UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE BIOCIÊNCIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA

Tese de Doutorado

Planejamento e avaliação de medidas mitigadoras de impactos de ferrovias e rodovias na fauna

Bibiana Terra Dasoler

Porto Alegre, junho de 2025

PLANEJAMENTO E AVALIAÇÃO DE MEDIDAS MITIGADORAS DE IMPACTOS DE FERROVIAS E RODOVIAS NA FAUNA

Bibiana Terra Dasoler

Tese de Doutorado apresentada ao Programa de Pós-Graduação em Ecologia, do Instituto de Biociências da Universidade Federal do Rio Grande do Sul, como parte dos requisitos para obtenção do título de Doutora em Ecologia.

Orientador: Dr. Andreas Kindel
Coorientadora: Dra. Fernanda Zimmermann Teixeira

Comissão Examinadora
Drª. Sara Santos
Drª. Marina Zanin
Dr. Vinicius Alberici

Porto Alegre, junho de 2025

CIP - Catalogação na Publicação

Dasoler, Bibiana Terra
Planejamento e avaliação de medidas mitigadoras de impactos de ferrovias e rodovias na fauna / Bibiana Terra Dasoler. -- 2025.
89 f.
Orientador: Andreas Kindel.

Coorientadora: Fernanda Zimmermann Teixeira.

Tese (Doutorado) -- Universidade Federal do Rio Grande do Sul, Instituto de Biociências, Programa de Pós-Graduação em Ecologia, Porto Alegre, BR-RS, 2025.

1. Ecologia de rodovias. 2. Atropelamentos de animais silvestres. 3. Conectividade. 4. Efetividade de mitigação. I. Kindel, Andreas, orient. II. Zimmermann Teixeira, Fernanda, coorient. III. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

Agradecimentos

Em primeiro lugar, gostaria de agradecer às diferentes instituições que me permitiram desenvolver essa tese:

- À UFRGS e ao PPG Ecologia, pela infraestrutura e pela qualidade do ensino proporcionado, que permitiram a minha qualificação profissional.

- Ao CNPq e à CAPES, pelas bolsas de doutorado e doutorado sanduíche, que garantiram meu sustento no Brasil e durante meu período em Portugal.

- Ao Zoológico de Sapucaia do Sul e ao CECLIMAR, pela solicitude no empréstimo dos indivíduos de quelônios, possibilitando o desenvolvimento de um dos capítulos desta tese.

- À Sociedade Sinhá Laurinha, pela disponibilização de dados, que também viabilizaram parte dessa tese.

Além das instituições, eu gostaria de agradecer pessoas que foram fundamentais para que essa tese chegasse até aqui:

- Ao meu orientador, Andreas, por sempre me lembrar do meu potencial e por sempre tentar tornar o percurso do doutorado mais leve e com mais motivação.

- À minha coorientadora, Fernanda, por ter me puxado pela mão para dentro do NERF e ter me incentivado nessa trajetória, sempre valorizando as minhas realizações.

- Aos colegas do NERF, que virtual ou fisicamente, estiveram presentes ao longo desses anos, permitindo inúmeras trocas de conhecimento e momentos de alegria. Meu muito obrigada, em especial a Tali, Ingridi e Lari, pelo suporte quase diário nesse processo.

- Ao Vinicius Bastazini e ao Fernando Ascensão, por me receberem em Portugal e me auxiliarem no desenvolvimento de parte dessa tese.

- Às amigas e aos amigos, dos diferentes grupos, pelo carinho, pelos momentos de descontração e pelas mais variadas conversas. Como diz Emicida, “quem tem um amigo tem tudo”. E que bom que eu tenho vários!

- À minha mãe, pelo apoio incondicional e por ser meu maior exemplo de esforço e determinação.

- Ao Nico, pelo companheirismo, paciência, incentivo e cuidado ao longo de mais essa etapa. Sou muito feliz em dividir a vida e as conquistas contigo e com o Chico.

Por último, alguns agradecimentos menos habituais mas igualmente importantes:

- À Cris, minha psicóloga, por me ouvir e me auxiliar nos momentos mais difíceis desse processo. A pós-graduação tem um enorme potencial para o frequente desenvolvimento da síndrome do impostor, por isso, façam terapia!

- Ao jiu-jitsu, por todas amizades que ele me trouxe, tanto aqui quanto em Portugal, e por ser uma oportunidade de ocupar a mente quando nada mais parecia funcionar.

- Aos profissionais da saúde que atuaram na linha de frente do combate à Covid-19 e ao pesquisadores que desevolveram uma vacina em tempo recorde para que vivenciássemos o fim desse momento histórico.

- A todos que atuaram nas enchentes do Rio Grande do Sul em maio de 2024, doando mão-de-obra, tempo, alimentação e dinheiro para ameanizar o sofrimento na vida das pessoas e animais atingidos nessa situação.

A todos vocês, meu sincero e carinhoso muito obrigada!

Resumo

As infraestruturas lineares, como rodovias e ferrovias, causam impactos diretos e indiretos sobre a fauna, como o aumento das fatalidades, a alteração da conectividade e da qualidade do habitat. Para reduzir esses impactos, diversas estratégias têm sido implementadas globalmente, como cercas e estruturas de travessia de fauna superiores e inferiores. No entanto, a efetividade dessas medidas varia, tornando essencial a avaliação do seu desempenho para garantir uma otimização do recurso disponível. Diante as diferentes oportunidades existentes relacionadas à mitigação durante as fases de uma infraestrutura linear, distintas abordagens e fontes de dados podem ser utilizadas no planejamento e na avaliação das medidas. Nesta tese, explorei três abordagens (experimentação, observação e simulação) para auxiliar nesse planejamento e avaliação. No Capítulo I, utilizei experimentos comportamentais para testar o uso de canaletas para evitar o aprisionamento de quelônios em ferrovias. Concluí que a simples remoção do lastro entre dormentes nos trilhos das ferrovias pode evitar o aprisionamento de tartarugas semiaquáticas. Enquanto os jabutis apresentaram evitamento às canaletas. No Capítulo II, analisei o efeito de sistemas de mitigação compostos por cercas e passagens de fauna na redução das fatalidades, monitorando as mudanças no número de mortes e de cruzamentos ao longo do tempo. Ainda que as estruturas de travessia tenham apresentado um aumento no número de cruzamentos dos animais ao longo dos anos, ficou evidente que o sistema não é efetivo para reduzir as mortes, provavelmente devido ao uso de cercas inadequadas e à carência de manutenção. No Capítulo III, utilizei modelos baseados em indivíduos para simular como a estrutura da paisagem influencia e características de movimentação influenciam a agregação de cruzamentos em estradas. Identifiquei variações nas agregações dos cruzamentos, com maior agregação em paisagens com quantidades intermediárias de habitat ou com poucas manchas de habitat e em simulações com baixa permeabilidade da matriz ou animais com maior visão (alcance da percepção ou ângulo da visão). Os resultados encontrados nessa tese qualificam o entendimento sobre a mitigação em diferentes fases das ferrovias e rodovias, podendo subsidiar a tomada de decisões futuras. Avançar no conhecimento das medidas é crucial para facilitar sua aplicação e aumentar sua efetividade na redução dos impactos das infraestruturas lineares na fauna.

Palavras-chave: colisões entre veículos e fauna, aprisionamento de animais, movimentação animal, conectividade, efetividade da mitigação.

Abstract

Linear infrastructures, such as roads and railways, cause direct and indirect impacts on wildlife, including increased fatalities, changes in connectivity, and habitat quality. To mitigate these impacts, different strategies have been implemented worldwide, such as fencing and wildlife crossings structures (overpasses or underpasses). However, there is a variation in effectiveness of these measures, making an evaluation of their performance essential to optimize the available resource. Given the different opportunities for mitigation during the phases of a linear infrastructure, distinct approaches and data sources can be used in planning and assessing mitigation measures. In this thesis, I explored three approaches — experimentation, observation, and simulation — to support this planning and evaluation. In Chapter I, I used experiments to test the use of trenches to prevent the entrapment of chelonians within tracks. I found that simply removing the ballast between railroad ties can prevent the entrapment Black-bellied slider while Red-footed tortoise exhibited high trench avoidance. In Chapter II, I analyzed the effectiveness of mitigation clusters composed of fencing and underpasses in reducing roadkill by monitoring changes in crossing and roadkill numbers over time. Although the crossing structures showed an increase in crossing numbers over the years, the clusters were not effective in reducing roadkill, likely due to innapropriated fencing and lack of maintenance. In Chapter III, I used individual-based models to simulate how landscape structure and movement traits influence the aggregation of wildlife crossings on roads. I identified variations in crossing aggregation, with higher aggregation in landscapes with intermediate amounts of habitat or few habitat patches and in simulations with low matrix permeability or animals with greater field of view (perceptual range or vision angle). The findings of this thesis enhance the understanding of mitigation at different phases of roads and railways, providing valuable insights for future decision-making. Advancing knowledge on mitigation measures is crucial to facilitate their implementation and improve their effectiveness in reducing the impacts of linear infrastructures on wildlife.

Keywords: roadkill, animal entrapment, animal movement, connectivity, mitigation effectiveness.

Sumário

Resumo **6**

Abstract **8**

Introdução Geral **10**

Impactos das infraestruturas lineares na fauna *10*

Medidas de mitigação de impactos *10*

Disponibilidade de dados e a estrutura dessa tese *11*

Capítulo I **14**

Experimental evaluation of the use of trenches by chelonians on railways *15*

Capítulo II **27**

Increased crossing structures use does not mean a decrease in roadkill numbers *28*

Capítulo III **53**

Prioritising road mitigation efforts based on landscape structure and species navigation traits: insights from mechanistic modelling *54*

Considerações Finais **84**

Referências **87**

Introdução Geral

Impactos das infraestruturas lineares na fauna

A fatalidade de animais silvestres, principalmente por colisões entre veículos e fauna, é um dos impactos mais antigos a serem documentados e um dos mais visíveis (van der Ree *et al.*, 2011), sendo uma das principais causas antropogênicas de mortalidade direta de vertebrados (Hill; DeVault; Belant, 2019). Além disso, em ferrovias, também já foram registradas mortes por barotrauma e desidratação, oriundas do aprisionamento de animais entre os trilhos (Budzik; Budzik, 2014; Dornas *et al.*, 2019; Kornilev; Price; Dorcas, 2006). Taxas elevadas de fatalidade podem resultar em declínio populacional, seja por mecanismos demográficos ou genéticos (Fahrig; Rytwinski, 2009; Holderegger; Di Giulio, 2010).

Além das fatalidades, as infraestruturas lineares funcionam como uma barreira ao movimento dos animais e reduzem a permeabilidade do ambiente, seja pelo evitamento das estradas ou pela impossibilidade de atravessá-las de maneira segura (Ascensão *et al.*, 2019; Rytwinski; Fahrig, 2015; Smith; Van Der Ree; Rosell, 2015). As rodovias são reconhecidas como um importante fator de mudança no comportamento e nos padrões de movimento de diferentes animais (Doherty; Hays; Driscoll, 2021; ig *et al.*, 2023). O grau de inibição da movimentação não é homogêneo entre as espécies (Assis; Giacomini; Ribeiro, 2019) e os diferentes componentes do movimento animal, como o alcance da percepção e a permeabilidade da paisagem, variam de acordo com o ambiente em que os animais se encontram (Lima; Zollner, 1996; Nathan *et al.*, 2008; Zeller; McGarigal; Whiteley, 2012). Alterações no movimento podem afetar as taxas reprodutivas, o fluxo gênico, a probabilidade de sobrevivência e outros processos populacionais (Cosgrove; McWhorter; Maron, 2018; Fahrig, 2007).

Medidas de mitigação de impactos

Visando reduzir os impactos das rodovias e ferrovias, diferentes estratégias de mitigação têm sido implementadas ao redor do mundo. Entre elas estão: placas sinalizadoras, refletores luminosos, sistemas de detecção de fauna, cercas bloqueadoras e estruturas de travessia de fauna superiores e inferiores (Glista; DeVault; DeWoody, 2009; Van Der Ree; Smith; Grilo, 2015). Entretanto, a efetividade em atingir o objetivo de

mitigação proposto varia entre as medidas (Rytwinski *et al.*, 2016; Soanes *et al.*, 2024). Sabe-se que as cercas são efetivas em reduzir as colisões entre veículos e animais (Rytwinski *et al.*, 2016), enquanto as estruturas de travessia, popularmente conhecidas como passagens de fauna, possibilitam o deslocamento seguro entre os lados da estrada (Soanes *et al.*, 2024).

Apesar da necessidade de minimizar ao máximo os impactos das estradas, os recursos financeiros disponíveis são limitados, e algumas decisões precisam ser tomadas de forma a priorizar os tipos de medida de mitigação e os locais para a sua implementação. Assim, o conhecimento sobre a efetividade de medidas já implantadas é fundamental para que os recursos aplicados em novas instalações não sejam desperdiçados e sejam escolhidas as soluções com a melhor relação custo-benefício (van der Grift *et al.*, 2013). Por conta disso, é importante identificar se as medidas estão atingindo os objetivos propostos no seu planejamento e que melhorias são necessárias para maximizar seu desempenho. Por exemplo, cercas comumente utilizadas nas estradas não bloqueiam mamíferos de pequeno porte e anfíbios e precisam ser adaptadas (Conan *et al.*, 2022), já a instalação de passarelas secas em dutos de água pode ampliar seu uso pela fauna (Villalva *et al.*, 2013).

Em uma rodovia, desde seu planejamento até a operação, existem diferentes oportunidades para a redução dos impactos. A Hierarquia da Mitigação é uma ferramenta de boas práticas que estabelece uma sequência nas possibilidades de intervenção para a redução de impactos ambientais identificadas como evitar, minimizar, recuperar e compensar (Ekstrom; Bennun; Mitchell, 2015). Além das ações mitigadoras serem planejadas e executadas nessa ordem de prioridade, o avanço para a etapa seguinte só acontece quando se esgotarem as alternativas da etapa em análise (Arlidge *et al.*, 2018). Para exemplificar, quando uma rodovia ainda está na fase de planejamento do seu traçado, podem ser criadas rotas alternativas a fim de evitar fragmentar uma mancha de vegetação importante naquela paisagem. Quando o evitamento já não é mais uma possibilidade, como em uma rodovia já operante em que foi identificado um local de agregação de cruzamento de animais, podem ser implementadas estruturas de travessia a fim de minimizar o impacto na movimentação animal acompanhadas de cercas para a redução da mortalidade.

Disponibilidade de dados e a estrutura dessa tese

Dadas as diferentes oportunidades existentes relacionadas à mitigação durante as fases de uma infraestrutura linear, distintas abordagens e fontes de dados podem ser utilizadas no planejamento e na avaliação das medidas, buscando também a otimização dos recursos disponíveis. Sem uma avaliação adequada, os recursos podem ser utilizados de maneira ineficiente, resultando na instalação de medidas que não são efetivas como acreditávamos (van der Grift *et al.*, 2013). Antes da proposição de uma medida de mitigação, é crucial antecipar se ela tem potencial para atingir o objetivo de minimizar o impacto para o qual ela está sendo planejada (Rytwinski *et al.*, 2015). A realização de experimentos permite a avaliação da performance potencial das medidas em mitigar o impacto em questão, por ser realizada em condições específicas, controlando influências externas (van der Ree *et al.*, 2015).

Em muitos casos, há situações em que se almeja avaliar o desempenho da mitigação depois que ela já foi implementada. Caso a medida se mostre ineficiente, é necessário compreender o porquê e buscar adaptações para qualificá-la (Smith; Van Der Ree; Rosell, 2015). Para realizar essa avaliação, deve-se obter dados que reflitam a efetividade do sistema ao objetivo proposto (van der Grift *et al.*, 2013), como avaliar o número de colisões entre veículos e animais quando se almeja reduzir as fatalidades de fauna. Além disso, devem ser utilizados desenhos amostrais apropriados para o contexto que será avaliado (Rytwinski *et al.*, 2015), como o uso do desenho controle-impacto quando não se tem informações anteriores a instalação de uma estrutura de mitigação.

Por outro lado, na etapa anterior à construção de novas infraestruturas lineares, em geral, não há informação prévia sobre a movimentação dos animais na paisagem, tampouco sobre as fatalidades. Modelos computacionais, como os Modelos Baseados em Indivíduos, podem ser utilizados para simulação de diferentes paisagens e espécies (Grimm; Railsback, 2005), antecipando a potencial efetividade ou não de determinadas medidas ou identificando sob quais condições uma medida é provavelmente mais efetiva. No contexto de rodovias, as simulações vêm sendo aplicadas tanto para predizer os locais de colisão entre veículos e animais (Quaglietta; Porto; Ford, 2019) quanto para avaliar a potencial efetividade de medidas de mitigação (Ascensão *et al.*, 2013).

O objetivo nessa tese foi explorar a experimentação, a observação e a simulação como abordagens para gerar evidências para o suporte do planejamento e a avaliação de medidas mitigadoras de impactos de ferrovias e rodovias sobre a fauna. No capítulo I tive como objetivo avaliar o uso de canaletas para evitar o aprisionamento de animais em ferrovias. A observação de mortes de quelônios mesmo em locais com pouco tráfego de

trens motivou, juntamente com concessionárias ferroviárias, a busca por mitigação para esse impacto. Assim, por meio de experimentos comportamentais, avaliei o sucesso e a rejeição das canaletas para diferentes espécies de quelônios. No capítulo II, avaliei o efeito de sistemas de mitigação compostos por cercas e passagens de fauna. O acesso aos dados de monitoramento das mortes na rodovia e de cruzamentos em estruturas de travessia, disponibilizados pela concessionária responsável pela rodovia, me permitiu avaliar as mudanças no número de registros observados ao longo do tempo e a efetividade dos sistemas na redução da mortalidade. Por fim, no capítulo III busquei compreender como a estrutura da paisagem influencia o cruzamento dos animais nas estradas. A partir de simulações computacionais de modelos baseados em indivíduos espacialmente explícitos, identifiquei quais características da paisagem e da movimentação animal resultam em maior agregação de cruzamentos nas estradas e discutimos como essa abordagem e as informações resultantes podem ser utilizadas para orientar a localização de medidas que mantenham a conectividade ou reduzam a mortalidade de fauna.

Capítulo I

Experimental evaluation of the use of trenches by chelonians on railways

Esse capítulo será submetido a revista "*Ecological Engineering*" e foi realizado em colaboração com Tatiane Moreira, André Soller, Andreas Kindel, Talita Menger, André Moreira Ramos, George Rangel, Leandro Brenner Fernandes, Luana Gobbo Mamede, Nathan Sarmento, Patricia Ruth Ribeiro, Sheron Sanches Sierakowski, Stefani Gabrieli Age Vailati e Fernanda Zimmermann Teixeira.

Experimental evaluation of the use of trenches by chelonians on railways

Abstract

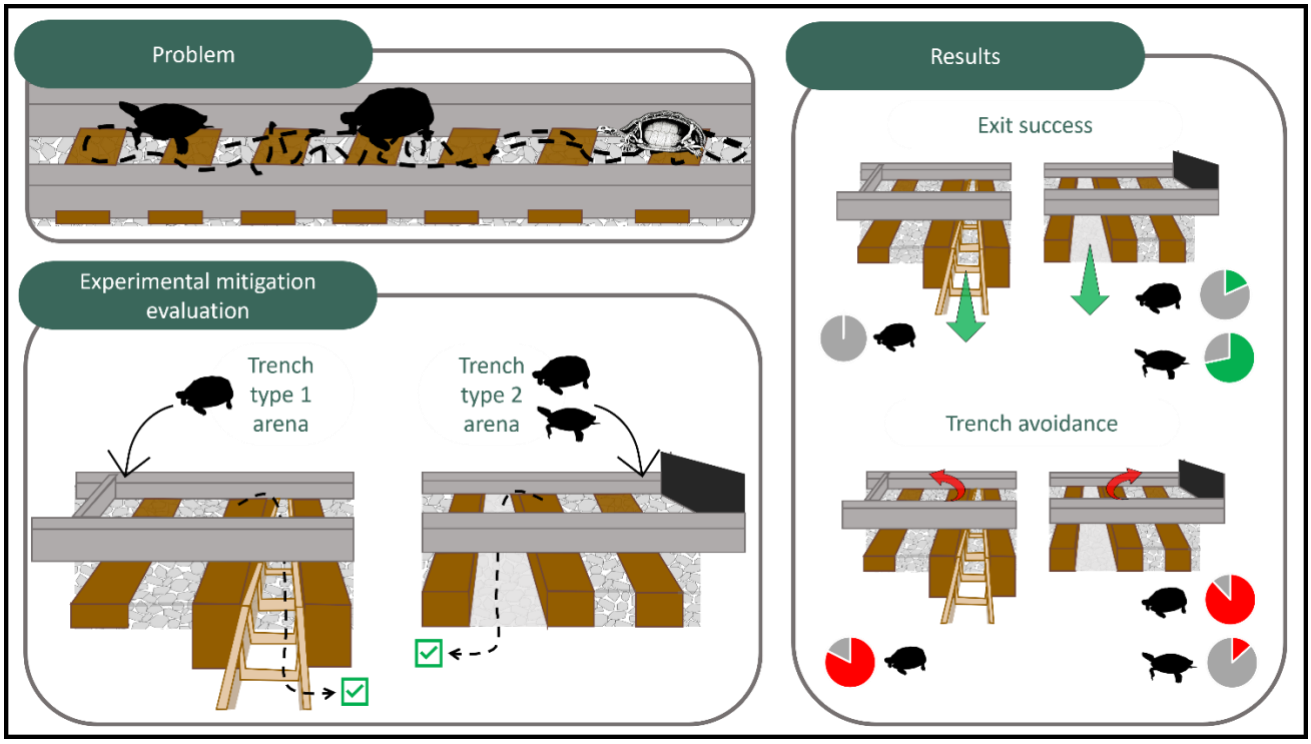
Railways can act as traps for some animals, such as amphibians and chelonians, that can become trapped between the rails and end up dying from overheating and dehydration, or barotrauma. Fences are expensive to implement and maintain, and one potential measure to prevent chelonian entrapment within rails involves creating trenches between the railway ties. In Brazil, railway companies have reported chelonian fatalities, even in places with minimal or no train traffic. Here, we aimed to evaluate the use of two trench types to prevent chelonian entrapment by allowing animals to escape under the rails. We used experimental arenas to test escape success and trench avoidance in individuals of Red-footed tortoise and Black-bellied slider, species with contrasting habitat and movement capacities. Our results showed significant differences between these two species. Sliders successfully used the type 2 trench as an exit route out of tracks, while tortoises did not frequently use either trench type and showed higher avoidance to them. These findings indicate that trench use for escape may depend on species-specific behaviors and morphology, which emphasizes the importance of evaluating mitigation measures for each target species. Furthermore, trenches may serve as dual function to prevent entrapment if installed on both sides of level crossings: as escape route, allowing species to exit the rails, or as barriers, guiding them back to their previous location. This measure offers potential benefits for conservation, and further research is needed to provide valuable evidence for broader application across species and taxa that also face entrapment issues.

Keywords: entrapment, tortoises, freshwater turtles, mitigation measures.

Highlights

- Trenches allowed the sliders to exit rails
- Tortoises presented high trench avoidance behavior
- Tortoises are more likely to be trapped between rails than sliders
- Trench is a simple and inexpensive mitigation for railway entrapment

Graphical abstract



1. Introduction

Studies on railway ecology are still less frequent than those on road ecology, probably because railways are less visible to the public (Popp and Boyle, 2017). Although understudied, railways impact wildlife similarly to roads, with collisions being the primary cause of fatalities of large mammals on railways (Santos et al., 2017). Most studies on animal mortality on rails focus on this group (Santos et al., 2017), but railways also substantially affect other species (Dasoler et al., 2020; Dornas et al., 2019; Godinho et al., 2017; Popp and Boyle, 2017). Beyond collisions, railways have been suggested to act as a trap for animals. Amphibians and chelonians can become trapped between the rails and end up dying from overheating and dehydration, or barotrauma (Budzik and Budzik, 2014; Dornas et al., 2019; Kornilev et al., 2006).

