crossing or forage being prone to be hit by an incoming vehicle; and b) detectability is the combination of the probability of an individual being hit by a vehicle and, if so, its carcass being detectable. Our main objective was to assess the roadkill risk along roads and relate it to land cover information. We conducted roadkill surveys over 114 km in nine different roads, biweekly, for five years (total of 484 surveys), and developed a Bayesian hierarchical occupancy model to assess spatial patterns of WVC occurrence for the six most road-killed taxa. Overall, we found a higher roadkill risk in road segments near urban areas and with higher cover of open habitat. Detectability tended to be higher for four-lane roads and in rainy season. We show that occupancy models can be used to access the roadkill risk along roads while accounting for imperfect detection. From a conservation perspective, our results highlight the need to upgrade road stretches near urban areas and with higher cover of open habitat.

Introdução Geral

A ecologia de estradas é uma ciência recente, e os estudos na área têm crescido exponencialmente diante da preocupação com a preservação das populações de fauna silvestre sob efeito do impacto das rodovias. O termo ecologia de estradas foi instituído pelo pesquisador Richard Forman e refere-se a uma ciência que investiga o impacto das rodovias nos componentes, processos e estrutura do ecossistema (Forman *et al.* 1998). O autor infere que as causas desses impactos estão relacionadas com a paisagem, planejamento do uso do solo e os meios de transporte. A ecologia de estradas é uma ciência que engloba ecologia, geografia, engenharia e planejamento urbano (Forman *et al.* 2003).

Impacto das Rodovias sobre a Fauna

As estradas causam uma variedade de efeitos danosos, incluindo a fragmentação do habitat, degradação no entorno da rodovia, poluição proveniente da pavimentação e dos veículos que trafegam, erosão, sedimentação dos corpos hídricos, alteração química dos solos, mudança no comportamento de algumas espécies, atropelamento de fauna e ainda funcionam como corredores de dispersão de espécies exóticas (Trombulak & Frissell 2000).

O atropelamento de fauna é reconhecido como a principal causa direta de mortalidade de vertebrados, superando impactos como a caça (Forman & Alexander 1998). Nos Estados Unidos foram estimados 365 milhões de atropelamentos/ano (década de 60), na Espanha 100 milhões (década de 90) e na Alemanha 32 milhões (1987-1988) (Seiler & Helldin 2006). Segundo o Centro Brasileiro de Estudos de Ecologia de Estradas – CBEE (2015), estima-se que 475 milhões de animais silvestres são atropelados por ano no Brasil. De acordo com o CBEE, a grande maioria dos animais mortos por atropelamento (90%) é composta por pequenos vertebrados, como sapos e pequenas aves.

É fato que as estradas ocasionam inúmeros efeitos negativos nas populações de animais silvestres (Trombulak & Frissell 2000) e estes impactos são similares em magnitude a outros, como por exemplo, a própria perda de habitat (Forman *et al.* 2003). As rodovias podem afetar a vida silvestre de diferentes maneiras: (1) as populações podem ter sua abundância reduzida devido ao aumento da mortalidade por colisões com veículos; (2) os distúrbios devido ao tráfego de veículos (ruído, por exemplo) reduzem a qualidade do habitat próximo a rodovias, afetando o sucesso reprodutivo de determinadas espécies; e (3) o efeito barreira provocado pelas estradas pode afetar o comportamento natural de inúmeras espécies, o que significa um decréscimo de acessibilidade de novos habitats e redução no fluxo gênico entre fragmentos (Laurance, Goosem & Laurance 2009).

Para muitas espécies, as estradas são vistas como corredores e são então utilizadas como rotas de deslocamento (Forman *et al.* 2003). Dessa maneira, um elevado número de espécies está suscetível à mortalidade via colisão com veículos (Laurance *et al.* 2008). A rodovia afeta diretamente a dinâmica fonte-sumidouro, contribuindo para a redução no fluxo gênico, endogamia e até mesmo extinções locais, ou mesmo transformando a própria rodovia em sumidouro, uma vez que as populações não conseguem colonizar ou migrar para novas áreas, devido o atropelamento (Woodroffe & Ginsberg 1998). O modelo fonte-sumidouro considera o movimento dos indivíduos entre os fragmentos de tal maneira que as populações fonte, aquelas cuja taxa de natalidade excede a taxa de mortalidade, estão em fragmentos maiores e de melhor qualidade de habitat. Os sumidouros, aquelas espécies cuja taxa de mortalidade excede a taxa de natalidade. Por sua vez, apresentam uma área menor, baixa qualidade de habitat e a menor probabilidade de persistência das espécies (Pulliam 1988).

A grande maioria dos artigos de atropelamento de fauna em estradas trata basicamente dos efeitos negativos (Clevenger, Chruszcz & Gunson 2003; Forman *et al.* 2003; Laurance, Goosem & Laurance 2009), mas existem respostas positivas ou neutras ante a implementação de uma rodovia (Fahrig & Rytwinski 2009; Rytwinski & Fahrig 2013). Na revisão bibliográfica de Fahrig e Rytwinski (2009) foi observado que três tipos de espécies podem apresentar respostas positivas a estradas: (1) espécies que são atraídas pelas estradas devido à disponibilidade de recurso, mas que evitam a proximidade com veículos; (2) espécies que não evitam áreas que apresentam os distúrbios ocasionados pelo tráfego, mas evitam as estradas, ou seja, a espécie pode frequentar a borda da estrada, mas não a estrada, e (3) aquelas espécies cujo principal predador apresenta uma redução na abundância em função da malha viária.

Unidades de Conservação e Estradas

O efeito das rodovias sobre as áreas protegidas no Cerrado ainda não é bem relatado e poucos são os estudos que englobam especificamente os impactos deste empreendimento linear nesse bioma (Caceres 2011; Rosa & Bager 2012; Freitas, Souza & Bueno 2013; Santos *et al.* 2016). As áreas especialmente protegidas tem prioridade em ações de conservação e compreender o impacto das rodovias nesses locais é fundamental para preservação da fauna e mitigação dos efeitos negativos deste tipo de empreendimento. O manejo e a conservação de áreas do Cerrado têm relevância mundial, especialmente depois que esse bioma foi considerado um dos 25 *hotspots* para a conservação do mundo (Myers *et al.* 2000).

Alguns estudos demonstraram que as áreas protegidas, apesar do seu *status* de conservação, estão sujeitas aos impactos das rodovias tanto quanto fragmentos isolados de vegetação circundados por rodovias. Em um estudo realizado no Parque Nacional de Everglades na Flórida, Estados Unidos, foi observado que as atividades sazonais (período de reprodução e dispersão) das serpentes coincidiam com as maiores taxas de atropelamento (Bernardino & Dalrymple 1992). Essa maior taxa de atropelamento das serpentes na época de reprodução corresponde com o período em que o parque recebe maior número de turistas. Outro estudo observou que diferenças no número de atropelamentos de fauna estavam correlacionadas com o status de proteção da área, sendo constatado que quanto maior era o *status* de proteção de uma determinada unidade de conservação, maior era o registro de colisões entre animais e veículos (Garriga *et al.* 2012). Ainda segundo os autores, as unidades de conservação recebem com frequência muitos visitantes e esse aumento do tráfego no entorno de unidades de conservação é provavelmente o fator preponderante no aumento das taxas de atropelamento no entorno de áreas protegidas.

O Método de Amostragem de Fauna Atropelada e o Erro Associado

Compreender e avaliar os atropelamentos de fauna é requisito fundamental para mitigar os efeitos negativos das estradas. No entanto, para quantificar a mortalidade de fauna em uma rodovia é importante considerar e mensurar os erros da metodologia de amostragem (Slater 2002). Alguns estudos assumem que diferenças entre rodovias ou trechos são decorrentes de diferenças entre as áreas de estudo, quando na verdade as estimativas de mortalidade por atropelamento são afetadas principalmente por dois fatores: a persistência das carcaças dos animais atropelados na rodovia e a detectabilidade das carcaças pelo observador em campo (Slater 2002; Teixeira *et al.* 2013b; Korner-Nievergelt *et al.* 2015). O tempo de persistência é a probabilidade da carcaça ainda estar disponível para detecção na rodovia durante os monitoramentos de campo e pode ser influenciada pelo clima, abundância e diversidade de carniceiros, tráfego de veículos e tamanho da carcaça (Slater 2002; Korner-Nievergelt *et al.* 2015). Grande parte da remoção ocorre por ação dos carniceiros que se deslocam para a estrada em busca de alimentos, já que a busca por recurso num ambiente onde há uma alta mortalidade de animais, ou alta disponibilidade de recurso, é mais eficiente e fácil do que em um ambiente natural (Devault, Rhodes & Shivik 2003). A atividade dos carniceiros pode ainda estar relacionada com o tráfego de veículos, sendo observado que um aumento desse último fator pode reduzir o acesso de carniceiros na rodovia, aumentando o tempo de persistência (Slater 2002; Santos, Carvalho & Mira 2011). No entanto, a relação carniceiros-remoção-tráfego não é tão simples, uma vez que em rodovias de alto fluxo de veículos a prensagem provocada pelo tráfego pode reduzir o tempo de permanência na pista, ou mesmo inibir o acesso dos carniceiros ao local (Slater 2002; Santos, Carvalho & Mira 2011; Planillo, Kramer-Schadt & Malo 2015). Além dessas variáveis, a paisagem no entorno da rodovia pode estar relacionada com a atividade dos carniceiros. Em um estudo realizado em uma ilha da Carolina do Norte-EUA a persistência dos animais atropelados foi significativamente menor em áreas florestadas do que em áreas não florestadas (Degregorio *et al.* 2011).

Já a detectabilidade da carcaça consiste na probabilidade da carcaça ser encontrada pelo observador e pode ser afetada por inúmeros fatores como: o método utilizada na amostragem (carro, a pé ou bicicleta, por exemplo), a eficiência do pesquisador de campo em encontrar um animal atropelado, o tamanho, a cor e a idade da carcaça (Slater 2002; Gerow *et al.* 2010). As amostragens realizadas a pé apresentam maior probabilidade de detecção do que os experimentos conduzidos por automóveis, sendo que o estudo com veículo se torna interessante quando se trata de um trecho de muitos quilômetros a ser monitorado (Slater 2002; Gerow *et al.* 2010; Guinard, Julliard & Barbraud 2012).

De uma maneira geral, há uma subestimação nos levantamentos de fauna atropelada, fato este que pode afetar diretamente os padrões espaciais e temporais de atropelamento. Embora seja fácil predizer que o tempo de persistência de uma carcaça seja maior em animais maiores, poucos estudos analisaram como a probabilidade de permanência das carcaças no tempo vai afetar a taxa de detecção em diferentes grupos taxonômicos/funcionais, e sob diferentes condições ambientais (Slater 2002; Antworth, Pike & Stevens 2005; Santos, Carvalho & Mira 2011; Teixeira *et al.* 2013a; Santos *et al.* 2016). Incorporar as informações sobre detectabilidade e persistência das carcaças se tornou um assunto de grande relevância na área, e alguns autores sugerem que todo programa de monitoramento deveria incluir esses fatores na metodologia, ajustando assim as estimativas de animais atropelados registrados (Teixeira *et al.* 2013a).

Fatores que influenciam no atropelamento de fauna – Identificando *Hotspots* e *Hot-moments*

Compreender os principais fatores que se relacionam com os atropelamentos de fauna é necessário para fornecer subsídios tanto para pesquisadores como para gestores na proposição de medidas que auxiliem na redução das colisões entre veículos e animais (Malo, Suárez & Díez 2004; Ramp *et al.* 2005; Morelle, Lehaire & Lejeune 2013). Inúmeros estudos na área de ecologia de estradas têm buscado compreender os padrões de distribuição dos atropelamentos, e os resultados demonstram que as variações na taxa de atropelamento estão ligadas a dois fatores principais: (1) fatores intrínsecos ou características biológicas das espécies como horário de atividade, idade, sexo, dieta, época de reprodução, capacidade de deslocamento e dispersão (Clevenger, Chruszcz & Gunson 2003; Forman *et al.* 2003; Jaeger *et al.* 2005) e (2) características da própria estrada como tráfego de veículos, desenho da rodovia, velocidade da via e a paisagem do entorno (Trombulak & Frissell 2000; Clevenger, Chruszcz & Gunson 2003; Malo, Suárez & Díez 2004; Grilo, Bissonette & Santos-Reis 2009; Gunson, Ireland & Schueler 2012).

Avaliar os padrões espaciais e temporais de atropelamento nas rodovias, identificando os locais (*hotspots*) e períodos (*hot-moments*) com elevado número de colisões, constitui uma ferramenta fundamental para identificar áreas prioritárias para implementação de medidas mitigadores (Clevenger, Chruszcz & Gunson 2003; Malo, Suárez & Díez 2004). Inúmeras pesquisas mostraram que os atropelamentos não acontecem de forma randômica, mas de maneira agregada em determinados pontos do ambiente e períodos do ano (Malo, Suárez & Díez 2004; Ramp *et al.* 2005; Coelho, Kindel & Coelho 2008).

Além de determinar os locais de atropelamento, é importante compreender a influência da sazonalidade nos padrões de mortalidade. Variações temporais no atropelamento estão intimamente relacionadas ao comportamento e padrões de atividade das espécies, tais como forrageamento, acasalamento e dispersão de juvenis (Morelle, Lehaire & Lejeune 2013). Inúmeros estudos já constataram que há uma relação entre a sazonalidade e a mortalidade de fauna nas estradas (Coelho, Kindel & Coelho 2008; Smith-Patten & Patten 2008; Gomes *et al.* 2009; Carvalho & Mira 2011; Morelle, Lehaire & Lejeune 2013). Répteis e anfíbios apresentam forte influência sazonal, com aumento dos atropelamentos nas estações reprodutivas. Durante eventos migratórios em massa há aumento considerável das colisões de veículos com animais desses grupos (Parris, Velik-Lord & North 2009). Para aves, sabe-se que a sazonalidade e a dispersão de juvenis após eventos reprodutivos podem incrementar o número de indivíduos e espécies atropeladas (Coelho, Kindel & Coelho 2008; Luis *et al.* 2012; Rosa & Bager 2012). Já os mamíferos estariam mais vulneráveis aos atropelamentos na estação com menor disponibilidade de recurso, pois alteram seus padrões de deslocamento e percorrem áreas maiores. Bueno e Almeida (2010) observaram uma frequência de atropelamentos de mamíferos significativamente maior na estação seca, onde supostamente há menor oferta de recursos.

É fundamental que os gestores e tomadores de decisão tenham informações confiáveis para identificar quando e onde espécies de particular interesse estão mais susceptíveis ao atropelamento, a fim de implementar medidas mitigadoras durante ou pós implantação da rodovia (Langen *et al.* 2007; Grilo, Bissonette & Santos-Reis 2009; Teixeira *et al.* 2013a). A partir dessas informações, ações direcionadas no tempo e espaço podem ser realizadas visando reduzir os custos do investimento. Uma vez que os atropelamentos estão concentrados em determinados pontos da estrada e estes pontos de agregação não se modificam ao longo dos anos, os gastos com medidas serão menores ao longo da estrada e ao longo dos anos. Além disso, se os atropelamentos da espécie alvo de preservação se concentram no verão, por exemplo, campanhas educativas podem ser intensificadas nesse período.

Modelos Preditivos e Distribuição Potencial de Atropelamentos

Trabalhos que se limitam a apenas quantificar os atropelamentos restringem a aplicação dos resultados de maneira prática e não permitem estimar a movimentação da fauna no ambiente. É interessante combinar o inventário básico com uma análise da paisagem do entorno da estrada, mapeando as conexões entre os diferentes habitats (Clevenger, Chruszcz & Gunson 2003; Jaeger *et al.* 2005; Langen *et al.* 2007). Apesar do crescente interesse e do número de estudos na área de ecologia de estradas, não é possível mapear toda a extensão viária, seus impactos e definir áreas prioritárias para preservação (Gomes *et al.* 2009). É importante que as pesquisas avancem no desenvolvimento de modelos preditivos que identifiquem áreas potenciais de atropelamento ou de corredores de passagem de fauna (Clevenger & Waltho 2005; Jaeger *et al.* 2005; Gunson, Ireland & Schueler 2012). Os modelos preditivos de atropelamento de fauna estimam a probabilidade de ocorrência de uma espécie em função de variáveis ambientais, estabelecendo a distribuição potencial do táxon como a área na qual esta probabilidade seja superior a um certo limite estipulado, definindo assim, locais com maior chance de ocorrência de um determinado evento (Malo, Suárez & Díez 2004). Gunson *et al.* (2012) desenvolveram uma ferramenta de modelagem de SIG baseada em características da paisagem, com o objetivo de modelar e indicar os locais de alto risco de mortalidade por atropelamento para espécies da herpetofauna. O intuito era criar uma ferramenta para ser utilizada pelas agências governamentais de transporte na priorização de *hotspots* de atropelamento ao longo de estradas.

Apesar de alguns estudos já terem desenvolvidos modelos preditivos para identificar áreas potenciais de atropelamento (Clevenger, Chruszcz & Gunson 2003; Jaeger *et al.* 2005; Langen *et al.* 2007; Gunson, Ireland & Schueler 2012), tais abordagens nunca consideraram a detecção imperfeita. A detecção imperfeita (ou as falsas ausências) ocorre quando a espécie não é detectada durante o levantamento/inventário, mesmo estando presente no sítio de interesse, ou seja, uma parcela da população pesquisada no estudo será perdida na análise dos dados (Royle & Nichols, 2003; Tyre *et al.* 2013). O não registro de uma espécie num determinado momento do inventário não garante que realmente a espécie esteja ausente na área. Pode ser simplesmente resultado de uma falha na detecção, gerando uma falsa ausência. Quando os estudos não consideram as falsas ausências na elaboração de modelos de distribuição de espécies os resultados obtidos podem levar a conclusões equivocadas que conduzem ao manejo errôneo da biodiversidade em estradas.

Uma abordagem promissora, que incorpora a detecção imperfeita nas análises, são os modelos de ocupação. Esses modelos são utilizados para estimar a probabilidade de ocupação de uma determinada espécie em relação à co-variáveis do ambiente (Mackenzie *et al.* 2002) e exigem amostragens constantes/repetidas para ajudar a contabilizar falsas ausências na área de interesse. Assim, os levantamentos devem ser realizados por meio de visitas aos sítios amostrais mais de uma vez, para estimar simultaneamente a probabilidade de ocupação e detecção (MacKenzie & Kendall 2002; Tyre *et al.* 2013). Com essas amostragens repetidas em sítios amostrais replicados espacialmente, a probabilidade de detectar a espécie é usualmente assumida como zero quando a espécie está verdadeiramente ausente, e as ausências observadas são assim uma mistura de não-detecções e ausências verdadeiras (Hanks *et al.* 2011). Os modelos de ocupação estão ganhando popularidade como ferramenta de manejo da biodiversidade, uma vez que uma das principais vantagens para estimar a distribuição das espécies é o uso de dados de incidência, que são usualmente menos onerosos (Coggins *et al*. 2014). Além disso, estudos de ocupação bem planejados permitem avaliar distribuições espaciais de espécies de grande alcance sem a necessidade de projetos de amostragem intensiva e de longo período, que são onerosos e às vezes ineficientes (MacKenzie *et al*. 2006; Karanth *et al*. 2011).

A premissa principal nos modelos de ocupação, de levantamentos/inventários repetidos no tempo e no espaço, é o protocolo de amostragem comumente utilizado nas pesquisas de atropelamento de fauna, onde os observadores conduzem o estudo na mesma estrada repetidas vezes, a fim de definir os locais com maior mortalidade. Este método permite que os pesquisadores de ecologia de estradas incorporem a detecção imperfeita ao estimar a distribuição de atropelamentos, isto é, inclui parâmetros que podem reduzir as incertezas na modelagem de distribuição potencial de atropelamentos.

Gestores e tomadores de decisão precisam conhecer os locais de maior probabilidade de atropelamento e direcionar as medidas para reduzir futuros incidentes, visando não apenas a segurança dos motoristas que trafegam na rodovia, mas também a manutenção da conectividade entre as populações de animais silvestres mais susceptíveis a este tipo de empreendimento (Forman *et al.* 2003). Dessa maneira, os modelos de distribuição tornam-se ferramentas importantes da biologia da conservação para definição de propostas de mitigação de atropelamento de fauna. Por fim, um bom modelo deve ser construído de maneira tal, que seja possível extrapolar o conhecimento adquirido para outras áreas para as quais não existem informações (Malo, Suárez & Díez 2004; Ramp *et al.* 2005; Seiler & Helldin 2006).

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Capítulo I - Carcass Persistence and Detectability: Reducing the Uncertainty Surrounding Wildlife-Vehicle Collision Surveys

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Abstract

Carcass persistence time and detectability are two main sources of uncertainty on road kill surveys. In this study, we evaluate the influence of these uncertainties on roadkill surveys and estimates. To estimate carcass persistence time, three observers (including the driver) surveyed 114 km by car on a monthly basis for two years, searching for wildlife-vehicle collisions (WVC). Each survey consisted of five consecutive days. To estimate carcass detectability, we randomly selected stretches of 500m to be also surveyed on foot by two other observers (total 292 walked stretches, 146 km walked). We expected that body size of the carcass, road type, presence of scavengers and weather conditions to be the main drivers influencing the carcass persistence times, but their relative importance was unknown. We also expected detectability to be highly dependent on body size. Overall, we recorded low median persistence times (two days) and low detectability (<10%) for all vertebrates. The results indicate that body size and landscape cover (as a surrogate of scavengers' presence) are the major drivers of carcass persistence. Detectability was lower for animals with body mass less than 100g when compared to carcass with higher body mass. We estimated that our recorded mortality rates underestimated actual values of mortality by 2-10 fold. Although persistence times were similar to previous studies, the detectability rates here described are very different from previous studies. The results suggest that detectability is the main source of bias across WVC studies. Therefore, more than persistence times, studies should carefully account for differing detectability when comparing WVC studies.

Introduction

Roads and associated traffic promote a variety of negative effects on biodiversity, including habitat degradation and pollution, dispersal of exotic species, and barrier effects [1-5]. Wildlife-vehicle-collisions (WVC), however, are often recognized as the most important source of non-natural animal mortality, exceeding other significant impacts such as hunting [2, 6, 7]. Population declines, inbreeding depression and local extinctions of some species may occur due to roadkills [1, 4, 8, 9]. In fact, virtually all species using road vicinities are negatively affected by WVC, from insects [10] to all terrestrial vertebrates [11-15]. To avoid these negative effects, mitigation measures such as faunal passages and drift fencing [2,4,5,6] are generally applied at road sections with higher frequencies of roadkills [14]. Because these mitigation measures are often expensive, it is crucial that roadkill rates along the road network are properly quantified for a correct identification of most problematic road sections [16-18]. Besides, correcting mortality estimates is very important to assess the effects of roadkills on population depletion. This, requires accurate WVC estimates, correcting for the two main sources of bias: carcass persistence time and carcass detectability [16-18]. Yet, the use of such unbiased estimates has barely been used[16, 18, 19].

Persistence time is the period up to which a carcass remains detectable, i.e. before it is decomposed by traffic or removed by scavengers [20], and is influenced by several factors, including the size of the carcass, traffic volume, and weather conditions [18, 21-27]. Larger carcasses are expected to remain for longer periods, while roads with higher traffic volume are expected to reduce carcass persistence given the faster degradation of more vehicles passing by [18,23,26]. Regarding weather, during the rainy season it is expected that carcasses show shorter persistence times, since heavy rain also promotes faster degradation of carcass, and washes away carcass debris [23, 26]. On the other hand, in drier days and at higher temperatures carcass may suffer desiccation therefore increasing the persistence time [23, 26]. Another important source of variation in persistence time is the scavenging activity, which is naturally related to the abundance and diversity of scavengers inhabiting the roads' vicinity areas [1,18,26]. The main difficulty in assessing the importance of scavenging for carcass persistence is obtaining reliable estimates of abundance and activity of scavengers in the vicinity of roads. One option to circumvent this difficulty is to use proxies for scavengers presence. The abundance and diversity of scavengers is expected to be higher in areas with better habitat quality and availability [28-30]. In fact, raptors and mammalian communities vary in relation to habitat transformations in several biomes (e.g. forests, deserts, savannah) [28-32]. For example, in Cerrado, the typical savannah in central Brazil, studies have shown that populations of raptors, including scavengers, are more abundant and have more species in areas dominated by natural habitat [29, 32]. Hence, communities of scavengers are expected to be more diverse and rich in road sections surrounded by natural and semi-natural habitats [28-31, 33-35].

Carcass detectability, i.e. the probability of a carcass being detected given it persists to the time of surveys, is highly dependent on the survey method (e.g. driving or walking), observer experience and the body size of carcass [18, 19, 36]. Surveys performed by car generally detect a lower proportion of carcass compared to walking surveys, and this is particularly evident for small-sized species [17, 18, 23]. Yet, disparate detectability values even for the same taxa, have been reported. For example, the detectability of bird carcasses can range between 1 and 67% (mean 26. 9%) [17, 18, 22, 23, 37].

The main objective of this study was to evaluate the influence of carcass persistence time and detectability when quantifying WVC rates. In particular, we aimed to 1) quantify carcass persistence time and assess how it is influenced by body mass of carcass, road-related characteristics, weather conditions and cover of (semi-)natural habitat (as a proxy of scavenger activity); and 2) estimate carcass detectability when performing road surveys by car. As a final goal, we wanted to (3) estimate the proportion of undetected carcasses after correcting for persistence and detectability bias in our studied roads. We expected the persistence time to be longer for large body-sized species, in roads with low traffic volume, and in periods without rainfall [26]. We also expected higher cover of natural habitat near roads to be related to a lower persistence time. The novelties of this study are the broad spatial scale of the study area and road types surveyed, as well the integration of persistence time and detectability for estimating the ‘true’ mortality rates [19, 26].

Materials and methods

No specific permissions were required for our study locations/activities, since it is not necessary field permit to monitoring wildlife-vehicle collision. In addition, the project was executed by the environmental agency of the state, responsible for the environmental monitoring. Lastly, it is not necessary authorization for the collection and transport of animals found dead, to scientific or educational use (Normative Ruling Nº 03 of September of 2014 - ICMBio, see Article 25). Our study did not involve endangered or protected species.

