UNIVERSIDADE FEDERAL DE PELOTAS
Instituto de Biologia
Programa de Pós-Graduação em Biologia Animal

Dissertação

Padrões espaço-temporais de atropelamentos da serpente *Helicops infrataeniatus* (Squamata, Dipsadidae) em rodovias do sul do Brasil

Guillermo Dávila Orozco

Pelotas, 2020

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Dissertação apresentada ao Programa de Pós-Graduação em Biologia Animal do Instituto de Biologia da Universidade Federal de Pelotas, como requisito parcial à obtenção do título de Mestre em Biologia Animal.

Orientador: Dr. Rafael Antunes Dias

Coorientador: Dr. Andreas Kindel

Pelotas, 2020

Universidade Federal de Pelotas / Sistema de Bibliotecas
Catalogação na Publicação

O63p Dávila-Orozco, Guillermo

Padrões espaço-temporais de atropelamentos da serpente *Helicops infrataeniatus* (Squamata, Dipsadidae) em rodovias do sul do Brasil / Guillermo Dávila-Orozco ; Rafael Antunes Dias, orientador ; Andreas Kindel, coorientador. — Pelotas, 2020.

53 f. : il.

Dissertação (Mestrado) — Programa de Pós-Graduação em Biologia Animal, Instituto de Biologia, Universidade Federal de Pelotas, 2020.

1. Estimativa de mortalidade. 2. Sincronia de atropelamentos. 3. Répteis. 4. Serpentes aquáticas. 5. Variações temporais. I. Dias, Rafael Antunes, orient. II. Kindel, Andreas, coorient. III. Título.

CDD : 598.12

Elaborada por Ubirajara Buddin Cruz CRB: 10/901

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Dissertação aprovada, como requisito parcial para obtenção do grau de Mestre em Biologia Animal, Programa de Pós-Graduação em Biologia Animal, Instituto de Biologia, Universidade Federal de Pelotas.

Data da defesa:

10 de setembro de 2020 às 14:00 h

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A meus filhos, que sem saber me
acompanharam neste processo e aprendemos
juntos a ser mais pacientes.

Aos familiares e amigos que com seu apoio
somaram tempo e esforço ao projeto.

Aos amigos e colegas que participaram no
projeto, sem eles teria sido tecnicamente
impossível.

Agradecimentos

Somos gratos a Igor Pfeifer Coelho, Larissa Oliveira Gonçalves, Alfonso Langle Flores e Renata Aires de Freitas pelas contribuições feitas durante a execução deste projeto.

Aos amigos Andrews Duarte da Cruz, Bruno Klotzel, Vinicus Mendes e Carolina Calegaro pelo auxílio na coleta de carcaças para os exercícios de detecção e remoção. A Vinicius Mendes e Andrews Duarte da Cruz pelo seu auxílio no preparo das carcaças. A Andrews Duarte da Cruz, Fernanda de Oliveira Rosas, Michele Buffon Camargo, Gustavo Machado Wallwitz, Bruno Klotzel, Vinicus Mendes, Elizabeth S. Farias, Carolina Voser e Marcelo Dias de Mattos Burns, que participaram nos experimentos de remoção e detecção de carcaças. Marcelo Dias de Mattos Burns, Cauê Canabarro, Bruno Klotzel, Andrews Duarte da Cruz, Garbiel Alejandro Garza Caro e Fernada Morura Fernandez, assim como a Fernanda de Oliveira Rosas e Gustavo Machado Wallwitz, pela disponibilização de seus veículos durante as saídas de campo para coletas e os exercícios de detecção e remoção de carcaças.

Ao Departamento Nacional de Infraestrutura Terrestre (DNIT) pela disponibilização dos bancos de dados da BR-392 e BR116, assim como a STE - Serviços Técnicos de Engenheira S.A., especialmente a Adriano Peixoto Panazzolo, Chaina Teixeira da Silva e Silvia Olinda Soares Aurélio por realizar a gestão dos dados e disponibilizar um espaço de trabalho. A todos meus ex-colegas de STE - Serviços Técnicos de Engenheira S.A. que participaram na coleta de dados da BR-392 e BR-116: Rodrigo Sousa Torres, Felipe Castro Bonow, Sonia Huckembeck, Cindy Nobre Coimbra, Marcelo Dias de Mattos Burns, Andrews Duarte da Cruz, Fernanda de Oliveira Rosas, Michele Buffon Camargo, Gustavo Machado Wallwitz, Bruno Klotzer, Vinicus Mendes, Elizabeth S. Farias, Karoline Pacheco Abilhôa Freitas, Sharon Aires Paiva, Solano Ferreira, Juliana Cunha, Mateus Tavares, Michel Ambos e Ivan Ribeiro. Aos colaboradores do NERF que coletaram na BR-101.

Agradecemos a qualquer pessoa que nos ajudou e que porventura não tenha sido citada, assim como a todas as pessoas que acompanharam no âmbito profissional ou pessoal a formação e atuação docente e de pesquisa, pois com eles o caminho é ainda mais satisfatório. Essa dissertação, como muitas outras, não seria possível sem pessoas como vocês!

Resumo

DAVÍLA-OROZCO, Guillermo. **Padrões espaço-temporais de atropelamentos da serpente *Helicops infrataeniatus* (Squamata, Dipsadidae) em rodovias do sul do Brasil** Orientador: Rafael Antunes Dias. 2020. 53 f. Dissertação (Mestrado em Biologia Animal) – Instituto de Biologia, Programa de Pós-graduação em Biologia Animal, Universidade Federal de Pelotas, Pelotas, 2020.

A estimativa de mortalidade anual de fauna por atropelamento em uma dúzia de países é maior que 400 milhões de indivíduos. Entender os padrões espaciais e temporais de atropelamento de fauna é de vital importância para a sua mitigação. Existem avanços notáveis na produção e adaptação de ferramentas para responder onde, quando e quantos indivíduos são mortos por atropelamentos. Pela complexidade de se obter suficiência amostral, estes padrões são pouco estudados a nível de espécie e a literatura que explora as mudanças interanuais e variações entre populações é praticamente inexistente. Ante estas incertezas, é comum assumir que os padrões de atropelamentos são semelhantes entre anos, estradas e inclusive entre espécies similares, pudendo restar eficácia à medida mitigadora. Nosso trabalho tem por objetivo estudar as variações espaço-temporais dos atropelamentos de fauna utilizando como espécie-modelo a serpente *Helicops infrataeniatus*. Desta forma, estudamos as mudanças anuais de *hotspots*, *hot moments*, e no número de indivíduos atropelados ao longo dos anos e as variações entre três rodovias da planície costeira da região sul do Brasil. Exploramos também a influência da escala de avaliação e do esforço amostral na identificação de *hotspots* ao longo dos anos, assim como medimos a sincronia de atropelamentos entre rodovias. O número de indivíduos atropelados foi estimado incluindo no cálculo a eficiência da detecção de carcaças e sua persistência na rodovia. Nós encontramos que os *hotspots*, *hot moments* e a estimativa de mortalidade correspondem aos padrões esperados pela dispersão, atividade e abundância da espécie. Porém, os padrões de atropelamentos podem apresentar mudanças ao longo dos anos e diferenças entre as rodovias. A localização de *hotspots* ao longo dos anos varia menos em escalas espaciais e temporais maiores, e não necessariamente pelo tamanho amostral. Nossa maior estimativa de mortalidade é na BR-392 (53km) com 61 atropelamentos/km/ano, porém a estimativa pode ser cinco vezes maior se considerado que o impacto se concentra num trecho de 10 km na Várzea do Canal São Gonçalo. Esta mesma estrada apresentou um padrão bienal no número de indivíduos atropelados. É possível que as variações nos padrões de atropelamentos detectadas estejam relacionadas a mudanças ambientais ou comportamentais em escala local e de períodos curtos. Neste sentido, é importante direcionar esforços para identificar a causa-efeito nas mudanças dos padrões de mortalidade. Entretanto, a complexidade do estudo em ecologia de estradas apresenta desafios consideráveis. Assim, nós recomendamos que na identificação de padrões de atropelamentos e sua mitigação, os tomadores de decisões implementem esforços de monitoramento e escalas de avaliação que incluam as potenciais variações espaço-temporais da biologia do táxon estudado.

Palavras-chave: Estimativa de mortalidade. Sincronia de atropelamentos. Répteis. Serpentes. Serpentes aquáticas. Detecção de carcaças. Persistência de carcaças. Variações temporais. Mudanças anuais. Padrão bienal.

Abstract

DÁVILA-OROZCO, Guillermo. **Spatio-temporal patterns road-kills of the snake *Helicops infrataeniatus* (Squamata, Dipsadidae) on roads in southern Brazil** Advisor: Rafael Antunes Dias. 2020. 53 f. Dissertation (Masters in Animal Biology) – Institute of Biology, Postgraduate Program in Animal Biology, Federal University of Pelotas, Pelotas, 2020.

The estimate of fauna mortality from road-kill in just a dozen countries is higher than 400 million individuals per year. Understanding the spatial and temporal patterns of road-kill is important for their mitigation. There are notable advances in the production and adaptation of tools to answer where, when, and how many individuals are killed by cars. Due to the complexity of obtaining sample sufficiency, these patterns are poorly studied at the species level and the literature that explores interannual changes and variations between populations is non-existent. In view of these uncertainties, it is common to assume that the road-kill patterns are similar between years, roads and even between species, reducing the effectiveness of the mitigating measure. We studied spatio-temporal variations in road-kill using the snake *Helicops infrataeniatus* as a model organism. We studied the annual changes in hotspots, hot moments, and the number of road-kill over the years and between three roads on the coastal plain of southern Brazil. We also explored the influence of the spatial scale and the sampling effort in detecting hotspots over the years, as well as measuring the synchrony of road-kill between roads. The number of road-kill was estimated including searcher efficiency and carcass persistence. We found that hotspots, hot moments, and mortality estimates correspond to the expected patterns of dispersion, activity, and abundance of the species. However, road-kill patterns can change over the years and differ between roads. The location of hotspots over the years varies less with larger scales and with yearly increases in sampling effort, and not necessarily with sample size. Our highest mortality estimate was on BR-392 (53 km), with 61 road-kill/km/year. However, this number can be five times higher if we consider that the impact is concentrated in a 10 km stretch of the Canal São Gonçalo floodplain. We found a biennial pattern in the number of road-kill on one road. It is possible that the variations in road-kill patterns are related to environmental or behavioral changes on a local scale and for short periods. In this sense, it is important to direct efforts to identify the cause-effect of changes in mortality patterns. However, the inherent complexity presents considerable challenges. Thus, we recommend that decision makers implement monitoring efforts and evaluation scales that include the potential spatio-temporal variations of the studied taxon in the identification and mitigation of road-kill patterns.

Keywords: Mortality estimate. Road-kill synchronicity. Reptiles. Snakes. Water snakes. Detection of carcasses. Carcass persistence. Temporal variations. Annual changes. Biennial pattern.

Sumário

1. Introdução geral

Infraestruturas de transporte são de vital importância para o desenvolvimento urbano, econômico e social, sendo o sistema rodoviário a modalidade da qual o ser humano mais depende (FORMAN et al., 2003). A perda, fragmentação e degradação do habitat, os efeitos de barreira e de corredor, assim como a mortalidade de fauna, são efeitos ecológicos resultantes da implantação de uma estrada (VAN DER REE; SMITH; GRILO, 2015; TEIXEIRA; RYTWINSKI; FAHRIG, 2020). Durante a operação da rodovia, os impactos mais destacados em nível populacional são a mortalidade por atropelamento e o efeito barreira (VAN DER REE; SMITH; GRILO, 2015; TEIXEIRA; RYTWINSKI; FAHRIG, 2020). A representatividade deste impacto pode ser percebida ao considerar que em uma dúzia de países a morte de fauna por atropelamento ultrapassa os 400 milhões de indivíduos por ano (SCHWARTZ; SHILLING; PERKINS, 2020). Contudo, esse impacto não atua só no número de indivíduos atropelados, mas também na redução da abundância e de diversidade genética das populações, aumentando o risco de extinção (FRANKHAM, 2005; JACKSON; FAHRIG, 2011). Assim, o atropelamento também adquire relevância ao considerar que a perda e o declínio das populações é um impacto que gera defaunação, alterando o funcionamento dos ecossistemas (YOUNG, et al, 2016).

O estudo de padrões espaciais e temporais de atropelamentos de fauna em nível de espécie requer a obtenção de amostras robustas para diferentes localidades e/ou ao longo dos anos. Para obter uma amostra satisfatória em estudos em nível de espécies pode ser necessário realizar amostragens de longa duração e ainda assim encontrar dificuldades para estudar padrões ao longo do tempo. Por exemplo, GILBERT et al. (2001), utilizaram 729 registros de acidentes veiculares com urso-preto (*Ursus americanus*) obtidos nas rodovias da Florida ao longo de 23 anos, identificando zonas de maior atropelamento, porém com limitada capacidade para identificar variações ao longo dos anos. Outros estudos, como o realizado por RUIZ-CAPILLAS; MATA; MALO et al. (2014) utilizam menores escalas temporais (e.g., 1 ano) e espaciais (e.g., 4 km), porém com utilidade incerta para extrapolar os resultados a escalas maiores (VAN DER REE et al., 2011).