Chelonians have limited body flexibility due to their carapace and plastron, which exacerbates the difficulty of climbing over the rails to escape (Engeman et al., 2007; Kornilev et al., 2006; Rautsaw et al., 2018). Different species have been reported to be trapped between the rails, including Eastern Box Turtles (*Terrapene carolina*), Gopher Tortoises (*Gopherus polyphemus*), Chicken Turtles (*Deirochelys reticularia*), Florida Softshells (*Apalone ferox*), Indian Flapshell Turtles (*Lissemys punctata)* and Indian Black Turtles (*Melanochelys trijuga*) (Abinesh et al., 2022; Kornilev et al., 2006; Rautsaw et

al., 2018). Entrapments might occur when the chelonians access the interior of the tracks through a gap below the railroad ties or on level crossings but fail to exit them (Helms and Stains, 1966; Kornilev et al., 2006). Entrapment then contributes to mortality and restricts their movement patterns (Heske, 2015; Iosif, 2012; Kornilev et al., 2006).

With the aim of reducing fatalities in linear infrastructure, mitigation measures, such as fences that prevent animal access to the infrastructure, have been proposed for roads and railways (Carvalho et al., 2017; Rytwinski et al., 2016). Fences are expensive for implementation and maintenance and other specific solutions to prevent entrapment of chelonians within railway rails remain underexplored. One potential measure involves excavating the ballast between the railway ties to create trenches (Dorsey et al., 2015), which allow animals to cross below both sides of the track (Pelletier et al., 2005; Rautsaw et al., 2018). Although the trenches have been tested with Gopher Tortoises (Rautsaw et al., 2018) and Spotted Turtles (Pelletier et al., 2005) for use as underpasses (to allow connectivity between both sides of the tracks), this type of mitigation has not been tested as an escape measure for trapped chelonians.

In Brazil, railway companies have reported chelonian fatalities, mainly involving Red-footed tortoise (*Chelonoidis carbonarius*) and Black-bellied slider (*Trachemys dorbigni*), even in places with minimal or no train traffic (Moreira and Soller, personal observation). This indicates that chelonians are likely facing entrapment in various railway sections, with access to the tracks potentially facilitated by level crossings. Our aim was to evaluate the use of two trench types designed to mitigate chelonian entrapment by allowing animals to escape under the rails. We used experimental arenas to test the escape success and trench avoidance of two species of chelonians with contrasting habitat selection and movement capacities, the Red-footed tortoise (*Chelonoidis carbonarius*) and Black-bellied Slider (*Trachemys dorbigni*).

2. Methods

2.1 Trench types

Type 1 is a wooden-structured trench with a slightly convex design, sloping from the center toward the edges of the rails (Fig. 1A). It is designed to guide individuals down a ramp while preventing them from climbing back into the tracks. Its dimensions and sloped design, combined with the wooden structure, ensure both animal possibility to exit and the robustness needed to withstand track forces. The inner part of the wooden trench is 19 cm wide, and the trench height starts at 10 cm in the middle of the ramp and

gradually increases to 38 cm at the end. Type 2 consists of a straight, uniform trench created below the rails by removing the ballast down to the base level of the railroad ties (Figure 1B). Type 2 has an average width between the railroad ties of 36 cm and an average height of 18 cm. Both trenches function as pitfalls, preventing animals from returning to the tracks after falling in them.

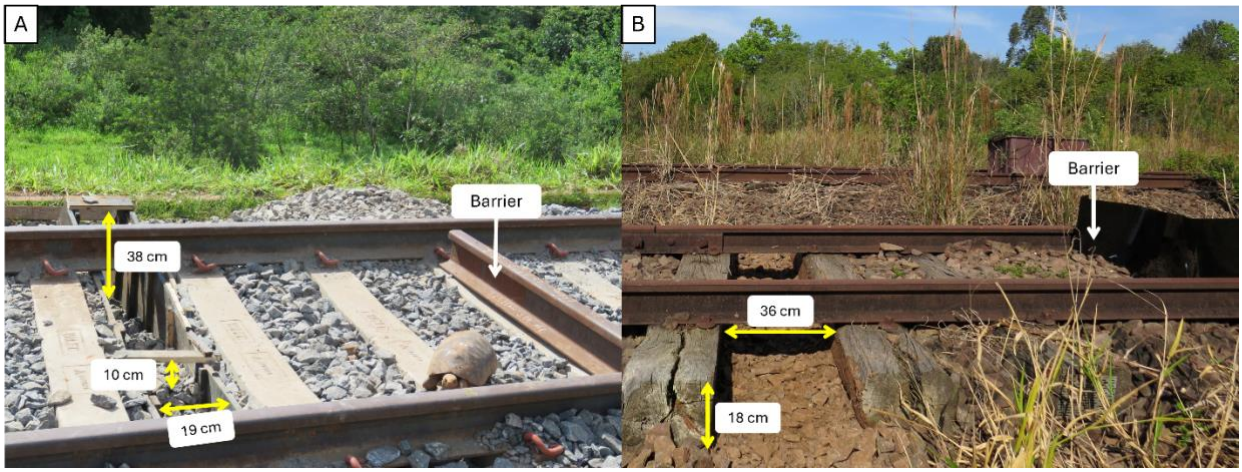


Figure 1. The two types of trenches that were experimentally tested. Type 1 on the left side (A) and type 2 on the right side (B).

2.2 Experiments

2.2.1 Species studied and general description of experiments

The Red-footed tortoise (*Chelonoidis carbonaria*) is a terrestrial tortoise distributed throughout Brazil except in the southern region, with a large dome-shaped carapace (Guedes et al., 2023; Wang et al., 2011). Its habitat involves forested and open areas, including the transition zone, and they are often found looking for shelter in burrows (Pingleton, 2006).

The Black-bellied slider (*Trachemys dorbigni*) is a freshwater turtle that occurs exclusively in the extreme southern region of Brazil, with an elongated and moderately convex carapace (Bujes, 2010; Guedes et al., 2023). Individuals inhabit aquatic environments such as lagoons and wetlands, and are frequently observed basking on logs, riverbanks, and floating vegetation, mainly to thermoregulate (Bujes, 2010). The basking behavior might be affected by other species or anthropogenic disturbances (Moore and Seigel, 2006; Rocha, 2005).

We choose these two species of chelonians to be tested on the trenches, as they are commonly found dead between tracks (Moreira and Soller, personal observations). The experiments were conducted in non-operating railway sections, selected according

to the availability of a research team and the possibility of borrowing the animals for the trials.

The individuals used in the experiments were borrowed from the Sapucaia do Sul Zoo, the Center for Coastal, Limnological, and Marine Studies (CECLIMAR), and the Wildlife Rescue and Rehabilitation Center (CETAS - Goiânia). The experiments were approved by the Ethics Committee of the Federal University of Rio Grande do Sul (process numbers: 35395 and 42333) and all guidelines provided by the responsible veterinarians were strictly followed.

We built experimental arenas with a trench on one end and a barrier on the other (Fig. 1). In experiments of type 1 trench, we used a piece of rail as the end barrier with a small gap between rails. Whereas in type 2 the end barrier was made of High-Density Polyethylene (HDPE) with 30 cm height and extended between rails. Each chelonian individual was released three times close to the barrier end. In each trial we recorded the individual's behavior. Each trial ended when the individual left the arena, or after a maximum of 15 minutes if the animal was unable to escape. In both experiments we recorded if or how often the individuals reached the railroad tie adjacent to the trench, if or how often they avoided the trench once they reached the tie and how individuals escape the tracks, if that happened.

2.2.2 Type 1 experiment

The experiment was carried out in Goiânia, Central-West Brazil, between November 27 and 29, 2018. The mean temperatures for these days were 24.6°C, 25.6°C, and 25.7°C, respectively. To test the type 1 trench, two arenas were built with a mean length of 120 cm. Due to transport and license logistics, we tested 19 individuals of Red-footed Tortoise; seven were tested on the first day, six on the second and the remaining six on the last day, in a total of 57 trials. The mean length of the carapace of these individuals was 33.11 cm (± 9.08 cm), and their mean height was 14.32 cm (± 4.25 cm) (detailed information about individuals on Supplementary Material 1).

2.2.3 Type 2 experiment

The experiment was conducted in Estrela, Southern Brazil, on August 23, 2022, and on May 23, 2023. The mean temperatures for these days were 15.5°C and 19.9°C, respectively. To test the type 2 trench, seven arenas were built with a mean length 160 cm. On the earlier date, we tested 20 individuals of Black-bellied slider, in a total of 60 trials. These individuals had a mean carapace length of 23.62 cm (± 3.71 cm) and a mean

height of 9.40 cm (± 1.82 cm) (detailed information about individuals on SM 1). On the latter date, we tested 11 individuals of Red-footed tortoise, in a total of 33 trials. These individuals had a mean carapace length of 34.9 cm (± 7.2 cm) and a mean height of 12.79 cm (± 2.74 cm). The differences in the number of individuals were due to the availability of animals in captivity to be borrowed from the institutions.

2.2.4 Arenas without trenches

To understand if these species could escape the rails in the absence of trenches, we also conducted experiments in arenas without trenches on May 23, 2023, at the Estrela site. We tested five individuals of each species to evaluate their behavior when moving in the arenas. These arenas were rectangular, with two sides delimited by the HDPE barrier and the other two by the rails, having a mean width of 175 cm between the barriers. The individuals of the Black-bellied slider tested had a mean carapace length of 24.84 cm (± 2.27 cm) and a mean height of 11.78 cm (± 0.47 cm), while the individuals of the Red-footed tortoise had a mean carapace length of 32 cm (± 9.28 cm) and a mean height of 11.24 cm (± 2.88 cm) (detailed information about individuals on SM 1). We recorded whether each individual escaped the arenas by climbing over the rails or if they were unable to leave.

3. Results

In the 57 trials conducted with Red-footed tortoises in the type 1 trench, only one individual entered the trench (Fig. 2). However, this individual got stuck in the trench because its body width exceeded the trench width, impeding it from escaping. In seven of the 57 trials, individuals escaped through the small gap in the experimental rail barrier from our arenas, and in one trial climbed over the rails.

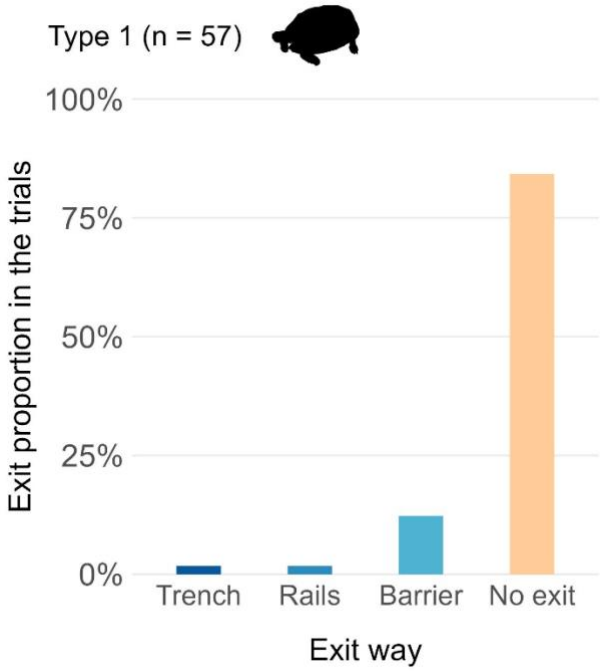


Figure 2. Proportion of the different exit ways from the arenas in the type 1 trench trials conducted with Red-footed tortoise individuals.

In the 60 trials conducted with Black-bellied sliders in the type 2 trench, most of the individuals successfully escaped from the arena through the trench (43 trials), but some of them also escaped by climbing over the rails (12 trials; Fig. 3A). In contrast, from the 33 trials of the type 2 trench experiment carried out with Red-footed tortoises, they were unable to escape in 24. They exited the tracks via the trench in only six trials, and in other 3 trials individuals crossed the HDPE barrier or climbed over the rails (Fig. 3B).

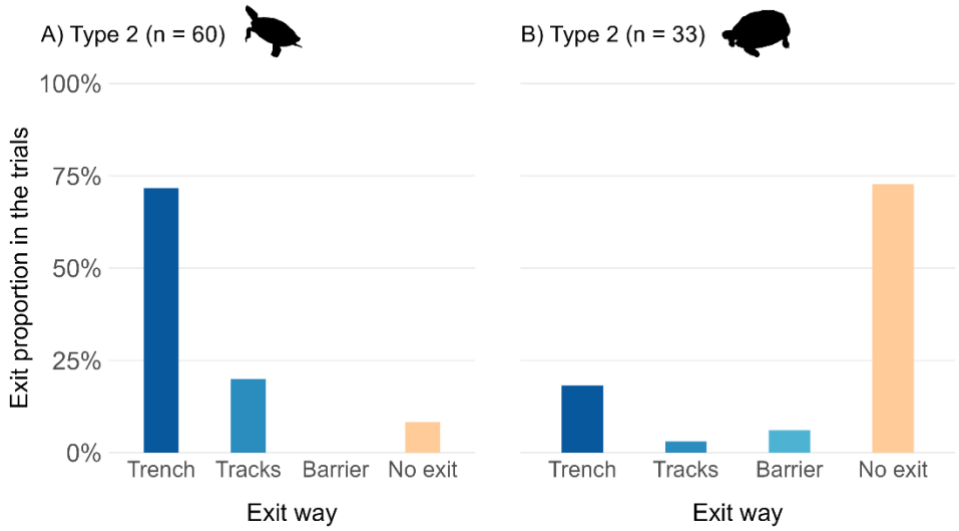


Figure 3. Proportion of the different exit ways from the arenas in the type 2 trench trials conducted with (A) Black-bellied sliders and (B) Red-footed tortoises.

Irrespective of the trench type, most tortoises reached the edge of the railroad tie adjacent to the trench during the trial, but almost all avoided jumping into the trench (Fig. 4). In type 2 trench experiments, the individual tortoises reached the railroad tie adjacent to the trench up to eight times (SM2), indicating multiple attempts to escape and repeated avoidance of the trench behavior. In contrast, the sliders reached the railroad tie fewer times (up to three; SM2), clearly not avoiding the trench. However, in some trials, neither tortoises nor sliders did not reach the tie at all (Fig. 4).

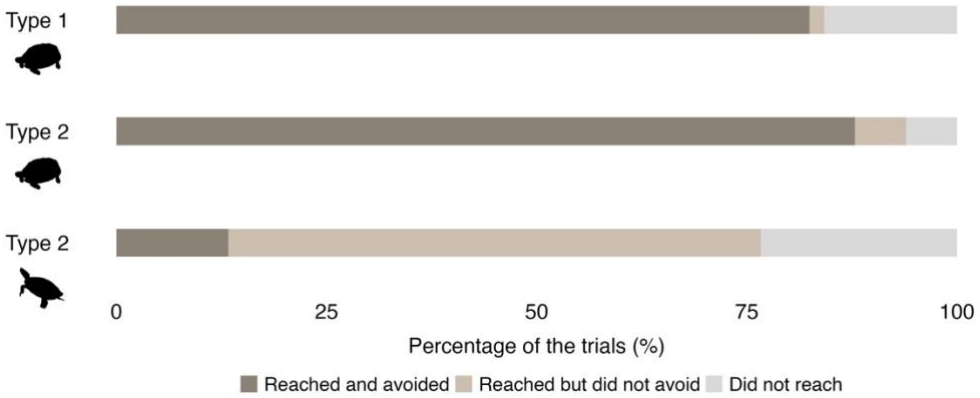


Figure 4. Proportion of trials of each species in which individuals reached or did not reach the railroad ties adjacent to the two types of trenches, and whether they avoided or did not avoid the trenches. From top to bottom: Red-footed tortoise on type 1 trench, Red-footed tortoise on type 2 trench and Black-bellied sliders on type 2 trench.

In the 30 trials conducted in the arenas without trenches, we observed contrasting results for each species. Sliders climbed over the rails in almost all trials (13 out of 15 trials), whereas Red-footed tortoises were unable to exit the arenas in all trials.

4. Discussion

We found contrasting results for both species in the experiments evaluating trench use. Black-bellied sliders successfully used type 2 trenches as an exit route out of railway entrapment, while for Red-footed tortoises neither trench type was frequently used to escape the tracks. The trench avoidance behavior, highly observed between tortoises, clearly demonstrates that this kind of mitigation will not be effective for this terrestrial species.

Differences of trench effectiveness between both species clearly resulted from the strong observed differences in the proportion of trench avoidance. Tortoises exhibited higher avoidance despite reaching the railroad tie adjacent to the trench more often compared to sliders on type 2 trench trials. This difference could probably be attributed to behavior differences between the two species. Emydidae turtles have the common behavior of sliding into the water when disturbed or threatened, interrupting their basking (Moore and Seigel, 2006). This may explain why most sliders readily entered the trenches upon facing it, using them as escape routes. In contrast, the use of trenches by tortoises is apparently constrained by plastron morphology or their willingness to slide as this behavior may not be within their repertoire. The observed reluctancy of red-footed tortoises to walk in or through trenches also highlights that analogous structures as railway drainages (like concrete ditches) may be an additional barrier for tortoise species, a potential overlooked impact of those infrastructures (Barrientos and Borda-de-Água, 2017).

Regarding the ability to climb over rails, the two species also showed distinct behaviors. Sliders showed a surprising capacity to climb, consistent with findings of a study of another freshwater turtle, the Eastern long-necked turtle (*Chelodina longicollis*), which was recorded crossing over many obstacles (Dowling et al., 2024). However, in some of our trials, some form of support was necessary for them to climb, such as ballast accumulation or rail-fastening components like bolts or Pandrol clips. In contrast, tortoises were unable to climb over the rails in all trials of arenas without trenches. Although few of them attempted to climb, they could not pull themselves over the rails. The ability of tortoises to climb varies among tortoise species; some of them seemed

unable to escape over the rails (Rautsaw et al., 2018), while others have been recorded climbing with a foothold in arenas with corners (Ruby et al., 1994). Interestingly, during type 2 trials, we observed some individuals trying to climb over the rails with the aid of the barrier, although they were unable to exit tracks.

Our findings emphasize the importance of evaluating mitigation measures for each target species, given that differences in behavior and morphological traits could strongly influence the outcomes. Escape routes like trenches may be effective, at least for some species, to prevent chelonian entrapment, potentially reducing their mortality, although this remains to be demonstrated. Type 2 trenches may also work as a crossing structure between railway sides, as observed for Gopher tortoises (Rautsaw et al., 2018) and Spotted turtles (Pelletier et al., 2005), although we did not evaluate this aspect. Furthermore, trenches should be considered particularly at level crossings with rural or minor roads, such type 2 trench, could serve a dual function in preventing entrapment if installed on both sides of level crossings. They can act as escape routes, allowing species such black-bellied slider to exit the rails, or as barriers, guiding species such red-footed tortoise back to their previous location. To our knowledge this kind of solution has not yet been tested and is strongly necessary. Furthermore, these measures should be tested for other chelonian species and extended to other taxa, such as amphibians, which also face entrapment issues (Budzik and Budzik, 2014; Dornas et al., 2019). These efforts will provide valuable evidence for the broader applicability of such mitigation measures for various species and habitats.

Acknowledgements

We sincerely thank the Sapucaia do Sul Zoo, the Center for Coastal, Limnological, and Marine Studies (CECLIMAR), and the Wildlife Rescue and Rehabilitation Center (CETAS - Goiânia) for providing the Red-footed tortoise and Black-bellied slider individuals for our experiments. We also thank Ismael V. Brack for his assistance with the type 2 trench experiments and Marina Zanin, Sara Santos, and Vinicius Alberici for their suggestions in a previous version of this manuscript which helped us to improve it.

Data availability

The data of trenches experiments, and all codes of analyses and figures are available at https:// https://github.com/bibianadasoler/Trenches_chelonians_railways.

References

Abinesh, A., Maharaja, R., Vishnu, C.S., 2022. Mortality of freshwater turtles on a railway track in Puducherry, India. Reptiles & Amphibians 29, 345–347. https://doi.org/10.17161/randa.v29i1.17589

Barrientos, R., Borda-de-Água, L., 2017. Railways as Barriers for Wildlife: Current Knowledge, in: Railway Ecology. Springer International Publishing, Cham, pp. 43–64. https://doi.org/10.1007/978-3-319-57496-7_4

Budzik, K.A., Budzik, K.M., 2014. A preliminary report of amphibian mortality patterns on railways. Acta Herpetol 9, 103–107. https://doi.org/10.13128/Acta_Herpetol-12914

Bujes, C.S., 2010. Os Testudines continentais do Rio Grande do Sul, Brasil: taxonomia, história natural e conservação. Iheringia Ser Zool 100, 413–424. https://doi.org/10.1590/S0073-47212010000400016

Carvalho, F., Santos, S.M., Mira, A., Lourenço, R., 2017. Methods to Monitor and Mitigate Wildlife Mortality in Railways, in: Railway Ecology. Springer International Publishing, Cham, pp. 23–42. https://doi.org/10.1007/978-3-319-57496-7_3

Dasoler, B.T., Kindel, A., Beduschi, J., Biasotto, L.D., Dornas, R., Gonçalves, L.O., Lombardi, P.M., Menger, T., de Oliveira, G.S., Teixeira, F.Z., 2020. The need to consider searcher efficiency and carcass persistence in railway wildlife fatality studies. Eur J Wildl Res 66, 81. https://doi.org/10.1007/s10344-020-01417-7

Dornas, R., Teixeira, F.Z., Gonsioroski, G., Nóbrega, R.A.A., 2019. Strain by the train: Patterns of toad fatalities on a Brazilian Amazonian railroad. Science of the Total Environment 660, 493–500. https://doi.org/10.1016/j.scitotenv.2018.12.371

Dorsey, B., Olsson, M., Rew, L.J., 2015. Ecological Effects of Railways on Wildlife, in: van der Ree, R., Smith, D., Grilo, C. (Eds.), Handbook of Road Ecology. Wiley Blackwell West Sussex, pp. 219–227. https://doi.org/10.1002/9781118568170.ch26

Dowling, J.M., Bower, D.S., Nordberg, E.J., 2024. Ninja turtles: an experimental evaluation of potential anthropogenic barriers to movement for a freshwater turtle. J Zool. https://doi.org/10.1111/jzo.13236

Engeman, R., Smith, H., Kaufman, G., 2007. Gopherus polyphemus (Gopher Tortoise) Mortality. Herpetol Rev 38, 331–332.

Godinho, C., Marques, J.T., Salgueiro, P., Catarino, L., de Castro, C.O., Mira, A., Beja, P., 2017. Bird Collisions in a Railway Crossing a Wetland of International Importance (Sado Estuary, Portugal), in: Railway Ecology. Springer International Publishing, Cham, pp. 103–115. https://doi.org/10.1007/978-3-319-57496-7_7

Guedes, T., Entiauspe-Neto, O., Costa, H., 2023. Lista De Répteis Do Brasil: Atualização De 2022. Sociedade Brasileira de Herpetologia 12.

Helms, D., Stains, H., 1966. Kill of mammals and birds and entrapment of turtles on railroad tracks. Transactions of the Illinois Academy of Sciences 59, 297.

Heske, E.J., 2015. Blood on the Tracks: Track Mortality and Scavenging Rate in Urban Nature Preserves. Urban Naturalist 4, 1–13.