Study area

This study was conducted in Brasília, within the Federal District, Brazil (Fig1). The vegetation in the study area is typical of Cerrado biome, and is dominated by savanna forest (“Cerradão” and "Mata de Galeria"), open savanna (“Cerrado *sensu stricto*") and grasslands [38, 39]. The climate is tropical savanna (Köppen-Geiger classification) [40], with an average annual rainfall of 1540mm [41]. The region has distinct dry and wet seasons. During the wet season (October-March), monthly rainfall averages 214mm, monthly temperatures average 21.6ºC, and monthly relative air humidity averages 72% [41]. During the dry season (April to September), the monthly rainfall average drops to 41.9mm, monthly temperatures to 19.9ºC, and monthly relative air humidity averages 56%, dropping to less than 30% in some periods of the year [41].

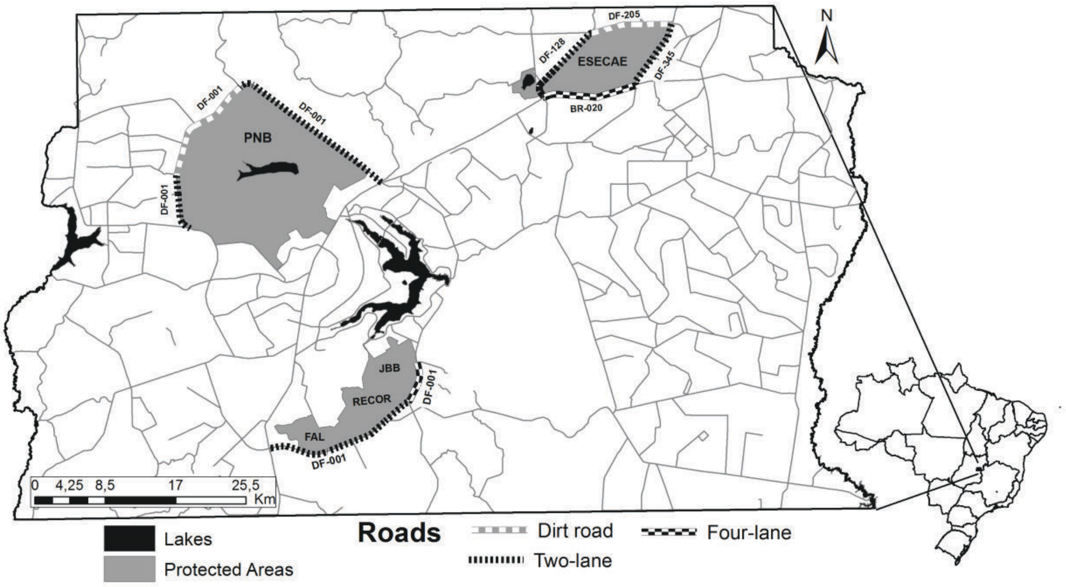

Fig 1. Study area with location of monitored roads and protected areas. Reprinted from Brasilia Environmental Institute (IBRAM) under a CC BY license, with permission from the head of the management of environmental information of IBRAM, original copyright 2016.

The surveys were conducted along nine roads (total 114 km), including four-lane (BR-020 and DF-001, 16 km), two-lane (DF-001, DF-345 and DF-128, 74 km), and dirt roads (DF-205 and DF-001, 24 km) (Fig 1). Both four-lane and two-lane sections were paved (with shoulders). The four-lane roads have the highest traffic volumes (5,000 to 7,000 vehicles/day), the dirt roads have the lowest (33 to 775 vehicles/day), while the two-lane roads have intermediate traffic volumes (775 to 4,000 vehicles/day, with a stretch of 10km reaching 8,000 vehicles/day) [42]. These road sections delimit five protected areas, namely Ecological Station of Águas Emendadas - ESECAE (10,000 ha), National Park of Brasília-PNB (44,000 ha), Botanical Garden of Brasilia-JBB (4,000 ha), Experimental Farm of

University of Brasília FAL/UnB (4,000 ha), and IBGE Biological Reserve-RECOR (1,300 ha) (Fig 1). UNESCO recognizes all these protected areas as core areas of the Cerrado Biosphere Reserve in the Federal District.

Data collection

Carcass persistence time

Road surveys were performed on a monthly basis, between March 2013 and April 2015, with each survey consisting of five consecutive sampling days (total 26 surveys, 130 sampling days). Three observers (including the driver) in a vehicle at ca. 50km/h sampled repeatedly the five consecutive days searching for carcasses. The vehicle stopped for each carcass found on the road. The observers identified the carcass to the lowest possible taxonomic level, and collected information of the position on the road (lane or shoulder) and the geographic coordinates using a hand-held GPS with 5 m-accuracy. All carcasses were left in the same position in which they had been initially found, and during subsequent sampling days their presence was rechecked to determine persistence time. Hence, carcasses found on the first, second and third days were monitored up to four, three or two days, respectively. Since the surveys were dependent on the technical staff of the local road agency, carcass monitoring could not be performed for more days. However, 5-year data from 484 roadkill surveys in the same roads (5,164 road-killed animals recorded) showed that 60% of carcasses weight less than 100g [43] and, thus, are unlikely to persist on the road for more than three days [17, 19, 26, 44, 45].

Carcass detectability

In order to estimate carcass detectability, we randomly selected 500m stretches of the studied roads to be additionally surveyed on foot. These walking surveys were performed independently by another two observers, and began 20 minutes after the car-based team (two observers and one driver in a vehicle at ca. 50km/h) had passed through the selected stretches to avoid visual contact between the car-based and walking teams. Each observer walked along one of the road shoulders looking for carcasses. The same protocol as that of the car-based team for data collection was followed when a carcass was detected. Walking surveys were also performed every month, between May 2013 and April 2015 (total 24 surveys). We surveyed 11 to 12 road stretches in each survey (total 292 stretches, 146 km walked). All carcasses found in the detectability assessment were removed from the road afterwards. The detectability assessment was performed after persistence assessment survey, to avoid removing carcasses that could be recorded in these surveys.

Explanatory variables

To assess what factors influence carcass persistence time, we collected additional information on species characteristics, weather conditions and land cover (Table 1). We obtained the mean body mass for each species (S1 Dataset) from bibliographic references [46-52]. Daily rainfall and air humidity were obtained for each survey day from a weather station located at ca. 15 km from the study area, in Brasilia [41]. We used the weather information of the first day a carcass was encountered to characterize the average meteorological conditions during the period of carcass persistence on the road.

Table 1. List of explanatory variables and their range values related to the animal, road, weather and land cover used to explain variations in carcass persistence.

Variable	**Range**
Animal	
Body mass (g) [b]	3-10,000
Road	
Position on Road	1: Lane [a] 2: Shoulder

Road Type	1: Dirt road (unpaved) [a] 2: Two-lane road (paved) 3: Four-lane road (paved)
Weather	
Rainfall	0: No rain [a] 1: Rain event
Air humidity (%) [c]	0.19-0.92
Land cover	
Proportion of savannah[c] (includes Cerrado *sensu strictu*, open savanna and dense Cerrado)	0.07-0.93
Proportion of forest [c] (includes Gallery Forest and "Cerradão")	0.00-0.15
Grasslands and pastures	0.00-0.24
Agriculture	0.00-0.70
Site	
Protected area (site) near which was recorded the roadkill [d]	1 - ESECAE 2 - PNB 3 - JBB/RECOR/FAL

[a] Reference level in Cox models, see main text.

[b] Logarithmic transformation.

[c] Arcsine square root transformation.

[d] Names of protected areas in study area description.

Land cover information was obtained from a map provided by the Brasília Environmental Institute [53], originated from the multispectral RapidEye satellite image from 2011 (spatial resolution of 5m). From this map we extracted the proportion of each land cover type with a circle centered at each carcass location, using buffer sizes of 2, 3 and 4-km radius, which correspond to a total area of ca. 12 to 50 km^2. We established these buffer sizes in order to capture the variation, in the adjoining areas, of the abundance of the three most common scavengers (obligate or otherwise), namely the southern crested caracara (*Caracara plancus*), the black vulture (*Coragyps atratus*), and the crab-eating fox (*Cerdocyon thous*). These species have estimated home ranges of ca. 7, 15 and 123 km^2, respectively [54, 55, 56].

Data analyses

We tested for an association between taxonomic Class and body mass using Kruskal-Wallis test. The result obtained revealed a strong relationship ($K = 110.03$, df = 2, p-value < 0.001), with mammals presenting higher body mass than birds and reptiles. Hence, we preferred to work with body mass instead of taxonomic Class, as persistence and detectability of carcasses are more likely similar across similar body sizes than across broad taxonomic levels as Class. To proceed with the analyses, the dataset was divided in carcasses with less than 100g and higher than 100g. This division was based on the dataset of the carcass detectability experiment (see Results and S1 Dataset for detectability experiment dataset). The carcasses that persisted up to the 5th day were classified as right-censored data (i.e., carcasses for which the true persistence time is longer than the study period).

Carcass persistence time and influence of environmental variables

The median carcass persistence probability was estimated using the Kaplan-Meier estimator [57], per body mass class and for all records combined. We considered a significant difference if the 95% confidence intervals of median persistence times did not overlap among classes.

Before examining the influence of the explanatory variables (Table 1) on the persistence probability of carcass we checked for pairwise multicollinearity using exploratory plots and Pearson correlations [58]. For each pair of variables exhibiting high correlation (>0.7) [59], the strongest explanatory variable in the simple Cox proportional hazard models was retained for further models (see S2 Table for correlations between variables). We applied, when necessary, arcsine or logarithmic transformations to achieve normality of data [58].

Multivariate mixed Cox models [60] were then fit using all possible combinations of the uncorrelated variables. Model averaging procedures were used to combine results from similarly ranked models (ΔAICc < 2) [61], and to calculate unconditional standard errors for averaged coefficients. Finally, the relative importance of each variable was obtained by summing the Akaike weights for all models (ΔAICc < 2) containing that variable [61]. To evaluate the goodness-of-fit of each model, we used the overall likelihood ratio (LR) test and the proportion of variance explained (R^2) after visual inspection of model residuals and proportional hazard assumptions.

Carcass detectability

To estimate the detectability of carcass surveys performed by car we applied a generalized linear model with binomial error distribution to model the number of detected and non-detected carcasses by the car team, using the function 'search.efficiency' available in the R package *carcass* [20]. Body mass was used as explanatory variable. We assumed that the ability to detect carcasses was not remarkably different between observers of both survey teams. This was assessed in joint preliminary surveys, by car and on foot. In all cases, no observer showed a greater capacity or difficulty in detecting carcass on the road.

Estimating the 'real' number of roadkills

Carcass persistence (s) and detectability (*f*) biases were combined to estimate the detection probability *p* of carcasses following Korner-Nievergelt et al. [62]:

$$p = \frac{f\left(s\frac{1-s^{d}}{1-s}\right)\left(\sum_{i=0}^{n-1}(n-i)\left((1-f)s^{d}\right)^{i}\right)}{nd} \qquad \text{(eq. 1)}$$

where *n* is the number of searches in the study and *d* is the search interval, i.e. the number of days between consecutive searches. We applied Monte Carlo simulations to account for the uncertainty on the estimation of *p*, using the Korner estimator as implemented in the

"Carcass" package [20]. We then estimated the 'real' number of carcasses (*N')* during the survey period, given *p* [20] using the equation 2, which corresponds to the Horvitz-Thompson estimate [62]:

$$N = \frac{\sum_{i=1}^{n} c_i}{p} \quad \text{(eq. 2)}$$

where: $c_i$is the number of carcass counted during search *i*. N' was estimated separately for the different body mass classes (i.e., with more or less than 100g).

We did not consider domestic species in the analysis as carcass persistence may have been affected by human action, for example the recovery by owners of road-killed dogs and cats (*pers. obs*.). All calculations and plots were performed within the R environment [63]. The R packages *survival* [64] and *coxme* [64] were used in Kaplan-Meier and Cox models, while *carcass* [20] was used in detectability and mortality estimates.

Results

We collected persistence data for 532 non-domestic road-killed animals, of which 2% were amphibians (n=14, 2 species), 19% reptiles (n=101, 31 species), 71% birds (n=374, 44 species), and 8% mammals (n=43, 12 species). Three quarters of records (n=381) were of small size (body mass < 100g) (S1 Dataset). We excluded amphibians from further analyses given the low number of records.

Carcass persistence time and influence of environmental variables

Overall, the median persistence time of carcasses was 2.2 days, with a persistence probability after one day of 0.43 (0.39-0.48, Confidence Interval), dropping to 0.30 (0.27-

0.35) in the second day, and reaching a persistence probability of 0.07 (0.05-0.10) in the fourth day. These values indicate a low persistence probability, with a substantial drop beyond the first day (Fig 2 and S3 Table). As expected, the median persistence time was significantly different (no overlapping confidence intervals) between smaller and larger carcasses, being approximately two days for those carcasses with less than 100g and four days for larger ones (S3 Table).

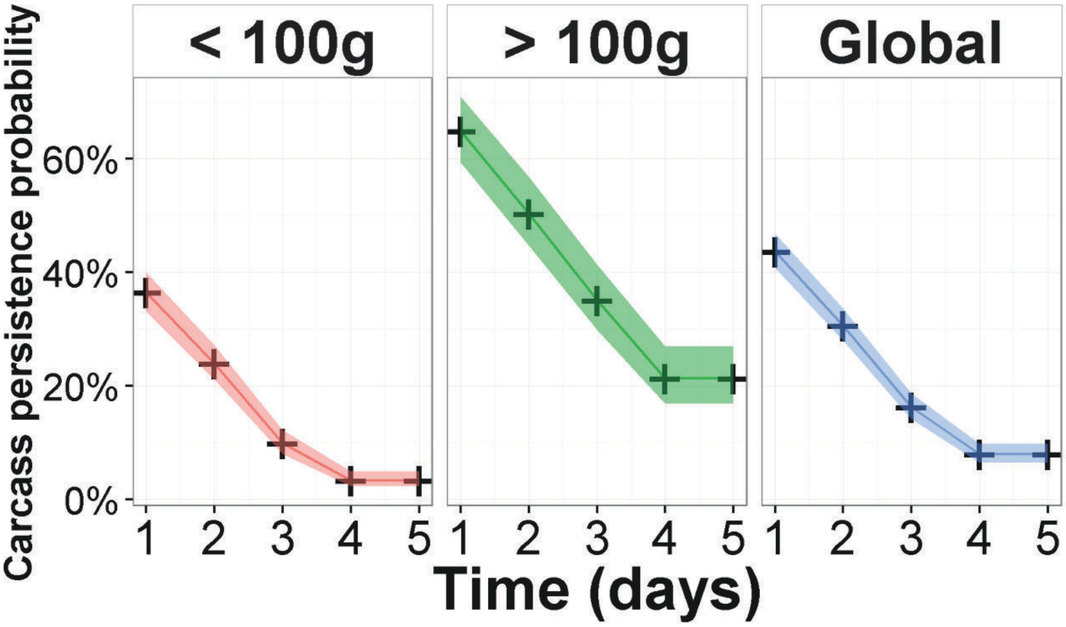

Fig 2. Survival curves from Kaplan-Meier models and corresponding 95% confidence intervals for global data, and body mass classes.

We retained 21 mixed Cox models (ΔAICc<2) relating the persistence time and environmental variables using the information from 3-km buffer radius (Table 2 and Table 3). Each model explained an average of 13.1% (range of 12.1-14.5%) of the variance, a low explanatory value. Graphical diagnostics based on the scaled Schoenfeld residuals showed evidence of proportional hazards for all buffers sizes (see S4 Fig). Likewise, the test for proportional hazards was not significant (see S4 Table). Results from models using information for 2 and 4 km buffer radius were similar and are presented in supplementary information S5 and S6, respectively.

Table 2. Summary of the top Akaike's Information Criterion models (ΔAICc<2.0) of the mixed Cox proportional hazard function for persistence data with 3-km buffer radius. All models included site as random effect. LogLik: maximum likelihood value; R^2: variance explained by the model; ΔAICc: Akaike's Information Criterion rank; *w*: AIC model weights.

Model	**LogLik**	**R^2**	**ΔAICc**	***w***
s+t+b	-2496.05	0.1285	0	0.09
s+r+t+b	-2495.15	0.1317	0.091	0.08
s+h+t+b	-2495.37	0.1309	0.622	0.06
s+g+b	-2496.88	0.1257	0.890	0.06
f+s+r+t+b	-2494.26	0.1347	0.952	0.05
s+b	-2497.98	0.1218	0.980	0.05
f+s+t+b	-2495.29	0.1312	1	0.05
f+s+a+r+t+b	-2493.17	0.1385	1.06	0.05
f+s+a+t+b	-2494.24	0.1348	1.18	0.05
f+s+h+t+b	-2494.44	0.1341	1.39	0.04
s+g+r+b	-2496.15	0.1282	1.48	0.04
f+s+a+h+t+b	-2493.34	0.1379	1.49	0.04
s+a+t+b	-2495.82	0.1293	1.55	0.04
s+g+t+b	-2495.75	0.1296	1.65	0.04
f+s+g+b	-2496.17	0.1281	1.65	0.04
s+a+r+t+b	-2494.95	0.1324	1.67	0.04
s+g+r+t+b	-2494.83	0.1328	1.74	0.04
s+r+b	-2497.37	0.124	1.79	0.04

s+g+h+b	-2496.32	0.1276	1.83	0.03
s+r+h+t+b	-2494.99	0.1322	1.83	0.03
s+t+p+b	-2496	0.1287	1.98	0.03

Legend for models: a - agriculture; b - body mass; f - forest habitat; g - grasslands; h - air humidity; p - position; r - rainfall; s - savannah; t - road type.

Table 3. Model-averaged coefficients (β), respective confidence intervals from unconditional standard errors (95% LCI and 95% UCI), estimates of the hazards ratio (e^{β}), and importance value of the top mixed Cox models (ΔAICc<2.0) to 3-km buffer. Variables are ordered according to their importance.

Variable	**β**	**95% LCI**	**95% UCI**	**e^{β}**	**Importance**
Savannah*	0.803	0.180	1.426	2.26	1.00
Body mass*					1.00
(>100g)	-0.192	-0.252	-0.132	0.822	
Road type					0.740
(Two-lane)	0.007	-0.533	0.551	1.007	
(Four-lane)	-0.225	-0.870	0.264	0.795	
Rainfall	0.048	-0.065	0.323	1.05	0.370
Forest habitat	-0.363	-2.907	0.692	0.690	0.330
Grasslands	0.115	-0.362	1.306	1.12	0.240
Agriculture	-0.077	-1.002	0.297	0.924	0.220
Air humidity	0.068	-0.264	0.890	1.07	0.220
Position on road					0.030
(Shoulder)	0.001	-0.183	0.224	1.001	

* Significant variables (95% confidence limits)

All 21 models included proportion of savannah habitat and body mass, which were also the variables that attained the highest importance (Table 2 and 3). According to the averaged model, the persistence time is lower for carcass located in areas with a high cover of savannah habitat nearby and of smaller body mass (<100g) (Table 3). Savannah habitat had the strongest effect on persistence times, with a hazard ratio of 2.26 (Table 3), suggesting a strong effect of the availability of this land use on persistence times. For carcasses with body mass less than 100g, the persistence probability was lower, being 0.36 (0.32-0.41) and 0.03 (0.02-0.05) for the first and fourth days, respectively. For carcasses with larger body mass (>100g), the persistence times were 0.71 (0.65-0.78) and 0.27 (0.22-0.34) for the same time frames (S3 Table).

The remaining variables had no significant coefficient estimates (Table 3). However, the road type was ranked as the third most important variable in model averaging procedures, despite its confidence interval on beta estimate crossing zero (Table 3). Interestingly, most of the top ranked models containing this variable showed a positive effect of the 4-lane road type, when compared to the dirt road. That is, results suggest that persistence time is higher in 4-lane roads relatively to dirt roads.

Carcass detectability

The walking team detected 117 carcasses, of which 16% were amphibians (n=19, 2 species), 28% reptiles (n=33, 12 species), 42% birds (n=49, 8 species), and 14% mammals (n=16, 3 species). Of these, only 10 carcasses (6 birds, 2 reptiles and 2 mammals) were also detected by the car-team, corresponding to an overall detectability (*f*) of 10% (6-19% CI). The detectability was apparently lower for carcasses with lower body mass (<100g), 7% (2-15%) relatively to 13.3% (4-29%) for carcasses of larger body mass. However, these results should be considered with caution as their confidence intervals overlapped zero.

Estimating the ‘real’ number of roadkills

We estimated a *N*’ of 55,906 roadkills/year of small sized species (<100g), which represents a mortality rate of 1.3 roadkills/day/km (Table 4). This estimate was 10 fold higher than the observed value of roadkills. For carcasses of higher body mass, we estimated a *N’* of 5,222 roadkills/year representing 0.12 roadkills/day/km, i.e., a two-fold increase in roadkills numbers. Overall, we estimated a mortality rate of 0.83 roadkills/day/km on our studied roads, representing an annual mortality of 34,536 animals along the 114 km surveyed (Table 4).

Table 4. Estimates of total roadkills corrected for biases introduced by carcass persistence and survey method. f – detectability (%), s – estimated median carcass persistence time (days), p – probability of a carcass being detected after one day. N' – mortality estimate with correction for detectability and carcass persistence (roadkills/day/km). C’ – mortality estimates without correction for detectability and carcass persistence (roadkills/day/km). Confidence intervals are provided when available.

Group	**f**	**s**	**p**	**C’**	**N'**
Carcass < 100g	6.8 (2-15)	1.80	0.36 (0.32-0.41)	0.13	1.32 (0.62-3.94)
Carcass > 100g	13.3 (4-29)	4.14	0.71 (0.65-0.78)	0.06	0.12 (0.06-0.41)
Global data	10 (6-19)	2.15	0.43 (0.39-0.48)	0.15	0.83 (0.47-1.17)

Discussion

With this study we aimed to evaluate the influence of carcass persistence time and detectability biases in quantifying roadkills. Our results confirm that carcasses persist on roads for about two days, which is in line with previous studies [17, 19, 26, 65]. This is a short persistence period when considering that the periodicity of most roadkill surveys is weekly to monthly. Moreover, our results support that the persistence is largely influenced by environmental variables and characteristics of the road itself, besides the size of the carcass.

The amount of cover of savannah surrounding the roads was the most important predictor explaining the persistence times, hence suggesting a significant effect of scavengers' activity. We considered that areas with higher savannah coverage have a more diverse and abundant scavenger community and therefore the removal of carcasses by scavengers is likely to be more accentuated in areas of (semi-)natural habitats than in anthropogenic areas (agriculture). This is in agreement with the lower persistence times detected in areas dominated by savannah habitat. Regarding the carcass body size, the persistence time was smaller for small-sized carcasses (<100g), which is in accordance to published literature [19, 26, 66-68]. This lower persistence time of smaller carcasses is likely to be due to a more rapid degradation by passing vehicles [19, 21, 69]. The effect of the remaining predictors was generally imprecise as confidence intervals of estimates in model averaging procedures overlapped zero. However, our results suggest a higher persistence for carcasses laying in the four-lane roads when compared to those in dirt roads, which have much less traffic. We suspect that a higher persistence time in 4-lane roads is due to the limited access of scavengers to carrion. That is, higher traffic volume probably inhibit scavengers from attempting to access the carcasses [18, 70]. In fact, a recent study recorded a maximum abundance and diversity of birds of prey along roads with medium traffic volume, when compared to highways with higher traffic volumes [71]. On the other hand, the dirt roads studied are embedded in areas with higher forest cover, hence increasing the chance of carcasses being detected by scavengers. These results stress that the influence of the scavenger-traffic volume relationship on carcass persistence time may not be straightforward [27]. Overall, our results highlight that the road mortality rates, as estimated by roadkill surveys, ought to be corrected for scavenger activity, species body mass and road type/traffic volume.

Regarding carcass detectability, our results reveal a low search efficiency of car surveys relatively to walking surveys, particularly for small-sized animals. The detection of smaller animals was two times lower than for larger animals. This difference in detectability between teams is unlikely to be observer-related, as all members received equal training. On the other hand, the car team moved at an average speed of 50km/h, which is probably too fast to detect most small carcasses. Interestingly, the literature reports a wide variability of detectability values, ranging between 1% and 67% [17, 22, 37, 72-74]. Even considering the different taxonomic groups targeted in those studies, the values are still highly discrepant: 4-23% (average 14%) for reptiles [17, 22, 25], 1-67% (27%) for birds [17, 18, 22, 23, 37], and 10-47% (26%) for mammals [17, 18, 22, 75]. Noteworthy, as previously referred the carcass persistence times estimates are similar across those studies, despite the different regions of the world and taxa [17, 21-26, 36, 69]. Hence, we stress the importance of accounting not only for the persistence bias, but perhaps more importantly, for the detectability bias as this latter is more variable across studies. Both are important to be accounted for, the difference is that detectability seems to be more variable and case-specific, so it should be estimated within each study, while persistence might be extrapolated from different areas.

Few studies in road ecology have taken into account carcass persistence and detectability to estimate a more accurate number of ‘real’ mortality rates [17, 18, 22, 23]. As a comparison with our results, a study conducted in the region of Atlantic Forest, in southern Brazil, estimated that corrected estimates for reptile and bird mortalities were 2 to 39 times greater than surveyed values [17]. Our results are in line with these studies and show that in our study region, after correcting for persistence and detectability bias, the actual number of roadkills is likely to be, at least, 2-10 fold greater than estimates based on roadkill surveys. We believe that a more ‘real’ estimate of mortality rates, i.e., corrected by detection and carcass persistence, is the first step to find out if the mortality by roadkills is additive or compensatory [76]. Compensatory mortality hypothesis predicts that no effect on annual survival must occur at low rates of harvest mortality up to a threshold, above which harvest mortality should be additive and with reductions in annual survival [77]. A second step is to identify those species that are likely to experience additive (as opposed to compensatory) mortality from vehicle collisions [76, 78]. The additive population mortality may have worse consequences such as population decreases at short-term [76] what makes conservation strategies priority to the affected species.

It is important to discuss some methodological limitations of our study. First, a low explanatory power of models does not mean that the influence of measured variables is not significant. WVC events are the result of several interrelated factors acting at different scales, from individual behavior responses and experience of both animals and drivers, to the influence of overall landscape connectivity and animal population dynamics. Hence, it is expected that a great proportion of variability is due to stochasticity or to unmeasured variables. Second, our study assumed that all roadkills were detected by walking surveys, but this assumption may not always stand, which could result in an overestimation of detection probabilities [22]. In fact, some road-killed animals are thrown off the lanes at the moment of impact by passing vehicles, and walking observers may fail to notice them [22]. Besides, higher height of the vegetation in shoulders may hide the carcasses and the experience and motivation of the observers may contribute to underestimate in walking surveys [78, 79]. However, we are confident that only a small number of carcasses was missed by the walking team, thus having a negligible effect on mortality estimates.