Ainda assim, existem avanços notáveis para responder onde, quando e quantos indivíduos são atropelados. Por exemplo, os *hotspots* (locais de maior agregação de atropelamentos do que o esperado ao acaso; COELHO et al., 2014) identificados no início da operação de uma estrada podem mudar posteriormente em função do impacto gerado pelo atropelamento nas populações originais (TEIXEIRA et al., 2017). Assim, determinar se *hotspots* mudam em populações com o impacto já consolidado é igualmente importante. A localização eficiente de *hotspots* depende do intervalo de amostragem (SANTOS; CARVALHO; MIRA, 2015) e da escala de avaliação (SANTOS et al., 2017), porém a representatividade do esforço amostral são outros elementos que podem influenciar a melhor identificação espacial das agregações ao longo dos anos. Assim como os *hotspots*, definir quando os atropelamentos ocorrem possibilita melhorar a efetividade das medidas mitigadoras a serem implementadas (GUNSON; TEIXEIRA, 2015). O reconhecimento de *hot moments* (momento no tempo com maior concentração de atropelamentos) é associado a elementos sazonais que podem influenciar o comportamento da fauna (ANDREWS, LANGEN; STRUIJK, 2015; GONÇALVES et al. 2018).

Quantificar a mortalidade por atropelamento é essencial para determinar a magnitude do impacto, e sua estimativa precisa ponderar elementos que influenciam no número de carcaças registadas (TEIXEIRA et al, 2013; CABRERA-CASAS; ROBAYO-PALACIO; VARGAS-SALINAS, 2020). Portanto, a comparação efetiva de atropelamentos entre populações depende de incluir no cálculo de mortalidade a detectabilidade, frequência de monitoramento e remoção de carcaças como elementos próprios de cada rodovia e espécie. COELHO et al (2014) considera a detectabilidade como a capacidade de detectar as carcaças pelo método empregado e a remoção como o tempo em que as carcaças são removidas da pista e, portanto, não sendo mais passíveis de registro.

Os padrões espaço-temporais dos atropelamentos ao longo dos anos e entre estradas ainda são largamente desconhecidos. Na prevalência dessas incertezas e ante a necessidade de efetivar medidas mitigadoras, é comum assumir que os padrões espaciais e temporais de atropelamentos são similares entre espécies, regiões ou ao longo do tempo. Assumir que os padrões observados se mantêm constantes pode ser contraproducente ao não ter o efeito esperados da medida mitigadora, quando na verdade o erro pode ser o uso inadequado das informações disponíveis (TEIXEIRA et al, 2017, 2013; VAN DER REE et al, 2011).

Para verificar se os padrões espaciais e temporais dos atropelamentos de fauna são constantes ao longo dos anos e entre rodovias, estudamos atropelamentos da serpente *Helicops infrataeniatus* em 540km de três rodovias da Planície Costeira do Rio Grande do Sul, no sul do Brasil. A espécie é atropelada de forma extensiva e em números expressivos nas rodovias do sul do Brasil com características ambientais semelhantes (GRILO et al., 2018). Considerando também que sua ocorrência está associada a ambientes específicos e sua atividade é fortemente sazonal (LEMA, 2002; CARREIRA; MANEYRO, 2013), esta espécie é um modelo adequado para estudar mudanças nos padrões espaço-temporais de atropelamentos ao longo dos anos.

Em relação ao padrão espacial dos atropelamentos, verificamos i) se as agregações se mantêm constantes ao longo dos anos e entre as rodovias, e ii) como o esforço amostral e a escala de avaliação influenciam o padrão observado. Em relação ao padrão temporal, verificamos iii) se a concentração temporal dos atropelamentos de cada rodovia é constante ao longo dos anos, e iv) se este é similar entre as rodovias. Também estimamos v) o número de atropelamentos para cada rodovia, e vi) verificamos se o número de indivíduos atropelados se mantém constante ao longo dos anos.

Esperamos que os padrões temporais, espaciais e que o número dos atropelamentos de cada rodovia mostre diferenças ao longo dos anos (obj. i, iii, vi), e que o padrão temporal seja similar entre as rodovias (obj. iv). Esperamos também que o padrão espacial dos atropelamentos seja mais estável ao longo dos anos o quanto maior o esforço amostral e maior a escala de avaliação (obj. ii). Finalmente esperamos alteração nos padrões espaciais e temporais quando os erros de amostragem são considerados (obj. v).

ARTIGO – Spatio-temporal patterns of *Helicops infrataeniatus* (Squamata, Dipsadidae) road-kill: are hotspots, hot moments and the number of road-kill constant over the years and similar between roads?

Manuscrito a ser submetido para a revista Science of the Total Environment

(Versão em Inglês)

Spatio-temporal patterns of *Helicops infrataeniatus* (Squamata, Dipsadidae) road-kill: are hotspots, hot moments and the number of road-kill constant over the years and similar between roads

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Spatio-temporal patterns of *Helicops infrataeniatus* (Squamata, Dipsadidae) road-kill: are hotspots, hot moments and the number of road-kill constant over the years and similar between roads?

Highlights

Hotspots, hot moments and the number of fatalities change over the years.
Hotspots and hot moments and the number of fatalities can vary between roads.
Smaller spatial and temporal analysis scales can influence the road-kill patterns.
Temporal patterns of road-kills are synchronous between roads.
A biennial pattern on the number of fatalities was observed on one road.
An estimated 11,712 snakes were killed annually in 540 km of three roads.

Abstract

The yearly estimate of road-killed animals in a dozen countries is higher than 400 million individuals. Understanding the spatial and temporal patterns of road-kill is essential to mitigate this impact. Today it is possible to answer where, when, and how many individuals died, but these patterns are seldom studied at species level and variations among years and between populations are unknown. We studied spatio-temporal variation of road-kill using *Helicops infrataneatus* as a model organism. We studied the annual changes in hotspots, hot moments, and the number of fatalities over the years and between three roads in southern Brazil. We explored the influence of the identification scale and sampling effort on the location of hotspots over the years. We also measured the synchronicity of road-kills between roads. The number of road-kills was calculated considering detectability and carcass persistence. Although the general patterns of hotspots, hot moments and mortality estimates follow the expected dispersal, activity and abundance patterns of the species. These patterns change over the years, but the position of hotspots over the years varies less at larger spatial scales and road-kill on one road. Also changes between roads, yet the road-kill are synchronous between roads. The spatio-temporal variations in road-kill patterns may be related to environmental or behavioral changes at local scales and for short periods of time. We recommend that decision makers implement monitoring efforts and assessment scales that include the potential spatial-temporal variations of the taxon studied to mitigate this impact.

Keywords: Road ecology. Mortality estimate. Synchronous. Spatial variations. Annual changes. Biennial pattern. Mitigation.

1. Introduction

Habitat degradation, habitat connectivity reduction and direct mortality are the main impacts of roads upon animals (Teixeira et al, 2020). At the population level, mortality from road-kill and the barrier effect are the most prominent impacts of roads (Jackson and Fahrig, 2011). Despite accumulated efforts to describe spatial and temporal patterns (Coelho et al., 2014; Gonçalves et al, 2018) even at the species level (Beaudry et al, 2018; Teixeira et al, 2017; González-Suárez et al, 2018), the pattern of where, when and how many individuals are killed in roads have seldom been evaluated for multiple locations over many years (But see Santos et al., 2017, for class level studies). Thus, it is highly uncertain whether known patterns remain constant between years and could be extrapolated between populations of a same species.

The efficient location of road-kill faunal hotspots (at multi species focus) is rooted in the sampling interval (Santos et al., 2015) and the scale of evaluation (Santos et al., 2017), but the representativeness of the sampling effort is other element that can improve the spatial identification of aggregations. Additionally, road-kill hotspots identified at the beginning of the operation of a road may change due to the impact generated by mortality on the original populations (Teixeira et al., 2017).

Defining when road-kill happens may be equally important to mitigate fatalities of animals on roads (Gunson and Teixeira, 2015). The recognition of hot moments (i.e., the time period in which number of fatalities is larger than expected by chance) helps to define the potential use and maintain of temporary management strategies (van der Ree and Tonjes, 2015; Spanowicz et al., 2020) in the period with the highest cost-benefit. If the temporal roadkill patterns are demonstrated to be synchronous between locations (Liebhold et al, 2004, Gouhier and Guichard, 2014), this can assist the management planning of temporal mitigation measures by extrapolating decisions from one road to another (Liebhold et al, 2004; Cusack, et al., 2017).

Quantifying road-kill is essential to determine the potential magnitude of the impact and prioritize road sections for mitigation within a road network. Unbiased estimates of roadkill numbers need to consider potential observation errors that influence carcass quantification on roads (Gerow et al., 2010 and Teixeira et al, 2013), like searcher efficiency, carcass persistence and sampling interval (Korner-Nievergelt et al., 2011). Although the imperfect detection of carcasses has been recognized in road ecology studies for fatality estimation at least since 2010 (Gerow et al., 2010) it has been seldom recognized in fatality estimation of single species studies (but see Winton et al., 2018)

If spatial and temporal road-kill patterns are relatively stable within and across study areas, it would be possible to use single year and/or single site studies to extrapolate outcomes and support mitigation planning along a whole region of a species occurrence and over time. However, if spatial and temporal patterns are inconstant, the extrapolation approach will be counterproductive (Teixeira et al, 2017; van Der Ree et al, 2011) and we would need to monitor them annually and perhaps opt for non-permanent spatial mitigation strategies.

To evaluate whether the spatial and temporal patterns of road-kill are constant over the years within and between locations, we used fatality data of the snake *Helicops infrataneatus* (Squamata, Colubridae) from three roads extending 540 km in the Coastal Plain of Southern Brazil. This species is extensively killed on southern Brazilian roads (Grilo et al., 2018), is highly dependent on aquatic environments, and has strong seasonal activity patterns (Lema, 2002; Carreira and Maneyro, 2013). Thus, it is a suitable model for studying spatio-temporal road-kill patterns over the years. Specifically, we asked: i) are road-kill aggregations constant over the years within roads?; ii) what is the influence of the sampling effort and the scale of evaluation on the spatial pattern of observed fatalities?; iii) is the temporal concentration of fatalities within each road constant over the years?; iv) is the temporal distribution of fatalities synchronous between roads?; and v) do unbiased fatality estimates remain similar between roads and over the years?

2. Methods

2.1 Study species

Helicops infrataeniatus (Jan, 1865) is an aquatic snake found in Argentina, Uruguay, Paraguay, and sout hern Brazil (Carreira and Maneyro, 2013; Costa and Bérnils, 2018). This abundant snake occurs in many types of freshwater habitats, including the temporary pools and extensive marshes typical of the coastal region of southern Brazil (Quintela and Loebmann, 2009). This species frequently uses hydrophilic vegetation or shrubs adjacent to waterbodies as a refuge (Carreira and

Maneyro, 2013), and preys on fish and frogs (Aguiar and Di-Bernardo, 2004). Breeding takes place in the spring and summer and females give birth to up to 25 individuals from late spring to early autumn (Lema, 2002). This species may reach 1 m long and has a characteristic ventral coloration (Lema, 2002, Quintela and Loebmann, 2009, Carreira and Maneyro, 2013), which facilitates the identification of carcasses (Figure 1).

2.2. Study area

Our study area comprises 540 km of three roads (BR-392 with 53 km, BR-116 with 211 km and BR-101 with 276 km) located in the coastal plain surrounding the Lagoa dos Patos system in Rio Grande do Sul state, southern Brazil (Figure 2; S 1). The three roads are representative segments of the road network that surrounds the Lagoa dos Patos (Initial and final coordinates for BR-392: 31° 46' 55.17'' S, 52° 21' 28.88'' W; 32° 06' 37.01'' S, 52° 06' 42.08'' W; Initial and final coordinates for BR-116: 30° 07' 09.75'' S, 51° 20′ 50.59'' W; 31° 37' 47.58'' S, 52° 19' 27.24'' W. Initial and final coordinates for BR-101: 30° 09' 01.20'' S, 50° 30' 49.33'' W; 32° 08′ 23.64'' S, 52° 02' 17.73'' W). This region is covered with grasslands, wetlands and rice and soy plantations (IBGE, 2004). Patches of forests occur on more rolling terrain and along watercourses (IBGE, 2004). Climate is temperate, with an average temperature of 10–15 °C and rainfall is evenly distributed throughout the year, with an average of 1500 mm (IBGE 2002).

2.3. Data collection

Data for BR-392 and BR-116 were provided by the National Department of Transport Infrastructure (DNIT), whereas data for BR-101 was obtained from Grilo et al. (2018). For each road killed individual of *H. infrateniatus* we accessed the date and the spatial coordinates from the databases. For BR-392, we used data from 36 surveys carried out between April 2011 and March 2017. Intervals between each survey averaged 64 days. The full stretch of that road was sampled by car in five consecutive days by three people (including the driver). For BR-116, we used data from 30 surveys carried out between October 2012 and March 2018 (intervals averaging 69 days). The road was divided into four ~50 km sections sampled by car by two teams, each with three observers (including the driver). Each team sampled one section per day, on two consecutive days. The entire sampling stretches of both BR-392 and BR-116 was traveled from start to end and back again per sampling day. On each traveled the respective line (or roadway on four-lane road) and shoulder was reviewed. The BR-101 data was based on 24 monthly surveys (intervals averaging 30 days) between September 2012 and August 2014 with (Gonçalves et al., 2018). The entire stretch of the road was surveyed in one or two days by car by two observers (including the driver) traveling in a single direction (Gonçalves et al., 2018) where the observation area included the roadway and shoulders. In all cases, sampling was carried out at an average speed of 40 km/h.