310 Iosif, R., 2012. Railroad-Associated Mortality Hot Spots for A Population of Romanian
311 Hermann's Tortoise (Testudo Hermanni Boettgeri): A Gravity Model for Railroad-
312 Segment Analysis. Procedia Environ Sci 14, 123–131.
313 https://doi.org/10.1016/j.proenv.2012.03.012
314 Kornilev, Y. V., Price, S.J., Dorcas, M.E., 2006. Between a rock and a hard place:
315 Responses of eastern box turtles (Terrapene carolina) when trapped between
316 railroad tracks. Herpetol Rev 37, 145–148.
317 Moore, M.J.C., Seigel, R.A., 2006. No place to nest or bask: Effects of human
318 disturbance on the nesting and basking habits of yellow-blotched map turtles
319 (Graptemys flavimaculata). Biol Conserv 130, 386–393.
320 https://doi.org/10.1016/j.biocon.2006.01.001
321 Pelletier, S.K., Carlson, L., Nein, D., Roy, R.D., 2005. Railroad crossing structures for
322 spotted turtles: Massachusetts Bay Transportation Authority–Greenbush rail line
323 wildlife crossing demonstration project.
324 Pingleton, M., 2006. Red-footed tortoises: natural history, behavior and their influences
325 on captive care.
326 Popp, J.N., Boyle, S.P., 2017. Railway ecology: Underrepresented in science? Basic
327 Appl Ecol 19, 84–93. https://doi.org/10.1016/j.baae.2016.11.006
328 Rautsaw, R.M., Martin, S.A., Vincent, B.A., Lanctot, K., Bolt, M.R., Seigel, R.A.,
329 Parkinson, C.L., 2018. Stopped Dead in Their Tracks: The Impact of Railways on
330 Gopher Tortoise (Gopherus polyphemus) Movement and Behavior. Copeia 106,
331 135–143. https://doi.org/10.1643/ce-17-635
332 Rocha, D.F.N. de B., 2005. Biologia termal das tartarugas Trachemys dorbigni (Duméril
333 & Bibron, 1835) e Trachemys scripta elegans (Wied, 1839) dos lagos de Porto
334 Alegre, RS, Brasil (Testudines, Emydidae).
335 Ruby, D.E., Spotila, J.R., Martin, S.K., Kemp, S.J., 1994. Behavioral Responses to
336 Barriers by Desert Tortoises: Implications for Wildlife Management.
337 Herpetological Monographs 8, 144. https://doi.org/10.2307/1467078
338 Rytwinski, T., Soanes, K., Jaeger, J.A.G., Fahrig, L., Findlay, C.S., Houlahan, J., Van
339 Ree, R. Der, Van Der Grift, E.A., 2016. How effective is road mitigation at
340 reducing road-kill? A meta-analysis. PLoS One 11, 1–25.
341 https://doi.org/10.1371/journal.pone.0166941
342 Santos, S.M., Carvalho, F., Mira, A., 2017. Current knowledge on wildlife mortality in
343 railways, in: Railway Ecology. Springer, Cham, pp. 11–22.
344

Capítulo II

Increased crossing structures use does not mean a decrease in roadkill numbers

Esse capítulo está em revisão na revista "*Transportation Research Part D: Transport and Environment*" e foi realizado em colaboração com Talita Menger, Andreas Kindel, Franciane Almeida da Silva, Ingridi Camboim Franceschi, Júlio Cezar Gonçalves Leonardo, Larissa Biasotto, Larissa Oliveira Gonçalves, Ricardo Miranda Braga e Fernanda Zimmermann Teixeira.

Increased crossing structures use does not mean a decrease in roadkill numbers

Abstract

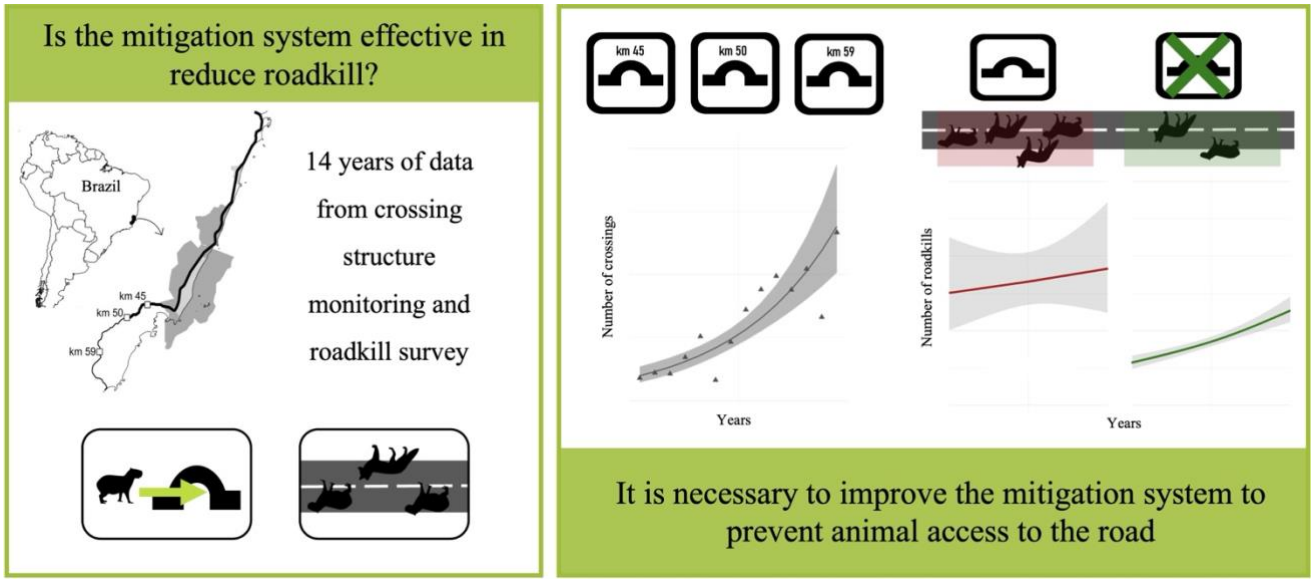
Roads significantly impact wildlife by increasing mortality and reducing connectivity. To mitigate these effects, mitigation measures like underpasses and fences are implemented worldwide. This study assesses the effectiveness of mitigation systems composed of clusters of underpasses and fences in southeastern Brazil, based on 14 years of data for ground-dwelling species. We analyzed whether crossing and roadkill records changed over the years and if roadkill hotspots were eliminated where mitigation was installed. Results showed a significant increase in underpasses use in all mitigation clusters. However, roadkill persisted at mitigated segments and remained higher than on non-mitigated segments. Roadkill hotspots overlapped all mitigation clusters but at other locations along the road. These results indicate that underpasses alone do not sufficiently reduce road mortality without appropriate fencing to prevent road access. Our findings highlight the need of well-designed mitigation strategies, reinforcing that fencing quality and extent are critical for reducing roadkill.

Keywords: mitigation measures; fences; underpasses; wildlife-vehicle collisions.

Highlights

- We used data from 14 years of daily monitoring of roadkills and underpasses
- Mitigation clusters showed an increase in the number of crossings over time
- Roadkill numbers have increased over the years, even in mitigated segments
- Roadkill hotspots overlapped mitigation locations
- Mitigation clusters are likely to fail to reduce roadkill due to absence of proper fencing

Graphical abstract



Introduction

Roads impact wildlife mainly by increasing mortality and reducing connectivity and habitat quality (Teixeira et al., 2020). Wildlife-vehicle collisions and the barrier effect of roads can limit or impede animal movement between roadsides, affecting population dynamics (Ceia-Hasse et al., 2018; Jackson and Fahrig, 2011). To minimize these impacts, different mitigation measures are being implemented around the world. For example, wildlife crossing structures have been shown to allow animal movement across roads (Soanes et al., 2024) and fences have been reported to reduce roadkill and guide individuals to these structures (Rytwinski et al., 2016).

For each road project, an evaluation of the effectiveness of mitigation is critical to assess if the measures are fulfilling their purpose. When a measure proves ineffective, it is crucial to understand the reason and explore the necessary adaptations (Smith et al., 2015). For example, the use of underpasses could be improved by adding a dry ledge (Villalva et al., 2013), while higher fence extensions are more effective than short sections to reduce roadkill (Huijser et al., 2016). Also, measurement endpoints must be carefully chosen. Roadkill numbers should also be used to assess mitigation effectiveness, because considering only the use of crossing records may not fully reflect the system's effectiveness (van der Grift et al., 2013). Therefore, the effectiveness evaluation must align with the mitigation structures adopted and their specific objectives, following the best available study designs.

Different study designs have been used to assess mitigation effectiveness, for example, control-impact (CI), before-after (BA), and before-after-control-impact (BACI)

(Christie et al., 2019). BACI is the preferable design, as it allows to control confounding factors that could influence the response variable beyond the treatment of interest (Christie et al., 2019; Roedenbeck et al., 2007). CI designs, however, are the alternative when data are unavailable, whether due to poor planning or logistic restriction. In these situations, the use of multiple temporal replicates is a recognized way to counteract some limitations of the CI design, controlling fluctuations and stochastic factors (after-control-impact cf. De Palma et al., 2018).

Here, we evaluate a mitigation system composed of three clusters of multiple underpasses and fences installed during improvements of a coastal toll highway in southeastern Brazil to reduce road mortality. We analyze the use of underpasses and roadkill data of ground-dwelling species weighing more than 1 kg collected daily over 14 years. Our aim was to assess whether the crossing and roadkill records changed over the years and if mitigation eliminated the roadkill hotspots where they were installed. We expected a gradual increase in the number of crossings, given the known time lag in habituation behavior for newly implanted mitigation measures (Bond and Jones, 2008; Clevenger and Waltho, 2003; van der Grift et al., 2013). Furthermore, if fences were effective in blocking animal access to the road, we should expect a significantly lower number of roadkill records on mitigated road segments compared to roadkill numbers on non-mitigated road segments and the disappearance of roadkill hotspots on mitigated segments (Rytwinski et al., 2016).

Materials and Methods

Study area

This study was carried out on the ES-060, a 67.5 km highway that connects the municipalities of Vitória (−20.3138°, −40.2904°) and Guarapari (−20.7394°, −40.5515°) in the state of Espírito Santo, Southeastern Brazil (Fig. 1). ES-060 crosses four protected areas, two of IUCN category V (Jacarenema and Paulo Cesar Vinha Natural Parks, World Database of Protected Areas IDs: 555576222 and 555682160) and two of IUCN category VI (Lagoa Grande and Setiba Environmental Protected Areas, World Database of Protected Areas IDs: 555576215 and 555636625) (Dudley, 2008; UNEP-WCMC and IUCN, 2024). The measured annual average daily traffic at a toll located halfway between Vitória and Guarapari (Fig. 1) was always higher than 10,000 vehicles from 2009 to 2017 (ES, 2023).

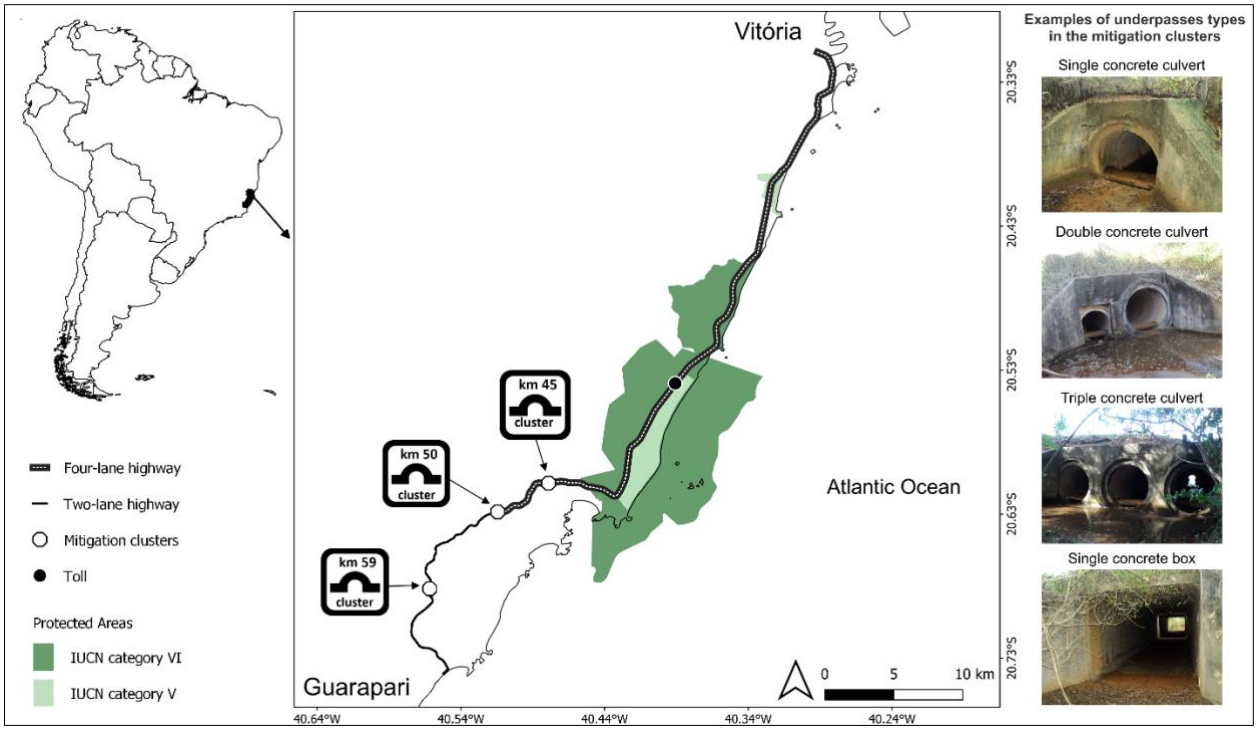


Figure 1. Location of highway attributes (toll, lane number, and mitigation clusters) and protected areas along the ES-060 highway, including examples of the underpasses.

This highway has been under a private concession (Rodovia do Sol S/A concessionaire; between 1998 and 2023), and between 2000 and 2003, two improvements were implemented. From kilometers 12 to 50, the highway was widened from two lanes to four lanes, and from kilometers 50 to 67.5 a new two-lane highway was constructed. During these constructions, culverts and bridges, expected to also function as wildlife underpasses, were installed in three locations (kilometers 45, 50 and 59), hereafter called mitigation clusters (Fig. 1). Each cluster has a different number of structures with different attributes (detailed information on each mitigation cluster is available in the Supplementary Material (SM) – Table 1). The cluster on km 45 has three kilometers of extension and is composed of a bridge and seven structures (four single, one double, and two triple culverts) installed to allow wildlife crossings and to connect remnants of restinga (coastal sandy soil vegetation) and mangroves. The cluster at km 50 has 500 meters of extension and two single concrete culverts installed primarily for drainage purposes, while the cluster at km 59 has one kilometer of extension and two structures (one single and one double concrete culvert) installed as wildlife crossings. Both clusters at km 50 and km 59 connect restinga remnants on both sides of the road. Fences were installed in mitigation clusters to guide animals to use crossing structures. At km 45, two

types of fences were implemented at both sides with 1.2 m of height: a galvanized welded fence with 15 cm horizontal wire opening, 6 lower wires with 10 cm between wires and 3 upper wires with 20 cm between wires (1,880 m on north-south roadside and 969 m on south-north roadside), and a chain link fence with 3" mesh and 14-gauge wire (length of 460 m on north-south roadside and 464 m on south-north roadside). In the other two clusters, only galvanized welded fencing (also 1.2 m in height) was installed, covering 400 m on the north-south roadside at km 50 and 800 m on both sides of road the road at km 59. Variations on fence types and extensions in each cluster were due to logistical constraints and local terrain characteristics.

Data collection

We used a dataset collected by the concessionaire after highway improvements. Data were collected in an environmental monitoring program created as part of the conditions required during the environmental permitting process of the ES-060 toll road.

To assess the use of underpasses by wildlife, sand track beds were installed at each underpass entrance with sandy clay soil to record animal tracks (SM – Fig. 1). Each sand bed was dimensioned to fit the width of the crossing structure. Eight of the 12 structures of all clusters were monitored with sand beds, checked every working day by a specialized technician between January 2004 and December 2017 (SM – Table 1). Even structures primarily set up for drainage were surveyed with sand beds, as they could also be used as a crossing structure by wildlife. For each track on each sand bed, the structure ID, date, species and movement trajectory (if inwards or outwards; SM – Fig. 1) were recorded. After checking the sand beds, their surfaces were homogenized to erase previous tracks. Crossings were considered successful only when tracks of the same species were registered at both entrances of the structure on the same occasion, indicating the same movement direction and a complete crossing (inwards at one side and outwards at the other).

Roadkill data was obtained from car surveys carried out daily by concessionaire road inspectors. Surveys covered the entire road extension every 3 hours, at a speed of 60 to 80 km/h, between January 2004 and December 2017. For each carcass, the inspector recorded the date and location (km marker on the highway every 100m). These locations were later converted to Universal Transverse Mercator coordinates for each 500 m segment of the road. All carcasses were collected and sent to the Rodovia do Sol Fauna

Laboratory, stored in freezers, and then identified by specialists to the lowest possible taxonomic level.

Data analysis

We analyzed data of ground-dwelling species larger than 1 kg, aiming to reduce detection errors in both underpasses and roadkill surveys. We considered ground-dwelling species any species that predominantly or frequently moves on the ground. We grouped the records of the species of boas (Boidae) and of armadillos (Dasypodidae) due to the possibility of misidentification of sympatric species from each family based on sand bed tracks.

We analyzed the number of crossings over time to assess whether the number of crossings varied since the mitigation structures were installed. We analyzed each cluster separately (km 45, km 50, and km 59) due to differences in the number and type of mitigation structures in each one. We used the total number of crossings recorded annually in each cluster as the response variable, considering all structures that make up each mitigation cluster.

We used the after-control-impact design (De Palma et al., 2018) to assess if roadkill numbers varied over time for mitigated and non-mitigated segments. We used the number of roadkills recorded on segments of 500 m of mitigated (impact) and non-mitigated (control) sites. Mitigated sites are the segments that encompass the mitigation clusters (n = 9 segments), while non-mitigated sites (n = 98 segments) include all other segments of the road except the segments immediately next to the mitigated clusters and the first 11 km of the road, due to high urbanization (SM – Fig. 2). We used the annual number of roadkills recorded in each segment as the response variable and the year as the predictor. Before this analysis, we performed a Moran I test and found no spatial autocorrelation for the number of roadkills in each segment (Moran I statistic = 0.041, p = 0.29).

To analyze changes in crossing and roadkill numbers over time as described above, we used Generalized Linear Models (GLMs), using Negative Binomial Distribution to deal with overdispersion. We performed the GLM analyses using the "glm.nb" function from the "MASS" package (Ripley et al., 2013) and checked the residuals using the "check_residuals" function from the "performance" package (Lüdecke et al., 2020). All analyses were performed in the R environment version 4.3.1 (R Core Team, 2023).

Furthermore, we evaluated whether roadkill hotspots spatially overlapped with mitigation clusters. We performed this analysis considering all species in the full survey time span and in three timeframes (T1– from 2004 to 2007; T2 – from 2008 to 2012; and T3 – from 2013 to 2017) to assess whether spatial patterns of roadkill were stable through time. We also individually analyzed the ten most recorded species in the roadkill and crossing surveys, which comprises 13 species in total, as some species are the most recorded in both surveys (SM – Table 2). To describe hotspot patterns, we adopted a two-step approach using the modified Ripley's K statistic followed by the 2D HotSpot Identification (Coelho et al., 2012), both analyses implemented in Siriema v.2.0 software (Coelho et al., 2014). The first analysis was used to identify whether roadkill records were significantly aggregated in space along the highway and, if so, at which spatial scales (Coelho et al., 2012). We used an initial radius of 500 m, radius increments of the 500 m, 100 simulations of random distribution of the same number of events, and a 95% confidence interval. The second analysis is used to identify the location of hotspots along the road (Coelho et al., 2012). It was only applied to the datasets that presented aggregation on the lowest scale (500 m radius) in the K statistics. For the hotspot location analysis, we used a radius of 500 m, 100 subdivisions of the highway (each segment with 675 m), 1,000 simulations of the random roadkill distribution of the same number of events, and a 95% confidence interval. We considered as hotspots all segments with a roadkill intensity value (number of observed carcasses - mean number of carcasses from simulations) higher than the upper confidence limit.

Results

During 14 years of surveys, 9,080 crossings were recorded in all mitigation clusters (detailed information about species in SM – Table 3). The cluster at km 45 presented 6,026 crossings, while the cluster at km 50 and 59 presented 643 and 2,411, respectively. As expected, we observed an increase in the number of crossings over time in each mitigation cluster ($p < 0.001$, df = 12; Fig. 2, SM – Table 4).

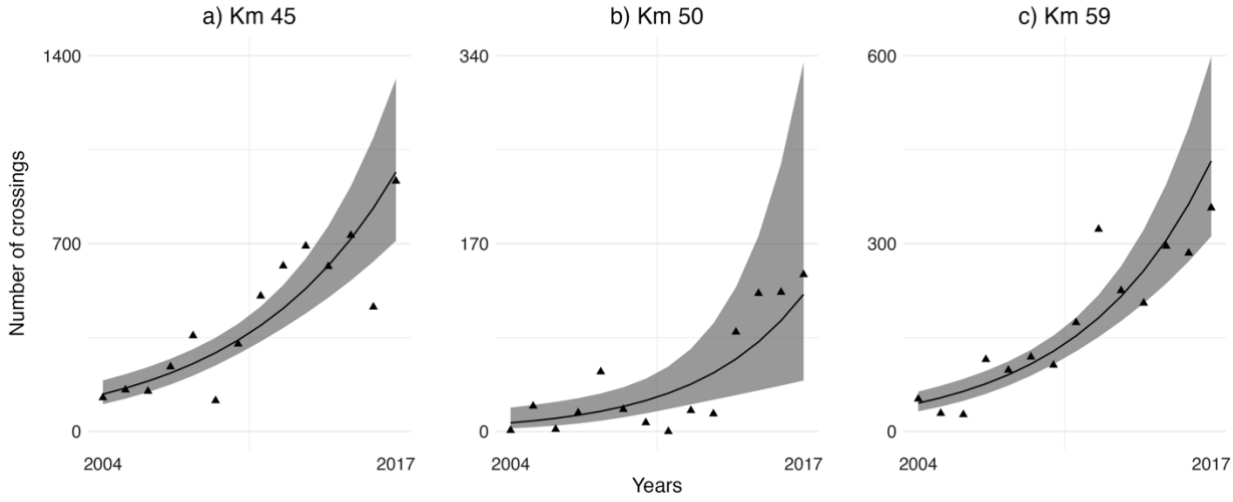


Figure 2. The number of crossings in the mitigation clusters at km 45 (a), km 50 (b), and km 59 (c) over time. The black lines represent the predicted number of crossings, and the dark gray shaded areas represent confidence levels (95%). The black triangles represent the number of crossings recorded each year. Note the difference in the y-axis scales for each cluster.

During 14 years of roadkill surveys, 1,493 carcasses were recorded on the entire highway (detailed information about species in SM – Table 3). A total of 210 roadkills were recorded at mitigated sites (1.67 ± 1.53 records per segment, n = 9; SM – Table 5) and 1,204 roadkills were recorded at non-mitigated sites (0.88 ± 1.33 records per segment, n = 98; SM – Table 5). We found a significant effect of year ($p < 0.001$, estimate = 0.0618, model df = 1494) and a non-significant effect of segment type ($p = 0.097$) on the number of roadkills, with the interaction of these variables also being non-significant ($p = 0.099$) (SM – Table 6). As expected, roadkills were not prevented, with numbers increasing over time at mitigated sites and remaining higher than those on non-mitigated sites throughout the study period (Fig. 3).

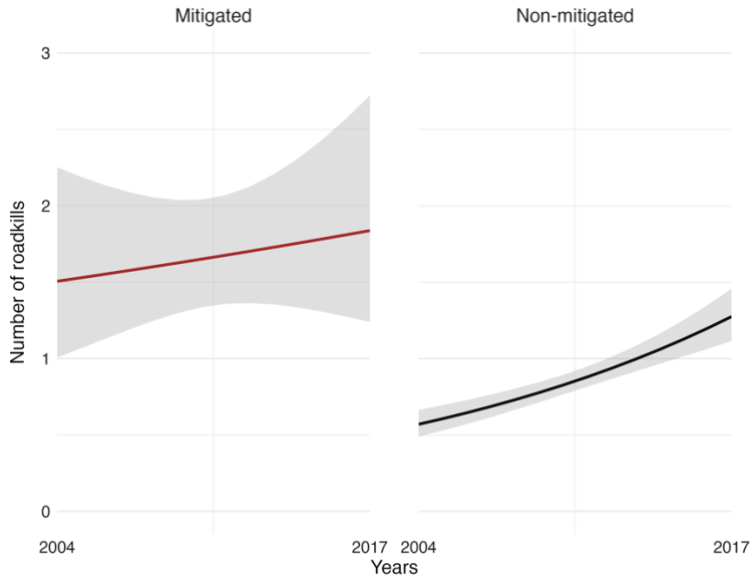


Figure 3. Predicted number of roadkills in 500 m segments of mitigated sites (red line) and non-mitigated sites (black line) along the study period. The gray-shaded areas represent confidence intervals (95%).

We observed that the roadkill hotspots overlapped all the mitigation clusters, considering the full survey time. This general pattern holds when we look at each timeframe, except for the cluster at km 50 in T3 (Fig. 4; complementary results on SM – Figs. 3 and 4). Roadkill hotspots were also present at other locations along the road, with the spatial distribution of the hotspots keeping irrespective of the timeframe analyzed.