Management implications

Our study suggests that if surveys are not corrected for carcass persistence and detectability, researchers will significantly underestimate mortality rates. When possible, surveys performed by car should be made at lower speeds. Collinson et al. [79] recommends monitoring by vehicle at speeds at 10-20 km/h. However, lowering the speed survey imply longer survey times, increasing the costs. For the same budget, one would survey less kilometers, which could reduce the generality of the study. These implications perhaps merit further study on ideal sampling design for roadkill surveys to maximize efficiency.

Overall, our results highlight that persistence time is generally concordant across studies, being about two days, although it can vary according to habitat and road type, together with body mass. More importantly, carcass detectability should be estimated for each study, in order to generate less biased mortality rates, as it is apparently the main bias in mortality estimates. We suggest performing an initial training period for observers participating in roadkills surveys to increase observers'efficiency.

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Author contributions

RALS and APF conceived the study and carried out the fieldwork. RALS, SMS and FA analyzed the data. RALS, SMS and FA wrote the paper. MSR, LMSA, AB and APF contributed to writing the paper. All authors read and approved the final manuscript.

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Supporting Information

S1 Dataset. All Dataset.

All Dataset are avaliable in PLOS ONE journal inhttp://dx.doi.org/10.1371/journal.pone.0165608.

S2 Table. Results for correlation test for variables with 2, 3 and 4-km buffer radius.

S2 Table A. Results for correlation test for variables with 2-km buffer radius.

Variable	**Rainfall**	**Air humidity**	**Savannah**	**Forest**	**Agriculture**	**Grasslands**	**Body mass**
Rainfall	1.000	0.253	0.027	-0.004	-0.038	0.005	-0.008
Air humidity	0.253	1.000	0.066	-0.012	-0.003	-0.090	-0.060
Savannah	0.027	0.066	1.000	-0.224	-0.327	-0.224	0.043
Forest	-0.004	-0.012	-0.224	1.000	-0.254	-0.134	-0.012
Agriculture	-0.038	-0.003	-0.327	-0.254	1.000	-0.114	-0.033
Grasslands	0.005	-0.090	-0.224	-0.134	-0.114	1.000	0.028
Body mass	-0.008	-0.060	0.043	-0.012	-0.033	0.028	1.000

S2 Table B. Results for correlation test for variables with 3-km buffer radius.

Variable	**Rainfall**	**Air humidity**	**Savannah**	**Forest**	**Agriculture**	**Grasslands**	**Body mass**
Rainfall	1.000	0.253	0.017	0.019	-0.007	-0.032	-0.008
Air humidity	0.253	1.000	0.069	0.057	-0.026	-0.075	-0.060
Savannah	0.017	0.069	1.000	-0.178	-0.221	-0.336	0.056
Forest	0.019	0.057	-0.178	1.000	-0.394	-0.042	0.006
Agriculture	-0.007	-0.026	-0.221	-0.394	1.000	-0.080	-0.029
Grasslands	-0.032	-0.075	-0.336	-0.042	-0.080	1.000	-0.006
Body mass	-0.008	-0.060	0.056	0.006	-0.029	-0.006	1.000

S2 Table C. Results for correlation test for variables with 4-km buffer radius.

Variable	**Rainfall**	**Air humidity**	**Savannah**	**Forest**	**Agriculture**	**Grasslands**	**Body mass**
Rainfall	1.000	0.253	0.009	0.004	0.005	-0.058	-0.008
Air humidity	0.253	1.000	0.057	0.029	-0.021	-0.040	-0.060
Savannah	0.009	0.057	1.000	0.015	-0.136	-0.516	0.055
Forest	0.004	0.029	0.015	1.000	-0.471	0.042	-0.003
Agriculture	0.005	-0.021	-0.136	-0.471	1.000	-0.207	-0.010
Grasslands	-0.058	-0.040	-0.516	0.042	-0.207	1.000	-0.013
Body mass	-0.008	-0.060	0.055	-0.003	-0.010	-0.013	1.000

S3 Table. Summary of results for persistence estimates.

S3 Table. Summary of results for persistence estimates for each body mass class and the ‘‘global data’’. N: sample size; Mean (95% CI): mean persistence time probabilities; T=1, T=2, T=3, T=4: estimate of persistence probability for 1-day (T=1), 2-day (T=2), 3-day (T=3) and 4-day (T=4) and corresponding 95% confidence intervals obtained with a Kaplan-Meier estimator.

Groups	**N**	**Mean Persistence Time (days)**	**T=1**	**T=2**	**T=3**	**T=4**
WVC < 100g*	316	1.80	0.36 (0.32-0.41)	0.24 (0.20-0.29)	0.09(0.07-0.13)	0.03(0.02-0.05)
WVC >100g**	199	4.14	0.71 (0.65-0.78)	0.57 (0.51-0.64)	0.42 (0.36-0.50)	0.27 (0.22-0.34)
Global data	515	2.15	0.43 (0.39-0.48)	0.30 (0.27-0.35)	0.16 (0.13-0.19)	0.07 (0.05-0.10)

* Carcass with body mass less than 100g

** Carcass with body mass higher than 100g

S4 Figures and Tables. Plots of residuals and results for test of proportional hazard assumptions.

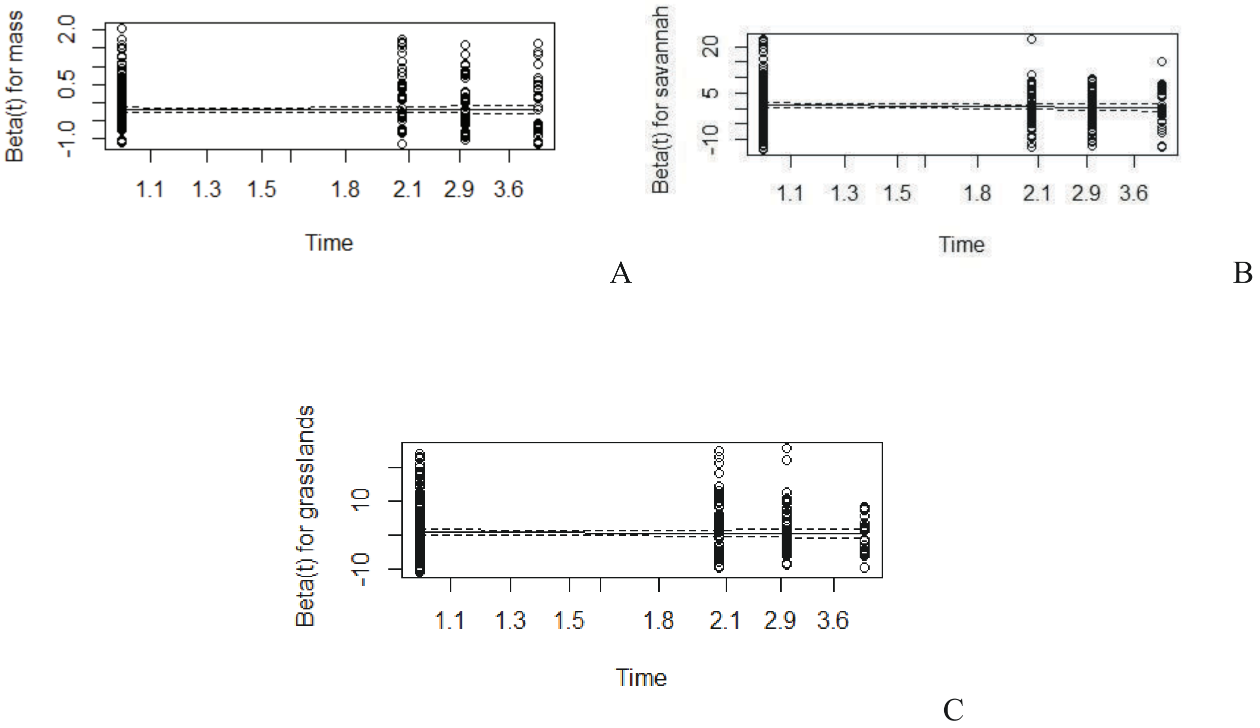

Figure S4 A. Plots of scaled Schoenfeld residuals against transformed time for each covariate to the best model with 2-km buffer-size. The solid line is a smoothing spline fit to the plot, with the broken lines representing a ± 2-standard-error band around the fit.

Table S4 A. Results for test of the proportional-hazards assumption to the best model with 2-km buffer-size. Chisq: Chi-square test.

	rho	**Chisq**	**p-value**
Body mass	0.0136	0.0819	0.775
Savannah	-0.0564	1.4884	0.222
Grasslands	-0.0184	0.1666	0.683
GLOBAL	NA	1.539	0.673

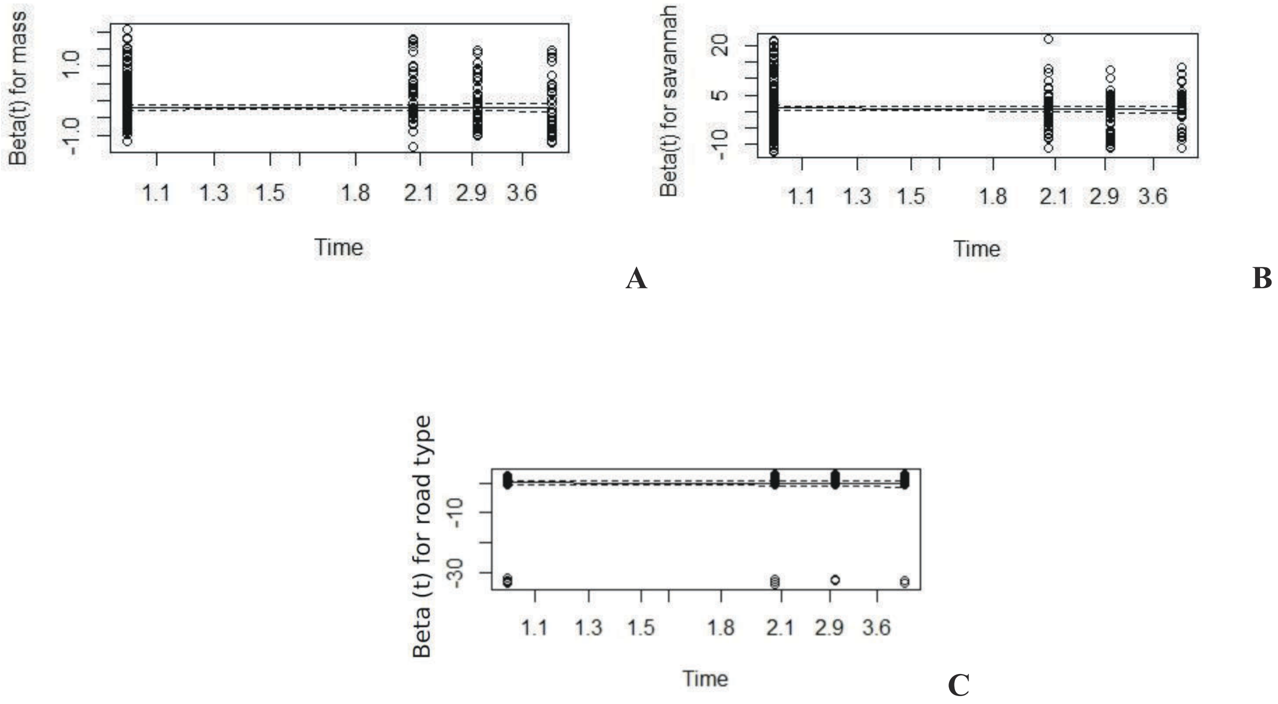

Figure S4 B. Plots of scaled Schoenfeld residuals against transformed time for each covariate to the best model with 3-km buffer-size. The solid line is a smoothing spline fit to the plot, with the broken lines representing a ± 2-standard-error band around the fit.

Table S4 B. Results for test of the proportional-hazards assumption to the best model with 3-km buffer-size. Chisq: Chi-square test.

	rho	**Chisq**	**p-value**
Body mass	0.003	0.005	0.945
Savannah	-0.041	0.715	0.398
Two-lane	-0.028	0.371	0.543
Four-lane	-0.010	0.042	0.837
GLOBAL	NA	1.685	0.793

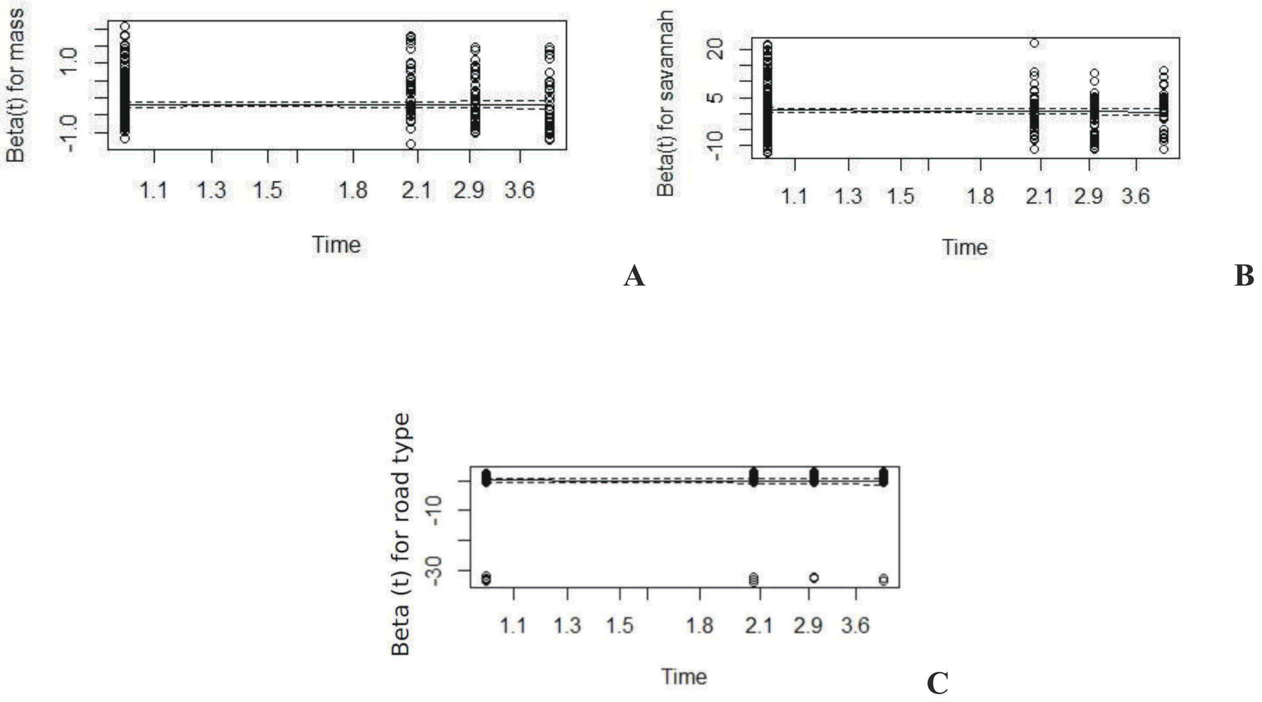

Figure S4 C. Plots of scaled Schoenfeld residuals against transformed time for each covariate to the best model with 4-km buffer-size. The solid line is a smoothing spline fit to the plot, with the broken lines representing a ± 2-standard-error band around the fit.

Table S4 C. Results for test of the proportional-hazards assumption to the best model with 4-km buffer-size. Chisq: Chi-square test.

	rho	**Chisq**	**p-value**
Body mass	0.00471	0.00961	0.922
Savannah	-0.04389	0.80955	0.368
Two-lane	-0.02975	0.40977	0.522
Four-Lane	-0.01038	0.05009	0.823
GLOBAL	NA	1.83229	0.767

S5 Table. Results for Cox Model to data with 2-km buffer radius.

S5 Table A. Summary of the top Akaike's Information Criterion models (ΔAICc<2.0) of the Cox proportional hazard function for persistence data with 2-km buffer radius. LL test: maximum likelihood test; R^2: variance explained by the model; ΔAICc: Akaike's Information Criterion rank; *w*: AIC model weights.

Model	**LogLik**	**R^2**	**ΔAICc**	***w***
s+g+b	-2495.53	0.1304	0	0.12
s+g+r+b	-2494.64	0.1334	0.27	0.11
s+g+h+b	-2494.76	0.133	0.53	0.09
f+s+g+b	-2494.91	0.1325	0.91	0.08
f+s+g+r+b	-2493.93	0.1359	0.99	0.07
s+g+r+t+b	-2493.47	0.1374	1.17	0.07
f+s+g+h+b	-2494.1	0.1353	1.35	0.06
s+g+t+b	-2494.58	0.1336	1.45	0.06
f+s+a+r+t+b	-2492.61	0.1404	1.68	0.05
s+g+h+t+b	-2493.69	0.1367	1.69	0.05
f+s+g+r+t+b	-2492.58	0.1405	1.71	0.05
s+a+g+b	-2495.44	0.1307	1.87	0.05
s+g+r+h+b	-2494.44	0.1341	1.93	0.05
s+r+t+b	-2495.12	0.1318	1.94	0.05
f+s+a+g+b	-2494.4	0.1342	1.98	0.05

Legend for models: a - agriculture; b - body mass; f - forest habitat; g - grasslands; h - air humidity; p - position; r - rainfall; s - savannah; t - road type.

S5 Table B. Model-averaged coefficients (β), respective confidence intervals from unconditional standard errors (95% LCI and 95% UCI), estimates of the hazards ratio (e^{β}), and importance value (Importance) of the top mixed Cox models (ΔAICc<2.0) to 2-km buffer radius. Variables are ordered according to Importance.

Variable	**β**	**95% LCI**	**95% UCI**	e^{β}	**Importance**
Savannah*	0.874	0.207	1.540	2.43	1.00
Body mass*	-0.194	-0.254	-0.134	0.820	1.00
Grassalands	0.692	0.030	1.506	2.02	0.90
Rainfall	0.061	-0.059	0.332	1.06	0.44
Forest habitat	-0.293	-2.172	0.554	0.741	0.36
Road type					0.33
(Two-lane)	-0.005	-0.556	0.528	0.994	
(Four-lane)	-0.093	-0.860	0.292	0.909	
Air humidity	0.082	-0.252	0.899	1.08	0.25
Agriculture	-0.039	-0.853	0.316	0.961	0.15
Position on road					
(Shoulder)	0.00	0.00	0.00	0.00	0.00

* Significant variables (95% confidence limits)

S6 Table. Results for Cox Model to data with 4-km buffer radius.

S6 Table A. Summary of the top Akaike's Information Criterion models (ΔAICc<2.0) of the Cox proportional hazard function for persistence data with 4-km byffer radius.LL test: maximum likelihood test; R^2: variance explained by the model; AICc: Akaike's Information Criterion; ΔAICc: Akaike's Information Criterion rank; *w*: AIC model weights.

Model	**LogLik**	**R^2**	**AICc**	**ΔAICc**	***w***
s+t+b	-2496.41	0.1273	5002.89	0	0.1
s+r+t+b	-2495.47	0.1305	5002.92	0.03	0.1
s+h+t+b	-2495.67	0.1299	5003.4	0.5	0.08
s+b	-2498.23	0.121	5003.56	0.67	0.07
s+g+b	-2497.26	0.1243	5003.79	0.9	0.06
f+s+r+t+b	-2494.74	0.1331	5003.96	1.07	0.06
f+s+t+b	-2495.76	0.1295	5004.01	1.12	0.06
s+r+b	-2497.58	0.1232	5004.29	1.4	0.05
s+g+r+b	-2496.52	0.1269	5004.37	1.47	0.05
f+s+h+t+b	-2494.96	0.1323	5004.45	1.56	0.04
s+h+b	-2497.68	0.1229	5004.5	1.61	0.04
f+s+g+b	-2496.58	0.1267	5004.57	1.68	0.04
s+r+h+t+b	-2495.29	0.1312	5004.62	1.72	0.04
s+g+h+b	-2496.7	0.1263	5004.71	1.82	0.04
s+g+t+b	-2496.27	0.1278	5004.73	1.84	0.04
s+a+t+b	-2496.35	0.1275	5004.76	1.87	0.04
s+g+r+t+b	-2495.34	0.131	5004.78	1.89	0.04

s+a+r+t+b	-2495.43	0.1307	5004.83	1.94	0.04
s+t+p+b	-2496.35	0.1275	5004.87	1.98	0.04

Legend for models: a - agriculture; b - body mass; f - forest habitat; g - grasslands; h - air humidity; p - position; r - rainfall; s - savannah; t - road type.

S6 Table B. Model-averaged coefficients (β), respective confidence intervals from unconditional standard errors (95% LCI and 95% UCI), estimates of the hazards ratio (e^{β}), and importance value (Importance) of the top mixed Cox models (ΔAICc<2.0) to 4-km buffer size. Variables are ordered according to Importance.

Variable	**Level**	**β**	**95% LCI**	**95% UCI**	**e^{β}**	**Importance**
Savannah*		0.859	0.175	1.542	2.39	1.00
Body mass*		-0.190	-0.250	-0.130	0.824	1.00
Road type						0.65
(Two-lane)		0.021	-0.510	0.575	1.02	
(Four-lane)		-0.178	-0.837	0.290	0.426	
Rainfall		0.046	-0.067	0.321	1.04	0.36
Grassalands		0.112	-0.483	1.327	1.12	0.26
Air humidity		0.073	-0.271	0.877	1.07	0.24
Forest habitat		-0.173	-2.725	0.976	0.838	0.20
Agriculture		-0.010	-0.809	0.552	0.989	0.07
Position						
(shoulder)		0.001	-0.183	0.224	1.001	0.04

* Significant variables (95% confidence limits)

Capítulo II - Assessing the consistency of hotspot and hot-moment patterns of wildlife road mortality over time

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Keywords: road segments; roadkill; aggregations; scale effect; mitigations.

Abstract

Spatial and temporal aggregation patterns of wildlife-vehicle collisions are recurrently used to inform where and when mitigation measures are most needed. The aim of this study is to assess if such aggregation patterns remain in the same locations and periods over time and at different spatial and temporal scales. We conducted biweekly surveys (n = 484) on 114 km of nine roads, searching for road casualties (n = 4422). Aggregations were searched different lengths of road sections (500, 1000, 2000 m) and time periods (fortnightly, monthly, bimonthly). Our results showed that hotspots and hot-moments are generally more consistent at larger temporal and spatial scales. We therefore suggest using longer road sections and longer time periods to implement mitigation measures in order to minimize the uncertainty. We support this finding by showing that the proportional costs and benefits to mitigate roadkill aggregations are similar when using different spatial and temporal units.

Introduction

Roads have a variety of ecological effects on their surrounding environment, and one of the most studied is wildlife-vehicle collisions (WVC) (Forman *et al.* 2003; Ree, Smith & Grilo 2015). Several researchers have demonstrated that roadkills are often spatially and temporally aggregated, hereafter referred as Wildlife-Vehicle Aggregations (WVA). WVA are generally related to species' biological traits (e.g. mating), road features (e.g. traffic volume), the surrounding landscape or climate conditions (Malo, Suárez & Díez 2004; Smith-Patten & Patten 2008; Gunson, Mountrakis & Quackenbush 2011). Therefore, WVA may indicate preferential targets (hotspots and hot-moments) for implementing mitigation measures (Malo, Suárez & Díez 2004; Morelle, Lehaire & Lejeune 2013; Ree, Smith & Grilo 2015). The identification of WVA is one of the most approaches used by researchers and decision makers to implement mortality mitigation on roads (Santos et al., 2015).

Mitigation measures must be planned to ensure effectiveness, due to the high cost of installation and maintenance (Ree, Smith & Grilo 2015). Thus, it is necessary to determine the best spatial scale(s) at which putative predictors indicate locations of WVA (Langen *et al*. 2007; *Ree et al.* 2015). Ideally, WVA need to be spatially restricted in length, since short road sections can be more easily mitigated by faunal passages and drift fencing than when WVA segments on road are distributed over a broader extent of the road (Langen *et al.* 2007). On the other hand, understanding the role of seasonality on road mortality allows the identification of possible WVA in certain periods (hot-moments), and decision makers can direct mitigation measures in time, reducing costs (Sullivan *et al.* 2004).

The aim of this study was to investigate if the spatial and temporal patterns of WVA were similar along time, for different taxonomic groups. If WVA occur consistently in the same location/time period, i.e. do not change over time, mitigation measures applied therein will probably be more cost-effective (Costa, Ascensão & Bager 2015). Additionally, we evaluated how different road segment length or time period affected the consistency of spatial and temporal patterns WVA. We considered that higher correlation of WVA patterns between consecutive years indicate higher reliability in using such locations as mitigation targets. Hence, we evaluated how cost-benefit effectiveness could vary when targeting mitigation to short/long road sections or time periods. Cost-benefit analysis can be complex in road ecology (Costa, Ascensão & Bager 2015). Here, we adopted a simple approach where we count the number of casualties that could have been prevented if road mitigation was implemented in WVA (assuming full effectiveness).

Materials and methods

Study area

We conducted the study in Brasília (Federal District), located in the Cerrado biome of Central Brazil. A total of 114 km pertaining to nine different roads were surveyed. More details of the study area, including weather conditions, traffic, roads, protected areas monitored and a map are provided in Text 1 in Appendix 1.

Data collection

We conducted road surveys biweekly (two surveys/week) for 5 years, surveying all 114 km by campaign (i.e, all road types were surveyed equally), between April 2010 and March 2015, totaling 480 roadkill surveys. One driver and two observers in a vehicle searched for roadkills, traveling at ca. 50 km/h. The observers recorded the location of carcasses using a hand-held GPS (5m accuracy). Carcasses were removed after data collection to avoid pseudo-replication and recounting carcasses. Domestic animals were not considered in further analyses.

Data analyses

WVC records were aggregated by class (amphibians, reptiles, birds, and mammals) and year, and separate datasets for the spatial and temporal information were created. For the spatial dataset, we aggregated the records by road segments of 500, 1000 and 2000 m length, The temporal dataset was aggregated using fortnightly, monthly and bimonthly time periods. We considered a year of survey as the time between April and March of the following year. Hereafter we will refer to the section lengths and time periods as units.