To produce unbiased estimates of snake fatalities and to assess the searcher efficiency and carcass persistence, we performed carcass placement experiments. These experiments are carried out by replicating the monitoring methodologies in representative segments of each road studied (detailed in Table 1). For BR-392 and BR-116, we used 99 *H. infrataeniatus* carcasses collected on BR-392. For BR-101, we used 51 multiple species snake carcasses but with similar sizes to *H. infrataeniatus* collected on the same road. In the searcher efficiency experiment, we placed snake carcasses on selected stretches and measured the number of detected and undetected carcass by each sampling team (combinations of observers who participated in the sampling of each road, detailed in Table 1). For carcass persistence, we checked which individuals placed in each stretch were removed in each time interval, obtaining a list of presence and absence per survey (detailed in Table 1).

2.4. Data analysis

We organized the data for each road in annual cycles, comprising six cycles for BR-392 (autumn 2011–summer 2017), five for BR-116 (autumn 2013–summer 2018) and two for BR-101 (spring 2012–winter 2014). For the synchrony analysis, we grouped the data for each road into seasons within annual cycles, comparing all road combinations using only data from the superimposed periods (details in Tables S 2.A and S 2.B).

2.4.1. Road-kill hotspots patterns

To study the spatio-temporal distribution of *H. infrataeniatus* hotspots, we split the pooled data set of each road into annual cycles and consecutive years data sets (year 1 to year 2, year 1 to year 3, and so on) up to the entire number of years monitored. We used 11 data sets for BR-392, nine for BR-116 and three for BR-101 (Table S 2.). For each data set, our analytical approach involved three steps. First, we used a modified Ripley's *K* statistics (Ripley's K-2D analysis; Coelho et al., 2014) to identify if there are spatial aggregations and the spatial scales at which they occur. Ripley's *K*-2D analysis evaluates if fatalities are distributed at random or not along a road, testing it at multiple scales delimited by a circle with increasing radius (scale of analysis), and controlling for road extension within that circle (Coelho et al, 2008). The *L*(*r*) is the output statistics and measures the intensity of aggregation for each studied scale (Ripley, 1981; Coelho et al, 2014). Values above the upper limit of the confidence interval are interpreted as significantly aggregated. We used an initial radius of 100 m and increments of 500 m up to the total size of the road and ran 1.000 simulations. For each data set, we checked if road-kills were aggregated or not in a 100, 500 and 1.000 m radius with 99%, 95% or 90% confidence intervals. We choose 100, 500, and 1000m radius sizes to study the road-kill spatial pattern over the years because mitigation measures for reptiles have been performed effectively on these scales (Baxter-Gilbert et al., 2015). In addition, Grilo et al. (2016) consider that the species' movement patterns are within these scales.

In a second step, we used Hotspots-2D analysis to verify where the road-kill aggregations occurred for all data sets that displayed an aggregated distribution (Coelho et al., 2014). In this analysis, the intensity of the aggregations for each road segment is given by the N_{events}-$N_{simulated}$ function, which is obtained from the number of road-killed individuals counted in a determined radius centered on segments with size equal or smaller than 2*r* (Coelho et al., 2014). Significant hotspots are considered the segments with aggregation intensity greater than the upper confidence limit values (95%) obtained from 1.000 simulations of carcass distribution with a uniform probability along a road. To explore the influence of the scale on the hotspot identification and distribution, we analyzed each data set with the three radius and segments sizes of interest (100, 500 and 1000 m). Both Ripley's K-2D and Hotspots-2D analyses were carried out in Siriema 2.0 (Coelho et al, 2014).

Finally, to explore the influence of both effort (i.e., the number of consecutive sampling years and the number of accumulated carcasses during this periods) and scale (radius size), we used a correlation test to compare the road-kill distribution of each temporal data set with the pooled data set (i.e., the totality of data for each road) used as our reference. This similarity analysis was restricted to BR-392 and BR-116, the only roads sampled for more than two years. We performed a Spearman correlation with the *rcorr* function of the *Hmisc* package (Harrell, 2020) for R (R Core Team, 2020) using hotspot presence/absence (significant and not significant hotspots) and hotspots intensity (given by the N_{events}-$N_{simulated}$ value described before, for each road segment) as variables.

2.4.2. Road-kill hot moment patterns

We used circular statistics with a seasonal scale to identify road-kill temporal aggregations for each road and for every annual cycle. Each sampling day was converted into an angle value within an annual cycle (each day representing 0.986°). The first day of our annual cycles began on December 22, which marks the start of the austral summer. A road-kill hot moment was described by the mean angle (μ), and its intensity by the concentration (r), where 0 represents a uniform distribution and 1 road-kill events that are entirely aggregated in time. We calculated the probability that the temporal data distribution is uniform via Rayleigh's test of uniformity or Z value (Kovach, 2011). These analyses were performed in Oriana 4.02 (Kovach, 2011).

2.4.3. Road-kill synchrony between roads

We used the activity index to compare the road-kill time series. This index is used in phenology studies to identify synchrony based on the percentage of individuals showing the same phase in a specified period (Bencke and Morellato, 2002). The higher the proportion of road-kill in a same given period, the higher is the synchrony between the roads. To apply this index, we calculated the percentage from the total road-kill per annual cycle recorded in each season.

Since the activity index does not formally quantify and test the synchrony (Bencke and Morellato, 2002), we used concordance analysis to measure the synchrony in snake road-kill between roads and test its significance (Gouhier and

Guichard, 2014). This technique verifies the average synchrony of the entire time series but tends to be sensible to the presence of noise that can mask both the presence or absence of synchrony (Gouhier and Guichard, 2014). Although concurrency analysis can overcome this issue, we were unable to use this alternative approaches because some of our temporal time cycles were not collected simultaneously and our sample effort was too small (Gouhier and Guichard, 2014). Concordance analysis was carried out using the *kendall.w* function of R package *synchrony* (Gouhier, 2019). This function uses the average Spearman correlation between each pair of time series to measure the degree of agreement (*w*) between the time series (ranging from 0 to 1, with 0 representing a null synchrony and 1 total synchrony) and employs a 1000 randomization test from the Kendall's W statistic to obtain the *P*-value (Legendre, 2005).

2.4.4. Road-kill numbers patterns

We estimated the road-kill numbers for each road and per annual cycle within each road with the *estimateN* function of the *carcass* package (Korner-Nievergelt et al., 2011, 2015) for R. To obtain the estimate of road-kill over the study period (*N*), the function uses, in addition to the observed records and the sampling interval, the probability of detection (*p*), searcher efficiency (*f*) and carcass persistence (*s*). We assumed that *f* and *s* are constant over time (Korner-Nievergelt et al., 2011).

The detection probability (*p*) is the probability that an individual killed during the study will be found during surveys (Korner-Nievergelt et al., 2011). The detection probability is calculated using the parameters of searcher efficiency, carcass persistence and the interval between surveys (Korner-Nievergelt et al., 2011). We used the *etterson* formula to calculate *p* because it allows for irregular time interval between surveys (Etterson, 2013; Korner-Nievergelt et al., 2015). Searcher efficiency (*f)* represents the proportion of carcasses observed among the total carcasses existing at the time of sampling (Korner-Nievergelt et al., 2011), obtained with data get on a field experiment (detailed in Table 1) using the search*.efficiency* function. Carcass persistence (*s*) refers to the road-kill proportion that is not removed from the road within a defined time interval (Korner-Nievergelt et al., 2011) and we obtained it with the *persistence.prob* function.

3. Results

We obtained 402 records of *H. infrataeniatus* from three databases for the years of interest (BR-392 = 190, BR-116 = 119 and BR-101 = 93). Of the 402 records, 374 were used for the hotspots, hot moments and road-kill analyses, and 378 for the synchrony analyses (details on Tables S 2.A and S 2.B).

3.1. Road-kill hotspots patterns

Using the pooled road-kill data set, the distribution of *H. infrataeniatus* was aggregated in all scales up to 40 km in all three roads and for BR-392 and BR -101 at even larger scales (Table S 3.A, Figures S 3.B and S 3.C). However, when analyzed by single annual cycle, the third year of BR-392 (n = 13) and the second year of BR-116 (n = 11) did not present significant aggregations with a 100 m scale, the first year of BR-101 (n = 31) did not present significant aggregations with 100 m and 500 m, and finally, the fifth year of BR-116 (n = 8) did not show significant aggregations for any scale of interest. The other analysis by annual cycle showed significant aggregations in the radius of interests with confidence intervals of 99, 95 or 90% (three with sampling size of 13 carcasses, one with 15 carcasses and five with > 37 carcasses). All analysis of data from two or more consecutive years data sets showed significant aggregations with a confidence interval of 99% (Table S 3.A, Figures S 3.B and S 3.C).

The hotspots are concentrated in a large segment of BR-392 and dispersed along the entire road on BR-116 and BR-101 (Figure 3; Figures S 3.D–2.H). Hotspots detected showed variations between annual cycles. Irrespective of the road, hotspot location similarity increased with both the scale of analysis and sampling effort (i.e., accumulated number of years or individuals). Also, a larger similarity was obtained with less effort when our interest was simply in locating hotspots rather than obtaining the location of the more intense hotspots (Figure 4). When the interest was on the location of the more intense hotspots, the scale of analysis had a strong effect with low similarities at finer scales.

3.2 Road-kill hot moment patterns

The majority road-kill of this species occur mainly in the spring / summer (89% on BR-392, 84% on BR-116 and 92% on BR-101). The circular statistics showed a concentrated distribution of fatalities in time for all three roads, with grand mean vector in spring for the BR-116 and BR-101 and in late spring–early for BR-392 (Figure 5). Therefore. Aggregation in time was more intense on BR-392 (r = 0.61; Z = 70.5; μ = 359.7°; P < 0.001) than on BR-116 (r = 0.55; Z = 27.4; μ = 301.7°; P < 0.001) or BR-101 (r = 0.52; Z = 24.3; μ = 323.7° P < 0.001). Although there was some yearly variation within each road, fatalities were still concentrated on those seasons (Figure 5), except for the second annual cycle of BR-116 that showed a uniform distribution (Table S 4).

3.3. Road-kill synchrony between roads

The activity index graph showed that the three roads displayed a similar pattern throughout time, with the largest number of fatalities in the spring–summer (Figure 7). Synchrony levels (concordance values) were statistically significant and above 0.75 for all possible road comparisons except BR-392 versus BR-101 (Table 2).

3.4. Road-kill numbers patterns

Mean searcher efficiency was relatively low and differed considerably between BR-101 (23%) and the other two roads (40% and 53% on BR-392 and BR-116, respectively; Table 3). The mean carcass persistence time was similar between BR-392 and BR-116 (0.74 and 0.78 days) and much lower than on BR-101 (5.25 days) (Table 3). When considering both sources of sampling error, estimated road-kill/km/year on each road was 103, 377 and 25 times larger than the observed values/km/year on BR-392, BR-116 and BR-101, respectively. The observed values of road-kill/km/year on BR-392 are 6.5 times larger than on BR-116 and 2.95 times larger than on BR-101. However, when the estimated values are used, estimated road-kill is 1.8 and 12.2 times larger, respectively (Table 3). Estimated fatalities displayed a biennial pattern for BR-392 and were constant for the second annual cycle on BR-116. For BR-101, road-kill doubled from the first to the second year (Figure 7).

Table 1. Characteristics of road stretches and data sets used for each searcher efficiency and carcass persistence trial on each road.

				Searcher efficiency			Carcass persistence		
Roads	Type of road	Average daily traffic**	Segment extent	Monitoring speed	N° survey teams	N° carcasses used per team	N° of checks	Mean time interval between checks	N° carcasses used
BR-392	Four-lane road	12531	20 km	40 km/h	2	21 and 19	3	0.125 d	49
BR-116	Two-lane road	22902	30 km	40 km/h	2	17 and 14	3	0.135 d	50
BR-101	Two-lane road	2900	30 km	40 km/h	6	42	6	1 d	36

**Average daily traffic of BR-392 (2015-2017) and BR-116 (2015-2018) from DNIT (2017); average daily traffic of BR-101 (2012-2015) from Gonçalves et al. (2018).

Table 2. Synchrony and *P*-values for each pairwise comparison of road-kill number between roads.

Arrangement	*kendall.w*	
	Synchrony	*P*-value
BR-116 vs BR-101	0.87	0.02
BR-392 vs BR-101	0.78	0.09
BR-392 vs BR-116	0.75	0.02
BR-392 vs BR-116 vs BR-101	0.77	<0.01

Table 3. Searcher efficiency, carcass persistence and observed and estimated fatalities per road. The credibility intervals are in parenthesis.

Roads	Searcher efficiency	Daily persistence probability	Fatalities	
			Observed/km/year	Estimated/km/year
BR-392	0.40 (0.27–0.54)	0.28 (0.14–0.43)	0.59	61 (31–141)
BR-116	0.53 (0.36–0.68)	0.16 (0.07–0.28)	0.09	34 (15–87)
BR-101	0.23 (0.16–0.34)	0.83 (0.76–0.87)	0.2	5 (3–9)

Figure 1. *Helicops infrataeniatus* pictures. Picture 1A shows variations in the ventral pattern (Regnet et al., 2017) and 1B and 1C shows road-killed individuals (Serviços Técnicos de Engenheira files).