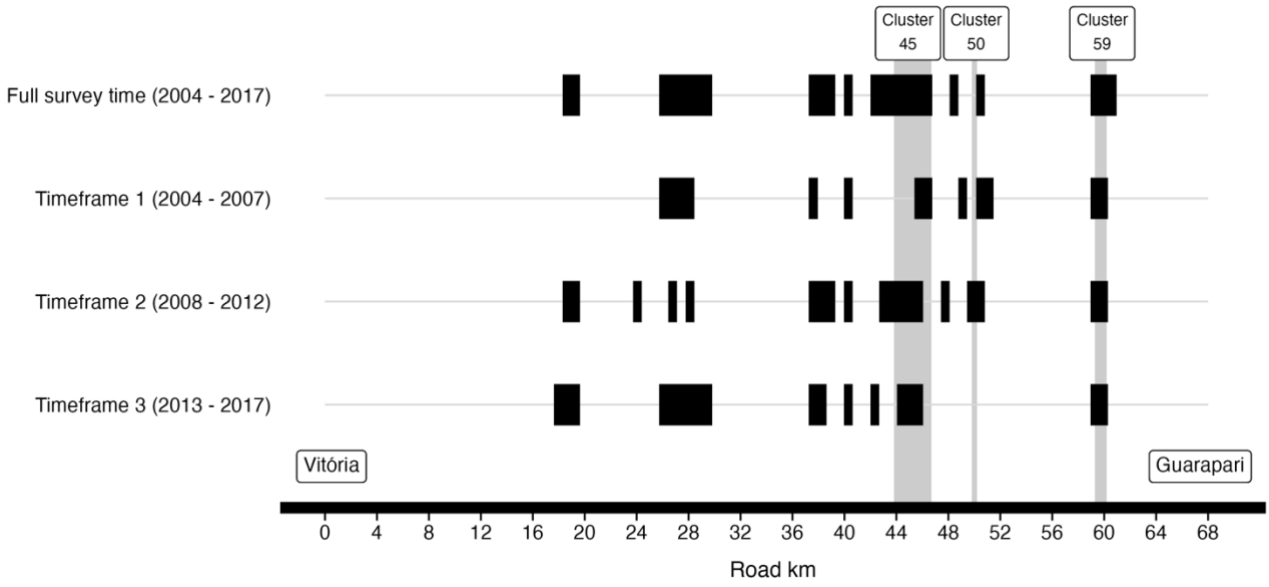


Figure 4. Spatial distribution of hotspot zones considering all species together along the highway for the full survey time span (14 years) and three timeframes (T1, T2, and T3). The black stripes

represent the hotspot zones identified in the 2D HotSpot Identification analyses. The gray shaded zones correspond to the location and extension of mitigation clusters.

Roadkill aggregations on the 500-m scale were observed for eight of the 13 recorded taxa, for which we performed the 2D HotSpot Identification analysis (Fig. 5; complementary results in SM – Figs. 3 and 4). Six of them showed hotspot zones that overlapped the cluster locations: Boidae, *Cerdocyon thous, Coendou insidiosus,* Dasypodidae, *Didelphis aurita,* and *Procyon cancrivorus* (Fig. 5). *Salvator merianae* and *Sylvilagus brasiliensis* did not show aggregation on the 500-m scale and *Cuniculus paca*, *Eira barbara*, and *Lontra longicaudis* had only a few roadkill records, so we did not perform the hotspot analysis for these species. Although we could not perform the hotspot analysis for *Cuniculus paca*, we highlight that its roadkill records overlap the cluster at km 45.

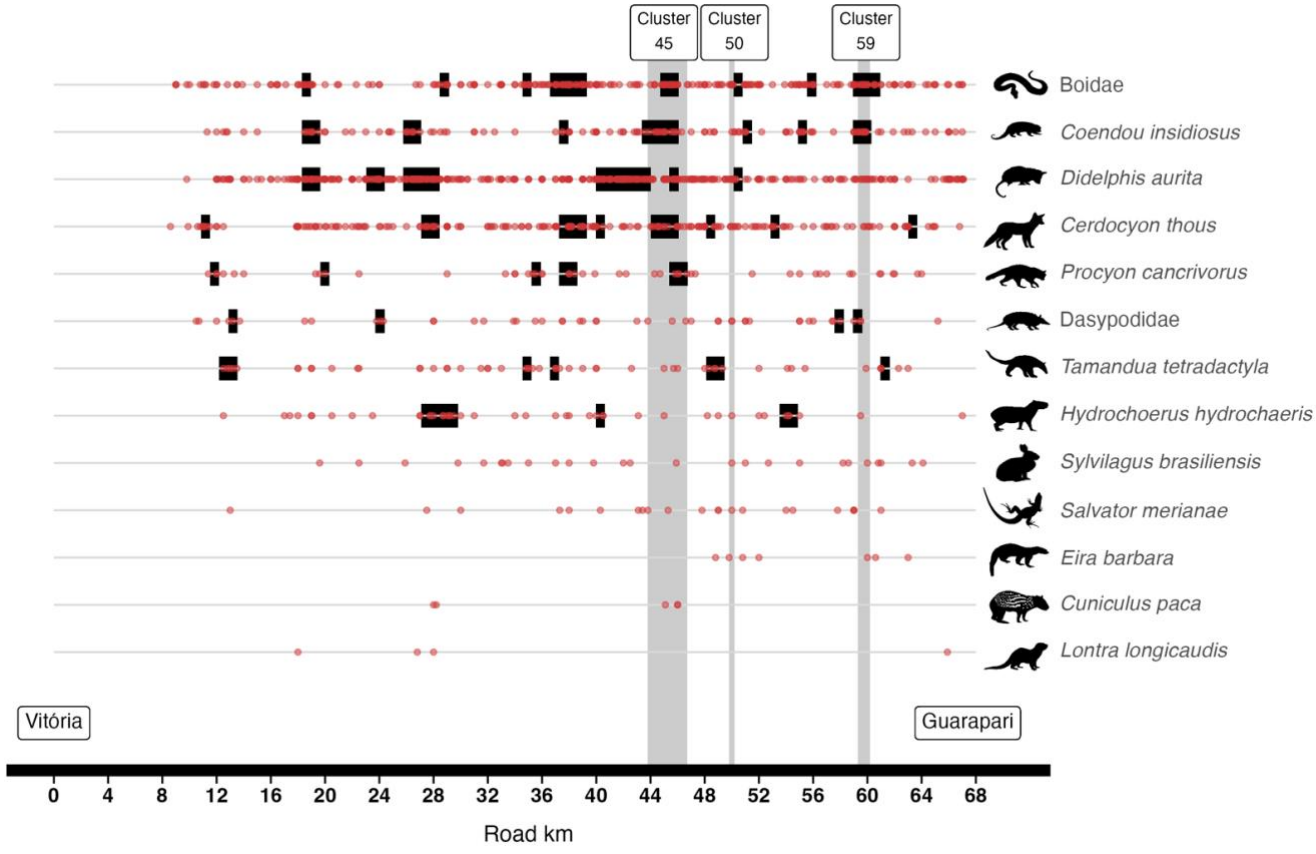


Figure 5. Spatial distribution of roadkill records (red dots) and hotspot zones (black stripes) identified in the 2D HotSpot analysis along the highway for the most recorded taxa on crossings and roadkill surveys. The gray shaded zones correspond to the location and extension of mitigation clusters. The bottom five species did not have enough data to perform the hotspot analysis.

Discussion

We found an increase in the number of crossings and roadkills over time. The number of crossings indicates that all mitigation clusters were used more over the years. However, our results illustrate that the increase in the use of underpasses does not mean a decrease in roadkill in segments with mitigation measures. Roadkill hotspots were still found in mitigated segments whether we look at the entire survey duration or the more restricted timeframes and using a multi-species or single-species dataset.

The use of underpasses is expected to increase over the years after their installation as individuals become more habituated to the presence of these structures (van der Grift et al., 2013). An increase in use over time has been shown for different species at different locations, such as bears, wolves, and ungulates in the United States and Canada (Clevenger and Waltho, 2003; Gagnon et al., 2011), and wild boars, deer, hares, and foxes in Poland (Mysłajek et al., 2020). Our results also showed this pattern for all mitigation clusters. This indicates that underpasses are being functional for animals to cross the road safely (Soanes et al., 2024).

In contrast, fences are clearly ineffective, not blocking target species from accessing roads. Roadkill numbers presented an undesired increase over time, and after years of mitigation, they were still higher on mitigated segments than on non-mitigated sites. Additionally, roadkill hotspots still overlapped mitigation clusters over time. These findings reinforce the known conclusion that even functional underpasses are not an effective mitigation measure against roadkill (Rytwinski et al., 2016) and fences that are not properly designed for target species do not reduce roadkill (Ciocheti et al., 2017; Elliott and Stapp, 2007).

Some recommendations can be made to improve the mitigation system to increase safe crossings and reducing roadkill for all species. First, the current fencing system should be revised, ensuring the installation of fences with extensions that account for the home range of the target species (Huijser et al., 2016). The effectiveness in reducing roadkill has been widely demonstrated if the right type of fence is being used to block animal access to the road (Huijser et al., 2016; Rytwinski et al., 2016; van der Ree et al., 2015). In addition to reducing roadkill, fencing also contributes to increasing wildlife use of underpasses to cross the road safely (Denneboom et al., 2021). For example, we could expect that improvements to fences designed to block large and climbing snakes of the family Boidae (such as metal mesh fencing with a lip and 100 cm of height; e.g., Macpherson et al., 2021) would not only reduce their roadkill rates, but also encourage

greater use of underpasses. Second, more mitigation clusters (fences and underpasses) should be implemented along the road, as the analyses indicated several hotspots on other road segments (e.g., km 28 and 38; Fig. 5). Finally, as some species that are seen as roadkilled on the road are rarely registered using underpasses, this could indicate the need to improve the fencing and eventually adapt the crossing structures to attract them. The use of underpasses could increase with the appropriate type of fence to block animal access to the road and guide them to the crossing structures. The underpasses could be assessed to identify factors affecting their use by different species, including structural and environmental characteristics.

A long-term survey provides an incredible opportunity to assess the performance of mitigation measures over time (Gagnon et al., 2011). Our findings offer important information about the effectiveness of the mitigation system in a tropical and highly diverse region, adding to the literature on mitigation effectiveness, highly biased towards larger mammals and the global North (Denneboom et al., 2021; Rytwinski et al., 2016; Soanes et al., 2024). Although we do not have data collected prior to mitigation implementation, 14 years of simultaneous monitoring of crossings and roadkill numbers gave us more confidence in evaluating the effectiveness of mitigation clusters. The trend of our results suggested that the three mitigation clusters are ineffective in avoiding roadkill, so adaptations in the mitigation system must be conducted, such as installing fences that in fact block animals from accessing the road. More studies must be developed for species in tropical and subtropical regions to improve the knowledge about structure types that are effective in reducing roadkill.

Conclusion

Our study highlights the importance of considering both crossing and roadkill indicators to improve understanding of mitigation effectiveness. Based on our findings, the mitigation clusters showed an increase in the number of crossings; however, the roadkill records also increased over time and were higher in the mitigation segments. Furthermore, our findings suggested a necessary improvement in the mitigation systems to avoid the impact of roadkill while allowing animals to cross the road. The increased use of underpasses suggests that the animals are habituated to them and efforts must be made to implement effective fencing, associated with underpasses, to improve the effectiveness of mitigation clusters in reducing roadkill.

Acknowledgements

We thank Rodosol company for sharing the detailed monitoring dataset. We also would like to thank Marina Zanin, Sara Santos, and Vinicius Alberici for their suggestions in a previous version of this manuscript which helped us to improve it.

References

Bond, A.R., Jones, D.N., 2008. Temporal trends in use of fauna-friendly underpasses and overpasses. Wildlife Research 35, 103. https://doi.org/10.1071/WR07027

Ceia-Hasse, A., Navarro, L.M., Borda-de-Água, L., Pereira, H.M., 2018. Population persistence in landscapes fragmented by roads: Disentangling isolation, mortality, and the effect of dispersal. Ecol Modell 375, 45–53. https://doi.org/10.1016/j.ecolmodel.2018.01.021

Christie, A.P., Amano, T., Martin, P.A., Shackelford, G.E., Simmons, B.I., Sutherland, W.J., 2019. Simple study designs in ecology produce inaccurate estimates of biodiversity responses. Journal of Applied Ecology 56, 2742–2754. https://doi.org/10.1111/1365-2664.13499

Ciocheti, G., de Assis, J.C., Ribeiro, J.W., Ribeiro, M.C., 2017. Highway widening and underpass effects on vertebrate road mortality. Biotropica 49, 765–769. https://doi.org/10.1111/btp.12480

Clevenger, A.P., Waltho, N., 2003. Long-term, year-round monitoring of wildlife crossing structures and the importance of temporal and spatial variability in performance studies.

Coelho, A.V.P., Coelho, I.P., Teixeira, F.Z., Kindel, A., 2014. Siriema: road mortality software. Manual do usuário V.2.0.

Coelho, I.P., Teixeira, F.Z., Colombo, P., Coelho, A.V.P., Kindel, A., 2012. Anuran road-kills neighboring a peri-urban reserve in the Atlantic Forest, Brazil. J Environ Manage 112, 17–26. https://doi.org/10.1016/j.jenvman.2012.07.004

De Palma, A., Sanchez-Ortiz, K., Martin, P.A., Chadwick, A., Gilbert, G., Bates, A.E., Börger, L., Contu, S., Hill, S.L.L., Purvis, A., 2018. Challenges With Inferring How Land-Use Affects Terrestrial Biodiversity: Study Design, Time, Space and Synthesis. pp. 163–199. https://doi.org/10.1016/bs.aecr.2017.12.004

Denneboom, D., Bar-Massada, A., Shwartz, A., 2021. Factors affecting usage of crossing structures by wildlife – A systematic review and meta-analysis. Science of the Total Environment 777, 146061. https://doi.org/10.1016/j.scitotenv.2021.146061

Dudley, N., 2008. Guidelines for applying protected area management categories. IUCN.

Elliott, D., Stapp, P., 2007. Effects of a purpose-built underpass on wildlife activity and traffic-related mortality in southern California: the Harbor Boulevard wildlife underpass.

ES, G. do E.S., 2023. Fluxo de Veículos - Rodovia do Sol.
Gagnon, J.W., Dodd, N.L., Ogren, K.S., Schweinsburg, R.E., 2011. Factors associated with use of wildlife underpasses and importance of long-term monitoring. J Wildl Manage 75, 1477–1487. https://doi.org/10.1002/jwmg.160
Huijser, M.P., Fairbank, E.R., Camel-Means, W., Graham, J., Watson, V., Basting, P., Becker, D., 2016. Effectiveness of short sections of wildlife fencing and crossing structures along highways in reducing wildlife–vehicle collisions and providing safe crossing opportunities for large mammals. Biol Conserv 197, 61–68. https://doi.org/10.1016/j.biocon.2016.02.002
Jackson, N.D., Fahrig, L., 2011. Relative effects of road mortality and decreased connectivity on population genetic diversity. Biol Conserv 144, 3143–3148. https://doi.org/10.1016/j.biocon.2011.09.010
Lüdecke, D., Makowski, D., Waggoner, P., Patil, I., 2020. Performance: assessment of regression models performance. R package version 0.4 4.
Macpherson, M.R., Litzgus, J.D., Weatherhead, P.J., Lougheed, S.C., 2021. Barriers for big snakes: Incorporating animal behaviour and morphology into road mortality mitigation design. Glob Ecol Conserv 26, e01471. https://doi.org/10.1016/j.gecco.2021.e01471
Mysłajek, R.W., Olkowska, E., Wronka-Tomulewicz, M., Nowak, S., 2020. Mammal use of wildlife crossing structures along a new motorway in an area recently recolonized by wolves. Eur J Wildl Res 66, 79. https://doi.org/10.1007/s10344-020-01412-y
R Core Team, 2023. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing.
Ripley, B., Venables, B., Bates, D.M., Hornik, K., Gebhardt, A., Firth, D., Ripley, M.B., 2013. Package 'mass.' Cran r.
Roedenbeck, I.A., Fahrig, L., Findlay, C.S., Houlahan, J.E., Jaeger, J.A.G., Klar, N., Kramer-Schadt, S., van der Grift, E.A., 2007. The Rauischholzhausen agenda for road ecology. Ecology and Society 12. https://doi.org/10.5751/ES-02011-120111
Rytwinski, T., Soanes, K., Jaeger, J.A.G., Fahrig, L., Findlay, C.S., Houlahan, J., Van Ree, R. Der, Van Der Grift, E.A., 2016. How effective is road mitigation at reducing road-kill? A meta-analysis. PLoS One 11, 1–25. https://doi.org/10.1371/journal.pone.0166941
Smith, D.J., Van Der Ree, R., Rosell, C., 2015. Wildlife crossing structures: an effective strategy to restore or maintain wildlife connectivity across roads, in: Handbook of Road Ecology. John Wiley & Sons Ltd Chichester, UK, pp. 172–183.
Soanes, K., Rytwinski, T., Fahrig, L., Huijser, M.P., Jaeger, J.A.G., Teixeira, F.Z., van der Ree, R., van der Grift, E.A., 2024. Do wildlife crossing structures mitigate the barrier effect of roads on animal movement? A global assessment. Journal of Applied Ecology 61, 417–430. https://doi.org/10.1111/1365-2664.14582
Teixeira, F.Z., Rytwinski, T., Fahrig, L., 2020. Inference in road ecology research: what we know versus what we think we know. Biol Lett 16, 20200140. https://doi.org/10.1098/rsbl.2020.0140

UNEP-WCMC, IUCN, 2024. Protected Planet: The World Database on Protected Areas (WDPA) [WWW Document]. Cambridge, UK: UNEP-WCMC and IUCN. URL www.protectedplanet.net (accessed 9.5.24).

van der Grift, E.A., van der Ree, R., Fahrig, L., Findlay, S., Houlahan, J., Jaeger, J.A.G., Klar, N., Madriñan, L.F., Olson, L., 2013. Evaluating the effectiveness of road mitigation measures. Biodivers Conserv 22, 425–448. https://doi.org/10.1007/s10531-012-0421-0

van der Ree, R., Gagnon, J.W., Smith, D.J., 2015. Fencing: A Valuable Tool for Reducing Wildlife-Vehicle Collisions and Funnelling Fauna to Crossing Structures, in: Handbook of Road Ecology. Wiley, pp. 159–171. https://doi.org/10.1002/9781118568170.ch20

Villalva, P., Reto, D., Santos-Reis, M., Revilla, E., Grilo, C., 2013. Do dry ledges reduce the barrier effect of roads? Ecol Eng 57, 143–148. https://doi.org/10.1016/j.ecoleng.2013.04.005

Supplementary Material

SM1. Detailed information of mitigation clusters

Table 1. Characteristics of each mitigation structure in each cluster at highway ES-060. The type, number of structures, and dimensions are provided for each cluster. Also, the exact location of each underpass and if it was surveyed using sand beds. The symbol ø means diameter and it is used for the circular underpasses. The underpass length refers to the extension under the road.

Structure type	Number of structures	Cell width (m) / cell diameter (m)	Height (m)	Length (m)	Location (km)	Surveyed
CLUSTER KM 45						
Single concrete box	2	2.5	2.5	26	45.3	Yes
				33	45.7	Yes
Single concrete culvert	2	ø 1.5	-	36.5	45.6	Yes
				38	46.2	No
Double concrete culvert	1	ø 1.5	-	51	44.8	No
Triple concrete culvert	2	ø 1.2	-	36	44.2	No
				39	45.4	Yes
Bridge over the Perocão River	1	3.5	22.5	21	46.4	No
CLUSTER KM 50						
Single concrete culvert	2	ø 1.5	-	116.9	50	Yes
CLUSTER KM 59						
Single concrete culvert	1	ø 1.5	-	53	59.7	Yes
Double concrete culvert	1	ø 1.5 and ø 1.2	-	62	59.9	No
		ø 1.5 and ø 1	-	59	60.2	Yes



Figure 1. Example of sand track beds construction and records of animals use.

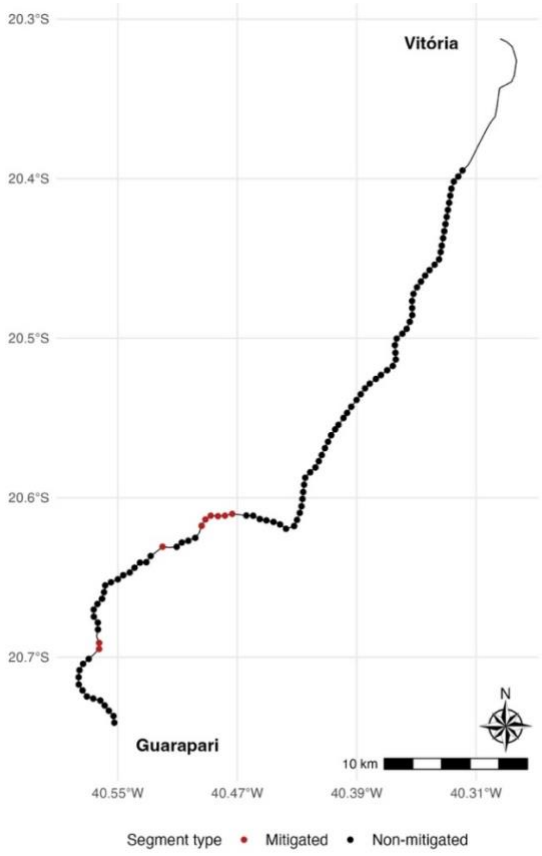


Figure 2. Location of mitigated (impact, n = 9) and non-mitigated (control, n = 98) road segments along ES-060.

433 Table 2. The ten most recorded species in crossing and roadkill surveys and their number of
434 records at each mitigation cluster. Species in bold represent the ones that were most recorded in
435 both roadkill and crossing surveys. The symbol (*) represents species with high roadkill but low
436 crossing records, while the symbol (¤) represents species with high crossing but low roadkill
437 records.

Species	Common name	Crossing records in each cluster			Roadkill records in each cluster			Roadkill records in the entire road
		km 45	km 50	km 59	km 45	km 50	km 59	
Didelphis aurita	Brazilian Common Opossum	776	37	367	34	13	10	540
Boidae*	Boas	9	3	2	26	10	11	285
Cerdocyon thous	Crab-eating Fox	279	127	51	16	5	5	226
Coendou insidiosus	Bahia hairy dwarf porcupine	198	4	69	14	3	11	137
Tamandua tetradactyla	Southern Tamandua	71	1	378	3	-	1	54
Procyon cancrivorus	Crab-eating Raccoon	1785	45	387	6	-	-	49
Dasypodidae	Armadillos	273	222	111	2	2	2	48
*Hydrochoerus hydrochaeris**	Capybara	86	11	28	1	1	1	44
*Sylvilagus brasiliensis**	Tapeti	64	1	10	-	-	-	26
Salvator merianae	Black-and-white Tegu	918	133	105	1	1	-	22
Eira barbara¤	Tayra	791	10	341	-	1	1	7
Cuniculus paca¤	Agouti	496	3	138	3	-	-	5
Lontra longicaudis¤	Neotropical Otter	130	28	358	-	-	-	4

438

439 Table 3. List of all species recorded in crossing and roadkill surveys and their number of records.
440 Boidae includes the species: *Boa constrictor*, *Epicrates cenchria* and *Corallus hortulanus*; and
441 Dasypodidae includes *Dasypus novemcinctus*, *D. septemcinctus* and *Euphractus sexcinctus*.
442

Species	Common name	Crossing records in all clusters	Roadkill records in the entire road
Boidae	Boas	14	285
Caiman latirostris	Broad-snouted Caiman	3	1
Cariama cristata	Red-legged Seriema	-	19
Cerdocyon thous	Crab-eating Ox	457	226
Chaetomys subspinosus	Bristle-spined Rat	-	14
Coendou insidiosus	Bahia Hairy Dwarf Porcupine	271	137
Cuniculus paca	Agouti	637	5
Dasypodidae	Armadillos	759	48
Didelphis aurita	Brazilian Common Opossum	1180	540
Eira barbara	Tayra	1142	7
Galictis cuja	Lesser Grison	-	1
Herpailurus yagouaroundi	Jaguarundi	24	8
Hydrochoerus hydrochaeris	Capybara	125	44
Leopardus sp.	Small Wildcat	53	1
Lontra longicaudis	Neotropical Otter	516	4
Nasua nasua	South American Coati	1	2
Procyon cancrivorus	Crab-eating Raccoon	2217	49
Salvator merianae	Black-and-white Tegu	1156	22
Sylvilagus brasiliensis	Tapeti	75	26
Tamandua tetradactyla	Southern Tamandua	450	54

443

444 Table 4. Model outputs of each cluster for the number of crossings annually recorded in relation
445 to the years.

Coefficients	Estimate	Std. Error	z value	Pr(>|z|)
Km 45				
Intercept	-293.77624	41.59607	-7.063	<0.0001
Year	0.14906	0.2069	7.205	<0.0001
Km 50				
Intercept	-424.26876	134.95362	-3.144	0.00167
Year	0.21274	0.06712	3.17	0.00153
Km 59				
Intercept	-343.89359	44.82627	-7.672	<0.0001
Year	0.17351	0.02229	7.783	<0.0001

446

447 Table 5. Number of roadkill at mitigated (impact) and non-mitigated (control) road segments used
448 in the analyses for each survey year.