For each class and year of survey we assumed that the observed number of roadkills per unit would follow a random Poisson distribution with a mean (λ) equal to the total number of roadkills divided by the total number of units. The probability of any unit having x number of collisions was therefore:

$$p(x) = \frac{\lambda^x}{x!\, e^\lambda}$$

A mean value (λ) for each taxa was calculated, and considering roadkills per year. As the mean (λ) varied across taxa, each 500 m of road section with three or more collisions, could be defined as WVA for Amphibians. Road sections with four or more collisions were classified as WVA for Reptiles, to birds seven or more collisions, and for mammals with three or more. These minimum values for WVA detection increased for longer road sections (1000 m and 2000 m) scales. For hot-moments, periods (fortnight) with five or more collisions could be defined as WVA for Amphibians. For Reptiles, periods (fortnight) with thirteen or more roadkills were classified as WVA, and to birds thirty three or more roadkills. These minimum values for WVA detection increased for longer time units (monthly and bimonthly time periods).

We considered a unit to be a WVA when $p(x) > 0.95$. We used the false discovery rate to reduce the likelihood of detecting false WVA (Type I error) due to multiple testing (Benjamini and Hochberg, 1995). We used the same approach of Malo et al. (2004) as it permits easy comparison among sampling schedules using a fixed spatial scale. Besides, this method seems to perform better than others to detect fatality hotspots (Gomes et al., 2009). We then transformed the consecutive units into a binary variable of presence/absence of WVA. Hence, for each year there is a hot-moment and a hotspot evaluation for each taxonomic class.

The similarity of WVA patterns over time was assessed using correlation tests between consecutive years using the Phi coefficient (r_{Phi}) (Zar 1999). The Phi coefficient measures the degree of association between two binary variables, and its interpretation is similar to the common correlation coefficients. This process was performed for each aggregation unit (spatial and temporal). Finally, the cost-benefit analysis was performed for each class, year and unit, by relating the proportion of road sections or time periods that were classified as WVA with the proportion of casualties potentially avoided if those WVA were mitigated. The proportion of road with mitigation was calculated by dividing the sum of all hotspots by the total number of sections. Meanwhile, the proportion of casualties potentially avoided was calculated by dividing the sum of roadkills in hotspots sections by the sum of all roadkills recorded. All calculations and plots were performed using R software (R Core Team 2015) and the R packages *Hmisc*, *vcd*, *cowplot* and *ggplot*.

Results

We recorded 4422 non-domestic road-killed animals, of which 5% were amphibians (n=274, 9 species), 15% reptiles (n=690, and 34 species), 71% birds (n=3009, and 91 species), and 9% mammals (n=448, and 24 species) (Tables S1 and S2 in Appendix 1). We detected several WVA in all classes for all spatial and temporal units considered, except for mammals hot-moments (Figure 1A and 1B).

Regarding the spatial dataset, when using units of 500 m and 1000m, most WVA were identified only once in each class (Figure 1A). However, this pattern was not consistent across the classes. For example, when using a unit of 1000 m, we detected only 4% of sections that were WVA for amphibians in more than one year, while for birds this proportion ascended to 14%. Nevertheless, we found overall low correlation values (r_{Phi}< 0.5) between consecutive years in WVA patterns for all classes for these smaller unit lengths (Figure 2A). Conversely, when using the longer unit length (2000 m) the number of sections that were classified as WVA more than once increased, e.g. 9% for amphibians and 23% for birds. Likewise, the similarity in WVA patterns was higher, particularly for amphibians and reptiles, with values of r_{Phi} well above 0.5 (Figure 2A and Figure S1 in Appendix 1). Surprisingly, the same WVA sections that occurred (km 10 and 38 for road split in 2000m, Figure 2A) for all taxa are located in four-lane roads (Figure S2 in Appendix 1). The cost-benefit evaluation suggests a similar pattern across unit length, within each class. For example, if mitigating 5-10% of the road one could potentially avoid 20-50% of casualties of amphibians, reptiles or mammals. In fact, for these classes, when using a unit length of 2000 m, the relation of the proportion of casualties potentially avoided (benefit) was generally 4 fold greater than the proportion of road mitigated (cost); while for birds the benefit was 2 fold greater (Figure 3A). Hence, planning mitigation using larger road sections is apparently more effective as it incorporates more WVA from different years, and yet does not represent a decrease in the cost-benefit relation.

Regarding the temporal dataset, we found higher similarity in WVA patterns in consecutive years when using the three different time units, except for mammals which was more evenly distributed throughout the year (Figure 1B). Higher correlations were detected when using longer time units (bimonthly), particularly for amphibians and birds (median rPhi> 0.75) (Figure 2B). The periods of highest roadkill for amphibians were between October and November; for reptiles between February and May (and peaks at December and

January); and for birds between October and March. These aggregation periods were consistently highlighted in the different units (Figure 2B and Figure S3 in Appendix 1). In general, using longer time units to detect WVA were also as effective as shorter units. For example, applying mitigation for about two and half months (20% of year) would potentially avoid ca. 50-75% of roadkills of amphibians. For reptiles, the identification of WVA using longer time unit (bimonthly) highlighted 2-6 months of higher mortality, which is probably related to the diversity of species included in this class that have different peaks of movement and therefore mortality throughout the year (e.g. turtles and lizards). In all cases, the relation between the proportion of casualties potentially avoided was twofold (or more) the proportion of year under mitigation (Figure 3B). Therefore, the use of longer time-periods is preferable as it potentially includes WVA from different years and again does not represent a decrease cost-benefit relation.

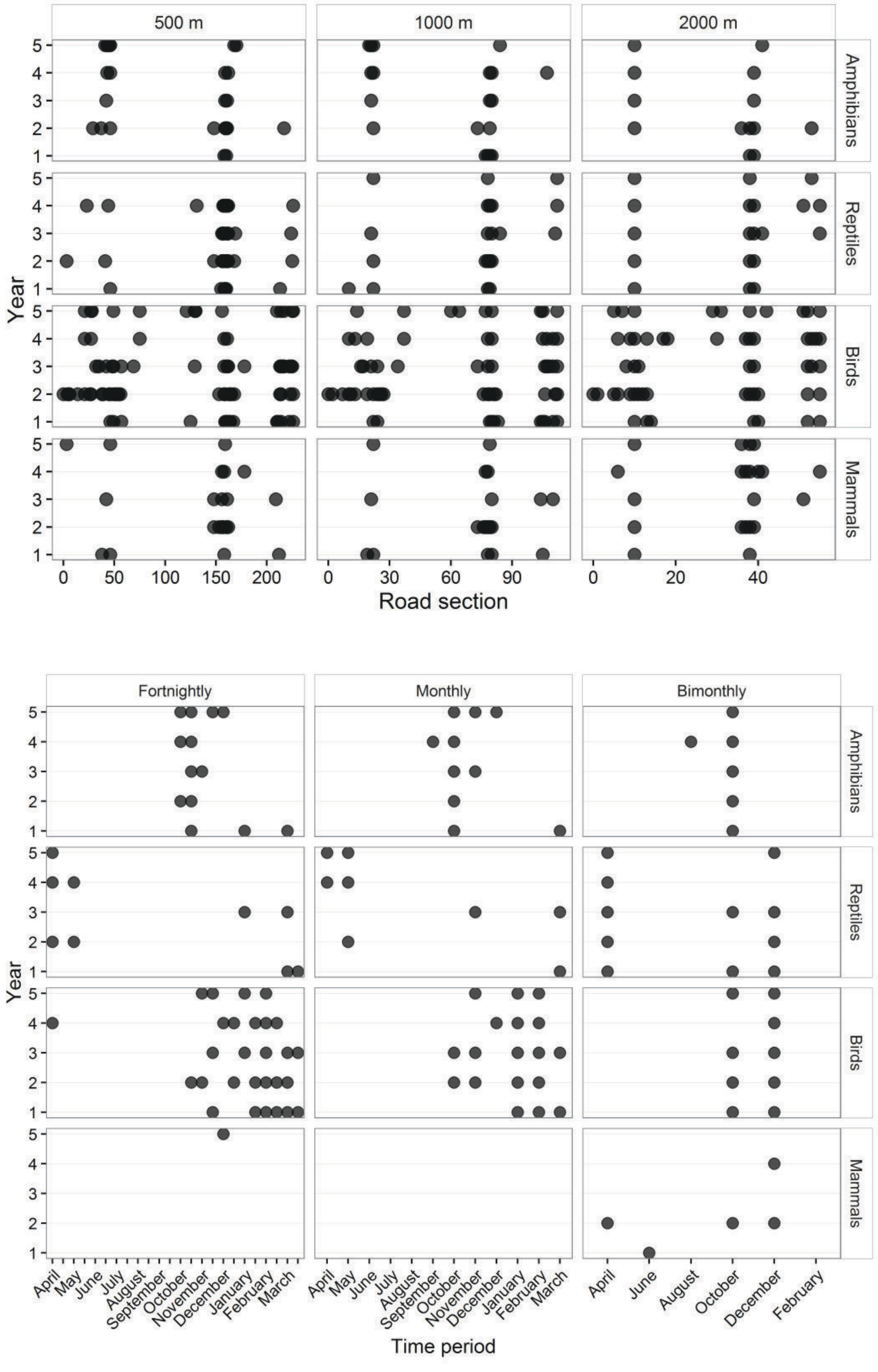

Figure 1. Location of wildlife-vehicle aggregations (WVA) per year and class, along the 114 km of road surveyed (A) and along the year (B). Each vertical panel presents the locations when using different spatial (A) or time (B) units to detect WVA.

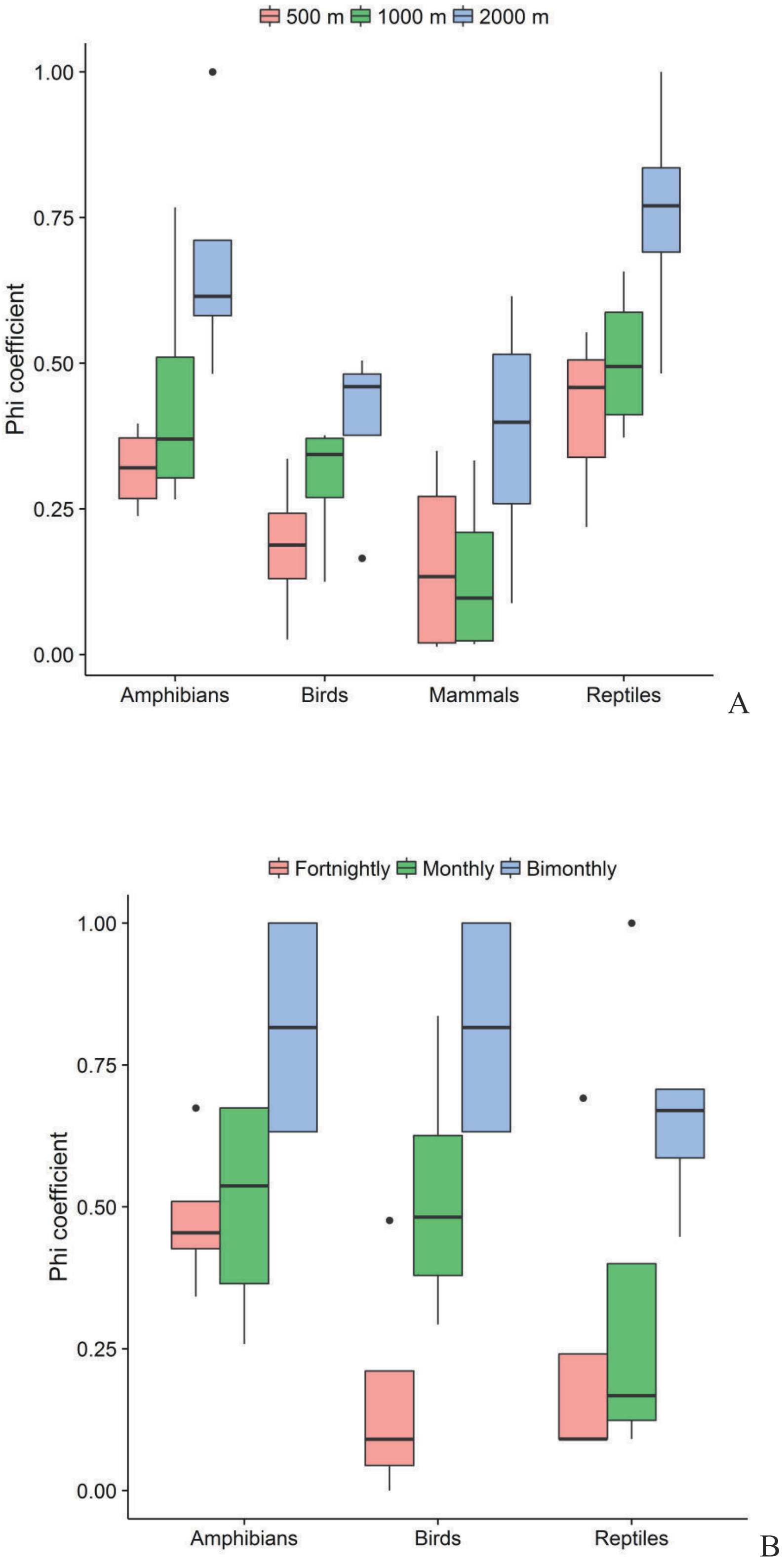

Figure 2. Phi correlations between consecutive years, per class and according to the spatial (A) or temporal (B) unit used to detect WVA.

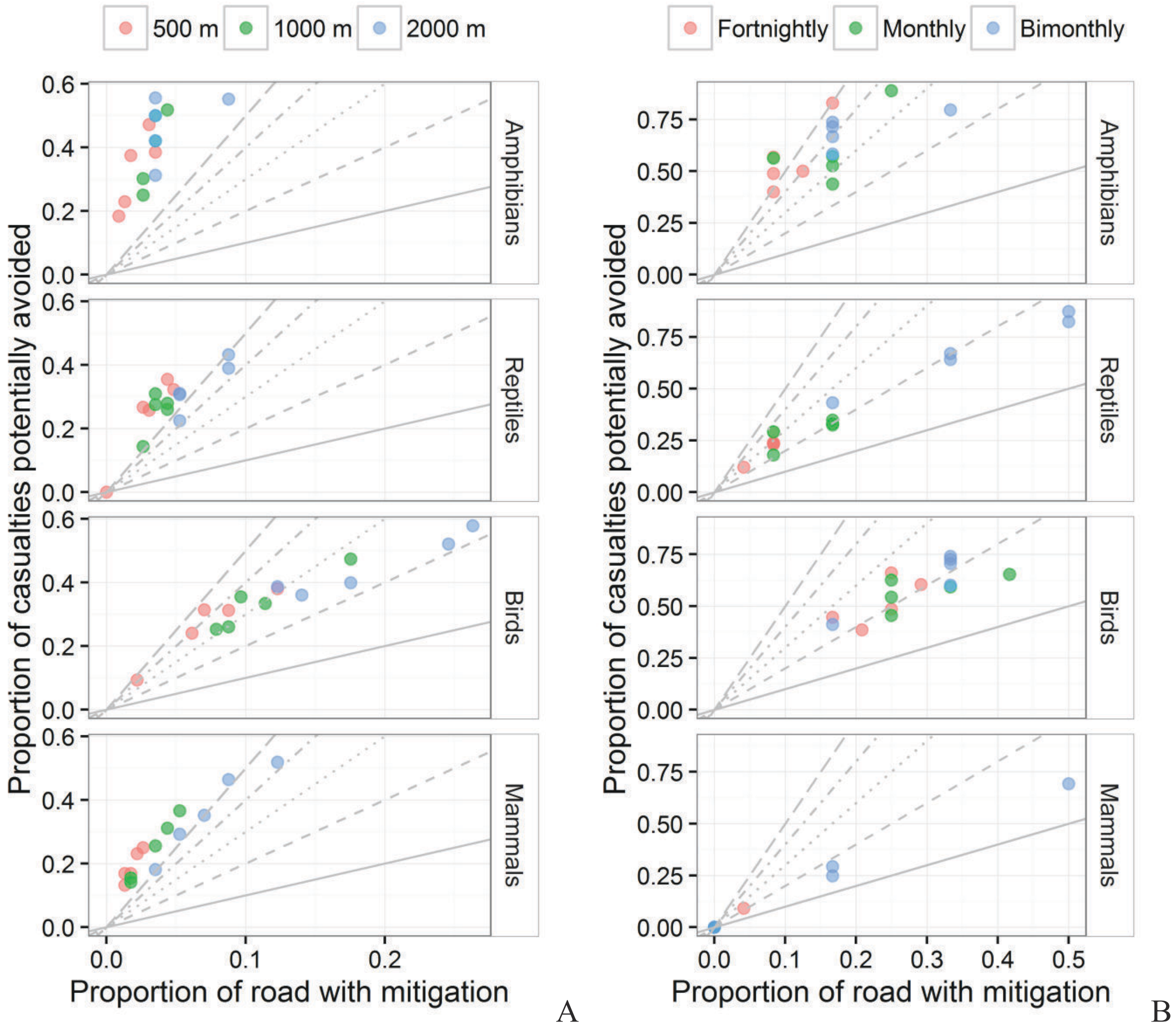

Figure 3. Cost-benefit assessment using the relation between the proportion of casualties that could potentially be avoided with the proportion of road (A) or year (B) that would be mitigated. Lines represent the gain in the proportion of casualties relatively to increase in mitigation. The straight line represents the 1:1 gain, i.e. when increasing the mitigation in 1% one would expect an increase in avoided casualties of 1%; the following lines represent, respectively, the gains 1:2, 1:3, 1:4 and 1:5.

Discussion

In this study we aimed to assess the consistency of hotspots and hot-moments overtime, i.e., we questioned if a significant proportion of WVA occur in the same sites/periods, and at what different scales such consistency is higher. Our results showed that WVA patterns are more consistent when using larger spatial and temporal units. Probably such variability in WVA patterns could be explained by a scale dependence affecting identification of consistent hot-moments and hotspots. Moreover, although intuitively one may think that mitigation plans should target well defined and short road sections or time periods to increase the cost-benefit resources, we show that the proportional costs and benefits when using different spatial and temporal units to detect WVA are similar. Although more resources are required when mitigating longer sections or time periods, the number of collisions potentially avoided is also higher. These patterns are well illustrated by the numerous sections classified as WVA when using smaller spatial or time units, many of which do not overlapped across years. Hence, larger units may guarantee more reliable information on where and when to allocate mitigation measures. Importantly, within each WVA, mitigation should cover the full extent of the road section or period as roadkills may occur at different points or moments in different years. Also, our results highlighted the four-lane sections as priority sections to mitigate, suggesting that the "true" WVA is a reflectance of high traffic, since these roads segments shows the highest traffic volumes in our study area.

Mitigation measures focused on single point locations (e.g., culverts) is unlikely to be sufficient to maintain the long-term viability of populations (Patrick *et al.* 2012). We suggest that mitigation should focus broad-scale measures deployed at longer road sections and time periods, although these are more expensive to build and maintain (Beaudry, deMaynadier & Hunter 2008; Patrick *et al.* 2012). Few measures can be implemented at large scales, such as the reduction of speed limits (Hobday & Minstrell 2008), velocity reducers and drift fences connecting to faunal underpasses (Ascensão *et al.* 2013; Ree *et al.* 2015). Different strategies can be adopted, which will depend on the financial resources available and the target species. For instance, many small crossings underground can be implemented if turtles are the target specie (Beaudry, deMaynadier & Hunter 2008).

The temporal analyzes revealed a strong association of WVA of amphibians, reptiles and birds with the rainy season (October to March in our study area). This period corresponds to the occurrence of migratory events and/or breeding season for many species here recorded (Sick 2001; Coelho *et al.* 2012). Previous works have also reported increased mortality rates during warm and wet seasons, while dry or cold seasons generally present lower values (Coelho *et al.* 2012; Langen *et al.* 2007; Morelle *et al.* 2013). Identifying hot-moments of WVC using larger temporal periods may provide important information to implement short-time mitigation measures such as temporary road closure or speed reduction (Sullivan *et al.* 2004; Hobday & Minstrell 2008). The lack of aggregation periods for mammals may stem from the fact that the dataset was composed mostly by highly mobile and generalist species. These traits lead to a more uniform distribution of roadkills and therefore minimized the chances of occurring WVA.

It should be noted that both spatial and temporal variation of roadkills may be related to differences in vehicle traffic during the year or fluctuations in population abundance (Coelho *et al*. 2012; Smith-Patten & Patten 2008). Unfortunately, to our knowledge, such data does not exist for our study area. Also, we worked at the taxonomic level of Class, thereby precluding more specific analyses. By analyzing at the species level, such patterns could probably be more stable over time. However, this would require a large volume of roadkill data for single species, which is rather unfeasible and it was not possible with our dataset. Finally, we chose not to analyze scales greater than 2000m, as the costs of implementing mitigation measures would become prohibitively.

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Appendix 1

Text 1 - Study Area.

Table S1 - Counts of wildlife-vehicle collisions (WVC).

Table S2 - Species list.

Figure S1 - Correlations for amphibians, reptiles, birds and mammals for hotspots.

Figure S2 - Hotspot that remain in the same place over the five years on our study.

Figure S3 - Correlations for amphibians, reptiles and birds for hot-moments.

Appendix 1

Text 1 - Study Area

The vegetation in the study area includes savanna forest (“Cerradão” and "Mata de Galeria"), open savanna (“Cerrado *sensu stricto*"), grasslands, and other less representative vegetation types of Cerrado biome (Ribeiro & Walter 2008). The region has a dry and a wet season well marked and the climate is tropical savanna (Köppen-Geiger classification) (Cardoso *et al*., 2014). During the wet season (October-March), relative air humidity reaches 75%, monthly rainfall averages 214 mm, and monthly temperature averages 21.6ºC (INMET 2015). During the dry season (April to September), relative air humidity drops to less than 30%, monthly temperatures to 19.9ºC, and average monthly rainfall drops to 41.9 mm (INMET 2015).

Nine road sections were surveyed (total 114 km): 16 km of four-lane paved roads (BR-020 and DF-001); 74km of two-lane paved roads (DF-001, DF-345 and DF-128), and 24 km of dirt roads (DF-205 and DF-001). The dirt roads have the lowest traffic volumes (33 to 775 vehicles/day), the four-lane roads have the highest (5,000 to 7,000 vehicles/day), while the two-lane roads have intermediate traffic volumes (775 to 4,000 vehicles/day, with a stretch of 10km reaching 8,000 vehicles/day) (DNIT 2015). Five protected areas were delimited by these road sections: Botanical Garden of Brasilia-JBB (4,000 ha), Experimental Farm of University of Brasília FAL/UnB (4,000 ha), IBGE Biological Reserve-RECOR (1,300 ha), National Park of Brasília-PNB (44,000 ha), and Ecological Station of Águas Emendadas-ESECAE (10,000 ha). All these protected areas are recognized as core areas of Cerrado Biosphere Reserve in the Federal District by UNESCO’s Man and the Biosphere Programme (MAB).

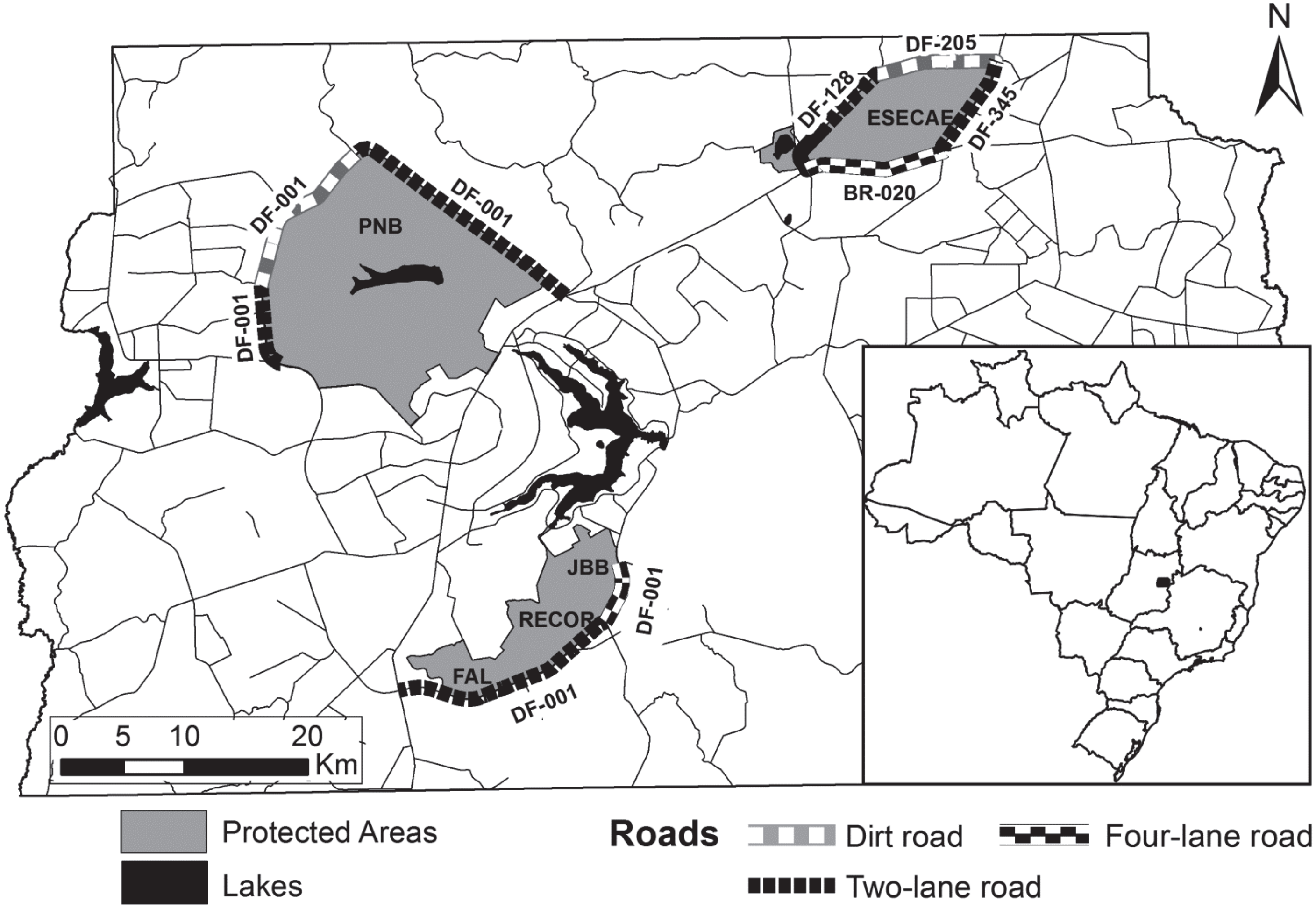

Figure S1. Study area with locations of monitored roads and protected areas.