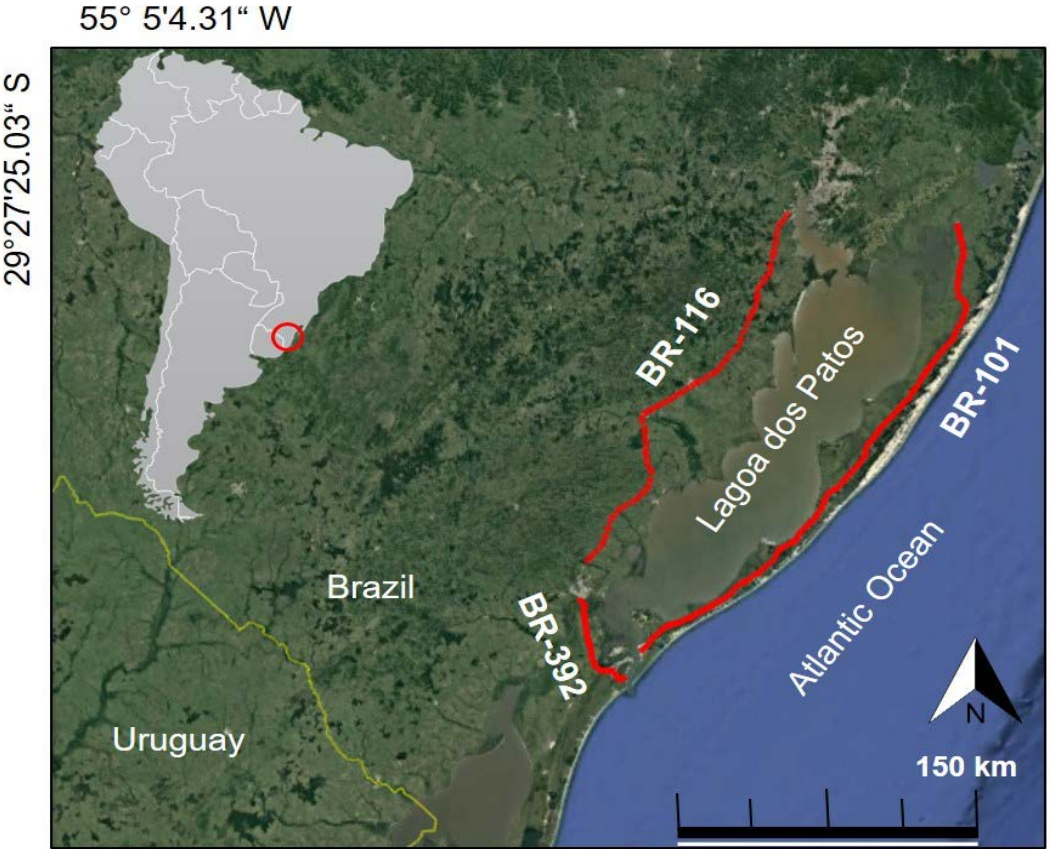

Figure 2. Location of the study area near the Brazil/Uruguay border (yellow line) and the sampled roads (red).

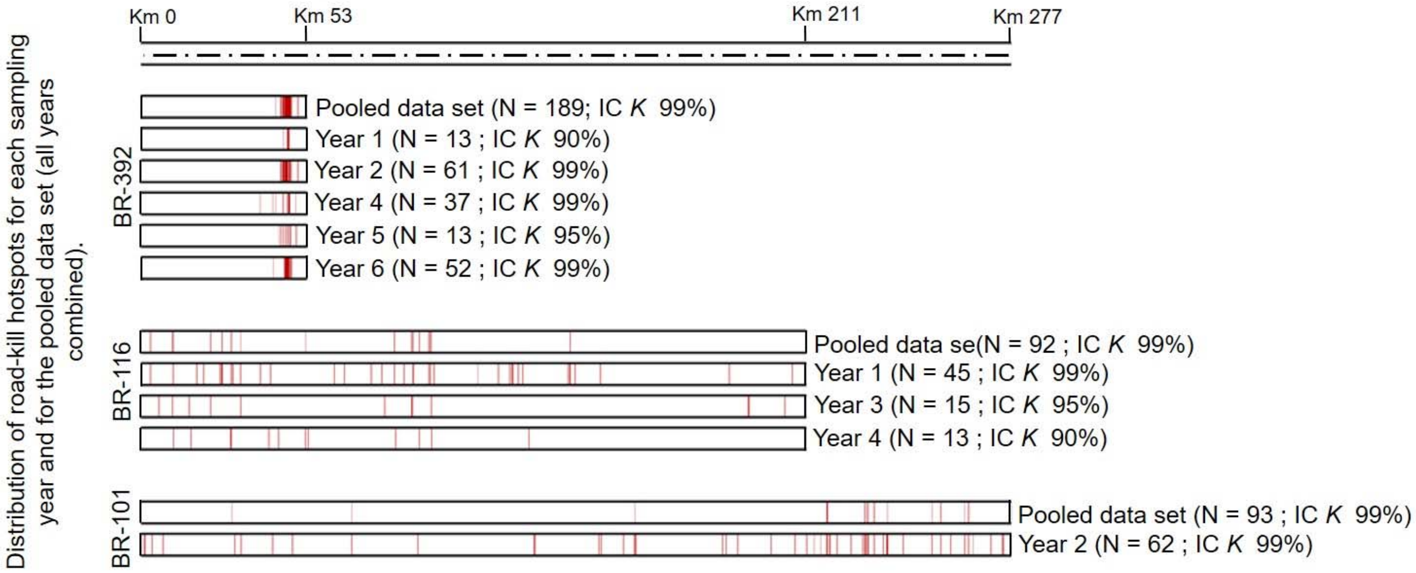

Figure 3. Single-line diagrams showing the hotspot location in each road (on equivalent linear distance scales) per sampling year. Hotspots were obtained from 2D Hotspot Identification analysis with a 100 m road segment size and 100 m radius. Data sets with random carcass distributions are not shown. Also indicated are the sample size (N) and the best Confidence Interval (90%, 95% or 99%) under which aggregations were significant for Ripley's K-2D.

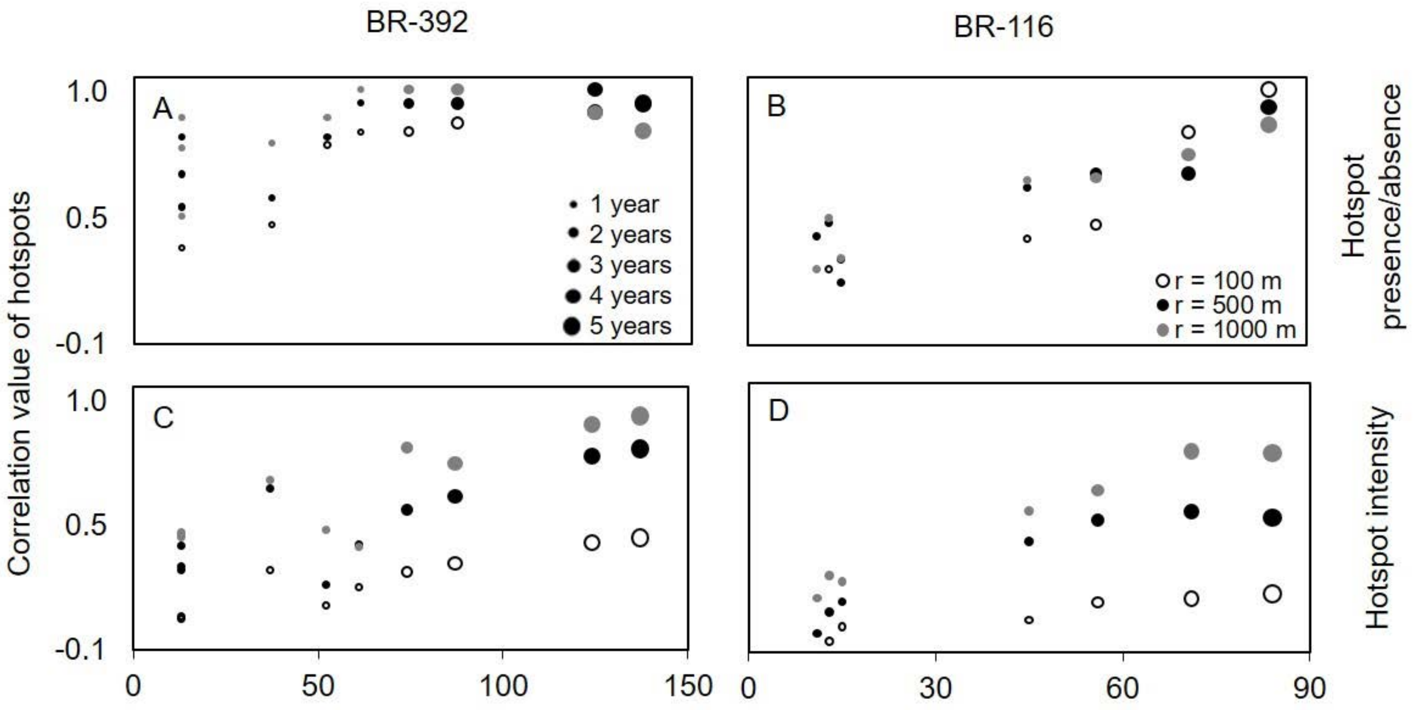

Figure 4. Hotspot similarity for all annual samples and accumulated year samples (up to 5 years for BR-392 and 4 years for BR-116) represented by increasing circle sizes (panel A) between the reference pooled data set, which corresponds to 6 year samples for BR-392 (panels A, C) and 5 years for BR-116 (panels B, D). The x-axis shows the number of accumulated carcasses for each data set (individuals and consecutive years). Spearman correlation values (y-axis) were used to analyze similarity for hotspot presence/absence (significant and non-significant hotspots; panels A, B) and hotspot intensity (the difference between the number of observed carcasses and the number of expected carcasses; panels C, D; see methods for details). Hotspot similarity is also compared for increasing radius sizes (shades of grey in panel B) to evaluate potential effects of scale on hotspots.

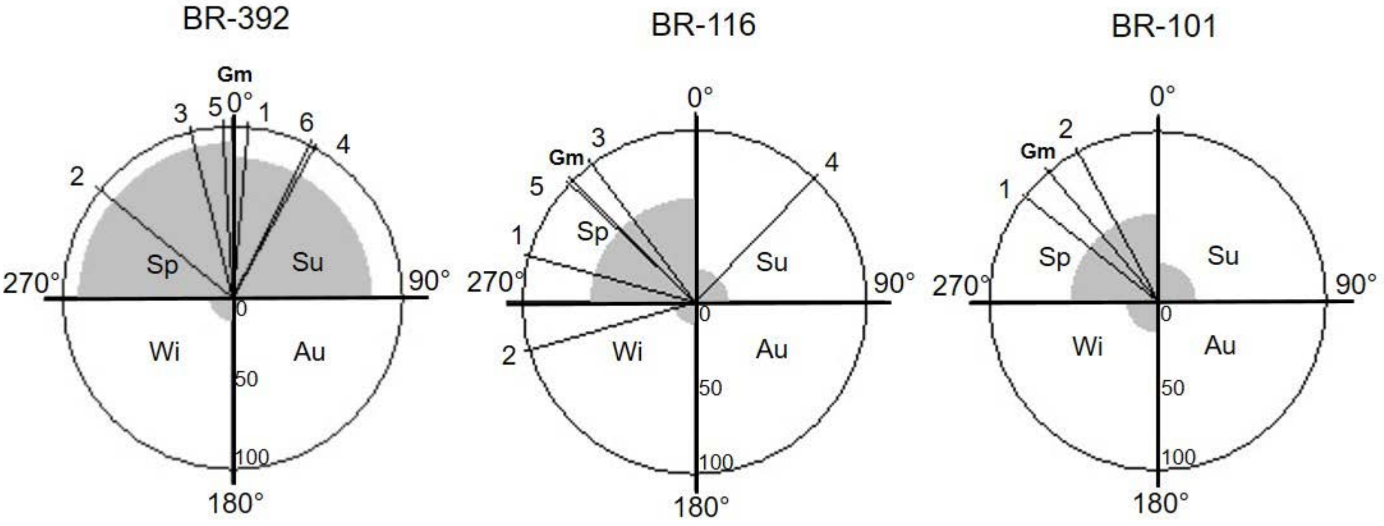

Figure 5. Observed fatality numbers of *H. infrateniatus* (grey) on each road per season (Su= summer; Au = autumn; Wi = winter; Sp = spring). Gm indicates the line of the grand mean vector, and lines accompanied by year number indicate the yearly means.