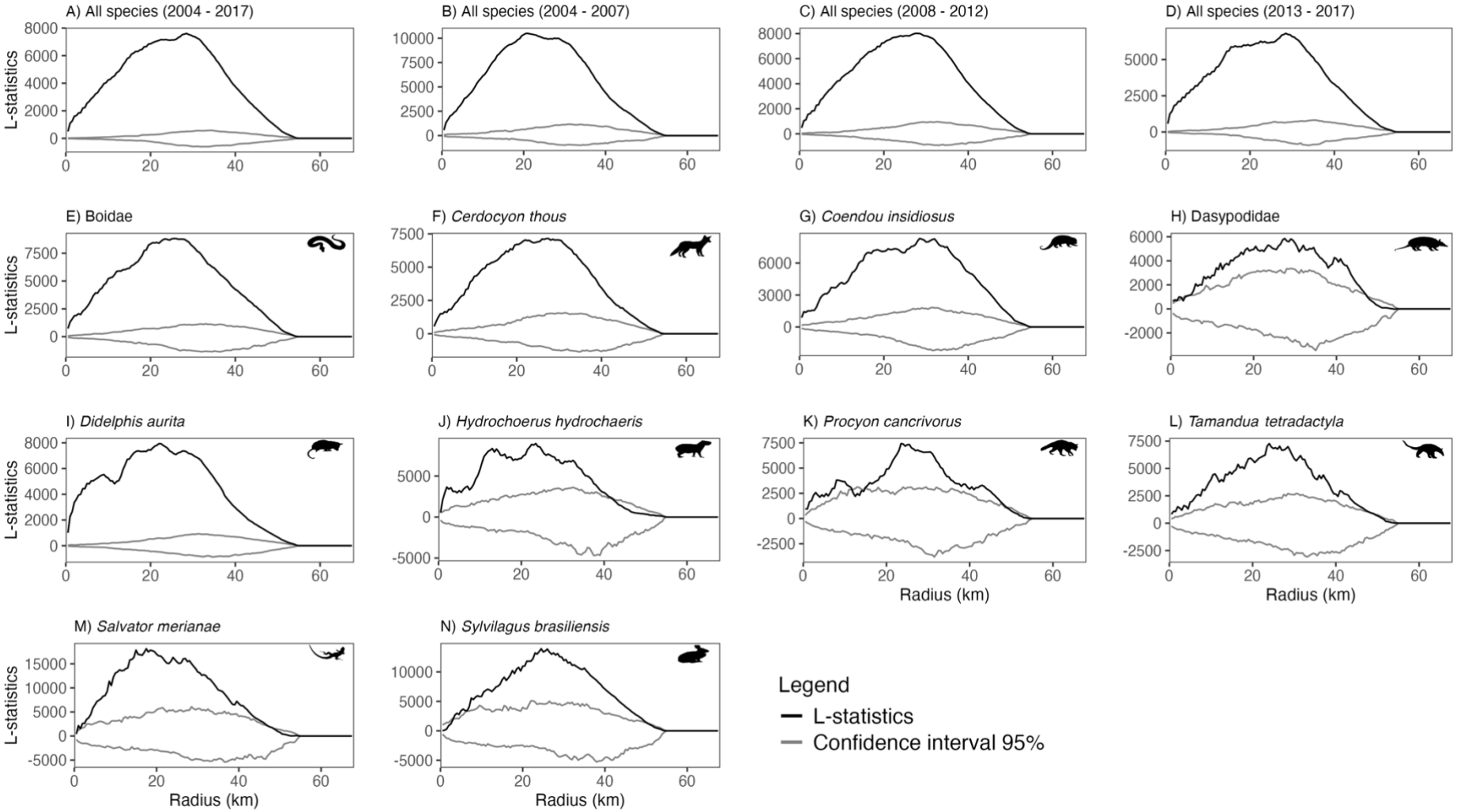
Year	Mitigated sites (n = 9)		Non-mitigated sites (n = 98)	
	Records per segment (500m)	Total records	Records per segment (500m)	Total records
2004	1.44 ± 1.67	13	0.52 ± 0.8	51
2005	1.44 ± 1.67	13	0.76 ± 1.13	74
2006	1.22 ± 0.97	11	0.56 ± 0.76	55
2007	1.22 ± 0.83	11	0.49 ± 0.93	48
2008	1.89 ± 1.45	17	0.48 ± 0.8	47
2009	1.44 ± 1.01	13	0.64 ± 0.83	63
2010	2.44 ± 2.46	22	0.56 ± 0.84	55
2011	0.78 ± 0.83	7	0.92 ± 1.11	90
2012	2.11 ± 1.76	19	1.64 ± 1.96	161
2013	2.56 ± 1.81	23	1.76 ± 2	172
2014	2.89 ± 1.05	26	1.68 ± 1.97	165
2015	1.89 ± 1.96	17	0.87 ± 1.27	85
2016	1 ± 0.87	9	0.71 ± 1.05	70
2017	1 ± 1.12	9	0.69 ± 1.09	68
All years	1.67 ± 1.53	210	0.88 ± 1.33	1204

449

450 Table 6. Model outputs of each cluster for the number of roadkills annually recorded in the
451 segments in relation to the years and the type of segment.

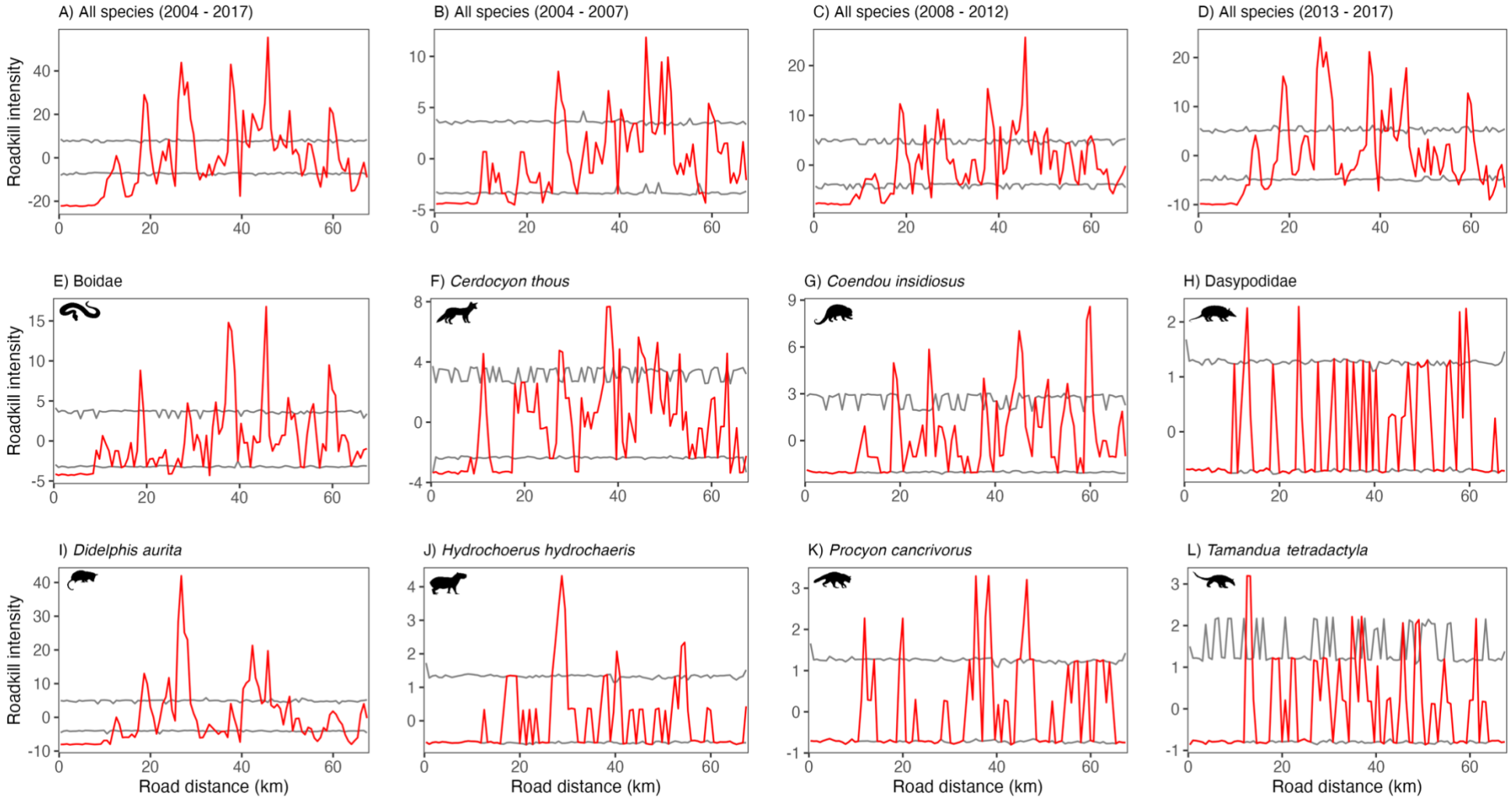
Coefficients	Estimate	Std. Error	z value	Pr(>|z|)
Intercept	-124.5	19.26	-6.467	<0.0001
Type impact	94.26	56.73	1.662	0.966
Year	0.0618	0.009575	6.46	<0.0001
Type impact*year	−0.04655	0.0282	-1.65	0.099

452


A) All species (2004 - 2017)
B) All species (2004 - 2007)
C) All species (2008 - 2012)
D) All species (2013 - 2017)
E) Boidae
F) Cerdocyon thous
G) Coendou insidiosus
H) Dasypodidae
I) Didelphis aurita
J) Hydrochoerus hydrochaeris
K) Procyon cancrivorus
L) Tamandua tetradactyla
M) Salvator merianae
N) Sylvilagus brasiliensis
L-statistics
Radius (km)
Legend
L-statistics
Confidence interval 95%

453

Figure 3. Results of modified Ripley's K analyses. L-statistics represents roadkill aggregation intensity and when black line exceeds the upper confidence limit (gray lines; 95%) indicate the occurrence of significant roadkill aggregations. *Salvator merianae* and *Sylvilagus brasiliensis* did not present aggregation in the 0.5 km scale (M and N).


A) All species (2004 - 2017)
B) All species (2004 - 2007)
C) All species (2008 - 2012)
D) All species (2013 - 2017)
E) Boidae
F) Cerdocyon thous
G) Coendou insidiosus
H) Dasypodidae
I) Didelphis aurita
J) Hydrochoerus hydrochaeris
K) Procyon cancrivorus
L) Tamandua tetradactyla
Roadkill intensity
Road distance (km)

458

459 Figure 4. Results of 2D HotSpot Identification analysis. Red lines exceeding the 95% upper confidence limit (top gray line) indicate a location of significant
460 roadkill aggregation (hotspot).

Capítulo III

Prioritising road mitigation efforts based on landscape structure and species navigation traits: insights from mechanistic modelling

Esse capítulo será resubmetido a revista "*Biological Conservation"* e foi realizado em colaboração com Fernanda Zimmermann Teixeira, Andreas Kindel, Fernando Ascensão e Vinicius A. G. Bastazini.

Prioritising road mitigation efforts based on landscape structure and species navigation traits: insights from mechanistic modelling

Abstract

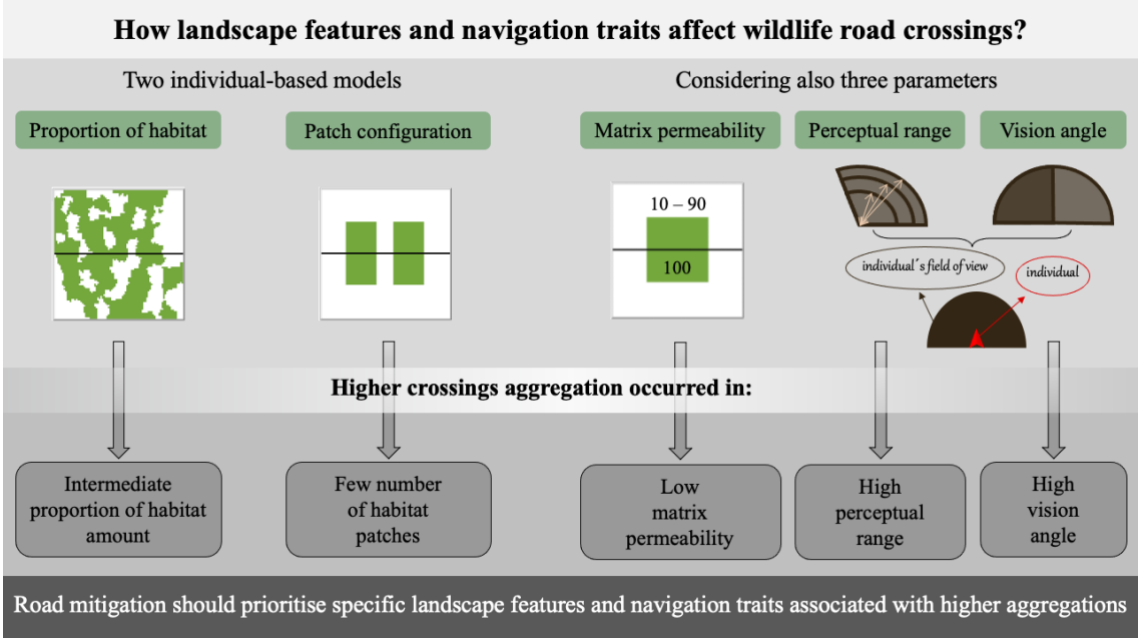
The expanding global road network increases habitat fragmentation, forcing wildlife to crossroads more frequently during their movements. Such impacts frequently modify wildlife movement patterns or wildlife-vehicle collisions. Mitigating these impacts is crucial, but since financial resources are limited, road mitigation must be spatially prioritised in places where it will be most effective. Our objective was to provide a mechanistic understanding of how landscape structure (habitat amount and configuration) affects wildlife road crossings, while considering matrix permeability and two navigation capacity traits: perceptual range and vision angle. We devised individual-based models to simulate the movement of diverse species across various landscapes. This allowed us to manipulate and analyse these parameters. A higher aggregation of road crossings was observed under two conditions: first, in landscapes characterised by intermediate amounts of habitat or fewer patches; and second, in simulations with lower matrix permeability or with larger fields of view (indicating higher perceptual range or vision angle). Our model enabled the identification of variations in wildlife road crossings aggregation and total number of crossings among different habitat amounts and configurations, as well as matrix permeability, perceptual range, and vision angle. The sets of landscape features and navigation capacity traits that resulted in higher aggregation of crossings should be prioritised in efforts to mitigate road impacts such as connectivity and wildlife-vehicle collisions.

Keywords: habitat amount, habitat configuration, wildlife road crossings aggregation, connectivity mitigation, wildlife-vehicle collision, agent-based modelling

Highlights

- Crossings aggregation values varied among landscape features and navigation traits
- Intermediate amounts of habitat or fewer patches showed higher aggregation
- Higher aggregation occurred in lower matrix permeability and larger field of view
- Mitigation should focus on landscapes and traits with higher crossing aggregations

Graphical abstract



1. Introduction

Wildlife-vehicle collisions occur when an animal is moving across a road. Different types of mitigation measures exist and are being implemented to re-establish movement between roadsides and to reduce roadkill (Rytwinski et al., 2016; Soanes et al., 2024). Animal movement is important for population persistence, species distribution and coexistence, food web dynamics and ecosystem functioning (Cosgrove et al., 2018; Jeltsch et al., 2013). Individuals move to search for food, escape from a predator or other environmental hazard, avoid competition, reproduce, migrate, or disperse (Fahrig, 2007; Nathan et al., 2008). Changes in animal movement patterns can affect reproduction rate, gene flow, survival probability and other population processes (Cosgrove et al., 2018; Fahrig, 2007). However, financial resources for implementing such measures are limited, and therefore we need to prioritise target locations for mitigation.

Understanding why an animal is choosing to cross the road in a given location may improve the cost-benefit of installing mitigation structures, maximizing their effectiveness (Lewis et al., 2011). The act of moving has several components, such as perceptual range and habitat or matrix permeability (Knowlton and Graham, 2010; Nathan et al., 2008). Perceptual range delineates the distance at which a landscape element can be perceived by an animal and can vary with land use type (Lima and Zollner, 1996; Nathan et al., 2008). Landscape permeability refers to the degree to which heterogeneous areas allow or impede the movement of organisms (Singleton et al., 2002;

Zeller et al., 2012). Hence, understanding how habitat amount and configuration, landscape permeability, and species' perceptual range interact to result in higher/lower concentration of wildlife road crossings could help determine where to install mitigation measures, and targeted for which type of species.

Individual-based models (IBMs) may provide good support for analysing different landscape scenarios and species conditions. IBMs are computational models in which the individuals can interact with each other and with the environment, through simple rules, resulting in the emergence of system properties (Grimm and Railsback, 2005). The individuals created are autonomous and may have explicit variance in spatial location, physiological traits, and behavioural traits (DeAngelis and Grimm, 2014). Advantages of IBMs include the possibility of considering stochastic processes to reproduce observed patterns and designing individuals to behave in response to environmental *stimuli* (Grimm and Railsback, 2005; McLane et al., 2011). IBMs have been used to simulate animal movement in different landscapes to understand the outcome patterns in the studied system (Tang and Bennett, 2010), such as to estimate landscape connectivity in scenarios of conservation planning (Allen et al., 2016), to predict wildlife-vehicle collision (Quaglietta et al., 2019), to investigate the variation in roadkill hotspot location through time (Teixeira et al., 2017), or to evaluate the performance of road mortality mitigation measures (Ascensão et al., 2013).

Using IBMs we assessed how habitat amount and habitat configuration affect the aggregation of wildlife road crossings, considering different levels of matrix permeability and individual navigation capacity traits (i.e., perceptual range and vision angle). We simulated landscapes formed by discrete habitat patches distributed along a nonhabitat matrix. We expected to observe a lower aggregation in landscapes with a higher habitat amount and higher number of patches. This is because animals are expected to move more uniformly across these kinds of landscapes, with crossings not concentrated in specific sections of the road. We expected lower aggregation in landscapes with higher matrix permeability due to the reduced resistance when transitioning between habitat and nonhabitat patches. However, we expect higher aggregation when the field of view is larger (higher perceptual range and higher vision angle), as animals will have a broader view of the landscape, leading to more directed movement to reach habitat patches. When planning mitigation, sets of landscape features and of navigation capacity traits (hereafter, also called contexts) resulting in higher crossings aggregation should be prioritised in efforts to mitigate road impacts.

2. Materials and methods

2.1 Model overview

We developed individual-based models (IBMs) using NetLogo version 6.0.4 (Wilensky, 1999). Models' details are provided in the Overview, Design concepts, and Details (ODD) protocol (Grimm et al., 2010) in Appendix (A1). Condensed information on the parameters of the models and their main processes is described below.

Our two IBMs simulate animal movement in a binary landscape composed of habitat patches dispersed in a non-habitat matrix. The landscapes in both models have the same sizes (grid of 84 x 84 pixels) and are divided by a uniform road (1 x 84 pixels) that does not affect animal movement; animals can cross the road without being killed. This is because we were interested in understanding what causes crossing aggregation, which can be related with roadkill probability, when considering traffic volume and animal behaviour (Jacobson et al., 2016; Visintin et al., 2016). The first model, which we refer to as 'habitat amount model', was developed to understand the relationship between crossings aggregation and habitat amount (Fig. 1 A). We tested the correlation between the proportion of habitat and the number of patches in random landscapes and found that these variables were negatively correlated (Pearson correlation = -0.79, A2). Therefore, we developed a second model, which we refer to as 'habitat configuration model', to evaluate if habitat configuration influences crossings aggregation. In this model, we kept the habitat amount constant in all simulations and created simplified conceptual scenarios that are symmetric on both sides of the road (landscapes differing in number and in configuration of patches) (Fig. 1 B).

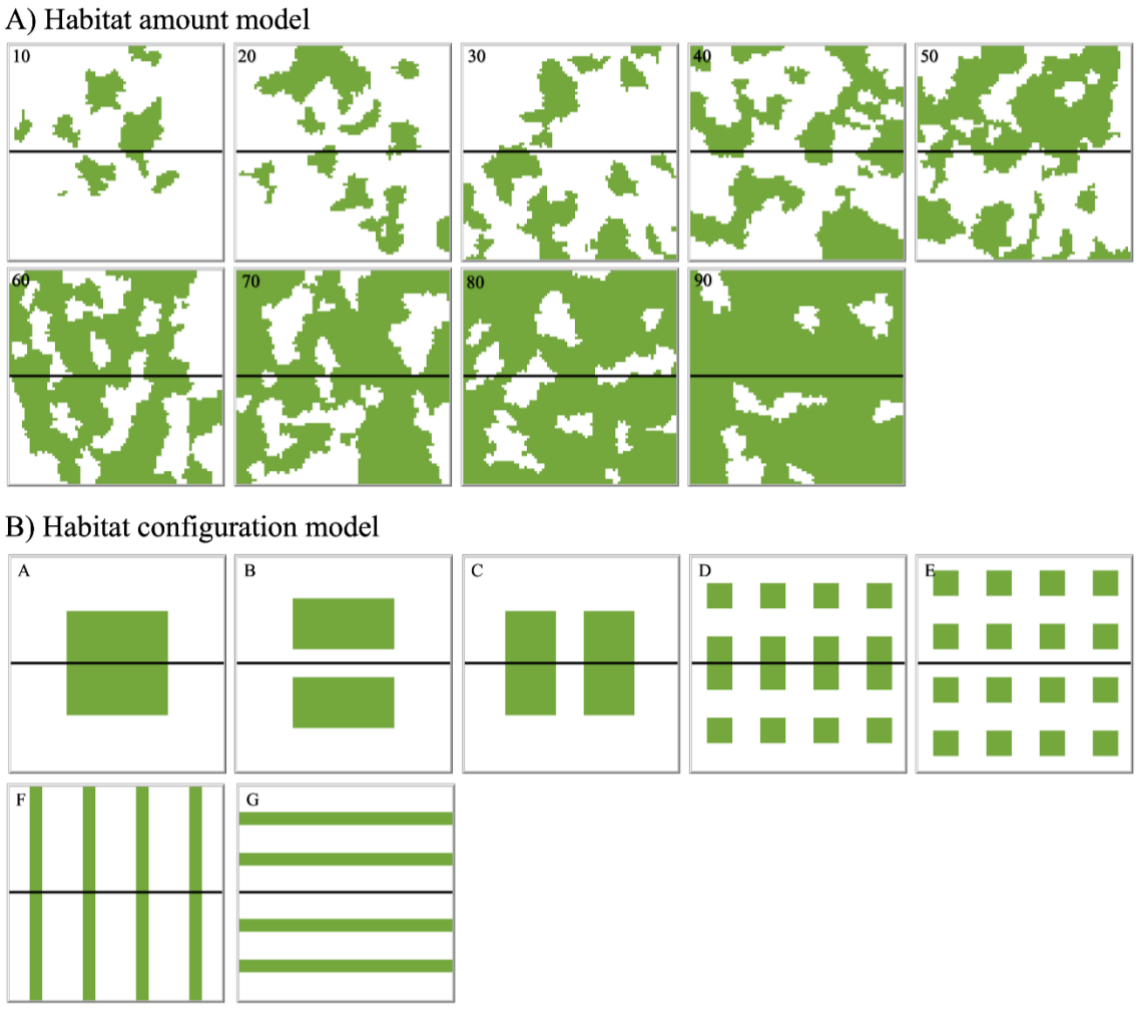


Figure 1. Landscapes created for each model. A) Examples of randomly generated landscapes for the habitat amount model; values in the upper left corner represent the habitat proportion (%). B) The seven different scenarios created for the habitat configuration model; the letters in the upper left corner identify each scenario.