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Table S1 - Counts of wildlife-vehicle collisions (WVC)

Table S1. Counts of wildlife-vehicle collisions (WVC) and roadkill mortality rates (roadkills/day/km in brackets) by year. Numbers of surveys was also split in dry season (April to September) and wet season (October to March).

	Year 1	**Year 2**	**Year 3**	**Year 4**	**Year 5**	**Total**
Amphibians	38 (0.003)	96 (0.008)	48 (0.004)	56 (0.005)	36 (0.003)	274 (0.003)
Birds	589 (0.05)	812 (0.07)	557 (0.05)	545 (0.04)	506 (0.04)	3009 (0.05)
Mammals	77(0.006)	112 (0.01)	82 (0.007)	106 (0.009)	71 (0.006)	448 (0.008)
Reptiles	127 (0.01)	161 (0.01)	136 (0.01)	155 (0.01)	111 (0.01)	690 (0.01)
Total	831 (0.07)	1181 (0.10)	823 (0.07)	862 (0.07)	724 (0.06)	4421 (0.08)
Surveys	98	95	95	98	94	480
Surveys – Dry Season	49	48	47	50	47	241
Surveys – Wet Season	49	47	48	48	47	239

Table S2 - Species list

Table S2. Species list.

Class	Order	Family	Species	Total
Amphibians	Anura	Bufonidae	*Rhinella cerradensis*	1
			Rhinella schneideri	15
			Rhinella sp	190
		Hylidae	*Hypsiboas albopunctatus*	1
			Scinax sp	1
		Leptodactylidae	*Leptodactylus labyrinthicus*	6
			Leptodactylus latrans	6
			Leptodactylus ocellatus	4
			Leptodactylus sp	1
			Not identified	21
			Rhinella rubescens	1
		Microhylidae	*Elachistocleis cesarii*	1
			Not identified	1
		Not identified	Not identified	19
	Gymnophiona	Caecilidae	*Siphonops paulensis*	6
Reptiles	Chelonia	Testudinidae	Not identified	1
	Not identified	Not identified	Not identified	1
	Squamata	Amphisbaenidae	*Amphisbaena alba*	103
		Anguidae	*Ophiodes striatus*	13
		Boidae	*Boa constrictor*	58
			Epicrates cenchria	26
		Colubridae	*Chironius exoletus*	1
			Chironius flavolineatus	3
			Chironius quadricarinatus	1
			Clelia sp.	1
			Not identified	1
			Simophis rhinostoma	1
			Spilotes pullatus	3
			Tantilla melanocephala	1
		Dipsadidae	*Apostolepis albicolaris*	1
			Boiruna maculata	10
			Erythrolamprus aesculapii	13
			Helicops modestus	1
			Not identified	6
			Oxyrhopus guibei	43
			Oxyrhopus rhombifer	1
			Oxyrhopus sp	52
			Oxyrhopus trigeminus	2
			Phalotris nasutus	1

			Philodryas agassizii	3
			Philodryas nattereri	3
			Philodryas olfersii	4
			Philodryas patagoniensis	18
			Philodryas sp	50
			Pseudoboa nigra	12
			Sibynomorphus mikanii	35
		Not identified	Not identified	47
		Polychrotidae	*Polychrus acutirostris*	16
		Teiidae	*Ameiva ameiva*	15
			Cnemidophorus ocellifer	2
			Cnemidophorus sp.	3
			Not identified	1
			Tupinambis duseni	2
		Tropiduridae	*Enyalius aff bilineatus*	1
			Tropidurus sp.	11
		Viperidae	*Bothrops moojeni*	1
			Bothrops sp.	9
			Crotalus durissus	94
			Not identified	1
			Xenodon merremii	3
			Xenodon neuwiedii	1
			Xenodon sp	2
	Testudines	Chelidae	*Phrynops geoffroanus*	12
Birds	Accipitriformes	Accipitridae	*Gampsonyx swainsonii*	1
			Geranoaetus albicaudatus	2
			Heterospizias meridionalis	3
			Rupornis magnirostris	8
	Apodiformes	Apodidae	*Streptoprocne zonaris*	1
			Tachornis squamata	1
		Not identified	Not identified	1
		Trochilidae	*Amazilia fimbriata*	11
			Amazilia sp.	1
			Chlorostilbon lucidus	2
			Colibri serrirostris	23
			Eupetomena macroura	13
			Heliothryx auritus	1
			Not identified	19
			Phaethornis pretrei	1
			Polytmus theresiae	2
			Thalurania glaucopis	1
	Caprimulgiformes	Caprimulgidae	*Antrostomus rufus*	5
			Chordeiles nacunda	1
			Chordeiles pusillus	4
			Hydropsalis albicollis	7

			Hydropsalis climacocerca	6
			Hydropsalis torquata	1
			Not identified	19
	Cariamiformes	Cariamidae	*Cariama cristata*	12
	Cathartiformes	Cathartidae	*Coragyps atratus*	4
	Charadriiformes	Charadriidae	*Vanellus chilensis*	9
	Columbiformes	Columbidae	*Columbina picui*	1
			Columbina sp	2
			Columbina talpacoti	21
			Not identified	3
			Patagioenas sp	12
			Zenaida auriculata	1
		Not identified	Not identified	1
	Coraciiformes	Alcedinidae	*Chloroceryle amazona*	2
	Cuculiformes	Cuculidae	*Crotophaga ani*	63
			Guira guira	55
			Piaya cayana	1
	Falconiformes	Falconidae	*Caracara plancus*	12
			Falco femoralis	1
			Falco sparverius	5
			Milvago chimachima	1
			Not identified	7
		Not identified	Not identified	3
	Galbuliformes	Bucconidae	*Nystalus chacuru*	17
	Not identified	Not identified	Not identified	156
	Passeriformes	Furnariidae	*Furnarius rufus*	4
			Not identified	1
			Phacellodomus ruber	3
			Phacellodomus rufifrons	9
		Hirundinidae	*Alopochelidon fucata*	2
		Icteridae	*Gnorimopsar chopi*	5
		Melanopareiidae	*Melanopareia torquata*	16
		Mimidae	*Mimus saturninus*	16
		Not identified	Not identified	547
		Thamnophilidae	*Thamnophilus torquatus*	2
		Thraupidae	*Ammodramus humeralis*	30
			Cypsnagra hirundinacea	2
			Emberizoides herbicola	19
			Lanio cucullatus	3
			Lanio pileatus	14
			Not identified	13
			Nemosia pileata	1
			Neothraupis fasciata	3
			Saltator similis	1
			Saltatricula atricollis	2
			Sicalis citrina	1

			Sicalis flaveola	4
			Sporophila caerulescens	13
			Sporophila leucoptera	1
			Sporophila nigricollis	21
			Sporophila plumbea	1
			Tangara sayaca	6
			Volatinia jacarina	1221
			Zonotrichia capensis	15
		Troglodytidae	*Troglodytes musculus*	14
		Turdidae	*Turdus amaurochalinus*	7
			Turdus leucomelas	1
			Turdus rufiventris	2
		Tyrannidae	*Camptostoma obsoletum*	2
			Elaenia chiriquensis	32
			Machetornis rixosa	19
			Not identified	1
			Pitangus sulphuratus	3
			Tyrannus albogularis	1
			Tyrannus melancholicus	11
			Tyrannus savana	61
			Xolmis cinerea	1
		Vireonidae	*Cyclarhis gujanensis*	7
	Piciforme	Picidae	*Colaptes campestris*	18
			Not identified	1
		Ramphastidae	*Ramphastos toco*	1
	Psittaciformes	Psittacidae	*Alipiopsitta xanthops*	3
			Amazona aestiva	2
			Amazona sp.	1
			Aratinga aurea	3
			Aratinga auricapillus	1
			Brotogeris chiriri	7
			Not identified	1
	Strigiforme	Strigidae	*Aegolius harrisii*	4
			Asio clamator	31
			Asio flammeus	1
			Athene cunicularia	114
			Glaucidium brasilianum	2
			Megascops choliba	19
			Not identified	8
		Tytonidae	*Tyto furcata*	56
	Tinamiforme	Tinamidae	*Crypturellus parvirostris*	37
			Not identified	5
			Nothura maculosa	14
			Rhynchotus rufescens	19
Mammals	Artiodactyla	Cervidae	*Mazama gouazoubira*	1
	Carnivora	Canidae	*Cerdocyon thous*	79

			Chrysocyon brachyurus	8
			Not identified	1
			Pseudalopex vetulus	17
		Felidae	*Leopardus sp.*	1
			Leopardus tigrinus	1
			Not identified	3
			Puma concolor	2
		Mephitidae	*Conepatus semistriatus*	31
		Mustelidae	*Galictis cuja*	33
		Procyonidae	*Nasua nasua*	3
			Procyon cancrivorus	9
	Chiroptera	Molossidae	*Molossops* sp.	2
			Not identified	5
		Not identified	Not identified	52
		Phyllostomidae	*Artibeus* sp.	2
			Glossophaga soricina	11
			Not identified	12
			Platyrrhinus sp.	2
			Sturnira lilium	1
	Cingulata	Dasypodidae	*Dasypus novemcinctus*	7
			Dasypus septemcinctus	6
			Dasypus sp.	1
			Euphractus sexcintus	5
			Not identified	1
		Not identified	Not identified	1
	Didelphimorphia	Didelphidae	*Didelphis albiventris*	61
	Lagomorpha	Leporidae	*Sylvilagus brasiliensis*	6
	Not identified	Not identified	Not identified	13
	Pilosa	Myrmecophagidae	*Myrmecophaga tridactyla*	1
	Primates	Atelidae	*Alouatta caraya*	1
		Cebidae	*Callithrix penicillata*	19
			Cebus libidinosus	1
	Rodentia	Cricetidae	*Calomys tener*	8
			Not identified	27
			Necromys lasiurus	10
		Dasyproctidae	*Dasyprocta sp.*	1
		Erethizontidae	*Coendou prehensilis*	2
		Hydrochoeridae	*Hydrochoeris hydrochaeris*	1
	Not identified	Not identified	Not identified	1
Total				**4422**

Figure S1 - Correlations for amphibians, reptiles, birds and mammals for hotspots

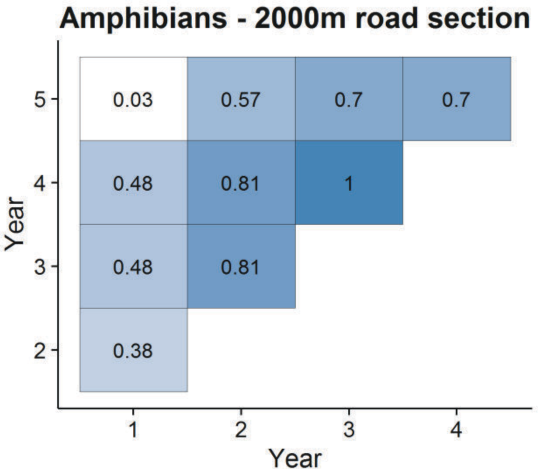
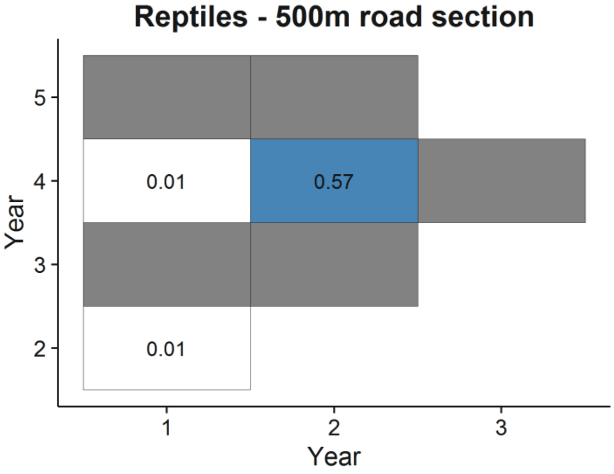
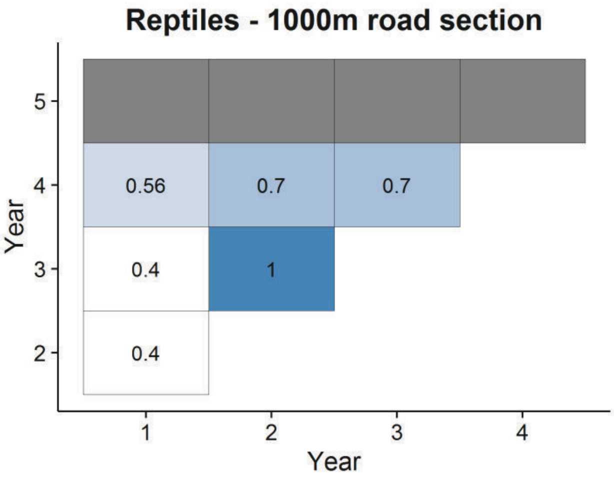


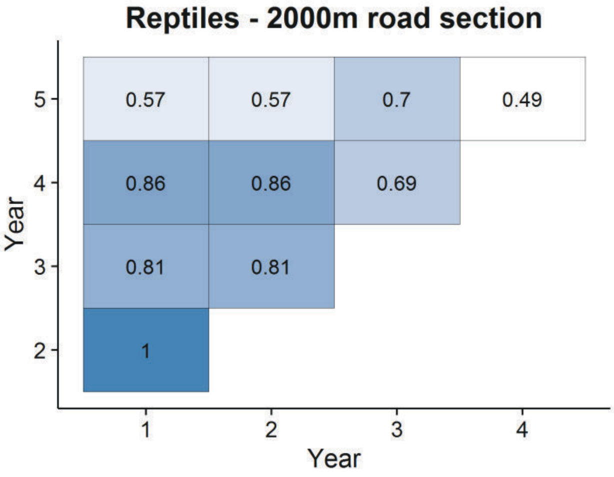
Figure S.1. Results of correlations for hotspots between years for amphibians considering road sections of 2000m. No correlations for road sections of 500m and 1000m are given as the data contained too many zeros. Years: 1 - April 2010 to March 2011; 2 - April 2011 to March 2012; 3 - April 2012 to March 2013; 4 - April 2013 to march 2014; 5- April 2014 to march 2015.



(A)



(B)



(C)

Figure S1.2. Results of correlations for hotspots between years for reptiles, considering road sections of size: (A) 500m, (B) 1000m and (C) 2000m. Grey boxes means that no value was calculated. Years: 1 - April 2010 to March 2011; 2 - April 2011 to March 2012; 3 - April 2012 to March 2013; 4 - April 2013 to march 2014; 5- April 2014 to march 2015.

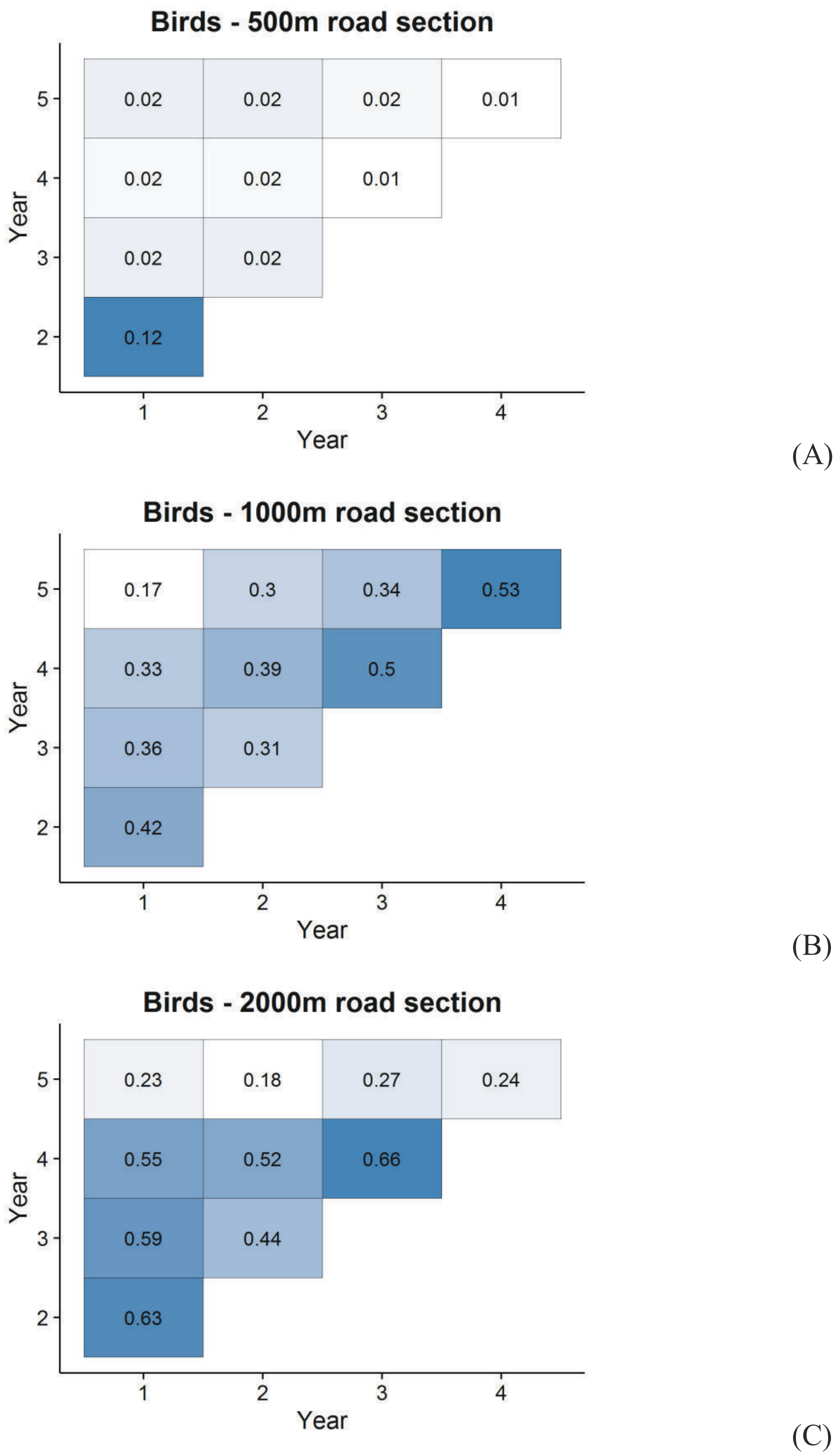
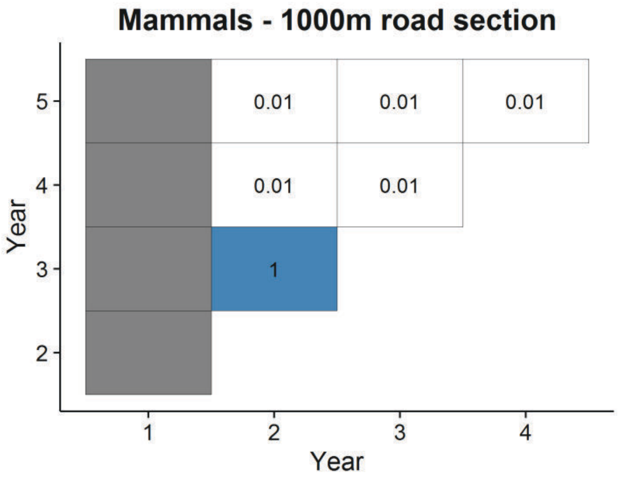
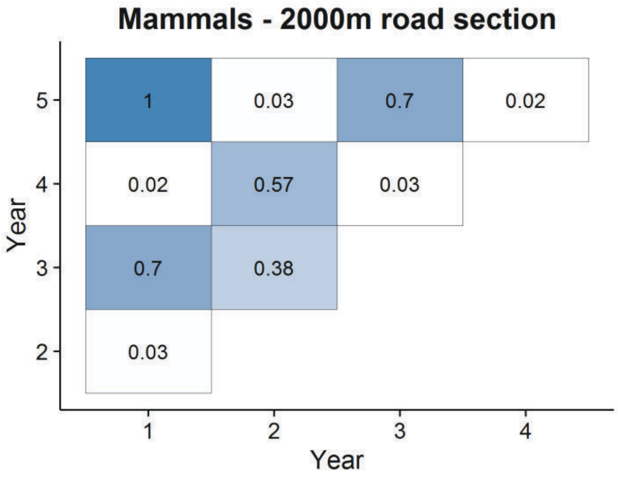


Figure S1.3. Results of correlations for hotspots between years for birds considering road sections of size: (A) 500m, (B) 1000m and (C) 2000m. Years: 1 - April 2010 to March 2011; 2 - April 2011 to March 2012; 3 - April 2012 to March 2013; 4 - April 2013 to march 2014; 5- April 2014 to march 2015.



(A)



(B)

Figure S1.4. Results of correlations for hotspots between years for mammals considering road sections of size: (A) 1000m and (B) 2000m. No correlations for road sections of 500m are given as the data contained too many zeros. Grey boxes means that no value was calculated. Years: 1 - April 2010 to March 2011; 2 - April 2011 to March 2012; 3 - April 2012 to March 2013; 4 - April 2013 to march 2014; 5- April 2014 to march 2015.

Figure S2 – Hotspots that remain in the same place over the five years.

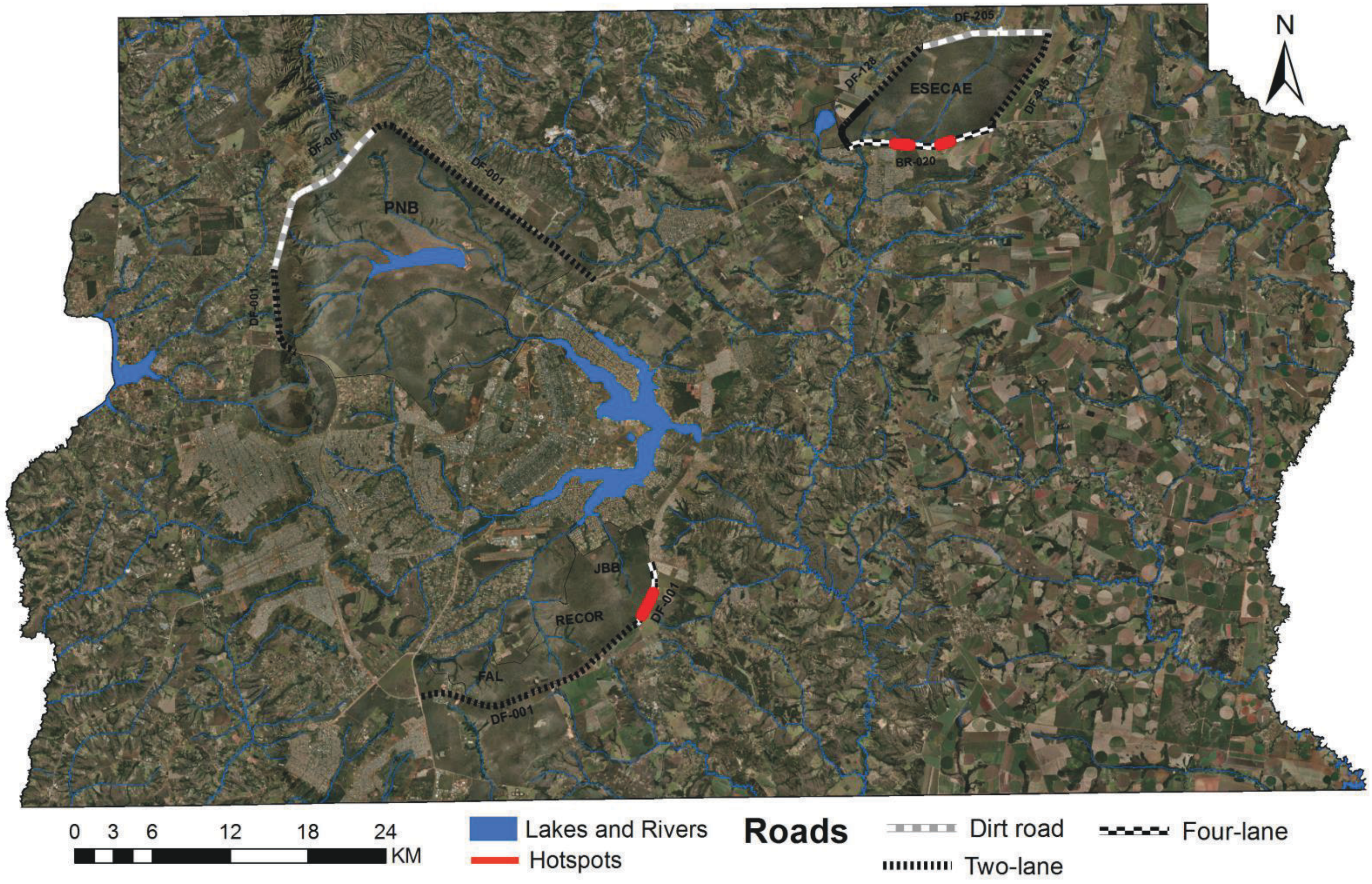
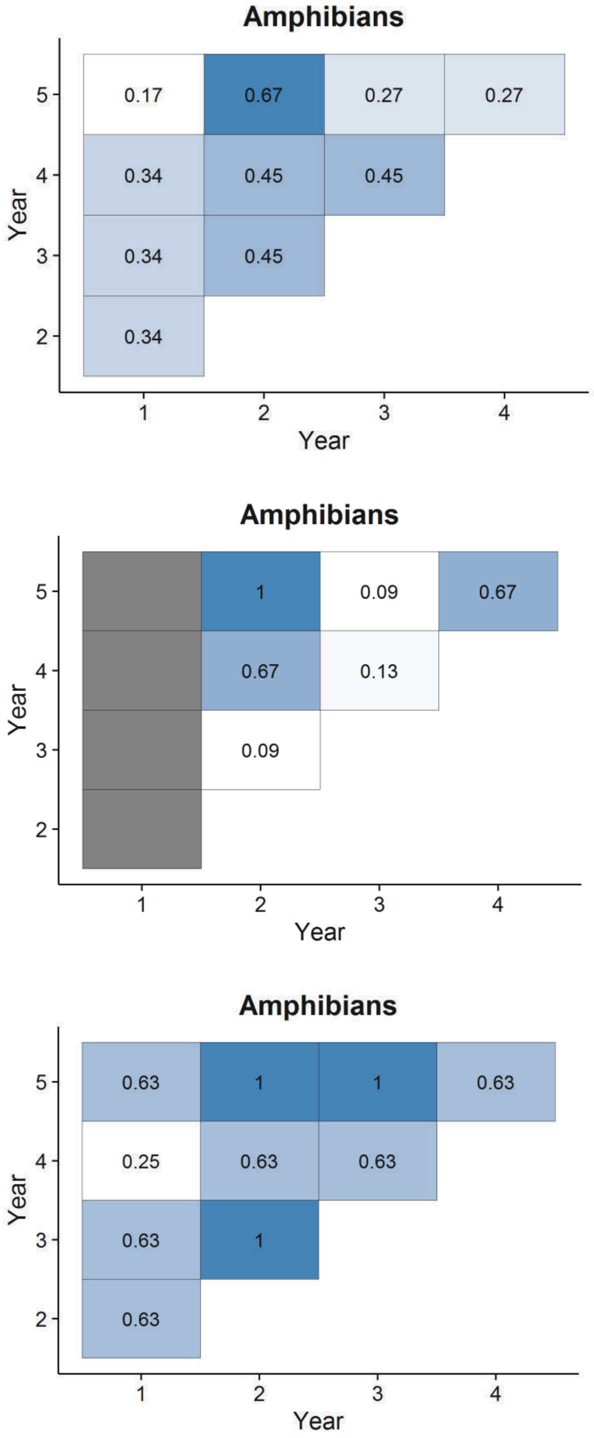


Figure S2. Hotspots that remain in the same place over the five years of study in the study area. DF-001 and BR-020 (four-lane road): hotspots for amphibians, reptile, birds and mammals.