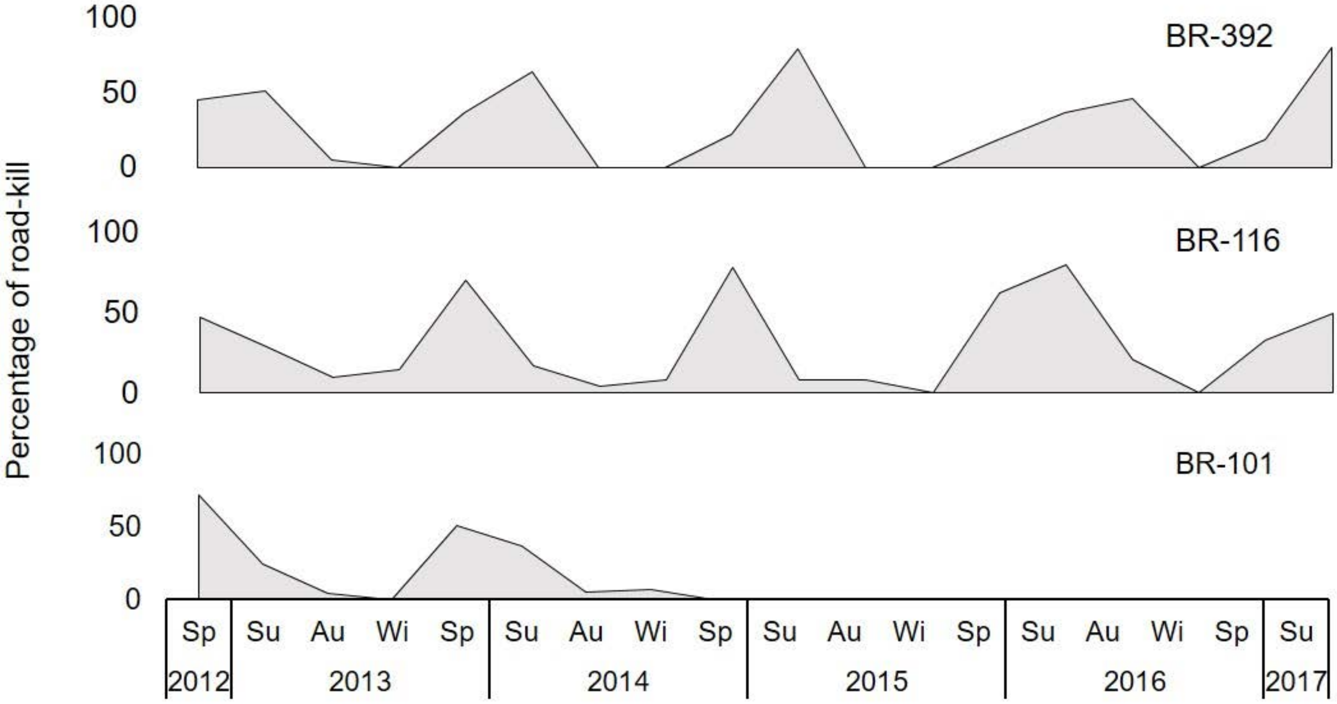

Figure 6. Activity index of *H. infrateniatus* fatalities throughout the study period in each road.

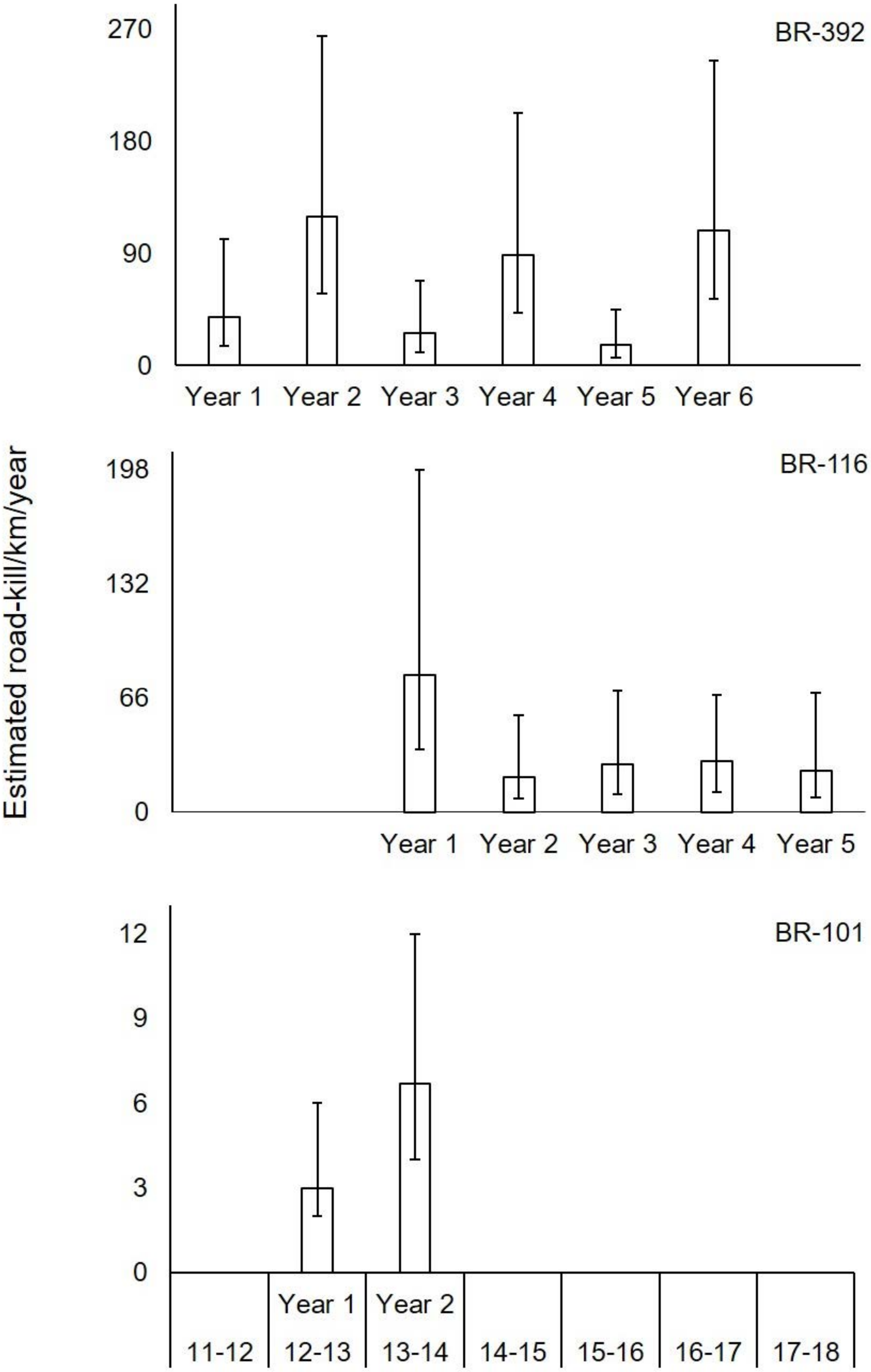

Figure 7. Mean and 95% credibility limits of estimated fatalities (roadkill/km/year) for each road between 2011-2018.

4. Discussion

We found different patterns of road-kill hotspots and hot moments, and in the number of road-killed snakes between roads. All these patterns varied between years, but hotspots were comparatively more stable as spatial and temporal scales increased. We also found that road-kill was synchronous between roads over the years. Accounting for carcass detection and persistence allowed us to demonstrate that the magnitude of the impact was highly underestimated with observed data. Our study highlights the importance of using single-species monitoring in road ecology, and of embracing spatio-temporal variation is assessing road-kill impacts and recommending mitigation measures.

4.1. Road-kill hotspots patterns

The occurrence of aggregations was relatively constant over the years at different spatial scales. Sample size however, could have inconsistent effects on aggregation identification. Despite the 31 records of road-killed snakes during the first year of sampling at BR-101, we found no significant aggregations at 100 and 500 m scales (90% CI). On the other hand, we found significant aggregations at the same scales (95% and 90% CI) at BR-392 and BR-116 with only 13 records. Spatial patterns may thus change between years, but not exclusively as an effect of sampling size.

The distribution of hotspots along roads could be concentrated in a specific zone or evenly spread. Hotpots on the BR-392 are concentrated in a 10 km stretch of the floodplain surrounding the *Canal São Gonçalo,* covered with extensive freshwater marshes. Wetlands along the BR-116 and BR-101 are much smaller and spread along these roads. Aggregations of road killed reptiles also are more frequent on roads that cross wetlands than on roads that border them (Langen et al. 2009). In this sense the pattern of road-marsh neighborhood given by the size, number and proximity of marsh influences the spatial distribution of hotspots aggregation.

The distribution of presence/absence or intensity of the hotspots also changed between years, even when they were concentrated in a larger zone such as the floodplain of the *Canal São Gonçalo*. However, this spatial variation is more noticeable at finer scales of analysis. Santos et al. (2017) also showed that hotspots are more consistent at larger spatial scales. We recommend testing for multiple spatial scales of analysis when planning for mitigation location to guarantee that fine spatial inter annual variation in hotspot location are encompassed within a target zone.

Our results indicate also that using data from at least two consecutive years of monitoring results in similar hotspot location when compared to much larger sampling periods. We recommend that monitoring mortality of this species for two consecutive years, with monthly or bimonthly sampling regimes, would be sufficient to detect patterns. Alternatively, Santos et al. (2015) demonstrated that sampling intervals shorter than a week could also be necessary to capture spatial patterns within shorter sampling periods (a year or less).

Large concentrations of *H. infrataeniatus* have been reported on mattresses of aquatic vegetation after major floods, especially during *El Niño* years (Lema, 2002; Regnet et al., 2017), and in remnant water bodies and muddy areas during droughts (Quintela and Loebmann, 2009; Carreira and Maneyro, 2013). Movements to and from these areas likely influence the probability of crossing roads (Roe et al., 2004; Siers et al., 2016). In this scenario, intra and inter annual variation in habitat use by *H. infrataeniatus* could influence movement and abundance. Therefore, atypical or cyclical climatic variations, which have a temporal effect on the configuration and suitability of habitat and on the dispersal of animals, could explain some of the above temporal contrasts in hotspot locations and need further investigation.

4.2. Road-kill hot moment patterns

Our temporal analysis indicates that road-kills are aggregated in the spring and summer. These seasons correspond to the main activity and breeding periods for *H. infrataeniatus*, for snakes in our study area in general (Lema, 2002), and for road-kill concentrations of multiple reptile species (Gonçalves et al., 2018). Although temporal patterns of snake mortality on roads are mostly related to variations in radiation, temperature and precipitation (Sosa and Schalk, 2016; Garriga et al., 2017), differences in the start or end of rainy periods that may trigger massive migrations or movements related with breeding activity are also important for aquatic species (Bernardino and Dalrymple, 1992; Roe et al., 2004). Such climatic variations could alter dispersal and breeding patterns at finer temporal scales and potentially change road-kill hot moments between years. To determine the best mitigation moment over the years, it is necessary to increase the effort in the number of monitored years and reduce sampling interval. Hot moments are more consistent at larger temporal scales, so an alternative to minimize this uncertainty would be to use longer time periods to implement mitigation measures (Santos et al., 2017). Also, our results clearly indicate that this monitoring can be restricted to the spring/summer months.

4.3. Road-kill synchrony between roads

Comparing the time series of snake road-kill between roads indicated a general synchrony in our study area. Seasonal dynamics in the biology of the species, climate-driven activity patterns and even temporal changes in traffic can produce similar temporal mortality patterns across large spatial scales (Bernardino and Dalrymple, 1992; Andrews et al., 2015; Siers et al., 2016; Gonçalves et al., 2018). Taken together, our results indicate that seasonal mitigation strategies (like temporary fencing near culverts, for example) can be developed simultaneously in different locations of southern Brazil. Synchrony studies can provide clues to understand the driving movement to road crossing and improve mitigation (Liebhold et al, 2004). For this reason, it is important to study synchrony patterns between localities at finer temporal scales (months or weeks) and include temporal variation in other variables.

4.4. Road-kill numbers patterns

The higher similarity between searcher efficiency and carcass persistence values between BR-392 and BR-116 when compared to BR-101 may be associated with differences between sampling techniques. At BR-392 and BR-116 there was an additional observer in relation to BR-101. Furthermore, at BR-392 and BR-116 observers traveled the road from start to end and back again per sampling day, increasing the probability of finding carcasses. The removal of snake carcasses on roads is primarily due to their degradation through the passage of vehicles, with higher volumes of traffic decreasing the persistence time of carcasses (Cabrera-Casas et al., 2020). Daily traffic volumes were notably higher at BR-392 and BR-116 and thus carcass persistence was much lower on that roads when compared do BR-101. Our fatality estimates were much higher than the observed number of carcasses, as already shown by Ruiz-Capillas et al. (2014) and Winton et al. (2018). We also demonstrated that differences between observed and estimated mortality may even alter the position of roads in a mortality rank and mitigation prioritization. Including these two sources of errors in roadkill studies is essential to obtain more accurate estimates of fatalities and to better prioritize the mitigation of this impact.

Snake road-kill (including *H. infrataeniatus*) is highly dependent on landscape characteristics (Langen et al., 2009; Meek, 2015; Grilo et al., 2016; Gonçalves et al., 2018). Differences in estimated mortality between roads may be linked with the distribution and suitability of habitats in each road. Despite such differences, *H. infrataeniatus* mortality was overall high in all studied roads. On BR-392, road-kill may be five times higher if we consider that the impact occurs mostly in a stretch of only 10 km across the *Canal São Gonçalo* floodplain. *H. infrataeniatus* is a key predator of small aquatic organisms and serves as prey for larger vertebrates (Lema, 2002; Aguiar and Di-Bernardo, 2004; Quintela and Loebmann, 2009). Species that are locally abundant are important for ecosystem functioning and their decline may introduce important changes in ecosystem dynamics (Wanger et al, 2014; Gaston and Fuller, 2008). Furthermore, road-kill may be a determining factor in reducing population size, even of common species (Cooke 2011). From a conservation perspective, whenever possible, the best option is to manage anthropogenic interference and driving forces like roadkills and minimize them to prevent the potential decline of the target species. Given their importance to ecosystem functioning, common species should perhaps also be recognized as target groups for roadkill mitigation.