Three input parameters are the same for both models: 1) matrix permeability: corresponds to the degree to which the landscape matrix promotes or impedes animal movement between habitat pixels; permeability of matrix pixels varies from 10 to 90, while permeability of habitat pixels is set to 100 (meaning that individuals can move freely in them); 2) perceptual range: corresponds to the animal's perception extend; varying from 5 to 42 pixels; and 3) vision angle: corresponds to the range of animal's vision; varying from 90° to 180°. The habitat amount model also has the proportion of habitat (varying from 10% to 90%) as an input parameter, while the habitat configuration model has seven different landscapes with different habitat arrangements as categorical scenarios (A to G). From each simulation for both models, we reported as output the

crossings aggregation value, which corresponds to the proportion of crossings in the 25% most crossed pixels of the road. To obtain this value, we sorted all road pixels in decreasing order by the number of crossings in each pixel and then divided the sum of crossings in the 25% most crossed pixels by the total number of crossings on the entire road. Although the total number of crossings on the entire road was not a primary focus, it was also recorded.

2.2 Model analysis

To perform the simulations of our IBM, before running the model, we set the number of individuals at 100, the number of steps at 500, and defined the remaining input values. Then the model was set up, with the landscape being generated according to each model. Individuals were allowed to move across the landscape, selecting their preferred pixels and going towards them. To select preferred pixels, the individuals consider the pixel permeability and the pixel distance of all pixels inside a cone in front of them, using perceptual range and vision angle to delimit the cone (detailed information of model steps are available in A1). This process is repeated until the model stops (stopping rule at 500 steps). We used the "*nlrx*" package (Salecker et al., 2019), in the R environment (R Core Team, 2023), to generate input values (from samples of parameters in their entire variation range), to run the simulations, and to save the results.

Using simulations for each model, we analysed the relationship among input parameter's values and output values to identify contexts (sets of landscape features and of navigation capacity traits) that are more important for mitigation. We evaluated the shape of the response curve between inputs and outputs by performing a model selection procedure considering the following candidate models: null (intercept-only), linear, exponential, asymptotic and Bragg (ie., unimodal response), using the "*aomisc*" package (Onofri, 2020). We measured the plausibility of each candidate model using the Bayesian Information Criterion (BIC; Claeskens & Hjort, 2008), using the package "*bbmle*" (Bolker and Bolker, 2017). In the result section, we present only the best-ranked model of input parameters individually for each model (model selection information is shown in A3).

We calculated the contribution of each input (varying them in a full range) to the variance of the model outputs using Sobol' method, a global sensitivity analysis, which is a quantitative method that can inform parameter sensitivity (Abreu and Ralha, 2018; Sobol' et al., 2007; Thiele et al., 2014). We performed one sensitivity analysis for each

model, using the function "sobol2007" from the "*sensitivity*" package (Pujol et al., 2015) in the R environment (R Core Team, 2023). We used 10,000 model evaluations (samples of parameters throughout their range), totalling 60,000 simulations, to obtain Sobol's first-order sensitivity index (or main effect, S_i) and Sobol's total-effects sensitivity index (S_{Ti}) for each input parameter in each output. The main effects are measures of each input factor contribution to the variance of the output, and their sum varies between 0 and 1, with less than 1 indicating presence of input interactions in the model (Saltelli et al., 2008).

3. Results

Modifying the input parameters in both models resulted in different intensities of crossings aggregation. Aggregation values in the simulations varied from 0.329 to 0.972 in the habitat amount model, and from 0.328 to 1 in the habitat configuration model (except for two out 60,000 simulations that presented zero aggregation because they did not have any crossing). Higher crossings aggregation was observed in landscapes with intermediate (30 - 40%) habitat amount (Fig. 2 A) and with fewer habitat patches (scenarios A, B, and C) (Fig. 2 E). Lower matrix permeability resulted in higher crossings aggregation for both models (Fig. 2 B and F). Higher values of both perceptual range and vision angle resulted in higher crossings aggregation for both models (Fig. 2 C and G for perceptual range, and D and H for vision angle).

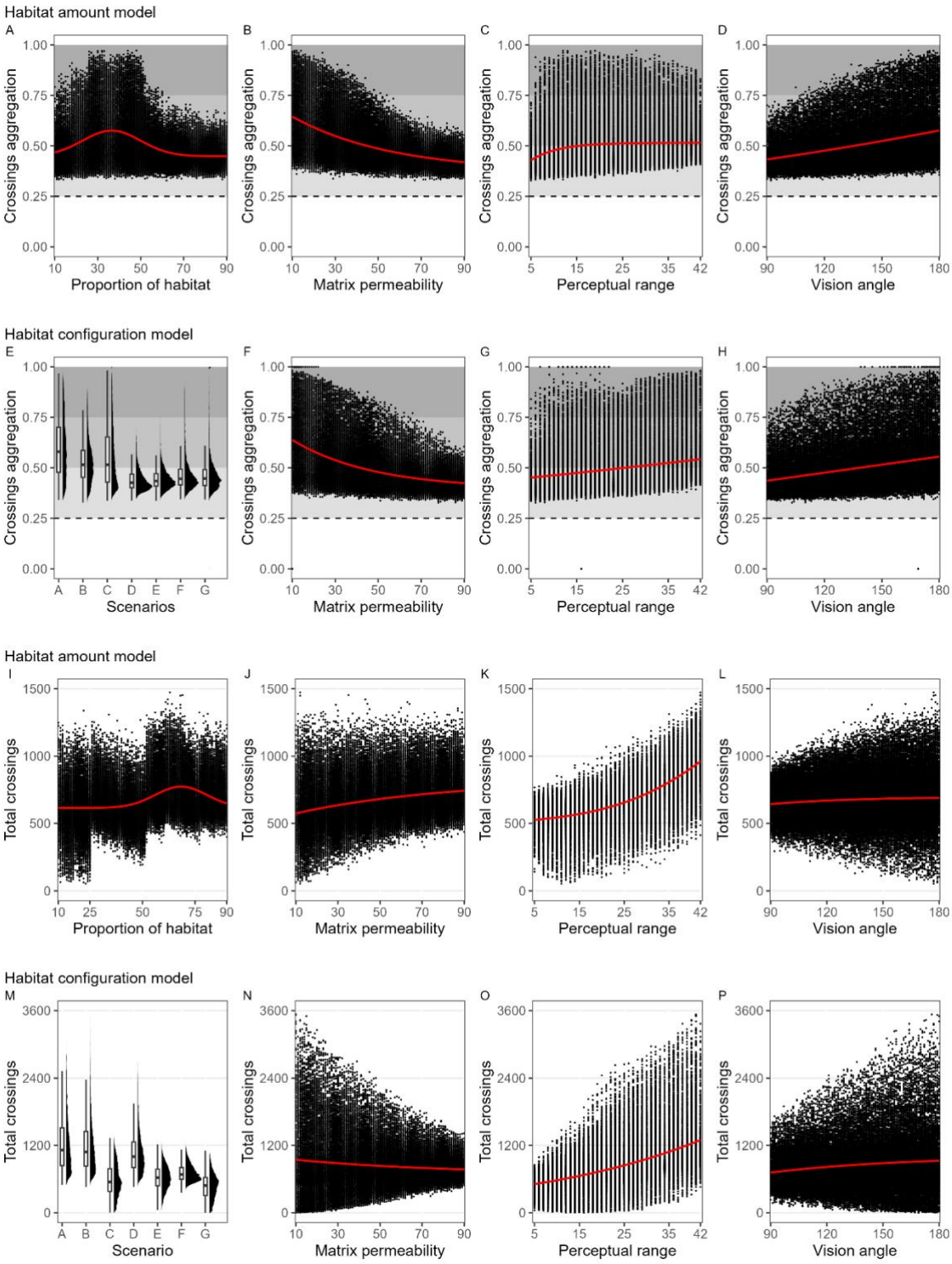


Figure 2. Simulations results for crossings aggregation values for each input parameter in habitat amount (A – D) and habitat configuration model (E – H), and for total number of crossings for each input parameter in habitat amount (I – L) and habitat configuration model (M – P). Red lines represent the best-model fitted for each parameter: Bragg (A, I, and K), asymptotic (B, C, F, J, L, N, and P), exponential (D, G, and O), and linear (H). Zero values for crossings aggregation

represent cases with zero total number of crossings. A – H: The stronger the shade of grey, the greater the crossings aggregation. Dashed lines show the minimum possible value for crossings aggregation according to its definition in our model. In E and M, black shapes indicate the parameter distribution for each scenario.

The total number of crossings on the entire road also varied according to input parameters. We observed a smaller variation in the total number of crossings in the habitat amount model (from 53 to 1,473) than in the habitat configuration model (from 0 to 3,533). The total number of crossings was higher for landscapes with 65 - 75% of habitat amount (Fig. 2 I) and in scenarios A, B and D in the habitat configuration model (Fig. 2 M). High matrix permeability resulted in a higher total number of crossings in the habitat amount model (Fig. 2 J), while we observed a slightly decrease in the total number of crossings as we increase matrix permeability in the habitat configuration model (Fig. 2 L). Higher values of perceptual range and vision angle resulted in a higher total number of crossings in both models (Fig. 2 K and O for perceptual range, and L and P for vision angle).

The most important input parameters driving the variation in crossings aggregation and in the total number of crossings differed for each model according to our sensitivity analysis. Matrix permeability was the parameter that most affected crossings aggregation individually (larger main effect) in the habitat amount model, followed by proportion of habitat when we consider its interaction with all other parameters (total effect) (Fig. 3 A). In contrast, perceptual range was the parameter with the highest main and total effects for the total number of crossings in the habitat amount model (Fig. 3 B). Matrix permeability was also the parameter with the highest main effect for crossings aggregation in the habitat configuration model, followed by the scenario parameter, but the latter was slightly more important than the former when we consider their interaction with all other parameters (total effect) (Fig. 3 C). Scenarios was the parameter with the highest main and total effects for the total number of crossings in the habitat configuration model, followed by the perceptual range (Fig. 3 D).

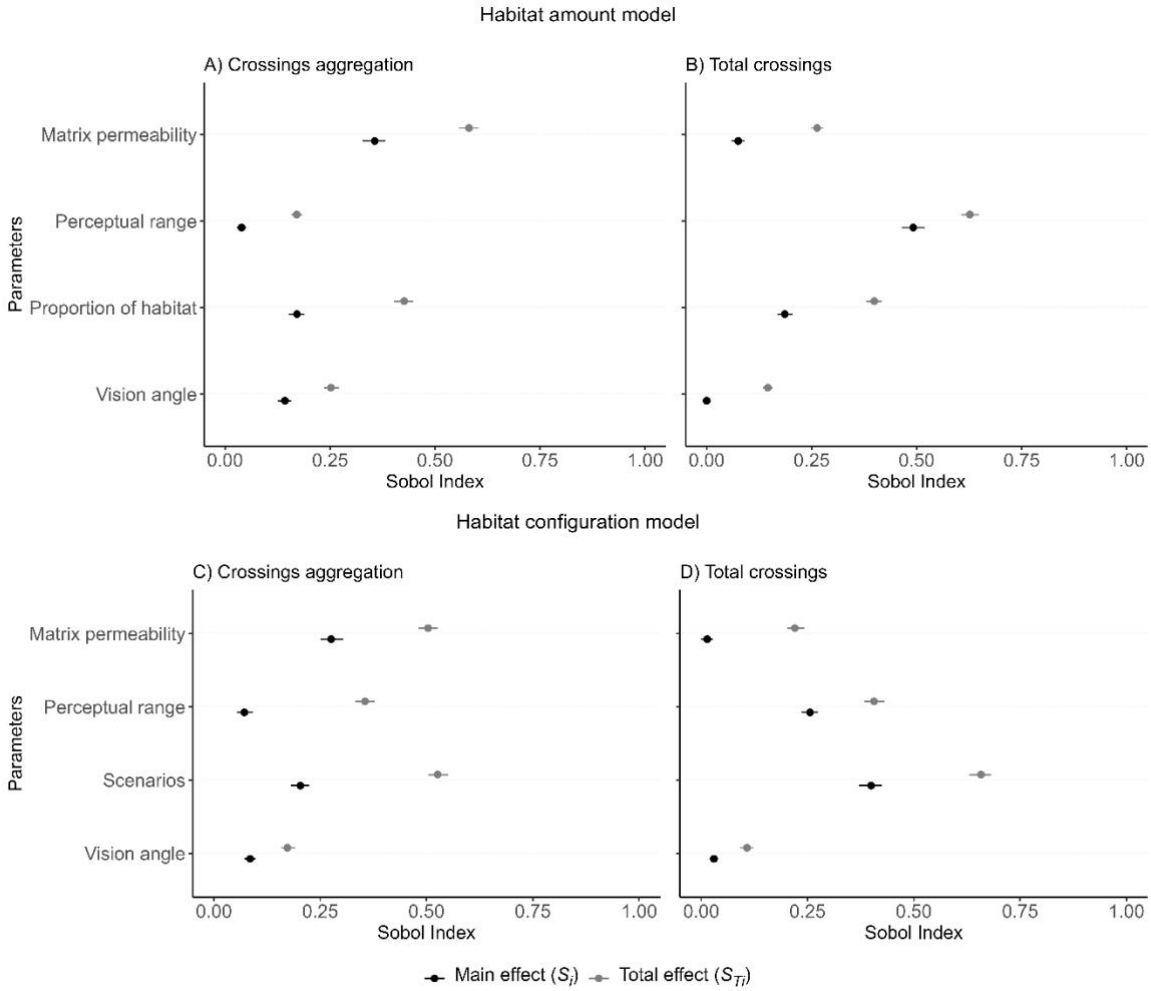


Figure 3. Sobol sensitivity analysis for crossings aggregation and total number of crossings of habitat amount model (A and B) and of habitat configuration model (C and D). Black circles and error bars show bias-corrected original main effect sensitivity index (S_i). Grey circles and error bars show bias-corrected original total effect sensitivity index (main and interaction effects, S_{Ti}).

The sum of the main effects from the sensitivity analysis was lower than 1 (Fig. 3), indicating that the interaction among parameters is also affecting model results. When exploring visually the relationship between model results and all parameters combined (A4), we identified that the relationship between crossings aggregation and perceptual range varies with habitat amount and habitat configuration in contexts of low matrix permeability.

4. Discussion

We used a mechanistic approach to understand how landscape and species traits can be used to prioritise mitigation of connectivity and wildlife-vehicle collision. We

found that habitat amount and habitat configuration drive crossings aggregation on the road. We showed that crossings aggregation varied with the input parameters: habitat amount, scenarios, matrix permeability, perceptual range, and vision angle. This variation in input variables was useful to identify sets of landscape features and navigation capacity traits (or contexts) where crossings are more aggregated and could be used to plan road mitigation.

The prioritisation for road mitigation in contexts with higher crossings aggregation would optimize the effectiveness of each mitigation unit or module (e.g., a given fenced road extension with its underpasses) due to concentration of wildlife use in specific road sections. Our results showed higher crossings aggregation on the road in simulations where landscapes presented intermediate amounts of habitat and lower number of patches, and when matrix permeability was lower, and perceptual range and vision angle were higher. Under these conditions individuals may have constrained their movement trajectories to habitat areas, and/or their wide perception allowed them to identify distant habitat areas, attracting crossings to specific road sections and resulting in more aggregated spatial patterns. These sets of traits could be considered a priority for connectivity and wildlife-vehicle collision mitigation, especially when real world movement data are not available, focusing on maintaining animals' movement between both roadsides but avoiding them to get exposed on the road.

Road crossings appear to be more random within landscapes with large amounts of habitat or high number of patches and when matrix permeability is high. This is probable because animals might move more freely in the landscapes. With crossings widely distributed along the road, it is more difficult to plan local mitigation, or the mitigation efficiency will be similar per spatial unit as the crossings are not concentrated. However, these contexts can be considered for mitigation when maintaining or restoring only a few safe crossroad movements is already a gain, for example for keeping genetic diversity and enhancing population viability (Benson et al., 2019; Van Der Ree et al., 2009). In contexts of high matrix permeability (resulting in lower crossings aggregation), planning to install several wildlife passages could enhance connectivity in the entire landscape (Karlson et al., 2017).

Our results showed a variation in the total number of crossings in the entire road when varying the parameters. This can be related to the spatial configuration of patches in the landscape, as this is known to lead to different movement patterns (Villard and Metzger, 2014). Higher number of road crossings results in more animal exposure to

vehicles on the road and, consequently, higher risk of collisions. In our simulations, higher number of crossings and higher aggregation were found when individuals had larger field of view. This is consistent with the evidence that species with higher spatial perception, which usually have higher body size and lower reproductive rates, are more susceptible to the negative effects of roads on animal abundance (Rytwinski and Fahrig, 2012). Therefore, as even simulations with few crossings resulted in crossings aggregation, a desirable mitigation planning should spatially prioritise locations where mitigation would be more efficient (higher crossings aggregation) but also where more animals are exposed to roads (higher total number of crossings).

We focused our individual-based models on the effects of landscape structure on wildlife road crossings, and for simplicity we assumed a road that does not include any specific effects (e.g., animal avoidance, traffic volume, sinuosity). This means that animals' movement is not affected by the road, and individuals do not die, due to the absence of road effects in our models. Either road avoidance or attraction (recorded for different species) might influence movement patterns, as well as behavioural response to roads and traffic (Jacobson et al., 2016; Rytwinski and Fahrig, 2015). Moreover, road mortality results from the combination of exposure (e.g. animal presence on a road) and hazard (e.g. traffic volume and speed) (Visintin et al., 2016). Further consideration of how spatial heterogeneity of road effects (avoidance and roadkill risk) influence wildlife movement patterns can improve the understanding of road impacts and their mitigation. Considering our model assumptions, our results could be applied to plan mitigation for connectivity loss and wildlife-vehicle collision in situations where we expect roads to have a uniform hazard, which can happen from local roads to highways and railways as long as they present low traffic variation regions.

Individual-based models offer a valuable theoretical framework for studying the interplay between animal movement ecology and road ecology, for example, providing guidance for effective management by simulating individual animal behaviour and its interactions with potential road impacts (Quaglietta et al., 2019). When landscape features and navigation capacity traits generate high total number of crossings but low crossings aggregation, preferably road construction should be avoided as animal movement is spread out along the entire road, or mitigation measures should be installed along the entire road. On the other hand, in contexts that result both in high total number of crossings and high crossings aggregation, priority road sections can be identified either to avoid (e.g., by smaller route deviations) or to minimize road impacts where crossings

are concentrated. To reduce the impacts in these specific sections, the installation of mitigation measures such as fences combined with crossing structures should be considered, to both maintain or restore connectivity and reduce wildlife-vehicle collision risk (Rytwinski et al., 2016; Soanes et al., 2024). Besides, landscape features and navigation capacity traits associated with low numbers of crossings, but high crossings aggregation could be considered for applying local measures to minimize impacts, although these contexts can be of less concern due to the low numbers of crossings. Therefore, our findings could be used to identify in which contexts mitigation planning should target to avoid, minimize, restore, or compensate road impacts, following the mitigation hierarchy framework (Arlidge et al., 2018).

5. Conclusions

Our model enabled the identification of crossings aggregation variations among habitat amount and configuration, matrix permeability and species navigation capacity traits. Our approach highlighted combinations of landscape features and species traits that can guide conservation strategies aiming to maximize ecological connectivity and minimize the risk wildlife-vehicle collision, especially when real data of these road impacts are not available. We demonstrated that higher aggregations of wildlife road crossings are expected in landscapes with intermediate proportion of habitat amount or few number of patches, with low matrix permeability and for animals with high perceptual range or vision angle.

Acknowledgements

We would like to thank Diego Bengochea, Érika Rocha, and Fernando Becker for their suggestions in a previous version of this manuscript which helped us to improve it; to Guilherme Korol for the computational assistance that allowed us to complete the simulations for sensitivity analysis.

Data availability

NetLogo model codes, analyses and model outputs are all available at https://github.com/bibianadasoler/ ibm-code.

References

Abreu, C.G., Ralha, C.G., 2018. An empirical workflow to integrate uncertainty and sensitivity analysis to evaluate agent-based simulation outputs. Environmental Modelling and Software 107, 281–297. https://doi.org/10.1016/j.envsoft.2018.06.013

Allen, C.H., Parrott, L., Kyle, C., 2016. An individual-based modelling approach to estimate landscape connectivity for bighorn sheep (Ovis canadensis). PeerJ 2016. https://doi.org/10.7717/peerj.2001

Arlidge, W.N.S., Bull, J.W., Addison, P.F.E., Burgass, M.J., Gianuca, D., Gorham, T.M., Jacob, C.D.S., Shumway, N., Sinclair, S.P., Watson, J.E.M., Wilcox, C., Milner-Gulland, E.J., 2018. A Global Mitigation Hierarchy for Nature Conservation. Bioscience 68, 336–347. https://doi.org/10.1093/biosci/biy029

Ascensão, F., Clevenger, A., Santos-Reis, M., Urbano, P., Jackson, N., 2013. Wildlife–vehicle collision mitigation: Is partial fencing the answer? An agent-based model approach. Ecol Modell 257, 36–43. https://doi.org/10.1016/j.ecolmodel.2013.02.026

Benson, J.F., Mahoney, P.J., Vickers, T.W., Sikich, J.A., Beier, P., Riley, S.P.D., Ernest, H.B., Boyce, W.M., 2019. Extinction vortex dynamics of top predators isolated by urbanization. Ecological Applications 29. https://doi.org/10.1002/eap.1868

Bolker, B., Bolker, M. Ben, 2017. Package 'bbmle.' Tools for General Maximum Likelihood Estimation 641.

Claeskens, G., Hjort, N.L., 2008. Model selection and model averaging. Cambridge University Press Cambridge.

Cosgrove, A.J., McWhorter, T.J., Maron, M., 2018. Consequences of impediments to animal movements at different scales: A conceptual framework and review. Divers Distrib. https://doi.org/10.1111/ddi.12699

DeAngelis, D.L., Grimm, V., 2014. Individual-based models in ecology after four decades. F1000Prime Rep 6. https://doi.org/10.12703/P6-39

Fahrig, L., 2007. Non-optimal animal movement in human-altered landscapes. Funct Ecol. https://doi.org/10.1111/j.1365-2435.2007.01326.x

Grimm, V., Berger, U., DeAngelis, D.L., Polhill, J.G., Giske, J., Railsback, S.F., 2010. The ODD protocol: A review and first update. Ecol Modell 221, 2760–2768. https://doi.org/10.1016/j.ecolmodel.2010.08.019

Grimm, V., Railsback, S.F., 2005. Individual-based modeling and ecology. Princeton university press.

Jacobson, S.L., Bliss-Ketchum, L.L., De Rivera, C.E., Smith, W.P., 2016. A behavior-based framework for assessing barrier effects to wildlife from vehicle traffic volume. Ecosphere 7, 1–15. https://doi.org/10.1002/ecs2.1345

Jeltsch, F., Bonte, D., Pe'er, G., Reineking, B., Leimgruber, P., Balkenhol, N., Schröder, B., Buchmann, C.M., Mueller, T., Blaum, N., Zurell, D., Böhning-Gaese, K., Wiegand, T., Eccard, J.A., Hofer, H., Reeg, J., Eggers, U., Bauer, S., 2013. Integrating movement ecology with biodiversity research - exploring new avenues to address spatiotemporal biodiversity dynamics. Mov Ecol. https://doi.org/10.1186/2051-3933-1-6

Karlson, M., Seiler, A., Mörtberg, U., 2017. The effect of fauna passages and landscape characteristics on barrier mitigation success. Ecol Eng 105, 211–220. https://doi.org/10.1016/j.ecoleng.2017.04.059

Knowlton, J.L., Graham, C.H., 2010. Using behavioral landscape ecology to predict species' responses to land-use and climate change. Biol Conserv. https://doi.org/10.1016/j.biocon.2010.03.011

Lewis, J.S., Rachlow, J.L., Horne, J.S., Garton, E.O., Wakkinen, W.L., Hayden, J., Zager, P., 2011. Identifying habitat characteristics to predict highway crossing areas for black bears within a human-modified landscape. Landsc Urban Plan 101, 99–107. https://doi.org/10.1016/j.landurbplan.2011.01.008

Lima, S.L., Zollner, P.A., 1996. Towards a behavioral ecology of ecological landscapes. Trends Ecol Evol 11, 131–135. https://doi.org/10.1016/0169-5347(96)81094-9

McLane, A.J., Semeniuk, C., McDermid, G.J., Marceau, D.J., 2011. The role of agent-based models in wildlife ecology and management. Ecol Modell. https://doi.org/10.1016/j.ecolmodel.2011.01.020

Nathan, R., Getz, W.M., Revilla, E., Holyoak, M., Kadmon, R., Saltz, D., Smouse, P.E., 2008. A movement ecology paradigm for unifying organismal movement research.