Figure S3 - Correlations for amphibians, reptiles and birds for hot-moments

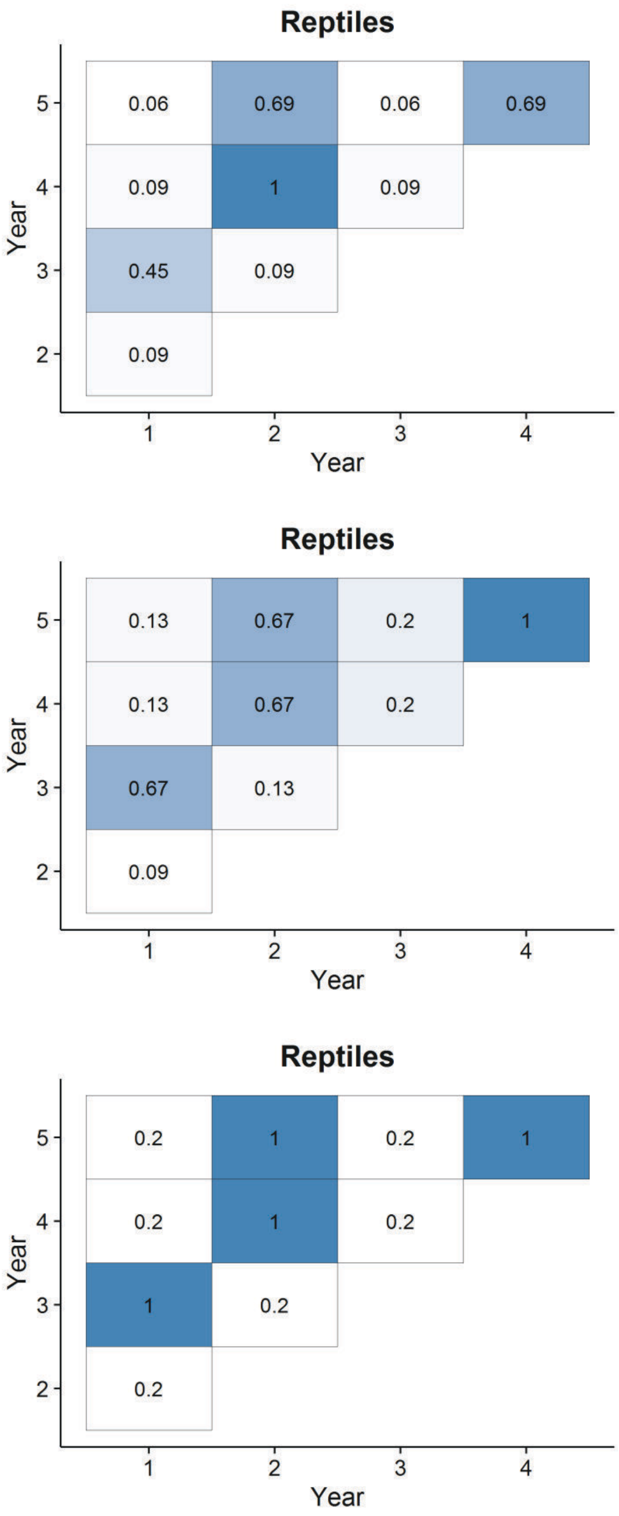


(A)

(B)

(C)

Figure 3.1. Results of correlations for hot-moments between years for amphibians considering data split into (A) fortnightly, (B) monthly and (C) bimonthly datasets. Grey boxes means that no value was calculated. Years: 1 - April 2010 to March 2011; 2 - April 2011 to March 2012; 3 - April 2012 to March 2013; 4 - April 2013 to march 2014; 5- April 2014 to march 2015.



(A)

(B)

(C)

Figure S3.2. Results of correlations for hot-moments between years for reptiles considering data split into (A) fortnightly, (B) monthly and (C) bimonthly datasets. Years: 1 - April 2010 to March 2011; 2 - April 2011 to March 2012; 3 - April 2012 to March 2013; 4 - April 2013 to march 2014; 5- April 2014 to march 2015.

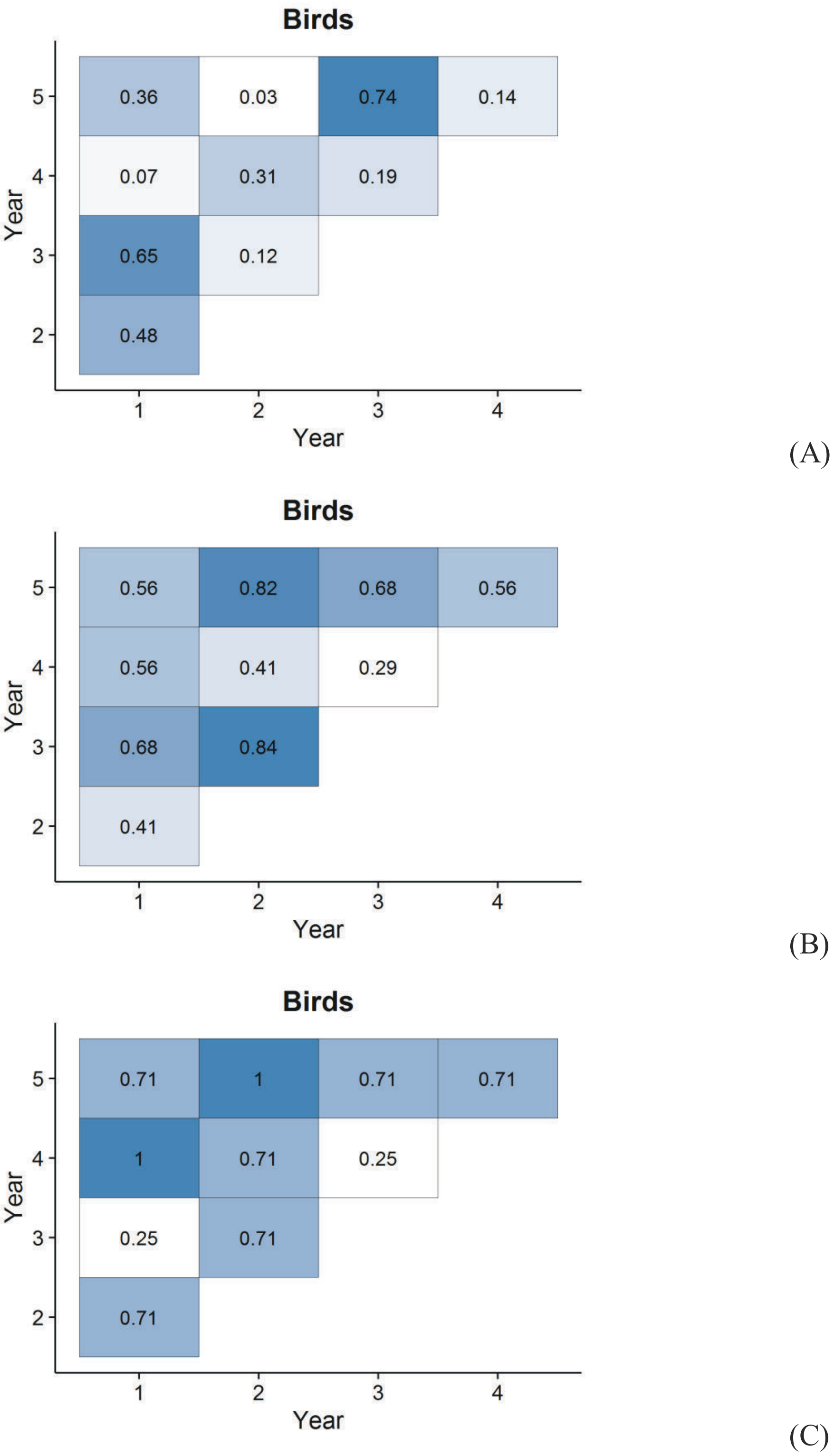


Figure S3.3. Results of correlations for hot-moments between years for birds considering data split into (A) fortnightly, (B) monthly and (C) bimonthly datasets. Years: 1 - April 2010 to March 2011; 2 - April 2011 to March 2012; 3 - April 2012 to March 2013; 4 - April 2013 to march 2014; 5- April 2014 to march 2015.

Capítulo III - Predicting the roadkill risk using occupancy models

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Abstract

Wildlife-vehicle collisions (WVC) represent a major threat for wildlife and understanding how WVC spatial patterns relate to surrounding land cover can provide valuable information for deciding where to implement mitigation measures. However, these relations may be heavily biased as many casualties are undetected in roadkill surveys, e.g. due to scavenger activity, which may ultimately jeopardize conservation actions. We suggest using occupancy models to overcome imperfect detection issues, assuming that: a) occupancy represents the roadkill risk, i.e. the animal uses a road section for crossing or forage being prone to be hit by an incoming vehicle; and b) detectability is the combination of the probability of an individual being hit by a vehicle and, if so, its carcass being detectable. Our main objective was to assess the roadkill risk along roads and relate it to land cover information. We conducted roadkill surveys over 114 km in nine different roads, biweekly, for five years (total of 484 surveys), and developed a Bayesian hierarchical occupancy model to assess spatial patterns of WVC occurrence for the six most road-killed taxa. For each focal taxon the data set is comprised of 10 seasons (five Dry and five Wet). Overall, we found a higher roadkill risk in road segments near urban areas and with higher cover of open habitat. Detectability tended to be higher for four-lane roads and in rainy season. From a conservation perspective, our results highlight the need to upgrade road stretches near urban areas and with higher cover of open habitat. The most important covariates were selected in almost all seasons (Wet and Dry), which support our close assumption of similar effects across seasons by co-variables and that our estimates for average response across seasons (ARS) were a good approach when using occupancy models. We show that occupancy models can be used to access the roadkill risk along roads while accounting for imperfect detection.

Key words: roadkill risk, imperfect detection, Bayesian models, road ecology.

1. Introduction

Roads are known to promote numerous negative impacts on natural populations and habitats worldwide (Trombulak & Frissell 2000; Forman *et al.* 2003; Ree, Smith & Grilo 2015). Perhaps the most important of such impacts is wildlife-vehicle collisions (WVC), which often represent a significant contributor to population depletion in the vicinity of roads, as reported for insects (Baxter-Gilbert *et al.* 2015), amphibians (Gibbs & Shriver 2002), reptiles (Beaudry, DeMaynadier & Hunter Jr. 2010), birds (Borda-de-Água, Grilo & Pereira 2014), and mammals (Ramp & Ben-Ami 2006). Additionally, WVC may aggravate the road barrier effect by blocking potential crossings, therefore restricting gene flow between roadside populations (Jackson & Fahrig 2011). Combined, population depletion and barrier effects may accelerate the loss of genetic variation due to random drift and increase inbreeding, which may result in local extinctions (Westemeier 1998; Reed, Nicholas & Stratton 2007). Hence, it is crucial to understand where WVC are more likely to occur, in order to delineate appropriate mitigation measures, e.g. road network design or implementation of mitigation measures such as road passages (Lesbarreres & Fahrig 2012).

WVC barely occur randomly in space (Crawford *et al.* 2014). In fact, it is expected that a higher number of WVC occur where species are more abundant (D'Amico *et al.* 2015) and where landscape facilitates the movement of individuals (Grilo *et al.* 2011). However, in many studies, the information regarding species' presence and abundance in road surroundings is absent. Therefore, the lack of roadkill records of a given species in a road segment can have multiple explanations: the species can in fact be absent from that area, or if the species was road-killed observers may fail to detect the carcasses. Such false absences may lead to biased conclusions on occurrence patterns that ultimately may result in incorrect biodiversity management decisions (Royle & Nichols 2003). Remarkably, there is a vast body of literature aimed at understanding the main drivers of WVC and predict where WVC are more likely to occur (Clevenger, Chruszcz & Gunson 2003; Malo, Suárez & Díez 2004; Ramp & Ben-Ami 2006; Beaudry, DeMaynadier & Hunter Jr. 2010; Crawford *et al.* 2014). However, to our knowledge, such approaches have never integrated the false absence issues.

We suggest using occupancy models (MacKenzie *et al.* 2002) to analyze WVC data. These models require repeated sampling to account for false absences, conducted at spatially-replicated sites, i.e. surveys made by visiting sites more than once, to simultaneously estimate occupancy and detection probability, thereby correcting for imperfect detection (MacKenzie & Kendall 2002; MacKenzie *et al.* 2006). With this approach, observed absences are integrated in the model as a mixture of non-detections and true absences (Hanks, Hooten & Baker 2011). Conveniently, the requisite of repeated surveys in time and space is also the typical sampling protocol employed in road mortality surveys, where observers drive the same road repeatedly searching for WVC. e considered that occupancy represents the probability of individuals using a given road section for crossing or foraging and be disponible for detection, and we assume as an estimate of the roadkill risk. We are assuming that animal behavior responses to traffic (Jacobson *et al.* 2016) have a minimum effect on animal mortality patterns. For the other hand, Detection is the probability to record a wildlife-vehicle-collision, once it has occurred and can be observed.. Hence, road sections with higher occupancy rates may indicate best locations to implement mitigation measures.

Occupancy models are gaining popularity as analytical tools (MacKenzie *et al.* 2006; Coggins, Bacheler & Gwinn 2014). Yet, to our knowledge, occupancy models have never been used in road ecology studies. We developed a Bayesian hierarchical occupancy model to assess patterns of WVC occupancy and applied it to a collection of taxa. Our main objective was to test if occupancy models are a viable alternative to assess the road kill risk along the road, and considering the distinct probabilities of being present and detected. Furthermore, we aimed to relate the roadkill risk to environmental variables, particularly land cover and road-related information, in order to provide guidelines for landscape and road management to reduce the roadkill risk. We believe this approach will allow researchers and road managers to account for false absence issues and therefore improve the estimation of the roadkill risk along surveyed roads, thereby providing more robust information to delineate and improve management practices.

2. Materials and methods

2.1 Study Area

We conducted the study in Brasília (Federal District), located in the Cerrado biome of Brazil (Fig. 1). The vegetation in the study area is dominated by open savannah ('Cerrado *sensu stricto*'), grasslands, and savannah forest ('Cerradão' and 'Mata de Galeria') (Ribeiro & Walter 2008). The climate is tropical savannah (Köppen-Geiger classification) (Cardoso, Marcuzzo & Barros 2014), with distinct dry and wet seasons, an average annual rainfall of 1540 mm (INMET 2015). During the dry season (April to September), the relative air humidity drops to less than 30%, monthly rainfall average drops to 41.9 mm, and monthly temperatures to 19.9 ºC (INMET 2015). During the wet season (October-March), relative air humidity reaches 75%, monthly temperatures average 21.6 ºC, and monthly rainfall averages 214 mm (INMET 2015).

Surveys were conducted along nine roads (total 114 km): dirt roads (DF-205 and DF-001; 24 km), two-lane (DF-001, DF-345 and DF-128; 74 km), and four-lane (BR-020 and DF-001; 16 km) (Fig.1). The four-lane roads had the highest traffic volumes (5000 to 7000 vehicles/day), followed by the two-lane roads (775 to 4000 vehicles/day, with a stretch of 10 km reaching 8000 vehicles/day), and dirt roads (33 to 775 vehicles/day) (DNIT 2009; IBRAM 2015). These roads delimit five protected areas recognized by UNESCO as core areas of the Cerrado Biosphere Reserve in the Federal District: National Park of Brasília-PNB (44,000 ha), Experimental Farm of University of Brasília FAL/UnB

(4000 ha), IBGE Biological Reserve-RECOR (1300 ha), Botanical Garden of Brasilia-JBB (4000 ha), and Ecological Station of Águas Emendadas-ESECAE (10,000 ha) (Fig.1).

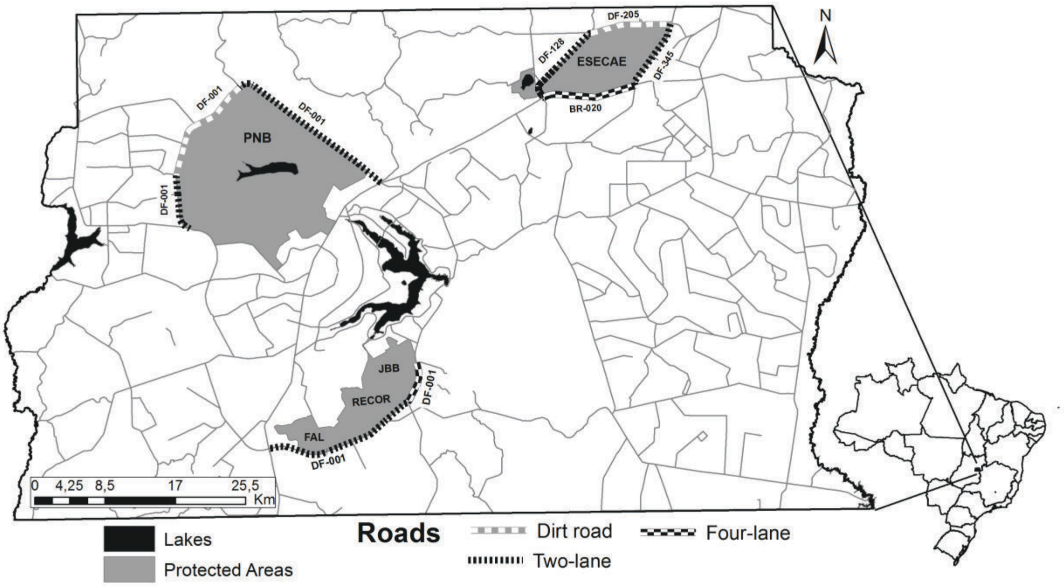

Fig.1. Study area with location of monitored roads and protected areas.

2.2 Roadkill Data

Road surveys occurred with two-day intervals (except for weekends) for 5 years, between April 2010 and March 2015, totaling 480 surveys. Three observers searched for WVC in a vehicle traveling at ca. 50 km/h. The observers identified each carcass to the lowest possible taxonomic level and collected the geographic coordinates using a hand-held GPS with 5m accuracy. The carcass was removed from the road to avoid double counting. Species having > 30 records were retained for model procedures.

2.3 Hypothesized Predictors for Occupancy and Detectability

We were interested in relating the roadkill risk (occupancy) to the land cover in order to provide management guidelines toward roadkill mitigation. Land cover information was provided by the Brasília Environmental Institute (IBRAM 2015), a map originated from a multispectral RapidEye satellite image from 2011 (spatial resolution of 5 m), using seven land cover classes. This map was aggregated to five main classes, of which we considered the three main classes - Savannah, Forest and Open areas (Table 1) - which together cover approximately 38% of the Federal District. For each road section (see below), we extracted the proportion of these classes within a 1-km buffer from the road. We further calculated the Euclidean distance to water (rivers, streams, water bodies) and to urban areas (Table 1).

Table 1. List of explanatory variables and their definitions and respective range of values.

Covariates	**Definition**	**Type**	**Range**
Occupancy			
SAVANNAH	% of areas of typical cerrado (cerrado *sensu strictu*)	Continuous	22-91
FOREST	% of areas of forested land (gallery forest and dense cerrado)	Continuous	0-15
OPEN	% of areas of non-forested vegetation (natural fields, pasture and farmland)	Continuous	0-61
DIST.WATER	Distance to nearest water body (m)	Continuous	340-1727
DIST.URBAN	Distance to nearest urban area (m)	Continuous	450-16.455
Detection			
ROAD TYPE (proxy for traffic volume)	Road pavement type	Categorical	1: 2-Lane (paved)*; 2: Dirt (unpaved); 3: 4-Lane (paved)
NATURAL (proxy for scavenger abundance)	% of areas of Savannah and Forest	Continuous	24-92
HUMIDITY	Air relative humidity (%) on the day at which the carcass is found	Continuous	19-92
DoY	Day of the year (mean of month)	Continuous	0-365

* Reference level

Regarding detectability, we expected that higher traffic volumes were likely to increase the number of roadkills, and therefore should have a positive effect on detectability. Because there are no regular traffic counts for the studied roads (only yearly estimates), we used the road type (IBRAM 2015) as a proxy of traffic volume (Table 1). Obtaining reliable estimates of abundance and activity of scavengers in the vicinity of roads is difficult. One option to circumvent this problem is to use proxies for scavenger presence (Santos *et al.* 2016). The abundance and diversity of scavengers is known to be higher in areas with better habitat quality (Crooks 2002; Eduardo, Carvalho & Marini 2007; Carrete *et al.* 2009). Thus, areas with greater coverage of natural habitat near roads are likely to have higher scavenger abundance. We therefore aggregated the land cover classes 'Savannah' and 'Forest' into a new class 'Natural habitat', and extracted the proportion of this new class within the same 1-km buffer from the road (Table 1). We considered that the cover of this land cover class would be directly related to scavenger presence.

To account for weather effect on carcass degradation and therefore detectability (Santos *et al*. 2016, 2011), we further included two more covariates in our model-based hypotheses to control for such effects: air humidity, which reflects the effect of both heat and precipitation (INMET 2015); and day of the year (DoY) as a measure of seasonality of overall weather conditions (Table 1). Air humidity was obtained for each survey from a weather station located in central Brasilia ca. 15 km from the study area (INMET 2015).

2.4 Data Analysis

DoY was transformed to circular data using the formula sin (π / 365* DoY), thus ranging between 0 and 1. All remaining continuous variables were standardized (mean=0 and standard deviation=1). Each year of monitoring was divided into two climatic seasons: WET, from October to March, and DRY, from April to September. Within each season, surveys were pooled into monthly data in order to reduce the excessive number of zeros (i.e. surveys with no WVC found in any section). We pooled the data into road sections of

2 km. Hence, for each focal taxon the data set is comprised of 10 seasons (five DRY and five WET), each with six surveys (monthly data) and 56 sites (road sections). Regarding explanatory variables, the models included five site-level covariates for the occupancy section: the three of the most representative land uses classes (Savannah, Forest and Open Area), Distance to Rivers and Distance to Urban Areas. For the detection section we included two site-level covariates: Natural Area (Savannah and Forest) and Type of Road, and two survey-level variables: Humidity and DoY (Table1).

2.5 Bayesian Hierarchical Occupancy Model

Our model is based on the community model proposed by Dorazio & Royle (2005) but instead of modeling several species in a community, we modeled several seasons for each taxa. In our model we assumed that the effect of each environmental predictor on occupancy and detectability is similar (not equal) across seasons within each season type (DRY and WET) and that this effect is taken from an unknown hyper-distribution represented by a normal distribution with a given mean and standard deviation. The advantage of such approach is that it improves the modeling of seasons with poor information, i.e., seasons with more observations lend strength to analyze seasons with fewer observations (Kéry & Royle 2008; Zipkin *et al.* 2010). Yet, some variation in the effect of the variables among seasons is allowed. For example, the effect of distance to water can be different between DRY and WET seasons and even among years due to differences in rain and drought periods. The average of each hyper-distribution is the Average Response across Seasons (ARS) for each predictor. ARS estimates with small credible intervals and not overlapping zero identify co-variables that consistently affect the occupancy and detectability. A detailed description of the model structure and code is shown in the Appendix A.

For each taxon, the model was run for three chains of 200,000 iterations after a burn in of 100,000, and then thinned by 50. We checked for convergence of the sub-models of occupancy and of detection using the Gelman-Rubin statistic (R-hat statistic), whereby values less than 1.1 indicate convergence (Gelman 2005). Model fit was assessed using posterior predictive checks based on standard Bayesian p-values (Gelman, Meng & Stern 1996). Extreme p-values (<0.05 or >0.95) are indicative of poor fit, whereas values near 0.5 indicate good-fitting models. Model discrimination ability was accessed by computing the area under the receiver operating characteristic curve (AUC) (Zipkin, Campbell Grant & Fagan 2012).

After accessing convergence and goodness of fit of the full models, we estimated the relative importance of each covariate for occupancy and detection probabilities. For this, we extended the linear equations for occupancy and detection by including an inclusion parameter (W) as a latent binary indicator with an uninformative prior [$W_i \sim$ *Bernoulli* (0.5)] (Congdon 2005; Royle & Dorazio 2008; Coggins, Bacheler & Gwinn 2014). For example, the equation for calculating the occupancy probability (Ψ_i) was:

$$\text{Logit}(\Psi_i) \sim \beta_0 + \beta_1 * W_1 * \text{SAVANNAH} + \beta_2 * W_2 * \text{FOREST} + \beta_3 * W_3 * \text{OPEN} + \beta_4 * W_4 * \text{DIST.WATER} + \beta_5 * W_5 * \text{DIST. URBAN}$$

When $W_1 = 1$, the co-variable SAVANNAH has an effect on the occupancy probability equal to β_1 (in the logit scale). Conversely, when $W_1 = 0$ this co-variable has no effect on the occupancy probability. The posterior probabilities of these inclusion parameters corresponded to the estimated probability that a particular covariate was included in the ‘‘best’’ model. Covariables with inclusion probabilities greater than 0.5 should be included in the “best” model (Barbieri & Berger 2004). Using this framework, we obtained occupancy and detection probabilities that were model-averaged, i.e. averaged across the different models included in the posterior sample. Finally, we obtained “model-averaged” estimates for the coefficients of the models by calculating the median and its 95% credible interval of the posterior samples with $W = 1$.