Like the spatial and temporal distribution of road-kills, the number of fatalities also varied over the years. We found a surprising biannual pattern in fatalities at BR-392. Although on BR-101 there was a sharp contrast in fatalities from one year to another, a biennial pattern was not detected perhaps because of the short monitoring period. In BR-116, the magnitude of fatalities was more stable, but with a notable difference in the first year compared to the subsequent four. Establishing the reasons behind the annual road-kill variations is fundamental to choose mitigation strategies and develop predictive models (Gunso and Teixeira, 2015). Although variation in traffic and climate can influence snake road-kill (Siers et al., 2016; Garriga et al., 2017; Gonçalvez et al., 2018), the biennial pattern we found may be related with the reproductive cycle of aquatic snakes (Blem, 1982; Brown and Weatherhead, 1999). Bellini et al. (2019) detected that biennial and multi-annual breeding is predominant in viviparous snakes (such as *H. infrataeniatus*), being adjusted by the hydrological cycle and temperature on aquatic species. Thus, increasing the number of monitoring years may be essential to establish the real magnitude of impacts on aquatic snakes. In a specific case like evaluating the effectiveness of a mitigation measure, monitoring to estimate mortality could be limited to the period where road-kill is higher.

5. Conclusion

Hotspots, hot moments and the number of fatalities changed in a yearly interval, more in some roads than others. These differences are likely influenced by environmental changes at finer spatial-temporal scales and by the breeding biology of the species. We demonstrated that with a multiyear sampling regime and testing scale effects on hotspot recognition, the uncertainties regarding mitigation planning of these fluctuations could be accommodated. However, more intensive survey regimes (monthly or weekly) should be also tested since they accumulate more carcasses and could be an alternative to multiyear sampling. Moreover, this could allow mitigation decisions to be anticipated. Independently of the intensity, we show that sampling and monitoring can be limited to periods of the year with the highest concentration of road kill. Furthermore, estimates of mortality and representativeness of the number of *H. infrataeniatus* killed on roads are significantly underestimated, as characteristics specific to the highway, traffic and methodology affect the probability to record fatalities. Estimates should include the temporal and spatial variations that affect the probability of finding road-killed snakes. Our results also demonstrated that the BR392 segment that crosses the *Canal São Gonçalo* floodplains is a regional priority for mitigation.

Acknowledgements

We are grateful to Daniel Loebmann and Larissa Oliveira Gonçalves for comments that improved the manuscript. We also thank the National Department of Transport Infrastructure (DNIT) and Serviços Técnicos de Engenheira (STE SA) for allowing access to the BR-392 and BR-116 databases and photographs. We are deeply gratefull to all colleagues that assisted during field surveys and experiments.

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Supplementary data

S 1. Google map with kmz file of:

- Sampled stretches of BR-392, BR-116 and BR-101.
- Road-kill records used to identify hotspots.
- Hotspots identified for each road and the spatio-temporal scales used.

S. 2. A. Data sets for hotspot tests, hot moments tests, and road-kill estimation. The X indicates analyzed data sets.

Road	Annual cycle (data group)	Sampling size	Rypley's K	Hotspot radius size			Similarity per radius size			Hot moments	Fatality
				100 m	500 m	1000 m	100 m	500 m	1000 m		
BR-392	Autumn 2011–Summer 2012 (Year 1)	13	X	X	X	X	X	X	X	X	X
	Autumn 2012–Summer 2013 (Year 2)	61	X	X	X	X	X	X	X	X	X
	Autumn 2013–Summer 2014 (Year 3)	13	X	-	X	X	-	X	X	X	X
	Autumn 2014–Summer 2015 (Year 4)	37	X	X	X	X	X	X	X	X	X
	Autumn 2015–Summer 2016 (Year 5)	13	X	X	X	X	X	X	X	X	X
	Autumn 2016–Summer 2017 (Year 6)	52	X	X	X	X	X	X	X	X	X
	Autumn 2012–Summer 2013 (Year 1–2)	74	X	X	X	X	X	X	X	-	-
	Autumn 2012–Summer 2014 (Year 1–3)	87	X	X	X	X	X	X	X	-	-
	Autumn 2012–Summer 2015 (Year 1–4)	124	X	X	X	X	X	X	X	-	-
	Autumn 2012–Summer 2016 (Year 1–5)	137	X	X	X	X	X	X	X	-	-
	Autumn 2012–Summer 2017 (Year 1–6; pooled data set)	189	X	X	X	X	X	X	X	X	X
BR-116	Autumn 2013–Summer 2014 (Year 1)	45	X	X	X	X	X	X	X	X	X
	Autumn 2014–Summer 2015 (Year 2)	11	X	-	X	X	-	X	X	X	X
	Autumn 2015–Summer 2016 (Year 3)	15	X	X	X	X	X	X	X	X	X
	Autumn 2016–Summer 2017 (Year 4)	13	X	X	X	X	X	X	X	X	X
	Autumn 2017–Summer 2018 (Year 5)	8	X	-	-	-	-	-	-	X	X
	Autumn 2013–Summer 2015 (Year 1–2)	56	X	X	X	X	X	X	X	-	-
	Autumn 2013–Summer 2016 (Year 1–3)	71	X	X	X	X	X	X	X	-	-
	Autumn 2013–Summer 2017 (Year 1–4)	84	X	X	X	X	X	X	X	-	-
	Autumn 2013–Summer 2018 (Year 1–5 pooled data set)	92	X	X	X	X	X	X	X	X	X
BR-101	Spring 2012–Winter 2013 (Year 1)	31	X	-	-	X	-	-	-	X	X
	Spring 2013–Winter 2014 (Year 2)	62	X	X	X	X	-	-	-	X	X
	Spring 2012–Winter 2014 (Year 1–2; pooled data set)	93	X	X	X	X	-	-	-	X	X

S. 2.B. Data sets for synchrony tests. The X indicates analyzed data sets.

Road	Annual cycle (data group)	Sampling size	Synchrony			
			BR-392 vs BR-116	BR-392 vs BR-101	BR-116 vs BR-101	BR-392 vs BR-116 vs BR-101
BR-392	Spring 2012–Winter 2013	60	X	X	-	X
	Spring 2013–Winter 2014	11	X	X	-	X
	Spring 2014–Winter 2015	37	X	-	-	-
	Spring 2015–Winter 2016	14	X	-	-	-
	Spring 2016–Winter 2017	52	X	-	-	-
BR-116	Spring 2012–Winter 2013	34	X	-	X	X
	Spring 2013–Winter 2014	42	X	-	X	X
	Spring 2014–Winter 2015	6	X	-	-	-
	Spring 2015–Winter 2016	17	X	-	-	-
	Spring 2016–Winter 2017	12	X	-	-	-
BR-101	Spring 2012–Winter 2013	31	-	X	X	X
	Spring 2013–Winter 2014	62	-	X	X	X

S 4. A. Ripley's *K*-2D per road per year, with the sample size (N) for each annual cycle per road, the confidence interval (IC) under which aggregations were significantly grouped with Ripley's K-2D statistic for 100, 500 and 1000 m radii, and the corresponding years in which each sample was collected.

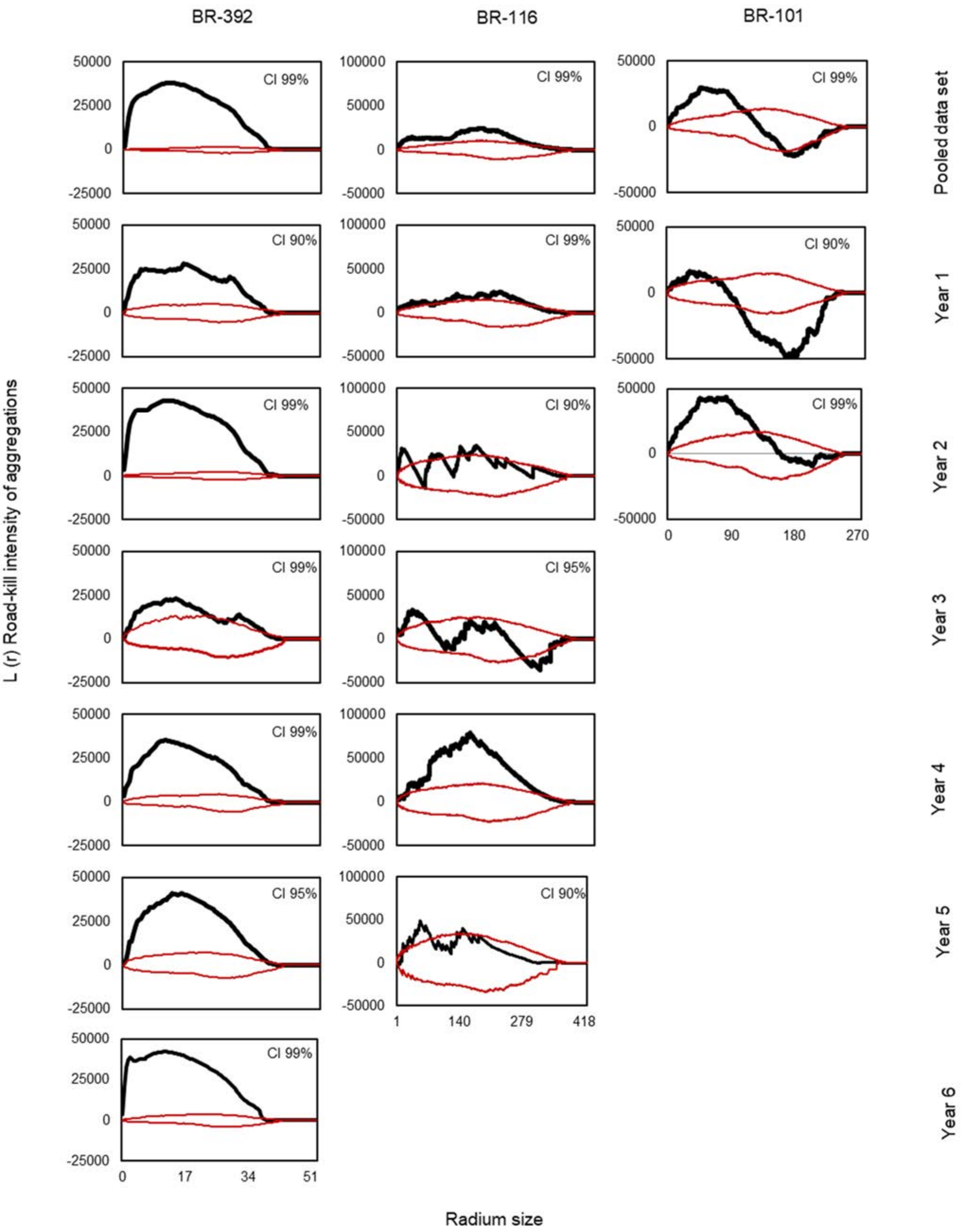
Annual cycle		11–12	12–13	13–14	14–15	15–16	16–17	17–18
	Dataset	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6	
BR-392	N	13	61	13	37	13	52	
	IC	90%	99%	99%[1]	99%	95%	99%	
	Dataset			Year 1	Year 2	Year 3	Year 4	Year 5
BR-116	N			45	11	15	13	8
	IC			99%	90%[1]	95%	90%	X
	Dataset		Year 1	Year 2				
BR-101	N		31	62				
	IC		90%[2]	99%				

"x" indicates no significant aggregation observed at any radius of interest.