Onofri, A., 2020. The broken bridge between biologists and statisticians: a blog and R package.

Pujol, G., Iooss, B., Iooss, M.B., DiceDesign, S., 2015. Package 'sensitivity.'

Quaglietta, L., Porto, M., Ford, A.T., 2019. Simulating animal movements to predict wildlife-vehicle collisions: illustrating an application of the novel R package SiMRiv. Eur J Wildl Res 65, 100. https://doi.org/10.1007/s10344-019-1333-z

R Core Team, 2023. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing.

Rytwinski, T., Fahrig, L., 2015. The impacts of roads and traffic on terrestrial animal populations. Handbook of road ecology 237–246.

Rytwinski, T., Fahrig, L., 2012. Do species life history traits explain population responses to roads? A meta-analysis. Biol Conserv 147, 87–98. https://doi.org/10.1016/j.biocon.2011.11.023

Rytwinski, T., Soanes, K., Jaeger, J.A.G., Fahrig, L., Findlay, C.S., Houlahan, J., Van Ree, R. Der, Van Der Grift, E.A., 2016. How effective is road mitigation at reducing road-kill? A meta-analysis. PLoS One 11, 1–25. https://doi.org/10.1371/journal.pone.0166941

Salecker, J., Sciaini, M., Meyer, K.M., Wiegand, K., 2019. The nlrx r package: A next-generation framework for reproducible NetLogo model analyses. Methods Ecol Evol 10, 1854–1863. https://doi.org/10.1111/2041-210X.13286

Saltelli, A., Ratto, M., Andres, T., Campolongo, F., Cariboni, J., Gatelli, D., Saisana, M., Tarantola, S., 2008. Global sensitivity analysis: the primer. John Wiley & Sons.

Singleton, P.H., Gaines, W.L., Lehmkuhl, J.F., 2002. Landscape Permeability for Large Carnivores in Washington: A Geographic Information System Weighted-Distance and Least-Cost Corridor Assessment.

Soanes, K., Rytwinski, T., Fahrig, L., Huijser, M.P., Jaeger, J.A.G., Teixeira, F.Z., van der Ree, R., van der Grift, E.A., 2024. Do wildlife crossing structures mitigate the

barrier effect of roads on animal movement? A global assessment. Journal of Applied Ecology 61, 417–430. https://doi.org/10.1111/1365-2664.14582
Sobol', I.M., Tarantola, S., Gatelli, D., Kucherenko, S.S., Mauntz, W., 2007. Estimating the approximation error when fixing unessential factors in global sensitivity analysis. Reliab Eng Syst Saf 92, 957–960. https://doi.org/10.1016/j.ress.2006.07.001
Tang, W., Bennett, D.A., 2010. Agent-based modeling of animal movement: A review. Geogr Compass 4, 682–700. https://doi.org/10.1111/j.1749-8198.2010.00337.x
Teixeira, F.Z., Kindel, A., Hartz, S.M., Mitchell, S., Fahrig, L., 2017. When road-kill hotspots do not indicate the best sites for road-kill mitigation. Journal of Applied Ecology 54, 1544–1551. https://doi.org/10.1111/1365-2664.12870
Thiele, J.C., Kurth, W., Grimm, V., 2014. Facilitating Parameter Estimation and Sensitivity Analysis of Agent-Based Models: A Cookbook Using NetLogo and R, Journal of Artificial Societies and Social Simulation.
Van Der Ree, R., Heinze, D., Mccarthy, M., Mansergh, I., 2009. Wildlife Tunnel Enhances Population Viability. Ecology and Society 14.
Villard, M., Metzger, J.P., 2014. REVIEW: Beyond the fragmentation debate: a conceptual model to predict when habitat configuration really matters. Journal of Applied Ecology 51, 309–318. https://doi.org/10.1111/1365-2664.12190
Visintin, C., Van Der Ree, R., McCarthy, M.A., 2016. A simple framework for a complex problem? Predicting wildlife–vehicle collisions. Ecol Evol 6, 6409–6421.
Wilensky, U., 1999. NetLogo.
Zeller, K.A., McGarigal, K., Whiteley, A.R., 2012. Estimating landscape resistance to movement: A review. Landsc Ecol. https://doi.org/10.1007/s10980-012-9737-0

Supplementary Material

A1. Objectives, Design concepts, and Details (ODD protocol)

Purpose

The main goal of this model is to understand how habitat amount and habitat configuration affects wildlife road crossings aggregation. To address this, we simulated animal movement in a landscape created based on two models: 1) habitat amount: varying the habitat proportion in the landscape; and 2) habitat configuration: varying the number and the arrangement of habitat patches with constant habitat amount. We expect to obtain the proportion of wildlife road crossings in the 25% most crossed patches of the road, based on how many times the animals cross each segment of the road.

Entities, state variables, and scales

The landscape

We have a binary landscape composed of patches of habitat dispersed in a non-habitat matrix, in which a road cuts the world horizontally. The environment in both models had the same sizes and are divided by a uniform road that does not influence animal movement; animals can cross the road but are not killed. The environment is a cell grid of 84 x 84 patches, and the landscape is generated according to the model chosen (see description in model the section). All variables relating to the patches are described below:

Static variables

- habitat: referring to the type of habitat of each patch. The values are the following: habitat = 1, matrix = 2, road = 0.
- permeability: referring to how permeable a patch is to the movement of individuals. The values vary between 10 to 90 to non-habitat (matrix) patches, obtained from matrix_permeability slider before setting up the model, and value of habitat patches is always defined as 100.
- proportion_of_habitat: referring to the habitat amount present in the landscape. The values range between 10% and 90%. This is only related to the habitat amount model (selecting "Habitat amount" in the chooser button) and is defined by the proportion_of_habitat slider before setting up the model.

Dynamic variables

- current_permeability: referring to the current permeability of a group of patches (candidate_pacthes). It is calculated considering patch permeability and the agent distance of each candidate_patches.
- visits: referring to the number of visits each patch receives. Each time that an animal passes through the patch is considered a visit.

The agents

All agents consider the following variables in their movement:

- candidate_patches: referring to the patches inside a cone in front of the agent. The cone has a radius size, obtained from perceptual_range value, and an angle degree, obtained from vision_angle value.

- chosen_patch: referring to the patch that has been chosen as the direction in which an agent will travel. At each tick, each individual has to choose its "chosen_patch" between one of candidate-patches.
- perceptual_range: referring to the radius distance of agent vision. This value varies between 5 and 42 and is defined in the slider button by the user before setting up the model.
- vision_angle: referring to the angle of vision of the agent. This value varies between 90° and 180° and is defined in the slider button by the user before setting up the model.

Process overview and scheduling

Before running the model, users must choose the number of individuals and steps. Users also must define the values for all parameters. Then, in the setup button, the landscape is generated, and the individuals are created in random locations weighted by patch permeability. After this, in the go button, individuals will be moving through the landscape until the number of ticks reaches the number of steps decided by the user. Figure 1 provides a representation of model steps.

In each tick, each individual identifies the candidate patches, which are all patches inside the cone of vision with radius size defined by perceptual range and angle defined by vision angle. For each candidate patch, the current permeability is calculated, considering the patch distance and the patch permeability (100 if a habitat patch and the value of matrix permeability for matrix patches). Then, considering this "current permeability" a patch is chosen to be faced, using a weighted probability function.

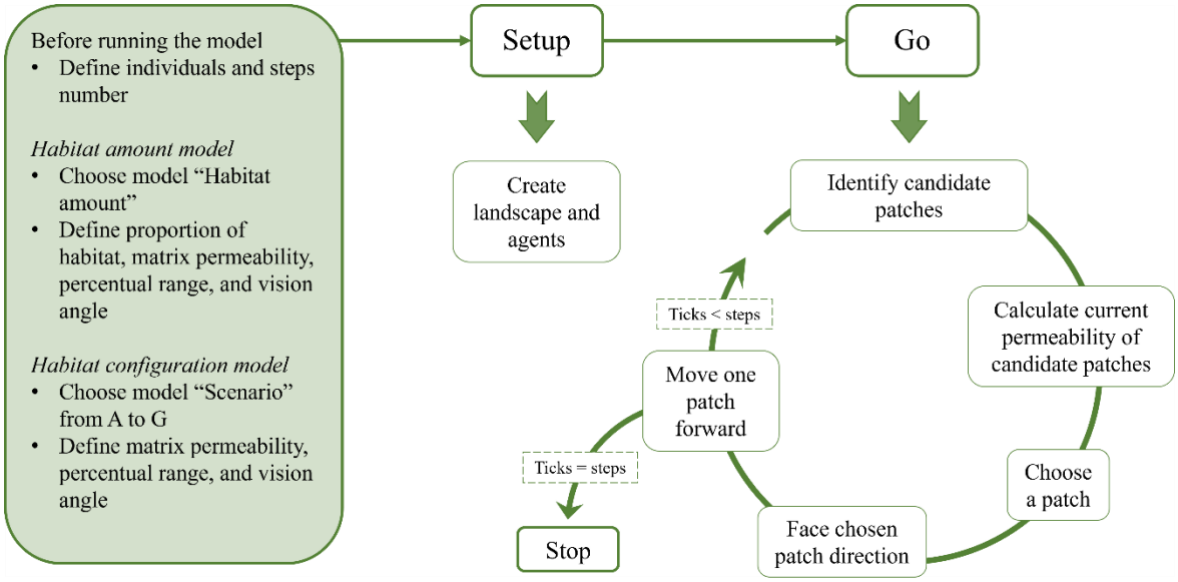


Figure 1. Diagram describing the steps in the model simulation.

Design concepts

Basic principles: this model aims to understand how changes in habitat amount and habitat configuration influence wildlife road crossings. Agents' movement is implemented as a correlated random walk, as they will choose a patch based on the current permeability (permeability value and the distance from the individual to each patch) of each patch inside a cone with an angle related to the last step. Individuals will keep moving until the number of ticks reaches the number of steps the users have chosen.

Emergence: the pattern of crossings emerges from individuals' movements influenced by habitat amount (model 1) or habitat configuration (model 2), patch permeability, perceptual range and vision angle.

Adaptation: individuals do not have adaptative traits, they make decisions to where to move based on the current permeability of candidate patches.

Objectives: there is no objective in the agent's movement.

Learning: agents do not learn neither from other agents nor from the past.

Prediction: agents cannot predict future information.

Sensing: agents can see all patches inside a cone (with radius based on perceptual_range and angle based on vision_angle) and evaluate the patch permeability value and their distance to each patch.

Interaction: not included. Each agent is independent from one another.

Stochasticity: in initialization, it is used to define agents' position. In the habitat amount model, it serves to determine which patches will be designated as habitats. Throughout the simulation, stochasticity is applied when individuals have to select a patch among the candidate patches.

Collectives: no collectives were implemented as the agents are independent of each other.

Observation: the simulation outputs are prop_top_sections (the proportion of crossings in the 25% most crossed road patches; obtained from the division of number of visits in 25% most crossed road sections by the number of visits in the entire road) and total_crossings (the total number of visits in the entire road). The outputs are recorded at the end of model running (when ticks are equal to steps number). Additional information of inputs parameters is also recorded with output to posteriori analyses.

Initialization

Before starting the model, the user should choose the number of individuals, the number of steps, matrix permeability (between 10 and 90), perceptual range (between 5 and 42), and vision angle (between 90° and 180°). For a habitat amount model, the user must choose model "Habitat amount" and define the proportion of habitat desired (between 10% and 90%). For habitat configuration model, proportion of habitat is constant, and the user must choose between model "Scenario - X" (X varying from A to G). Also, the user should indicate whether to save the results or not, and if so, indicate a path file and a filename. After making these decisions, the user should set up the model to generate the landscape and to create the individuals. Then, individuals start to move, and ticks number starts to rise.

Models

We developed two models to generate the landscapes. Both models have the same outputs and the same pattern of animal movement. The two outputs from the models are crossings aggregation and total crossings. Crossings aggregation corresponds to the proportion of crossings in the 25% most crossed patches of the road. To obtain this value, we sorted all road patches in decrescent order by number of visits in each patch, and then we divided the sum of crossings in the 25% most crossed patches by the number of visits in the entire road. Total crossings correspond to the sum of visits in all patches in the road. Models do, however, differ slightly in their inputs and how the landscapes are created.

Model 1: Habitat amount

This model was developed to understand the influence of *different proportions of habitat amount* in crossings aggregation and total number of crossings. In this model the user

must define the proportion of habitat (varying from 10 % to 90 %), and the landscape is randomly created.

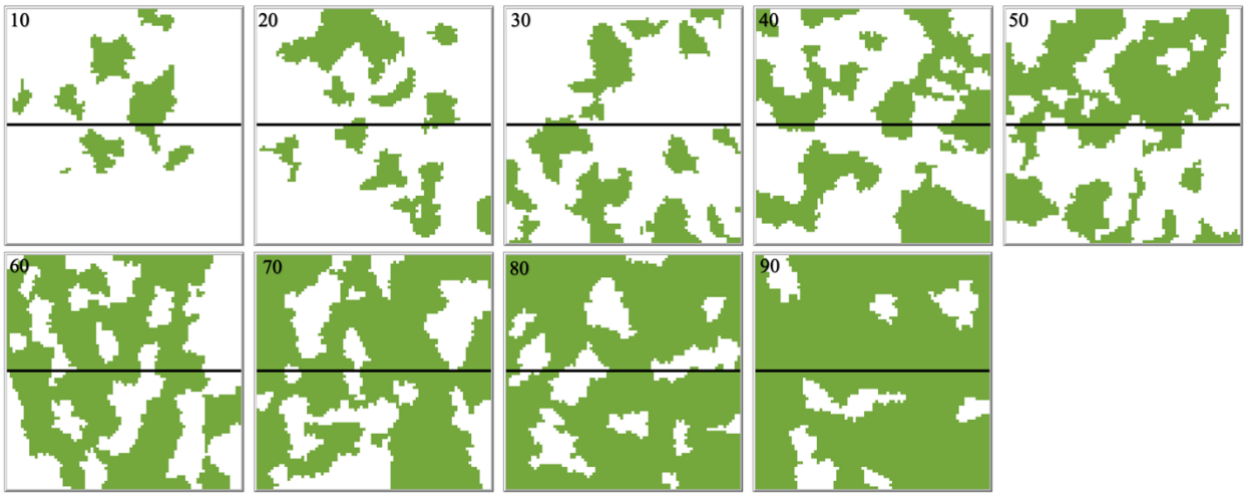


Figure 2. Examples of landscapes of habitat amount model. The values refer to proportions of habitat in each landscape (%).

Model 2: Habitat configuration

This model was developed to understand the influence of *different habitat patches configuration* in crossings aggregation and total number of crossings. It has seven scenarios (already defined), varying in patches number and their arrangement, that must be chosen by the user.

Scenario A – 1 patch

Scenario B – 2 vertical patches

Scenario C – 2 horizontal patches

Scenario E – 16 patches away from road

Scenario F – vertical lines

Scenario G – horizontal lines

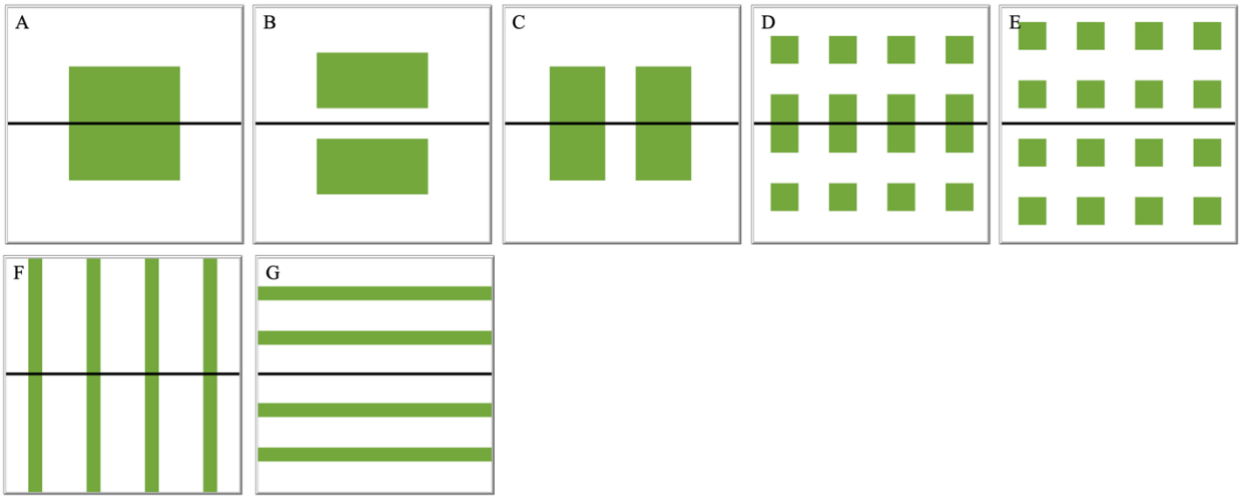


Figure 3. Landscapes created for habitat configuration model.

Input data

This model does not have input data.

Sub-models

The code procedures were developed to create and paint landscape and agents, to the animals move, to produce the output and to save the data.

Landscape and agents

Setup

The environment will be created depending on the option chosen by the user in the chooser button "model". If "Habitat amount" model was selected, the landscape will be created in the procedure "create_random", considering the proportion of habitat defined by the user in slider button. If one of the scenarios options were selected, the landscape will be generated using one of the "create_landscapes" procedures. The landscape patches are painted according to their habitat values (habitat, matrix or road in "paint_habitats" procedure) or to their visits number ("paint_patch_use" procedure). Also, the paint of road patches can be based only on the number of visits in road patches ("paint_road_crossings" procedure).

Setup_turtles

The agents will be created following the number of individuals defined by the user in random locations weighted by the patch permeability.

New_run

Another simulation can be run using the same landscape as before. All agents will be killed, and new ones will be created using the procedure "setup_turtles". Patches information will be restarted.

Update_permeability

Patches permeability can be reset to the initial values. Habitat patches will always be set on 100 and matrix patches will be set according to the value defined in the slider button "matrix_permeability".

Movements

Go

At each tick the individuals must identify their candidate patches ("check_move" procedure), which is any patch inside a cone with a radius of the size of the perceptual range and with an angle of the vision angle. For each candidate patch, a current permeability will be calculated considering the permeability of the patch and the distance between the agent and each candidate patch. Then, agents randomly choose patches weighted by the current permeability. After this, the individuals will face the chosen patch and move one patch towards the chosen patch ("move" procedure). This will be repeated until the number of ticks reach the number of steps defined by the user ("stop_simulation?").

Outputs

Prop_top_sections

To obtain the proportion of crossings in the 25% of most crossed patches of the, first, the crossings are sorted by the number of visits. Then, the crossings of 25% of road patches with higher crossing numbers road (in the top sections of the road) are summed and divided by the number of all crossings.

Total_crossings

The number of crossings in the entire road are calculated summing the number of crossings in all road patches.

Save data

Set_filename

This function either creates a new file (.csv) or accesses an existing file, as long as it is in the directory folder. If the file exists, it will be accessed by Netlogo for data storage; if not, a new file is generated to store the simulation information, such as the number of individuals, number of steps, model type, proportion of habitat, matrix permeability, perceptual range, vision angle, total crossings, and proportion of crossings in top sections. Then, the simulation information is saved when the model stopped, and the file is closed, using the "save_data" procedure. The file can be deleted using the "delete_file" procedure.

A2. Correlation analysis

We generated random landscapes to test the correlation between proportion of habitat and the number of habitat patches in the habitat amount model. We created 21,999 landscapes, varying the range of proportion of habitat from 10% to 90%, and saved them in raster format from NetLogo. Then, in the R environment (R Core Team, 2023), we extracted each patch number using "get_patches" function from "*landscapemetrics*" package (Hesselbarth et al., 2019). After that, we tested the correlation between the proportion of habitat, used to generate the landscapes, and the identified patches number, using function "cor.test", using Pearson's method, from the "*stats*" package. We found a negative correlation between these variables ($r = -0.7942091$, $p = 2.2e-16$). As proportion of habitat increases, the number of habitat patches decrease (Fig. 1).

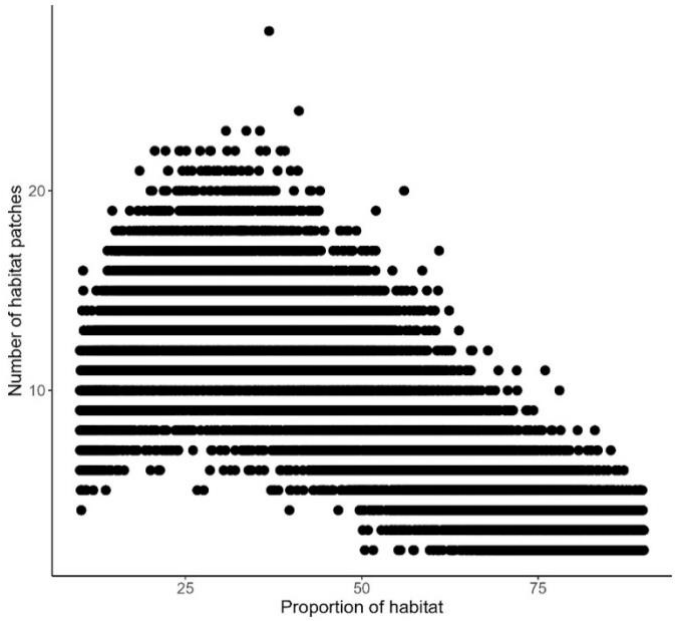


Figure 1. Correlation between proportion of habitat and the number of habitat patches.

Hesselbarth, M. H. K., Sciaini, M., With, K. A., Wiegand, K., & Nowosad, J. (2019). landscapemetrics: an open-source R tool to calculate landscape metrics. *Ecography*, *42*(10), 1648–1657. https://doi.org/10.1111/ecog.04617

R Core Team. (2023). R: a Language and Environment for Statistical Computing. In *R Foundation for Statistical Computing*. https://www.r-project.org/

Wickham, H. (2016). *ggplot2: elegant graphics for data analysis. R package version 3.4. 0*.

A3. Model selections

Table 1. Best-ranked models for crossings aggregation and total crossings in habitat amount model. Models were selected using Bayesian Information Criterion values (BIC) and weights. Values of coefficient of determination (R^2 or pseudoR^2) and of fitted parameters (a, b, c, d, e, k) are given. Only models with delta AIC (ΔBIC) <2 are showed.