Models were ran using JAGS (Plummer 2003) within the package jags UI (Kellner 2015) in R environment (R Core Team 2016). Model outputs were also handled in R environment.

3. Results

We recorded 5164 road-killed animals between April 2010 and March 2015. Of these, 742 were domestic animals. We developed occupation-detection models for red-tailed boa (*Boa constrictor*, n=58), blue-black Grassquit (*Volatinia jacarina*, n=1221), burrowing-owl (*Athene cunicularia*, n=114), hog-nosed skunk (*Conepatus semistriatus*, n=32) and crab-eating fox (*Cerdocyon thous*, n=79). The correct classification of carcasses of the common toad to the species level was often difficult, as it included three similar species: *Rhinella schneideri*, *R. cerradensis* and *R. rubescens*. Therefore, we aggregated these records and built a model for *Rhinella* sp. (n=207).

All occupation and detection sub-models for the six taxa converged to stable posterior distributions with values of the Gelman-Rubin statistic less than 1.1. The Bayesian p-values ranged from 0.32 (*Rhinella* sp.) to 0.49 (*A. cunicularia*) indicating good-fitting models (Table 2). The AUC median values estimated for the six taxa ranged from 0.58 to 0.69, denoting reasonable discrimination ability (Table 2). Most of the parameter' estimates tended to be widely distributed around their respective median, in some cases with credible intervals broadly overlapping zero (Fig. 2). Yet, ARS estimates are in line with the estimates of individual seasonal models, despite some variation in the effects across seasons (Appendix B). Overall, we considered that the models were robust to provide credible estimates of the roadkill risk along the surveyed roads.

Table 2. Average Response across Seasons (ARS) estimates and the corresponding 95% credible intervals (in brackets) for the six road-killed species models. Values are shown for each level of the hierarchical model. AUC is the area under the curve of the receiver operating characteristic for the full model. Highlighted in bold are parameters (except for the intercepts) with inclusion probability higher than 0.5 (IP> 0.5). BPvalue: Bayesian p-values.

Parameters	***Rhinella* sp.**		***Boa constrictor***		***Volatinia jacarina***	
	median (CI)	**IP**	**median (CI)**	**IP**	**median (CI)**	**IP**
OCCUPANCY						
Int. DRY	0.38 (-2.35 / 3.75)		0.28 (-2.38 / 3.87)		1.64 (0.09 / 4.09)	
Int. WET	1.88 (-0.07 / 5.64)		2.13 (-0.17 / 6.48)		5.09 (1.31 / 8.97)	
SAVANNAH	-0.13 (-3.17 / 3.68)	0.10	-1.1 (-5.07 / 2.68)	0.23	-0.13 (-1.79 / 1.77)	0.03
FOREST	-0.64 (-4.16 / 2.85)	0.38	-1.71 (-5.69 / 2.81)	0.30	0.26 (-0.83 / 1.51)	0.01
OPEN	-0.64 (-3.58 / 1.41)	0.06	0.6 (-3.42 / 4.41)	0.23	**1.57 (0.38 / 3.3)**	**0.88**
DIST.RIVERS	-0.1 (-1.79 / 2.54)	0.04	-2.36 (-6.15 / 0.54)	0.47	0.16 (-1.51 / 1.89)	0.07
DIST.URBAN	**-1.63 (-4.37 / -0.08)**	**0.79**	**-2.08 (-6.56 / 1.02)**	**0.61**	-1.41 (-2.82 / 0.23)	0.25
DETECTABILITY						
Int. DRY	-0.2 (-4.66 / 3.24)		-4.66 (-6.19 / -0.53)		-0.65 (-2.05 / 1.2)	
Int. WET	-3.49 (-4.96 / -0.73)		-3.56 (-4.17 / -2.78)		0.06 (-1.73 / 1.86)	
NATURAL	0.04 (-0.37 / 0.26)	0.00	-0.04 (-0.34 / 0.14)	0.00	-0.18 (-0.35 / -0.02)	0.05
DIRT	-0.81 (-2.04 / 0.26)	0.17	-0.79 (-3.34 / 0.84)	0.03	**-2.21 (-2.86 / -1.62)**	**1.00**
4-LANE	**1.77 (0.87 / 2.5)**	**1.00**	**1.32 (0.39 / 2.14)**	**0.95**	0.3 (-0.25 / 0.88)	0.05
HUMIDITY	**-0.26 (-1.64 / 1.12)**	**1.00**	0.5 (0 / 1.01)	0.02	**0.56 (-0.4 / 1.53)**	**1.00**
DoY	**-1.57 (-5.37 / 1.96)**	**0.97**	-0.21 (-2.01 / 1.75)	0.02	**-1.42 (-2.94 / -0.31)**	**1.00**
BPvalue	0.32		0.42		0.44	
AUC	0.66 (0.38 / 0.78)		0.65 (0.42 / 0.79)		0.59 (0.48 / 0.69)	

Parameters	***Athene cunicularia***		***Conepatus semistriatus***		***Cerdocyon thous***	
	median (CI)	**IP**	**median (CI)**	**IP**	**median (CI)**	**IP**
OCCUPANCY						
Int. DRY	0.78 (-0.67 / 3.3)		1.08 (-0.95 / 4.8)		0.8 (-0.56 / 4.41)	
Int. WET	1.28 (-0.58 / 4.39)		-0.95 (-3.6 / 1.85)		1.2 (-0.51 / 4.79)	
SAVANNAH	**-2.45 (-5.6 / -0.53)**	**0.92**	**-1.79 (-6.49 / 2.66)**	**0.98**	0.42 (-1.47 / 2.24)	0.06
FOREST	0.48 (-2.19 / 4.38)	0.28	0.61 (-2.47 / 3.78)	0.12	0.6 (-0.97 / 3.09)	0.03
OPEN	1.49 (-0.94 / 4.35)	0.18	**2.1 (-0.14 / 4.91)**	**0.56**	0.44 (-1.72 / 1.99)	0.03
DIST.RIVERS	-0.94 (-3.35 / 1.47)	0.25	0.22 (-2.37 / 4.86)	0.04	-0.33 (-1.8 / 0.71)	0.01
DIST.URBAN	**-2.06 (-4.69 / -0.63)**	**0.88**	-1.27 (-5 / 2.05)	0.11	-0.88 (-2.09 / 0.01)	0.11
DETECTABILITY						
Int. DRY	-2.81 (-3.35 / -1.69)		-3.96 (-4.93 / -2.09)		-3.32 (-4.02 / -2.22)	
Int. WET	-2.84 (-3.79 / -1.05)		-3.53 (-4.54 / -2)		-3.79 (-4.52 / -2.8)	
NATURAL	0.4 (-0.2 / 0.91)	0.02	-0.15 (-1.02 / 0.81)	0.01	-0.02 (-0.21 / 0.13)	0.00
DIRT	-1 (-2.21 / -0.07)	0.11	**-3.29 (-7.47 / -0.97)**	**0.94**	-1.06 (-2.37 / -0.11)	0.13
4-LANE	0.1 (-0.71 / 0.73)	0.01	0.75 (-0.48 / 1.69)	0.08	**1.01 (0.37 / 1.58)**	**0.80**
HUMIDITY	0.31 (-0.12 / 0.67)	0.01	0.63 (-0.02 / 1.37)	0.09	0.18 (-0.18 / 0.63)	0.01
DoY	-1.06 (-2.51 / 0.43)	0.06	-1.1 (-3.2 / 1.31)	0.04	-1.16 (-2.78 / 0.33)	0.03
BPvalue	0.49		0.46		0.42	
AUC	0.63 (0.45 / 0.73)		0.69 (0.47/0.86)		0.58 (0.42 / 0.70)	

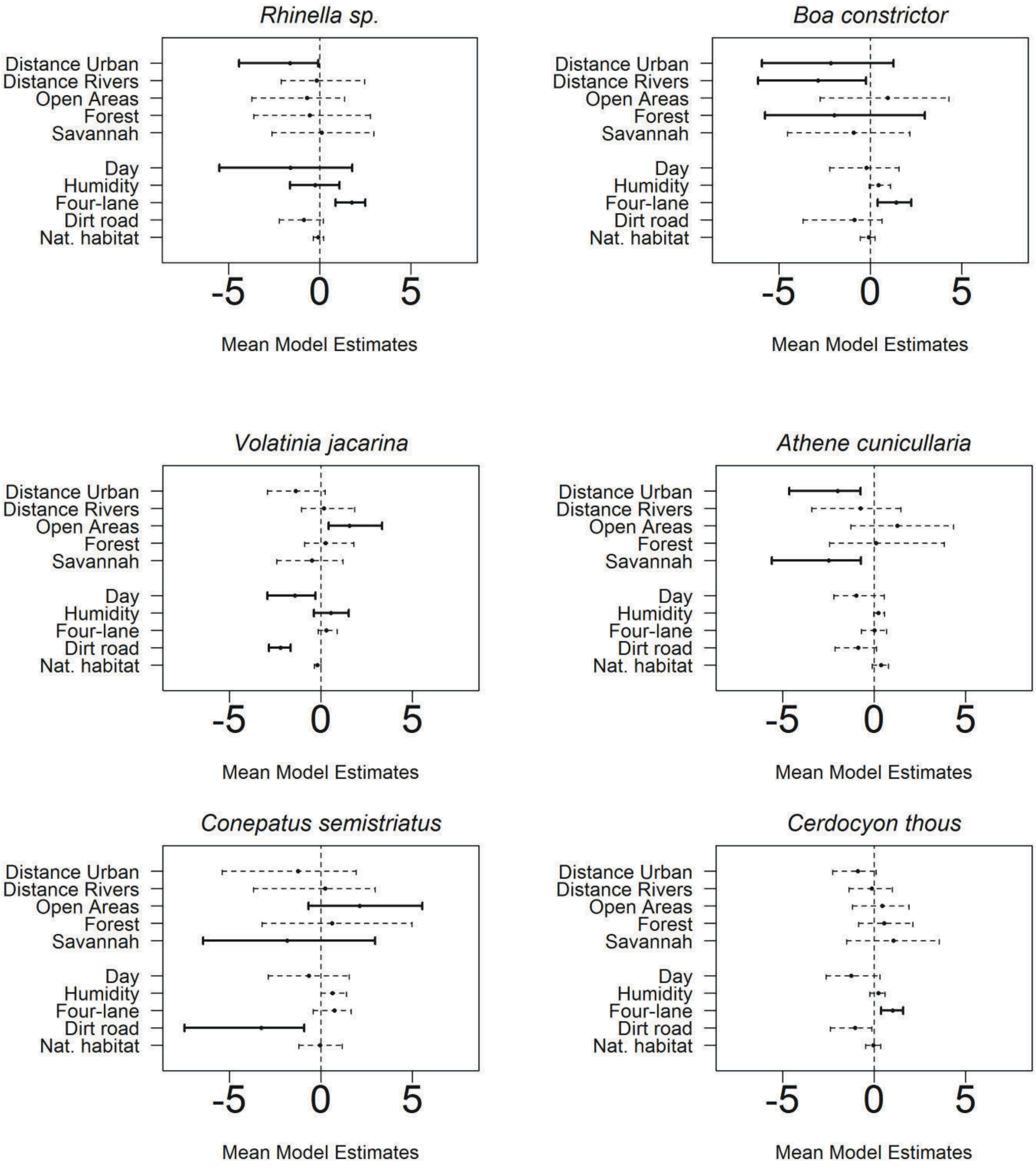

Fig. 2. Average Response across Seasons (ARS) estimates and the corresponding 95% credible intervals for the mean model of six road-killed species. The bold lines indicate the variables with inclusion probability above 0.5.

In general, we observed small differences in roadkill risk between seasons (Dry and Wet; Fig. 3). We identified three peaks of roadkill risk for *Rhinella* sp.; six major peaks for *B. constrictor*; a large proportion of the surveyed roads with a high risk for *V. jacarina* in the Dry season and a high risk along all road length in Wet season; several peaks for *A. cunicularia*; high risk in all road for *C. thous*; and several peaks for *C. semistriatus*. For this latter species, the roadkill risk across seasons was less clear, particularly for road sections between 20 and 40, where some seasons had a higher risk while other seasons estimated low risk. Hence, we considered that the uncertainty of the results for this species was higher.

The posterior inclusion probabilities for the occupancy sub-model indicated that the covariates most supported by the data were DIST.URBAN (a negative association for *Rhinella* sp., *B. constrictor* and *A. cunicularia*), OPEN (positive association for *V. jacarina* and *C. semistriatus*) and SAVANNAH (negative association for *A. cunicularia* and *C. semistriatus*) (Table 2). Posterior probabilities for detection covariates suggested a higher probability of carcasses being detected along the 4-lane highways relatively to the 2-lane roads for *Rhinella* sp., *B. constrictor* and *C. thous*; and a lower detectability in dirt roads for *V. jacarina* and *C. semistriatus* (Table 2). The variable DoY was also related to the detectability of *Rhinella* sp. and *V. jacarina,* being higher during the peak rainy season (December and January) (Table 2). Contrary to our primary hypothesis, there was no evidence of an effect of natural habitat in detectability.

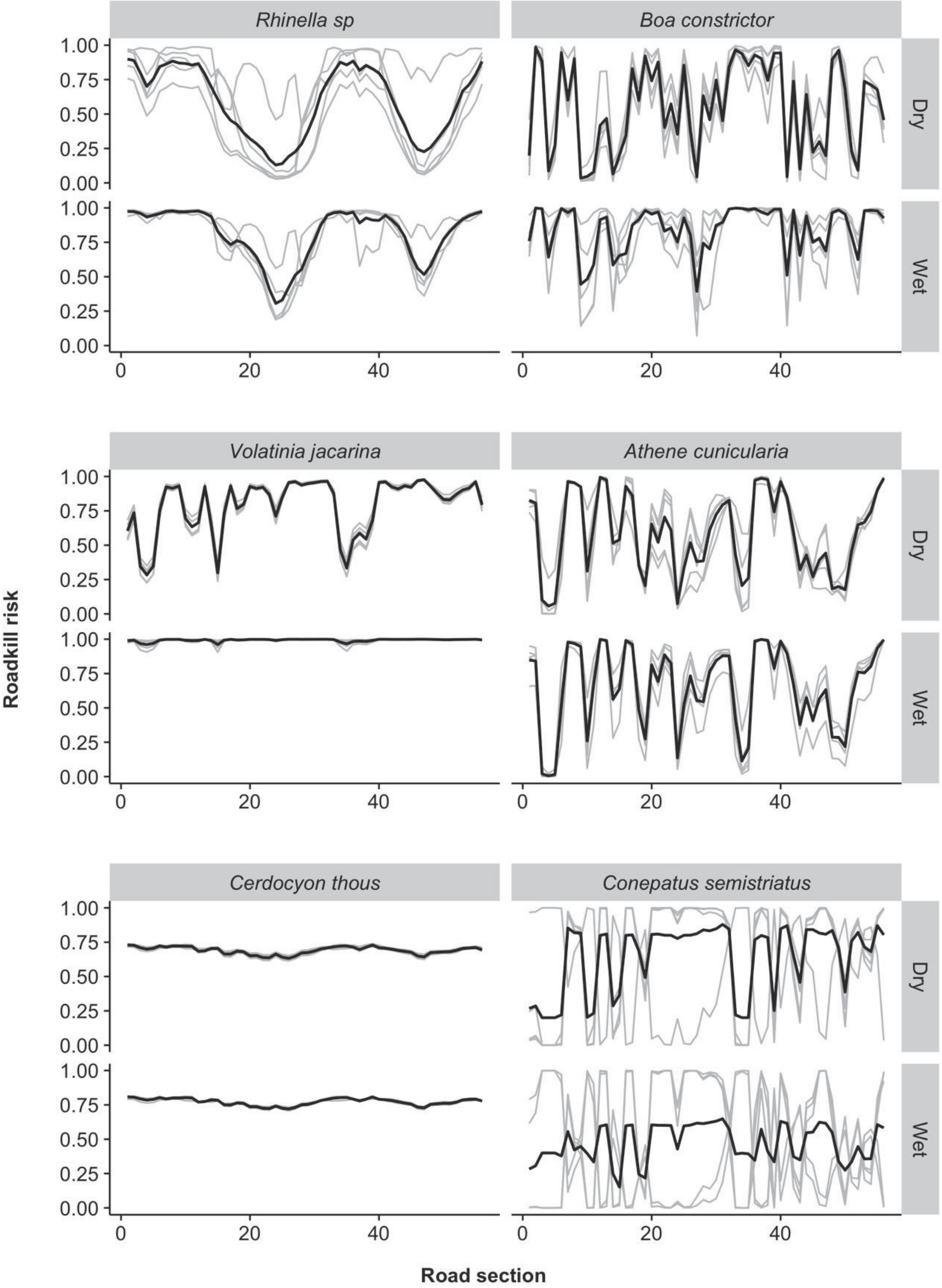

Fig 3. Roadkill risk along the road sections for each taxa and season. Grey lines are the individual seasons' response. Black lines represent the Average Response across Seasons (ARS).

4. Discussion

Our work expanded the use of occupancy models for road ecology studies and provided an insight on how these models can be applied to assess the roadkill risk along roads while accounting for imperfect detection. The roadkill risk can be used to prioritize the allocation of mitigating measures, in a similar manner as decisions based solely on roadkill numbers (Malo, Suárez & Díez 2004). However, our approach allows circumventing potential bias related to undetected casualties. Moreover, one may detect road sections with higher road kill risk, despite a low number of casualties found, as the model output reflects the variation on the potential occurrence of the species along the road. Therefore, known bias related to the use of roadkill aggregations can be minimized (Eberhardt, Mitchell & Fahrig 2013).

Our hierarchical models indicated that the roadkill risk was higher near urban areas for *Rhinella* sp., *B. constrictor* and *A. cunicularia*. This strong association with urban areas' proximity is probably due the fact that these species are very common and widespread, using a wide range of habitats including areas disturbed by human activities and urban areas (Sick 2001; Attademo *et al.* 2004; Coelho *et al.* 2012b). However, urban areas tend to have more traffic, therefore increasing the probability of wildlife-vehicle collision. We also found a positive association between open areas and the roadkill risk for *V. jacarina* and *C. semistriatus*. This indicates that natural fields and farmlands may be preferential areas for these species for road crossing or foraging in the verges. In fact, these species are commonly found in open areas, but seem to avoid dense forests (Sick 2001; Cuarón, Reid & Helgen 2012). Furthermore, there seems to be a lower risk of collision in areas with higher cover of savannah for *C. semistriatus* as well for *A. cunicularia*, therefore suggesting a low occurrence of these species in these areas, at least near the roads. Our data did not support any strong effect of habitat on roadkill risk for *C. thous*, evidencing its generalist characteristics (Trovati, De Brito & Duarte 2007), not selecting specific landscape characteristics for moving and feeding.

Road type was an important factor for the detection of five species. Recall that 'detection' in our model is a combined effect of at least one individual being hit with the chance of being detected in our surveys. Detections were significantly higher along four-lane highways for *Rhinella* sp., *B. constrictor* and *C. thous* whereas they were lower along dirt roads for *V. jacarina* and *C. semistriatus*. The higher traffic on the four-lane roads is likely to increase the occurrence of WVC (Fahrig *et al.* 1995), while not being sufficient to inhibit crossing movements (Jaeger *et al.* 2005). Moreover, roads with higher traffic volumes may prevent the access of scavengers to carrion, therefore contributing to higher detectability (Santos *et al*. 2016). A recent study recorded a maximum abundance of birds of prey, as well as richness and species diversity, along roads with medium traffic volume when compared to roads with higher traffic (Planillo, Kramer-Schadt & Malo 2015). Thus, we believe that detection was higher for four-lane roads because carcasses remain longer on this road type than they do on two-lane and dirt roads. On the other hand, dirt roads studied here have significantly lower traffic volumes and, therefore have a lower likelihood of occurring WVC. Furthermore, the low perturbation allows a fast removal of carcasses by scavengers.

The higher detectability estimated for December and January for *Rhinella* sp. and *V. jacarina* may be related to the higher mobility of individuals. In fact, this period corresponds to the peak rainy season in the region, with increased humidity, coincident with the breeding seasonality and dispersal of amphibians. Previous research have shown a greater number of roadkills of amphibians during rainy periods (Coelho, Kindel & Coelho 2008; Coelho *et al.* 2012b), which consequently increases detections during these periods. As expected, at this time, *Rhinella* sp. were more susceptible to WVC since individuals need to move from their territory through the landscape to find new places to establish or mates for reproduction. Likewise, several individuals of migrate, like *Volatinia jacarina*, to the study area between November and May (also the breeding season), when they form socially monogamous pairs (Almeida & Macedo 2001; Sick 2001). The higher density of this species in this time of year, together with the high number of juveniles, likely leads to higher mortality rates. Finally, contrary to expectations, our models did not point to a significant effect of natural habitat, implying that it is not a good proxy for scavenger activity or other predictors masked its effect.

Our results highlight the need to mitigate road stretches near urban areas and with higher cover of open habitat, with particular focus on the 4-lane highways. Drainage structures are known to provide safe crossing points for several species (Ascensão & Mira 2007; Lesbarreres & Fahrig 2012). Road managers could improve such structures already present along the studied roads to allow multiple taxa to use them. Also, these passages should be linked to drift fences to guide the animals to passage entrances (Clevenger, Chruszcz & Gunson 2001). The use of pole barriers can be a feasible mitigation measure to reduce bird roadkill, particularly when applied in open areas (Zuberogoitia *et al.* 2015).

The roadside vegetation should also be managed in order to prevent animals from staying or foraging in areas at greatest roadkill risk (Ascensão *et al.* 2012). Also according to our results, temporary mitigation measures may effectively reduce the number of WVC (Sullivan *et al.* 2004). We suggest installing temporary amphibian drift fences (Glista, DeVault & DeWoody 2009) connected to drainage passages. It should be noted that we modeled the most recorded taxa, which overall have generalist habits. However, any management actions targeting these species are likely to be used by several other species.

5. Conclusions

We believe that occupancy models can provide improved information for management guidelines. To our knowledge, this is the first study that attempts to infer roadkill risk using occupancy models. Yet, this approach can be substantially improved in future work by disentangling the detectability processes, namely the animal-vehicle collision *per se*, and its detection by roadkill surveyors. This, however, requires detailed information regarding the location of individuals hit and time of removal, e.g. by scavengers. On the other hand, we deliberately overlooked the effects of animal behavioral by assuming that the roadkill risk reflects the probability of individuals using a given road section for crossing or foraging and therefore being prone to be road-killed. Yet, it has been argued that different species or individuals manifest different behavioral responses to roads and vehicles (Jacobson *et al.* 2016). Hence, these models could be greatly improved by adding information on species' behavior. Likewise, the modeling framework here proposed would gain robustness by including detailed information regarding focal species' abundance, as well of abundance or at least occurrence of scavengers in road surrounding areas. However, the knowledge of road-related behavioral responses is still scarce or inexistent, and the distribution and abundance of wildlife species is generally unknown for our studied taxa.

We analyzed each season separately, from which we were able to estimate an average roadkill risk across seasons, assuming that the effect of the co-variables in the occupation of road sections and the detection of WVC is similar among seasons. For some taxa, particularly *C. semistriatus,* we detected differences in the roadkill risk between seasons. This is probably related to differences in population abundance and/or movement rates along the year. However, for most species, we observed little differences in roadkill risk between seasons. Moreover, the most important covariates were selected in almost all seasons (Wet and Dry), which support our close assumption of similar effects across seasons by co-variables and that our estimates for average response across seasons (ARS) were a good approach when using occupancy models.

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Supplementary material

Appendix A. Model Structure for Occupancy and Detection and JAGS Code.

Appendix B. Mean parameter estimates for season.

Research data

All roadkill data collected are available by Brasília Environment Institute - IBRAM in site http://www.ibram.df.gov.br/.

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Supporting Information

Appendix S1- Model Structure for Occupancy and Detection

Description

We developed a model based on the community model proposed by Dorazio & Royle (2005). In their approach, the authors model all species in a community as a series of stack-up models (one for each species) and models with more observations lend strength to models with fewer. Instead of modeling several species in a community, we modelled all seasons for each taxa in a similar way. In our model, we assumed that the effect of each environmental predictor on occupancy and detectability is similar (not equal) across seasons within each season type (DRY and WET) and that each effect is taken from an unknown hyper-distribution represented by a normal distribution with a given mean and standard deviation. The advantage of such approach is that it improves the modeling of seasons with poor information, i.e., seasons with more observations lend strength to analyze seasons with fewer observations (see Kéry & Royle 2008, 2016; Dorazio *et al.* 2010; Zipkin, Grant & Fagan 2012). This approach allows, yet, some variation in the effect of the variables among seasons (e.g.: we expect that the effect of distance to rivers should be different between DRY and WET seasons and even among years due to possibly of a dryer period). The mean of each hyper-distribution can be seen has an Average Response across Seasons (ARS) for each predictor. ARS estimates are a measure on how the co-variables consistently affect the occupancy and/or detectability.

True State

Let Z_i denote the true occurrence of a given species in a given season for road section i, with $Z_i = 1$ indicating a presence, and $Z_i = 0$ an absence. We modeled Z_i as an outcome of a Bernoulli trial:

$$Z_i \sim \text{Bernoulli}(\Psi_i) \text{ – Eq.1}$$

Here, Ψ_i represents the probability of the individuals of a given species using the road section i for crossing. We assumed that the state of occupancy doesn't change during the season and that the occupancy in the following seasons will not depend on the occupancy state in the previous seasons.