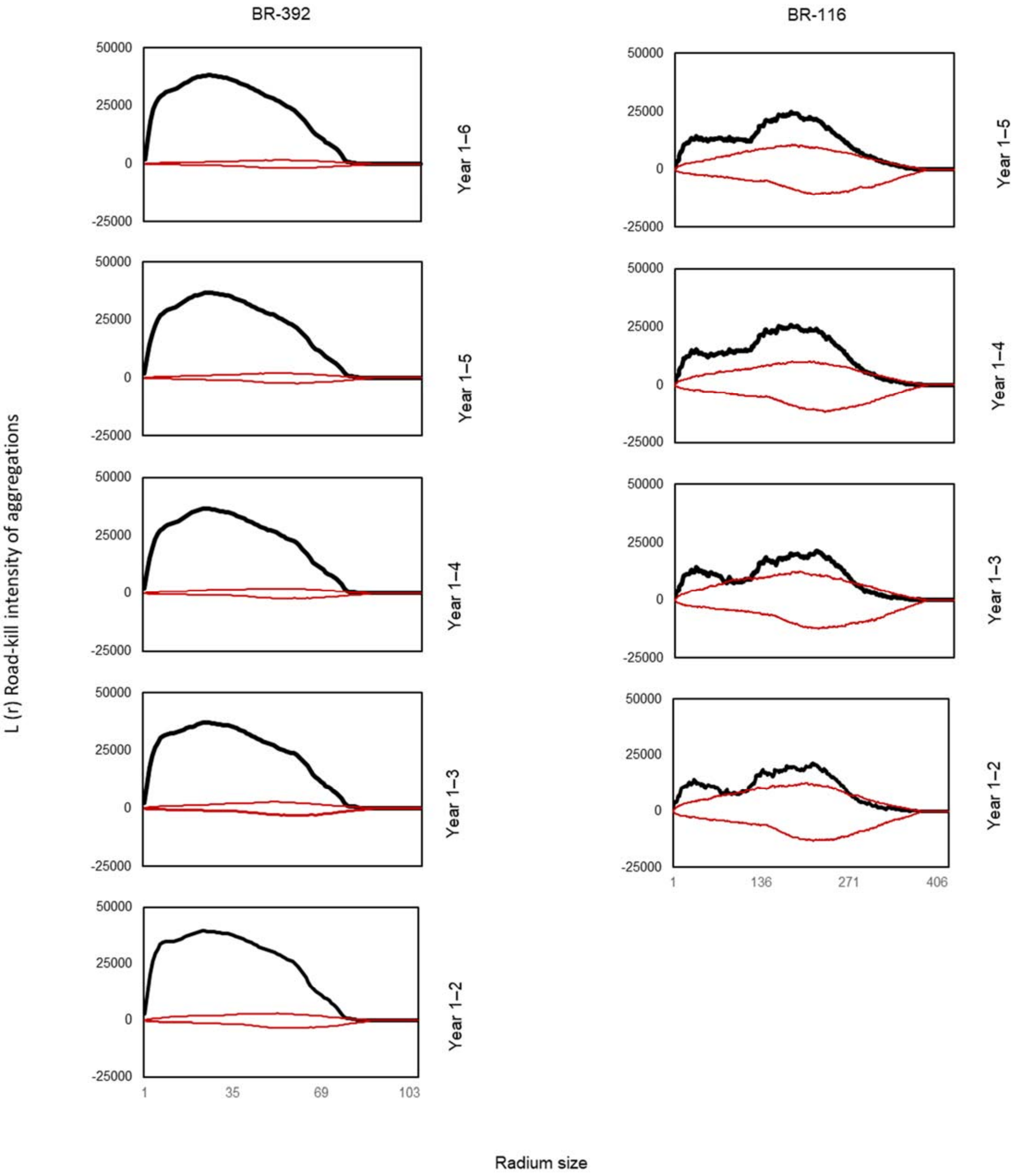
[1] Indicates no significant aggregation observed only in 100 m radium.

[2] Indicates no significant aggregation in 100 and 500 m radius.

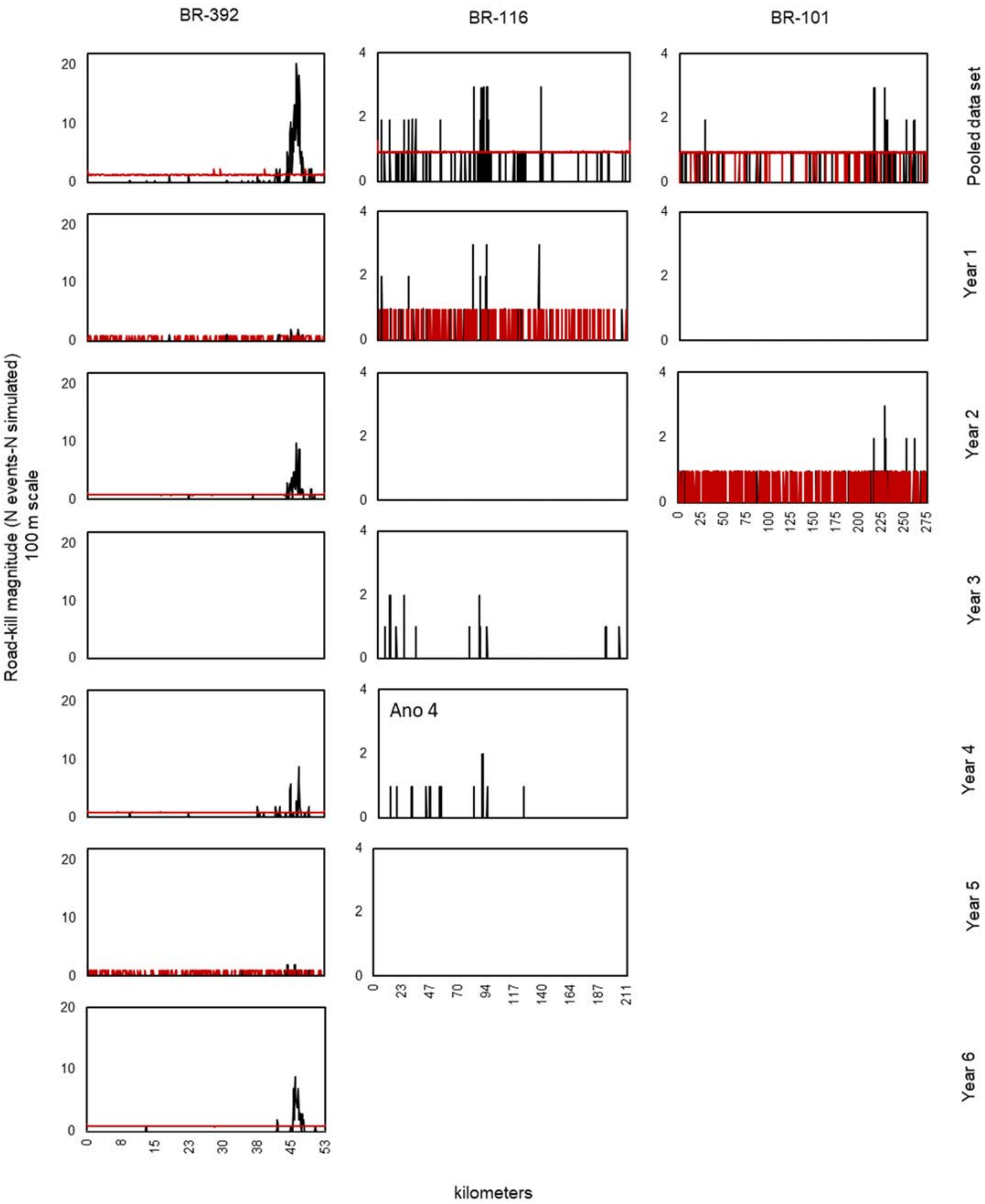
The aggregation was significant in any radius with a confidence interval of 99% for all data groups with one or more accumulated years.



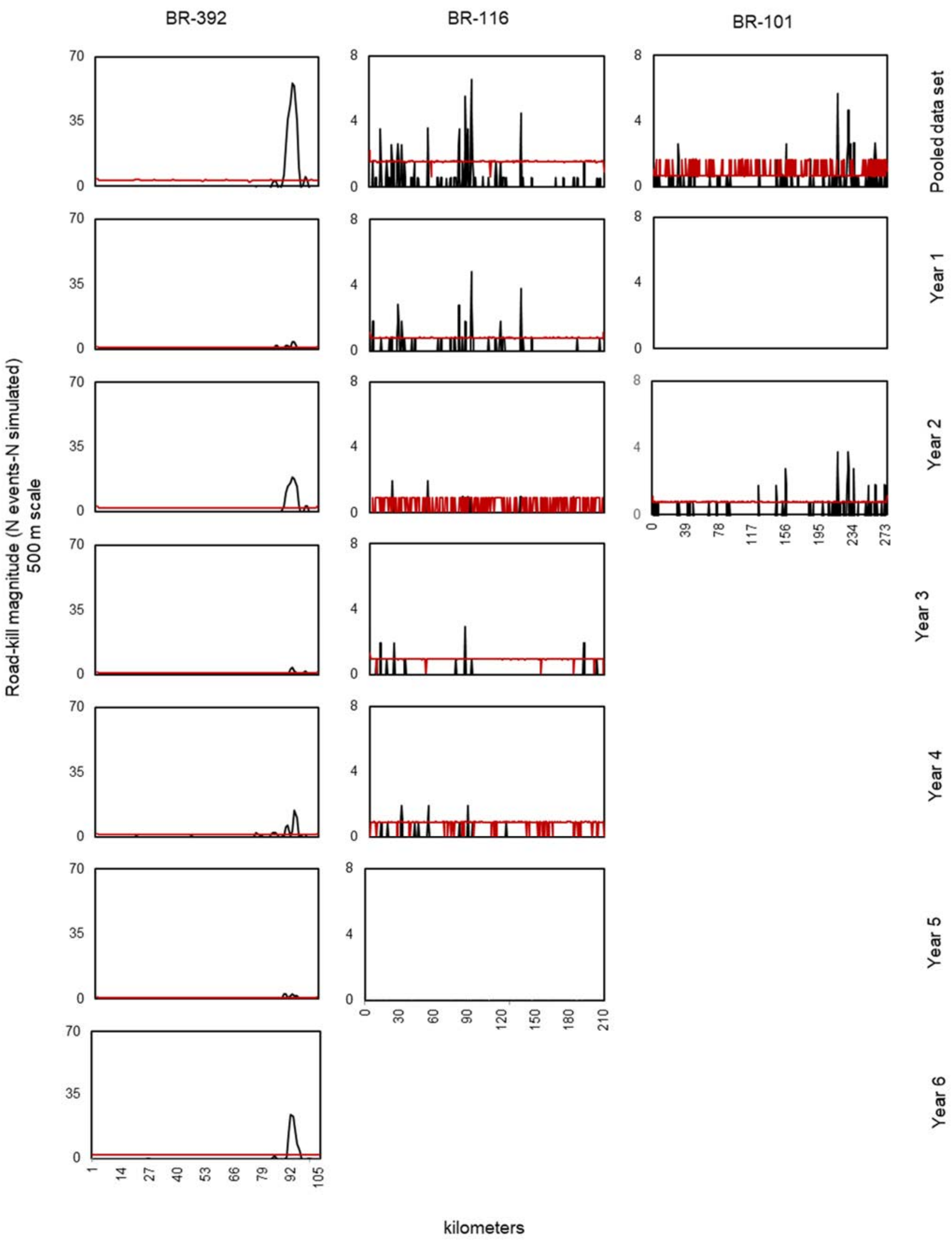
S 3.B. Graphics comparing Ripley's K-2D per year in the three sampled roads and their respective pooled data set. The intensity of the road-kill aggregations, given by the function *L*(*r*), is represented by the black line. Red lines represent the upper and lower confidence limits with 99, 95 or 90% confidence intervals. Road-kill aggregations were considered significant when values of *L*(*r*) were larger than the upper confidence limit.



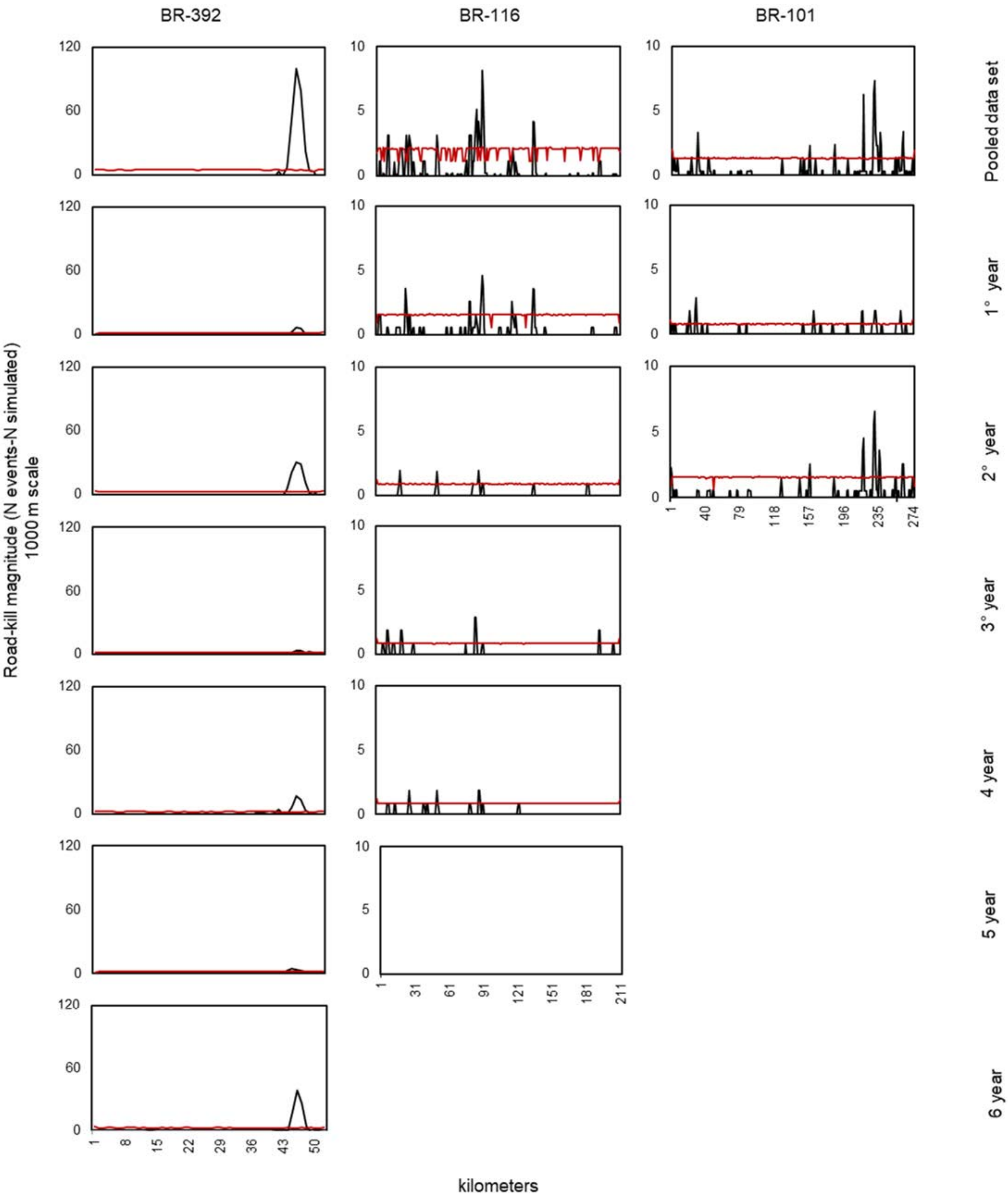
S 3.C. Graphics comparing Ripley's K-2D accumulated per year for BR-392 and BR-116. The intensity of the road-kill aggregations, given by the function *L*(*r*), is represented by the black line. Red lines represent the upper and lower confidence limits with 99, 95 or 90% confidence intervals. Road-kill aggregations were considered significant when values of *L*(*r*) were larger than the upper confidence limit.