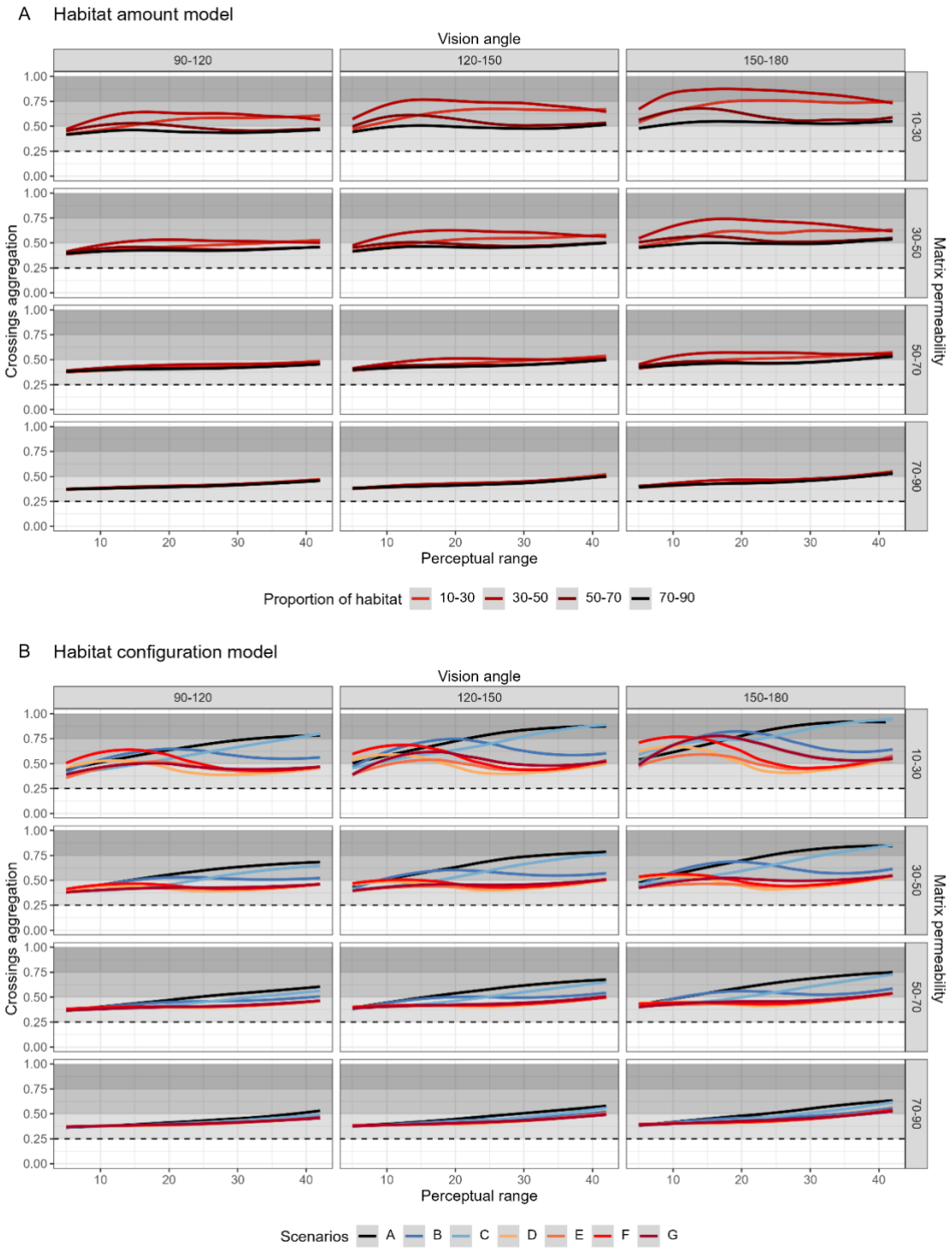
Model	BIC	weight	R^2/pseudo R^2	a	b	c	d	e	k
Crossings aggregation									
Proportion of habitat									
Bragg	-107864.8	1	0.1755029	-	0.0027	0.4488	0.5756	36.4284	-
Matrix permeability									
Asymptotic	-121644.0	1	0.3445615	0.3705	0.7112	0.02158	-	-	-
Perceptual range									
Asymptotic	-98447.8	1	0.0352124	0.5153	0.3166	0.1667	-	-	-
Vision angle									
Exponential	-105763.2	0.925	0.1457978	0.3252	-	-	-	-	0.003179
Total crossings									
Proportion of habitat									
Bragg	785329.2	1	0.1055766	-	0.0032	615.2373	773.2743	68.2916	-
Matrix permeability									
Asymptotic	787462.3	1	0.0730374	826.0763	537.4781	0.0138	-	-	-
Perceptual range									
Bragg	751541.3	1	0.4906947	-	0.0004	492.1779	2930.129	102.3736	-
Vision angle									
Asymptotic	791661.2	0.87	0.0058423	697.3634	307.9888	0.0219	-	-	-

Table 2. Best-ranked models for crossings aggregation and total crossings in habitat configuration model. Models were selected using Bayesian Information Criterion values (BIC) and weights. Values of coefficient of determination (R^2 or pseudoR^2) and of fitted parameters (a, b, c, d, e, k) are given. Only models with delta AIC (ΔBIC) <2 are showed.

Model	BIC	weight	R^2/pseudo R^2	a	b	c	d	e	k
Crossings aggregation									
Matrix permeability									
Asymptotic	-108054.0	1	0.26918	0.3952	0.7122	0.02655	-	-	-
Perceptual range									
Exponential	-92473.3	0.996	0.05231	0.4407	-	-	-	-	0.00497
Vision angle									
Linear	-94873.8	0.65	0.08949	0.3173	0.0013	-	-	-	-
Exponential	-94872.6	0.35	0.08946	0.3451	-	-	-	-	0.00266
Total crossings									
Matrix permeability									
Asymptotic	899434.0	0.515	0.01271	683.984	982.5109	0.01363	-	-	-
Exponential	899434.3	0.449	0.01252	954.5504					- 0.00252
Perceptual range									
Exponential	881612.8	1	0.26628	449.7172	-	-	-	-	0.02519
Vision angle									
Asymptotic	899052.9	1	0.018961	1012.5318	- 15.3241	-	-	-	-

A4. Exploratory visualization

We made an exploratory visualization of the relationship between the model outputs and all the parameters combined for both models. We categorized some variables (proportion of habitat, matrix permeability and vision angle) to be able to visually explore patterns of crossings aggregation and total number of crossings. Categories were arbitrarily defined to have the same range. Plots include a smoothing line from a generative additive model for categories of proportion of habitat (Fig. 1 A and Fig. 2 A) that was generated using the function "geom_smooth", with default arguments (method was automatically defined as "gam" and formula as "y ~ s(x, bs = "cs")", from the package "ggplot2" (Wickham, 2016). Plots include a smoothing line from a locally estimated scatterplot smoothing for scenarios (Fig. 1 B and Fig. 2 B) that was generated using the function "geom_smooth", with default arguments (method was automatically defined as "loess" and formula as "y ~ x", from the package "*ggplot2*" (Wickham, 2016).



719

720 Figure 1. Results of crossings aggregation values for all input parameters in habitat amount model
721 (A) and in habitat configuration model (B). The stronger the shade of grey, the greater the
722 aggregation. Dashed line shows the minimum possible value for crossings aggregation according
723 to its definition in our model.

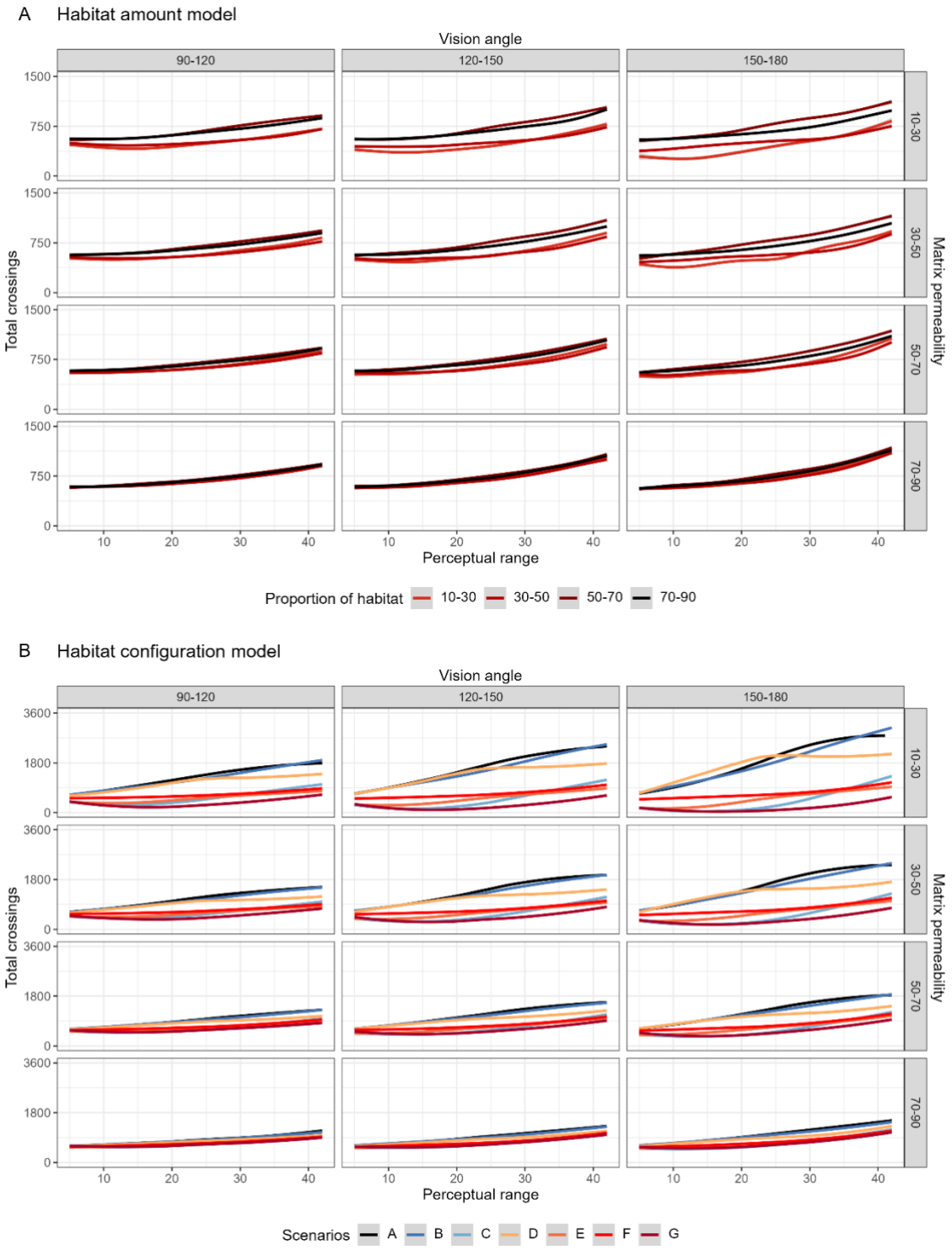


Figure 2. Results of number of total crossings for all input parameters in habitat amount model (A) and in habitat configuration model (B).

Considerações Finais

As infraestruturas lineares são fundamentais no nosso cotidiano, mas causam inúmeros impactos à biodiversidade. Medidas de mitigação são essenciais para reduzir os impactos que não podem ser evitados e deve-se otimizar a alocação dos recursos disponíveis para maximizar as oportunidades de redução dos impactos. Nessa tese, utilizei diferentes abordagens para auxiliar o planejamento e a avaliação de medidas mitigadoras de impactos de ferrovias e rodovias na fauna.

A partir dos experimentos realizados no capítulo I, concluí que a simples remoção do lastro entre dormentes nos trilhos das ferrovias pode evitar o aprisionamento de tartarugas semiaquáticas. Enquanto os jabutis apresentaram evitamento às canaletas. Resultados como esses permitem uma melhor compreensão quanto à funcionalidade de medidas de mitigação e são importantes para assegurar que sejam instaladas apenas medidas com potencial de evitar o impacto para os quais foram desenvolvidas.

No capítulo II, avaliei a efetividade de três sistemas de mitigação para redução das fatalidades de fauna. Com o acúmulo de dados observados ao longo de 14 anos, ficou evidente que o sistema não é efetivo, provavelmente devido ao uso de cercas inadequadas para bloquear aos animais da região e à carência de manutenção. Ainda que as estruturas de travessia tenham apresentado um aumento no número de cruzamentos dos animais ao longo dos anos, os resultados concordam com a literatura, que indica que as cercas são essenciais para a redução de colisões entre veículos e fauna. Nesse caso, são necessárias melhorias no sistema de mitigação, como a instalação de cercas apropriadas para as espécies mais observadas mortas na estrada e a realização de frequente manutenção das cercas.

No capítulo III, consegui compreender como esses fatores afetam o cruzamento dos animais nas estradas, por meio de simulações com diferentes características da paisagem e de movimentação animal. Os modelos desenvolvidos permitiram identificar variações nas agregações dos cruzamentos, com maior agregação em paisagens com quantidades intermediárias de habitat ou com poucas manchas de habitat e em simulações com baixa permeabilidade da matriz ou animais com maior visão (alcance da percepção ou ângulo da visão). As condições, das paisagens e dos animais, que resultaram em maior agregação podem ser consideradas para o planejamento da mitigação dos impactos das

estradas, como a conectividade e a fatalidade de fauna, quando não há dados locais disponíveis.

Os resultados dessa tese indicam que a aplicação de abordagens distintas como experimentação, observação e simulação qualificam o entendimento das medidas mitigadoras em diferentes fases das ferrovias e rodovias. Além disso, a colaboração entre diversos setores e profissionais é essencial para a obtenção de dados e a aplicação dessas abordagens. O capítulo I, por exemplo, foi realizado a partir da identificação das mortes dos quelônios e do desenvolvimento de canaletas por duas concessionárias ferroviárias. Já o capítulo II foi desenvolvido com dados obtidos com a execução de condicionantes da Licença de Operação de uma rodovia. Todos os resultados encontrados nessa tese podem subsidiar futuras tomadas de decisão no âmbito do planejamento e da avaliação da mitigação. A fim de obter respostas mais robustas, a integração dessas abordagens pode ser utilizada para fortalecer os resultados obtidos isoladamente, desde que haja disponibilidade de dados ou viabilidade para sua obtenção. Avançar na compreensão das medidas mitigadoras é crucial para a facilitar sua aplicação e aumentar sua efetividade.

Epílogo, ou: a minha motivação

Ao longo da elaboração dessa tese de doutorado, vivi diferentes fases em relação à motivação em fazê-la. O otimismo e o entusiasmo do início foram abruptamente interrompidos pela pandemia da Covid-19. Aos poucos, todos nos adaptamos e novas motivações surgiram. Quando parecia ser possível avistar o fim dessa tese, a energia para concluí-la foi levada pela enchente histórica que ocorreu no Rio Grande do Sul em maio de 2024. Foram muitos altos e baixos que, aos poucos, fui descobrindo fazerem parte do processo. Embora pareça incoerente começar uma sessão de motivação relembrando situações difíceis, elas mostram a necessidade de achar novas motivações e recomeçar em qualquer situação.

As rodovias e as ferrovias trazem inúmeros benefícios para nós, humanos, mas trazem impactos significativos à biodiversidade, muitas vezes despercebidos pela sociedade. Conversar com diferentes pessoas sobre isso – principalmente com quem não havia refletido sobre os impactos das estradas – sempre me trouxe motivação para avançar nessa tese. “Como vai tua pesquisa? É muito triste, tenho visto muitos animais mortos nas estradas.” “Vi dois bugios usando as passagens de corda, que incrível!” Frases assim servem como combustível para buscar respostas, aumentar a sensibilização sobre o

problema e mostrar que há soluções para reduzi-lo. Ter a oportunidade de questionar, investigar, compreender e buscar alternativas melhores na interação das estradas com a fauna me move. Tenho como motivação, e espero seguir tendo, a possibilidade de gerar informação que auxilie pesquisadores, órgãos ambientais e consultores na redução dos impactos das estradas.

Hoje, cinco anos depois de ter começado essa trajetória, eu finalmente cheguei ao que considero o fim dessa tese. Obviamente a sensação de que poderia ter feito mais e melhor existe, mas certa vez ouvi que "doutorado não se termina, doutorado se dá um fim" e cheguei ao fim do meu. Eu tive a sorte de desenvolver esse trabalho em um ambiente seguro, com a participação de pessoas que se importam com mais do que apenas a publicação de artigos científicos e que valorizam as diferentes etapas do percurso, me ajudando a achar motivação em momentos desmotivados. O caminho para me tornar Doutora em Ecologia foi percorrido além dos capítulos apresentados nessa tese, mas sem a motivação em concluí-los, não haveria como trilhar o caminho. Finalmente, eu chego ao final com o otimismo e o entusiasmo do início, motivada por perguntas que ainda seguem sem resposta e pela divulgação dos impactos das rodovias, mas principalmente pelas soluções para eles.

Referências

ARLIDGE, William N.S. *et al.* A Global Mitigation Hierarchy for Nature Conservation. **BioScience**, [*s. l.*], v. 68, n. 5, p. 336–347, 2018.

ASCENSÃO, Fernando *et al.* Beware that the lack of wildlife mortality records can mask a serious impact of linear infrastructures. **Global Ecology and Conservation**, [*s. l.*], v. 19, 2019.

ASCENSÃO, Fernando *et al.* Wildlife–vehicle collision mitigation: Is partial fencing the answer? An agent-based model approach. **Ecological Modelling**, [*s. l.*], v. 257, p. 36–43, 2013. Disponível em: https://linkinghub.elsevier.com/retrieve/pii/S0304380013001294.

ASSIS, Julia C.; GIACOMINI, Henrique C.; RIBEIRO, Milton Cezar. Road Permeability Index: Evaluating the heterogeneous permeability of roads for wildlife crossing. **Ecological Indicators**, [*s. l.*], v. 99, n. May 2018, p. 365–374, 2019.

BUDZIK, Karolina A.; BUDZIK, Krystian M. A preliminary report of amphibian mortality patterns on railways. **Acta Herpetologica**, [*s. l.*], v. 9, n. 1, p. 103–107, 2014. Disponível em: www.fupress.com/ah. Acesso em: 20 ago. 2020.

CONAN, Antonin *et al.* Effectiveness of wire netting fences to prevent animal access to road infrastructures: an experimental study on small mammals and amphibians. **Nature Conservation**, [*s. l.*], v. 47, p. 271–281, 2022.

COSGROVE, Anita J.; MCWHORTER, Todd J.; MARON, Martine. **Consequences of impediments to animal movements at different scales: A conceptual framework and review**. [*S. l.*]: Blackwell Publishing Ltd, 2018.

DASOLER, Bibiana Terra. **Campanhas educativas de trânsito e as colisões com a fauna silvestre**. 2021. - Universidade Federal do Rio Grande do Sul, Porto Alegre, 2021.

DASOLER, Bibiana Terra; GONÇALVES, Larissa O. Traffic education campaigns and animal-vehicle collisions in Brazil. **Anais da Academia Brasileira de Ciencias**, [*s. l.*], v. 95, n. 3, 2023.

DOHERTY, Tim S.; HAYS, Graeme C.; DRISCOLL, Don A. Human disturbance causes widespread disruption of animal movement. **Nature Ecology and Evolution**, [*s. l.*], v. 5, n. 4, p. 513–519, 2021.

DORNAS, Rubem *et al.* Strain by the train: Patterns of toad fatalities on a Brazilian Amazonian railroad. **Science of the Total Environment**, [*s. l.*], v. 660, p. 493–500, 2019. Disponível em: https://doi.org/10.1016/j.scitotenv.2018.12.371.

EKSTROM, Jon; BENNUN, Leon; MITCHELL, Robin. A cross-sector guide for implementing the Mitigation Hierarchy. **Cross Sector Biodiversity Initiative, Cambridge**, [*s. l.*], 2015.

FAHRIG, Lenore. **Non-optimal animal movement in human-altered landscapes**. [*S. l.: s. n.*], 2007.

FAHRIG, L; RYTWINSKI, T. Effects of roads on animal abundance: An empirical review and synthesis. **Ecology and Society**, [*s. l.*], v. 14, n. 1, 2009.

FRANCESCHI, Ingridi Camboim *et al.* Monitoring the use of a canopy bridge and underpasses by arboreal mammals on a Brazilian coastal road. **Folia Primatologica**, [*s. l.*], 2022.

GLISTA, David J.; DEVAULT, Travis L.; DEWOODY, J. Andrew. A review of mitigation measures for reducing wildlife mortality on roadways. **Landscape and Urban Planning**, [*s. l.*], v. 91, n. 1, p. 1–7, 2009.

GONÇALVES, Larissa Oliveira *et al.* **Entre os caminhos da ecologia de transportes: desafios para a conservação da fauna**. 1. ed. Guarujá: Fauna News, 2023a.

GONÇALVES, Larissa Oliveira *et al.* Estimativas e análises espaciais de fatalidades de fauna em rodovias com o programa Siriema. *In*: FREITAS, Simone; MENDONÇA, Renata; BAPTISTA, Marcela (org.). **Impacto dos transportes na fauna**. Santo André: Universidade Federal do ABC, 2023b. p. 91–112.

GRIMM, Volker; RAILSBACK, Steven F. **Individual-based modeling and ecology**. [*S. l.*]: Princeton university press, 2005.

HILL, Jacob E.; DEVAULT, Travis L.; BELANT, Jerrold L. Cause-specific mortality of the world's terrestrial vertebrates. **Global Ecology and Biogeography**, [*s. l.*], v. 28, n. 5, p. 680–689, 2019.

HOLDEREGGER, Rolf; DI GIULIO, Manuela. The genetic effects of roads: A review of empirical evidence. **Basic and Applied Ecology**, [*s. l.*], v. 11, n. 6, p. 522–531, 2010.

KORNILEV, Yurii V.; PRICE, Steven J.; DORCAS, Michael E. Between a rock and a hard place: Responses of eastern box turtles (Terrapene carolina) when trapped between railroad tracks. **Herpetological Review**, [*s. l.*], v. 37, n. 2, p. 145–148, 2006.

LIMA, Steven L.; ZOLLNER, Patrick A. Towards a behavioral ecology of ecological landscapes. **Trends in Ecology & Evolution**, [*s. l.*], v. 11, n. 3, p. 131–135, 1996. Disponível em: https://linkinghub.elsevier.com/retrieve/pii/0169534796810949.

NATHAN, Ran *et al.* **A movement ecology paradigm for unifying organismal movement research**. [*S. l.: s. n.*], 2008. Disponível em: www.pnas.orgcgidoi10.1073pnas.0800375105. .

QUAGLIETTA, Lorenzo; PORTO, Miguel; FORD, Adam T. Simulating animal movements to predict wildlife-vehicle collisions: illustrating an application of the novel R package SiMRiv. **European Journal of Wildlife Research**, [*s. l.*], v. 65, n. 6, p. 100, 2019. Disponível em: http://link.springer.com/10.1007/s10344-019-1333-z.

RYTWINSKI, Trina *et al.* Experimental study designs to improve the evaluation of road mitigation measures for wildlife. **Journal of Environmental Management**, [*s. l.*], v. 154, p. 48–64, 2015.

RYTWINSKI, Trina *et al.* How effective is road mitigation at reducing road-kill? A meta-analysis. **PLoS ONE**, [*s. l.*], v. 11, n. 11, p. 1–25, 2016.

RYTWINSKI, Trina; FAHRIG, Lenore. The impacts of roads and traffic on terrestrial animal populations. **Handbook of road ecology**, [*s. l.*], p. 237–246, 2015.

SMITH, Daniel J; VAN DER REE, Rodney; ROSELL, Carme. Wildlife crossing structures: an effective strategy to restore or maintain wildlife connectivity across roads. *In*: HANDBOOK OF ROAD ECOLOGY. [*S. l.*]: John Wiley & Sons Ltd Chichester, UK, 2015. p. 172–183.

SOANES, Kylie *et al.* Do wildlife crossing structures mitigate the barrier effect of roads on animal movement? A global assessment. **Journal of Applied Ecology**, [*s. l.*], v. 61, n. 3, p. 417–430, 2024.

TEIXEIRA, Fernanda Zimmermann *et al.* Predição de impactos na fauna: uma proposta para aprimorar estudos de impacto ambiental. **Boletim da Sociedade Brasileira de Mastozoologia**, [*s. l.*], v. 89, p. 146–153, 2020.

VAN DER GRIFT, Edgar A. *et al.* Evaluating the effectiveness of road mitigation measures. **Biodiversity and Conservation**, [*s. l.*], v. 22, n. 2, p. 425–448, 2013.

VAN DER REE, Rodney *et al.* Effects of Roads and Traffic on Wildlife Populations and Landscape Function Road Ecology is Moving toward Larger Scales. **Ecology and Society**, [*s. l.*], v. 16, n. 1, p. 48, 2011.

VAN DER REE, Rodney *et al.* Good Science and Experimentation are Needed in Road Ecology. *In*: HANDBOOK OF ROAD ECOLOGY. [*S. l.*]: Wiley, 2015. p. 71–81.

VAN DER REE, Rodney; SMITH, Daniel J; GRILO, Clara. **Handbook of road ecology**. [*S. l.*]: John Wiley & Sons, 2015.

VILLALVA, Pablo *et al.* Do dry ledges reduce the barrier effect of roads?. **Ecological Engineering**, [*s. l.*], v. 57, p. 143–148, 2013. Disponível em: http://dx.doi.org/10.1016/j.ecoleng.2013.04.005.

ZELLER, Katherine A.; MCGARIGAL, Kevin; WHITELEY, Andrew R. **Estimating landscape resistance to movement: A review**. [*S. l.: s. n.*], 2012.