Since not all animals present in the road suffer from vehicle collisions as well as not all road-killed animals are detected (Slater 2002) the true state (Z_i) is only partial observed. If no carcass were observed at road section i, this could be result of a true absence, no collisions, or collisions with no carcass detected. Let y_{ij} denote the observation of section i during survey j, with $y_{i\,j}= 1$ indicating at least one carcass detected during survey j at road section i an $y_{ij}= 0$ indicating no detections. Thus, for each season, at section i, we observed an encounter history indicating whether species was detected or not detected during each of the surveys j until all J surveys are completed. However, the detection is dependent whether the specie is present or not, i.e. the occupancy state Z_i. Thus, we modeled the detection at a separate Bernoulli process:

$$y_{ij} \sim Bernoulli\ (Z_i * p_{ij}) \text{ – Eq. 2}$$

Where p_{ij} is the probability of an animal being road-killed and detected at a road section i, survey j. Note that in sections that the species is absent ($Z_i = 0$), $y_{i,j}$ will be 0 for all J observations with probability 1. If the species is present, observations ($y_{ij}= 1$) with probability p_{ij}. We believed that the independence between surveys j were guaranteed since in each survey the observers removed the carcasses from road. We further assume that that WVC occurred at a site doesn't cause a local extinction thus changing the occupancy state.

Link Variables

We assumed that probabilities Ψ_i and p_{ijk} are function of the habitat, road type and weather. The model of occurrence for roadkill species that incorporated potential covariate effects using a logit link function (Mccullagh & Nelder 1989):

$$\text{logit}(\Psi_i) \sim \beta_0 + \beta_1\text{*savannah} + \beta_2 \text{* forest habitat} + \beta_3\text{*open areas} +$$

$$+ \beta_4 \text{*distance rivers} + \beta_5 \text{*distance urban areas} – \text{Eq.3}$$

where β_0 represents the intercept of the distribution sub-model and β_1, β_2,…, β_5 represent logit-scale effects of the associated covariates (Table 1 in the main text) on the probability of the occurrence. Similarly, we specified the detection model as:

$$\text{logit}(p_{ijk}) \sim \alpha_0 + \alpha_1\text{*natural habitat} + \alpha_2\text{*dirt road} + \alpha_3\text{*four lane} +$$

$$+ \alpha_4\text{*air humidity} + \alpha_5\text{*day} – \text{Eq.4}$$

where α_0 represents the intercept of the distribution sub-model and α_1 through α_5 are logit-scale effects of the respective covariates on detection.

Priors and Hyper-Parameters

By modelling each of the seasons separated we produce a model with many parameters and some of the species are detected infrequently, or not all in some seasons, making estimation of all the model parameters impossible unless we made further assumptions (Dorazio *et al.* 2010). We assumed that the effect in occupancy and detection were similar (not equal) across seasons and these effects were taken from an unknown distribution that report to hyper-parameters. This permits for seasons with more observations to borrow strength to seasons with lesser observations but still getting some flexibility in the effects of the variables between seasons. We assume that effects of the co-variables in each season were taken from a normal distribution with unknown mean and standard deviation that we can estimate:

$$\beta_{m,k} \sim \text{Normal}(\mu\beta_m,\ \sigma^2\beta_m) – \text{Eq.5}$$

The estimate of the effect $\beta_{m,k}$ of co-variable *m* in season *k* is taken from a normal distribution with $\mu\beta_m$ and standard deviation $\sigma^2\beta_m$. We gave to this hyper-parameters uninformative priors:

$$\mu\beta_m \sim \text{Normal}(0, 10) – \text{Eq.6}$$

$$\sigma^2\beta_m \sim \text{Uniform}(0, 10) – \text{Eq.7}$$

Conceptually, the mean of these hyper-distribution ($\mu\beta_m$) can be looked has an Average Response across Seasons (ARS) for each predictor. ARS estimates (and credible intervals) are a measure on how the co-variables consistently affect the occupancy and/or detectability.

In order to account for the phenology of the taxa the intersect for the occupancy and detections probabilities (base-line) for dry and wet seasons were taken from two different normal distributions (one for dry and other for wet seasons):

$$\beta0_k \sim \mathrm{Normal}(\mu\beta0_k,\ \sigma^2\beta0_k) \text{ – Eq.8}$$

Where:

$$\mu\beta0_k = \mu\beta0_{wet} * \mathrm{Wet}_k + \mu\beta0_{dry} *(1 - \mathrm{Wet}_k) \text{ – Eq.9}$$

$$\sigma^2\beta0_k = \sigma^2\beta0_{wet} * \mathrm{Wet}_k + \sigma^2\beta0_{dry} *(1 - \mathrm{Wet}_k) \text{ – Eq.10}$$

Were the intersect for season *k* was taken from a normal distribution with mean $\mu\beta0_k$ and standard deviation $\sigma^2\beta0_k$. These parameters are taken from the "wet distribution" or the "dry distribution" using the Wet_k as a latent variable indicating if season *k* is a wet season ($Wet_k=1$) or a dry season ($Wet_k=0$). These parameters also have uninformative priors, e.g.:

$$\mathrm{Logit}\ (\mu\beta0_{wet}) \sim \mathrm{Uniform}(0, 1) \text{ – Eq.11}$$

$$\sigma^2\beta0_{wet} \sim \mathrm{Uniform}(0, 10)\text{– Eq.12}$$

Inclusion Probability and Model Averaging

We estimated the relative importance of each covariate for occupancy and detection probabilities. For this, we extended the linear equations for occupancy and detection (Equations 3 and 4) by including an inclusion parameter (W) as a latent binary indicator with an uninformative prior [Wi ~ Bernoulli (0.5)] (Congdon 2005; Royle & Dorazio 2008; Coggins, Bacheler & Gwinn 2014). For example, the equation for calculating the occupancy probability (Ψi – Eq. 3) was modified as follows:

$$\mathrm{logit}\ (\Psi_i) \sim \beta_0 + \beta_1 * W_1 * \text{savannah} + \beta_2 * W_2 * \text{ forest habitat } + \beta_3 * W_3 * \text{open areas} +$$
$$+ \beta_4 * W_4 * \text{distance rivers} + \beta_5 * W_5 * \text{distance urban areas – Eq.3}$$

When $W_1 = 1$, the co-variable *savannah* has an effect on the occupancy probability equal to β_1 (in the logit scale). Conversely, when $W_1 = 0$ this co-variable has no effect on the occupancy probability. As the model updates, in each run, these indicators include or exclude variables in the model, resulting that some variables would be included more often than others. The mean of posterior probabilities of these inclusion parameters corresponded to the estimated probability that a particular covariate was included in the ‘‘best’’ model. Co-variables with inclusion probabilities greater than 0.5 (i.e. variables that were included in the model more than half of the runs) should be included in the “best” model (Barbieri & Berger 2004). Using this framework, we obtained occupancy and detection probabilities that were model-averaged, i.e. averaged across the different models included in the posterior sample. Finally, we obtained “model-averaged” estimates for the coefficients of the models by calculating the median and its 95% credible interval of the posterior samples with $W = 1$.

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Code

```
model {
# Hyper Parameters Priors
#Dry Season Hyper Parametters
mean.p_D ~ dunif(0, 1)          # Detection intercept mean on prob. scale
lp_D<- logit(mean.p_D)       #   same on logit scale
mean.psi_D ~ dunif(0, 1)        # Occupancy intercept mean on prob. scale
lPsi_D<- logit(mean.psi_D)      #   same on logit scale

lpSD_D ~ dunif(0,10)   # Standard Deviation for Hyper distribution of detections
lpPrec_D<- pow(lpsiSD_D,-2)
lpsiSD_D ~ dunif(0,10) # Standard Deviation for Hyper distribution of occupancy
lpsiPrec_D<- pow(lpsiSD_D,-2)

#Wet Season Hyper Parametters
mean.p_W ~ dunif(0, 1)          # Detection intercept mean on prob. scale
lp_W<- logit(mean.p_W )       #   same on logit scale
mean.psi_W ~ dunif(0, 1)        # Occupancy intercept mean on prob. scale
lPsi_W<- logit(mean.psi_W )      #   same on logit scale

lpSD_W ~ dunif(0,10)  # Standard Deviation for Hyper distribution of detections
lpPrec_W<- pow(lpsiSD_W ,-2)
lpsiSD_W ~ dunif(0,10)           # Standard Deviation for Hyper distribution of occupancy
lpsiPrec_W<- pow(lpsiSD_W ,-2)

for(a in 1:nX1){               # Loop over terms in detection model
alpha_m[a] ~ dnorm(0, 0.1)   #Average Response across Seasons (ARS) for detection
alphaSD[a] ~ dunif(0,10)
alphaPrec[a] <- pow(alphaSD[a],-2)
wa[a]~dbern(.5)
}

for(b in 1:nX2){              # Loop over terms in occupancy model
beta_m[b] ~ dnorm(0, 0.1)    # ARS for Occupancy
betaSD[b] ~ dunif(0,10)
betaPrec[b] <- pow(betaSD[b],-2)
wb[b]~dbern(.5)
}

for(c in 1:nX3){              # Loop over terms for survey variables
alpha_s_m[c] ~ dnorm(0, 0.1) #ARS for survey variables
alpha_s_SD[c] ~ dunif(0,10)
alpha_s_Prec[c] <- pow(alpha_s_SD[c],-2)
wa_s[c]~dbern(.5)
}
#Priors
for (k in 1:nseasons){
	#Choose parameter for intersect (Wet or Dry)
	lp[k] <- wet[k]*lp_W + (1-wet[k])*lp_D #Mean for Detection intercept
	lpPrec[k] <- wet[k]*lpPrec_W + (1-wet[k])*lpPrec_D #Standard Deviation for Detection intercept
	lPsi[k] <- wet[k]*lPsi_W + (1-wet[k])*lPsi_D #Mean for Occupancy intercept
	lpsiPrec[k] <- wet[k]*lpsiPrec_W + (1-wet[k])*lpsiPrec_D #Standard Deviation for Detection intercept
```

```
    alpha0[k] ~ dnorm(lp[k], lpPrec[k])       #   detection  intercept
    beta0[k] ~ dnorm(lPsi[k], lpsiPrec[k])      #  Occupancy intercept

    for(a in 1:nX1){                # Loop over terms in detection model
        alpha[a,k] ~ dnorm(alpha_m[a], alphaPrec[a])  # Covariates for detection
        alpha_w[a,k] <- alpha[a,k] * wa[a]        #Include or not the variable
    }

    for(b in 1:nX2){               # Loop over terms in occupancy model
        beta[b,k] ~ dnorm(beta_m[b], betaPrec[b])    # Covariates for occupancy
        beta_w[b,k] <- beta[b,k] * wb[b]           #Include or not the variable
    }

    for(c in 1:nX3){                # Loop over terms in detection model
        alpha_s[c,k] ~ dnorm(alpha_s_m[c], alpha_s_Prec[c])   # Covariates for Surveys
        alpha_s_w[c,k] <- alpha_s[c,k] * wa_s[c]           #Include or not the variable
    }

    # Likelihood
    for (i in 1:M) { # Loop over sites
        z[i,k] ~ dbern(psi[i,k]) #True state
        logit(psi[i,k]) <- beta0[k] + inprod(beta_w[,k], occDM[i,])  # Occ linear Model

        for (j in 1:J) {# Loop over surveys
            y[i,j,k] ~ dbern(z[i,k] * p[i,j,k]) #Detections

            logit(p[i,j,k]) <- alpha0[k] + # Detection linear Model
            inprod(alpha_w[,k], detDM[i,]) + # Site co-variables
            inprod(alpha_s_w[,k], SrvDM[j,k,]) #Survey co-variables

            q[i,j,k] <- 1 - p[i,j,k]      #Non-detections probability

        }
        p1[i,k] <- psi[i,k] * (1- prod(q[i, ,k])) #Conditional Observation probability
        Res[i,k] <- d[i,k] - p1[i,k] #residuals
        sq[i,k] <- pow(Res[i,k], 2) # Squared residuals for observed data

        d_rep[i,k]  ~ dbern(p1[i,k] ) #Generate replicate observations
        Res_rep[i,k]  <- d_rep[i,k]  - p1[i,k]  #Replicate residuals
        sq_new[i,k] <- pow(Res_rep[i,k], 2) # Squared residuals for replicated data
    }
}

fit <- sum(sq[,])              # Sum of squared residuals for actual data set
fit.new<- sum(sq_new[,])       # Sum of squared residuals for new data set
test <- step(fit.new-fit)  # Test whether new data set more extreme
bpvalue<- mean(test) }  # Bayesian p-value
```

Appendix S2 – Variation of co-variables effects across seasons

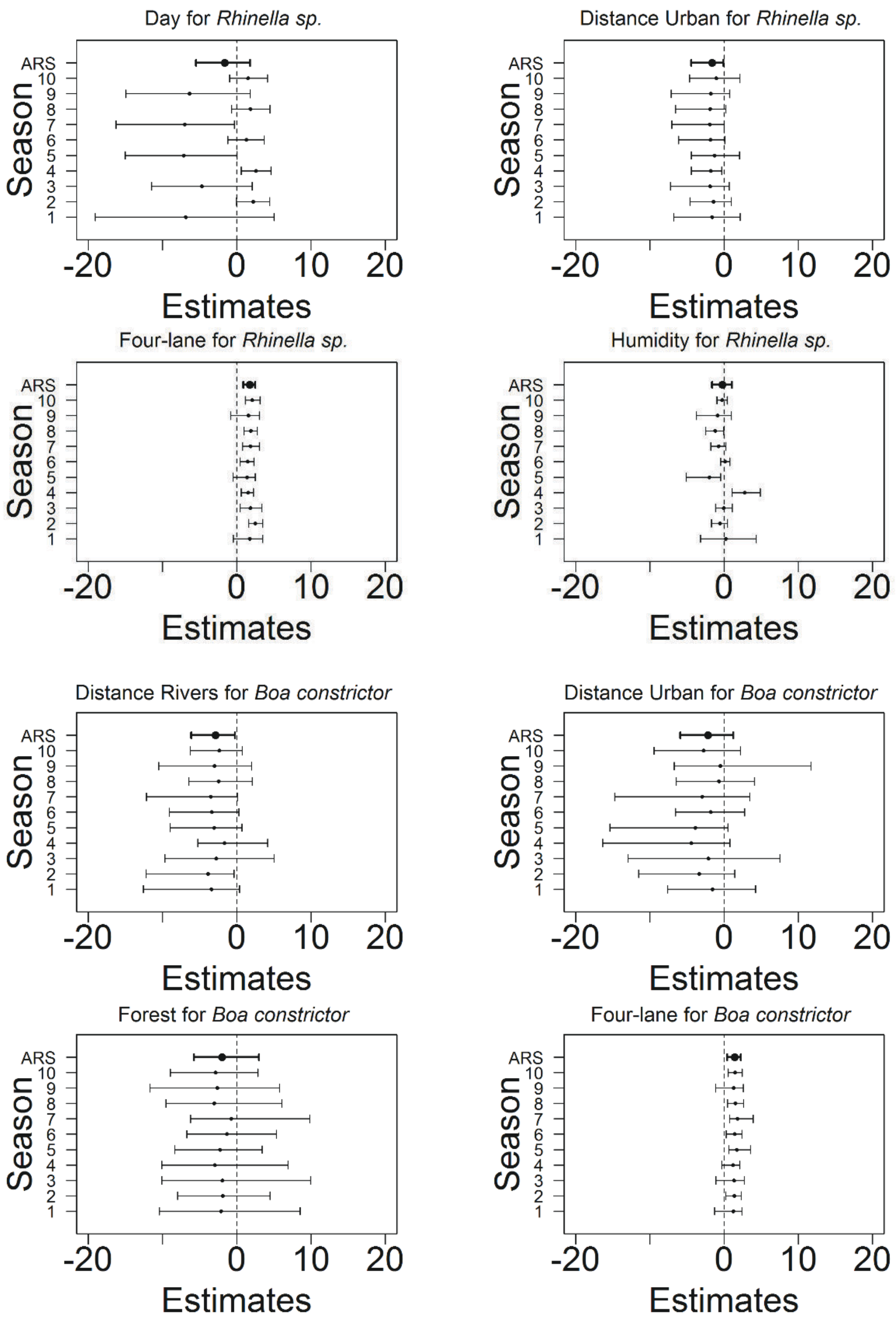

Figure S2-1. Median parameter estimates and the corresponding 95% credible intervals for the variables selected by the inclusion probability for all seasons for *Rhinella* sp. and *Boa constrictor*.

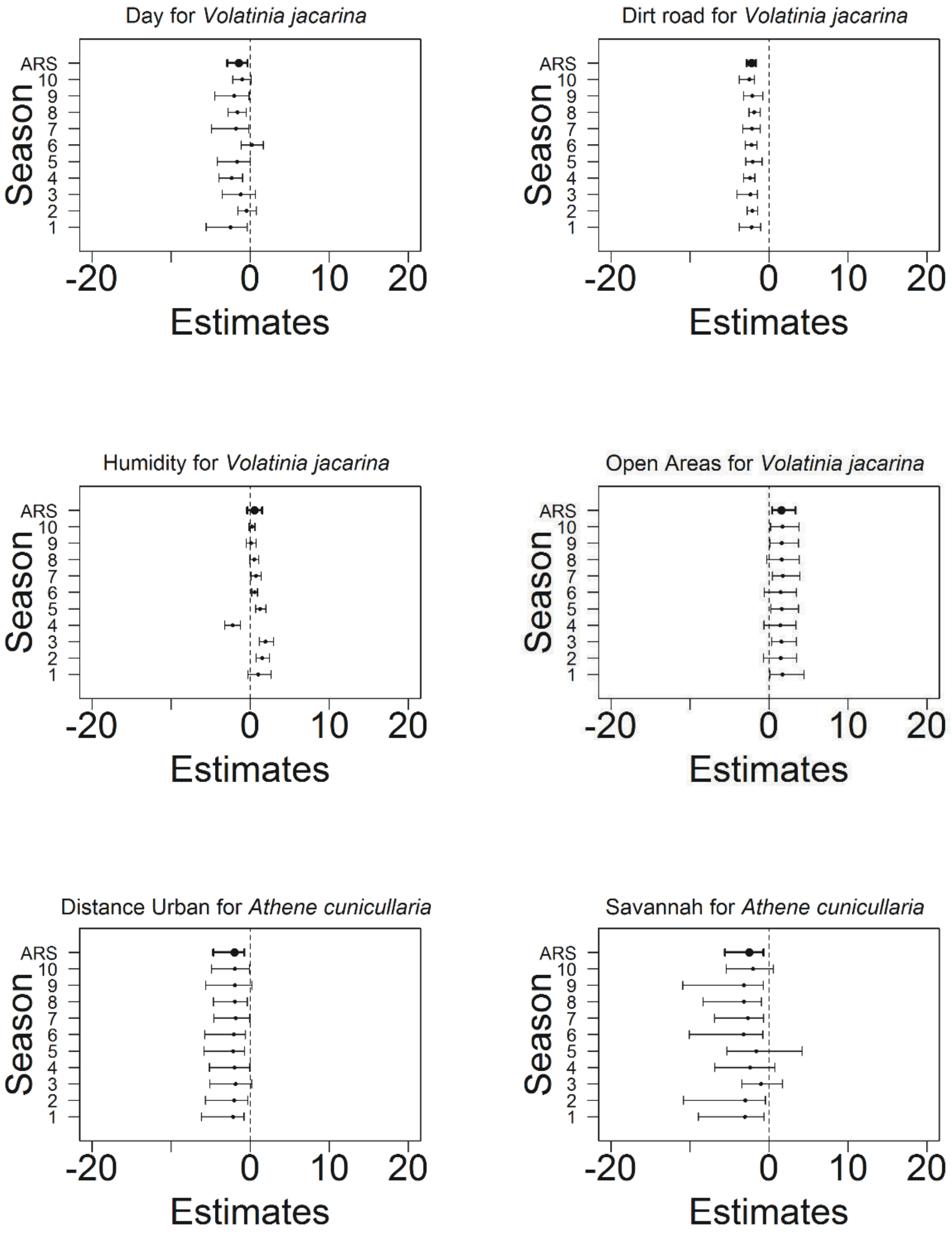

Figure S2-2. Median parameter estimates and the corresponding 95% credible intervals for the variables selected by the inclusion probability for all seasons for *Volatinia jacarina* and *Athene cunicullaria*.

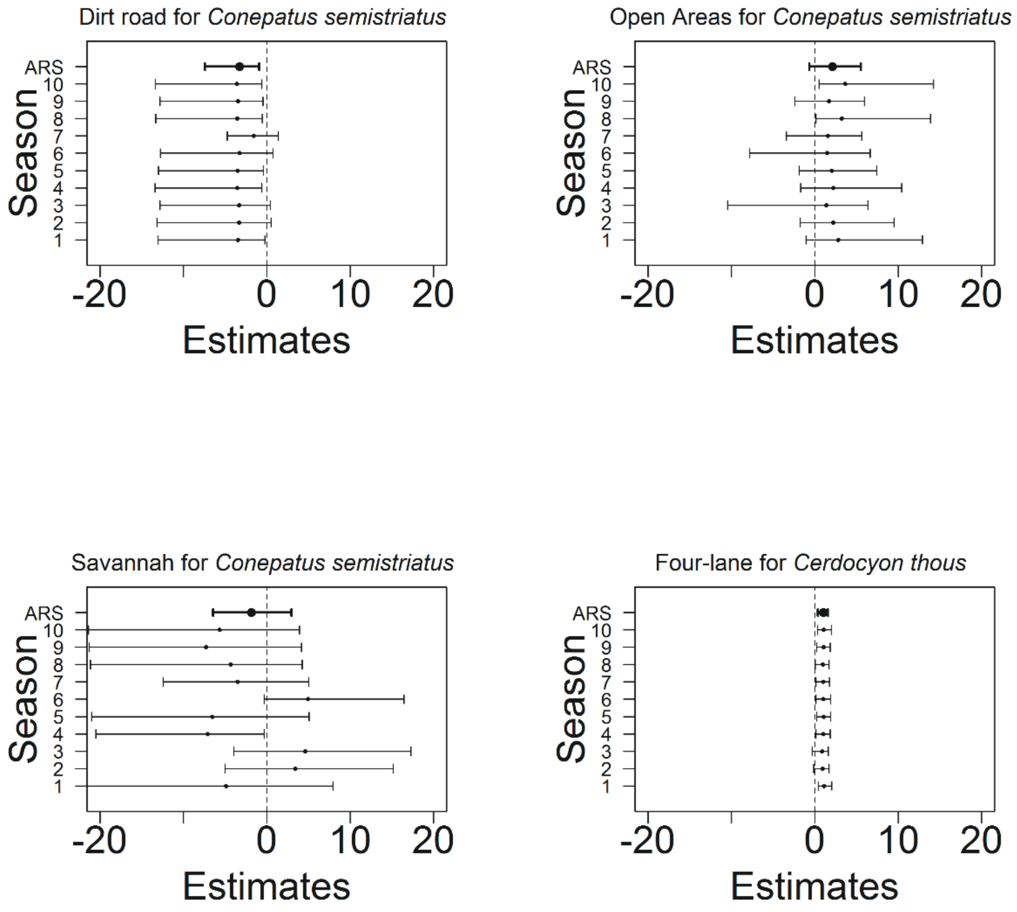

Figure S2-3. Median parameter estimates and the corresponding 95% credible intervals for the variables selected by the inclusion probability for all seasons for *Conepatus semistriatus* and *Cerdocyon thous*.

Considerações Finais

Os resultados obtidos nessa tese fornecem informações inéditas e relevantes para o manejo da biodiversidade no entorno de estradas. Os assuntos abordados tiveram como objetivo auxiliar no processo de licenciamento ambiental de rodovias indicando e sugerindo ferramentas de avaliação de atropelamento de fauna aos pesquisadores da área e aos tomadores de decisões.

Nessa pesquisa ficou claro que é fundamental que todo e qualquer estudo realizado a partir de veículos automotores deve proceder com um teste de correção da detecção do observador, corroborando assim com alguns estudos que já relataram a importância de corrigir esse viés. Foi possível constatar que a detecção do observador é a maior fonte de incerteza nos levantamentos de animais atropelados. De uma maneira geral, o tempo de persistência das carcaças é similar em diferentes regiões. É importante relatar que tal afirmação não implica na não execução de testes de persistência das carcaças, mas sim em testes realizados em locais com características peculiares da paisagem. Por exemplo, foi possível observar um efeito da vegetação no tempo de remoção da carcaça. Portanto, é interessante que estudos que englobem uma paisagem diversificada realizem experimentos de tempo de persistência e de preferência com a padronização na disposição das carcaças, ou seja, em intervalos regulares de espaçamento.

A identificação de *hotspots* e *hot-moments* tem se tornado um procedimento padrão de apresentação de resultados nos estudos de impacto ambiental de empreendimentos lineares. Porém, o uso indiscriminado dessa ferramenta por pesquisadores e empreendedores, inclusive sem a correta aplicação do método de amostragem, de esforço e análise dos resultados pode levar a conclusões equivocadas e manejo inadequado da biodiversidade. É importante que o pesquisador tenha em mente que essas ferramentas devem ser utilizadas, mas com o devido cuidado, e se possível complementado com outras estratégias de análise de informação, como por exemplo, uma análise da paisagem e sua relação com os atropelamentos. Dessa maneira, o leitor deve estar atento ao ler essa tese, uma vez que os capítulos II e III se complementam. É interessante trabalhar com escalas maiores para detecção de *hotspots/hot-moments*, mas há ainda uma incerteza atrelada ao método de identificação de agregações de atropelamento, e essa incerteza diminuirá com o aumento do esforço de amostragem. Os resultados aqui apresentados são fruto de uma amostragem intensiva e sistemática de longo tempo que nem sempre será replicada em outros estudos de impacto ambiental. É nessa lacuna de esforço amostral que se pode aplicar a análise de locais de maior risco de atropelamento utilizando os modelos de ocupação. A vantagem da aplicação desses modelos é lidar com uma baixa detecção de espécies/atropelamentos e gerar potenciais locais de ocorrência de colisões entre animais silvestres e veículos. Os modelos de ocupação tornam-se uma ferramenta interessante e de alta aplicabilidade na ecologia de estradas ao levarem em consideração a detecção imperfeita e as variáveis ambientais preditoras de atropelamentos.

Novas abordagens tem surgido com o intuito de aprimorar as análises de agregação de atropelamento, com a incorporação da interação entre as dimensões espaciais e temporais de forma simultânea nestas análises, ou corrigindo o efeito da heterogeneidade espacial na definição de *hotspots*. Diante do exposto, é primordial que o pesquisador procure adotar diferentes estratégias ou métodos para definir as áreas de mitigação de atropelamentos.