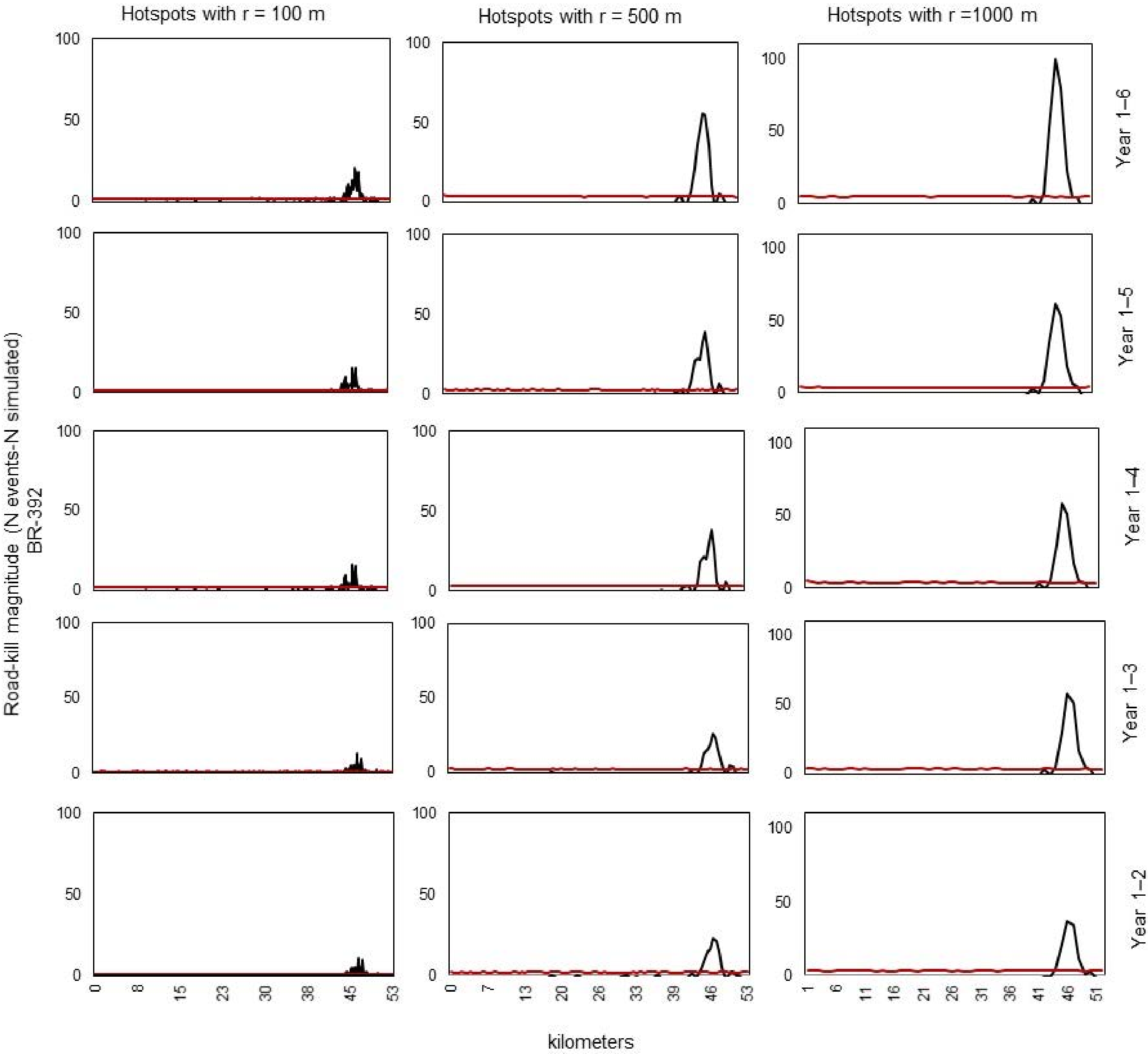
S 3.D. Graphics showing the distribution of hotspots per year. Hotspots were obtained from 2D Hotspot Identification analysis with a 100 m road stretch and 100 m radium. Data groups with random aggregations are not shown (Graphs without information). The red line represents the function N events- N simulated and the black lines represent confidence limits (95%). We considered hotspots the segments (x-axis) with road-kill magnitude greater than the upper confidence limit.



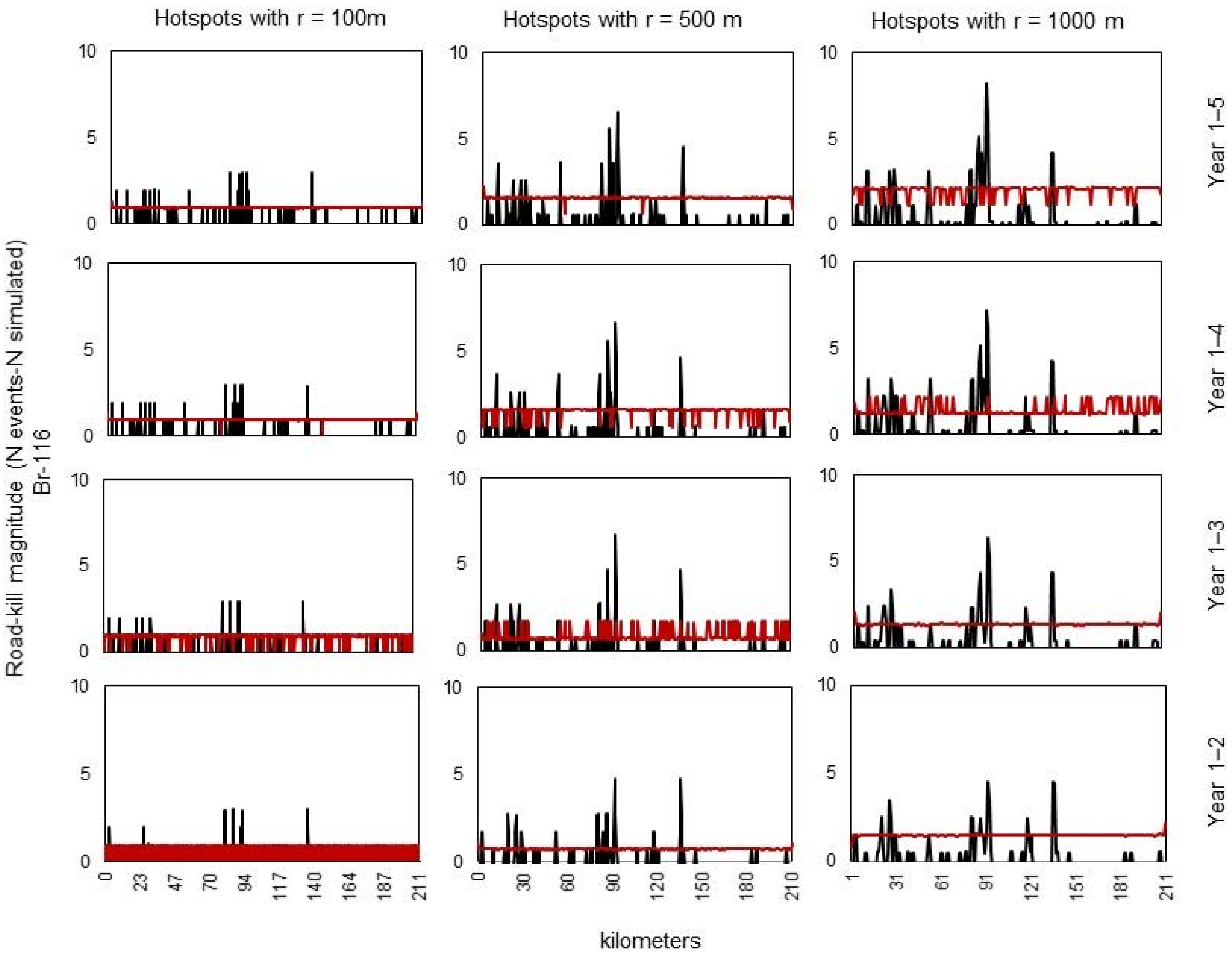
S 3.E. Graphics showing the distribution of hotspots per year. Hotspots were obtained from 2D Hotspot Identification analysis with a 500 m road stretch and 500 m radium. Data groups with random aggregations are not shown (Graphs without information). The red line represents the function N events- N simulated and the black lines represent confidence limits (95%). We considered hotspots the segments (x-axis) with road-kill magnitude greater than the upper confidence limit



S 3.F. Graphics showing the distribution of hotspots per year. Hotspots were obtained from 2D Hotspot Identification analysis with a 1000 m road stretch and 1000 m radium. Data groups with random aggregations are not shown (Graphs without information). The red line represents the function N events- N simulated and the black lines represent confidence limits (95%). We considered hotspots the segments (x-axis) with road-kill magnitude greater than the upper confidence limit



S 3.G. Graphics showing the distribution of hotspots per consecutive on BR-392. Hotspots were obtained from 2D Hotspot Identification analysis with a 100, 500 and 1000 m road stretch and radius size. The red line represents the function N events- N simulated and the black lines represent confidence limits (95%). We considered hotspots the segments (x-axis) with road-kill magnitude greater than the upper confidence limit



S 3.H. Graphics showing the distribution of hotspots per consecutive on BR-116. Hotspots were obtained from 2D Hotspot Identification analysis with a 100, 500 and 1000 m road stretch and radius size. The red line represents the function N events- N simulated and the black lines represent confidence limits (95%). We considered hotspots the segments (x-axis) with road-kill magnitude greater than the upper confidence limit

S 4. Results of the circular tests per year for each road and data group.

BR-392								
Annual cycle	2011–2012	2012–2013	2013–2014	2014–2015	2015–2016	2016–2017	2017–2018	2011–2017
Data group	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6		Year 1–6
Sample size	13	61	13	37	8	57		189
Length of the mean vector (*r*)	0.69	0.70	0.60	0.82	0.80	0.77		0.61
Mean vector (*μ*)	5.18	308.81°	345.62°	28.7°	356.68°	26.71°		359.69°
Lower 95% CI for *μ*	337.44°	296.73°	312.14°	17.06°	323.90°	16.09°		351.30°
Upper 95% CI for *μ*	32.93°	320.89°	19.11°	40.40°	29.46°	37.33°		8.08°
Rayleigh's Test (*Z*)	6.11	30.06	4.76	24.62	5.05	33.86		70.46
Rayleigh's Test (*P*)	0.001	< 0.001	0.006	< 0.001	0.003	< 0.001		< 0.001
BR-116								
Annual cycle	2012–2012	2012–2013	2013–2014	2014–2015	2015–2016	2016–2017	2017–2018	2013–2018
Data group			Year 1	Year 2	Year 3	Year 4	Year 5	Year 1–5
Sample size			45	11	15	13	8	92
Length of mean vector (*r*)			0.78	0.26	0.69	0.53	0.79	0.55
Mean vector (*μ*)			285.73°	253.86°	323.32°	43.68°	313.38°	301.72°
Lower 95% CI for *μ*			274.12°	112.12°	297.92°	3.60°	279.95°	287.83°
Upper 95% CI (+) for *μ*			300.99°	80.13°	356.69°	96.34°	357.31°	315.62°
Rayleigh's test (*Z*)			27.52	0.77	7.17	3.68	4.96	27.35
Rayleigh's test (*P*)			< 0.001	0.474	< 0.001	0.022	0.004	< 0.001
BR-101								
Annual cycle	2012–2012	2012–2013	2013–2014	2014–2015	2015–2016	2016–2017	2017–2018	2012–2014
Data group		Year 1	Year 2					Year 1–2
Sample size		31	62					93
Length of mean vector (*r*)		0.55	0.53					0.52
Mean vector (*μ*)		308.04°	331.80°					323.74°
Lower 95% CI for *μ*		284.11°	314.07°					309.14°
Upper 95% CI (+) for *μ*		9.22	17.15					338.34°
Rayleigh's test (*Z*)		< 0.001	< 0.001					25.35
Rayleigh's test (*P*)		< 0.001	< 0.001					< 0.001

3. Considerações finais

Os padrões observados de *hotspots*, *hot moments* e no número de atropelamentos de *H. infrataeniatus* apresentam mudanças ao longo dos anos e apresentam variações entre rodovias que podem estar relacionadas a alterações ambientais e comportamentais anuais em escala local ou em períodos de curta duração. Neste sentido, sempre será desejável entender a relação de causa e efeito dos padrões de atropelamento, seja para compreender melhor o impacto gerado como para sua mitigação. Mas estabelecer este tipo de relação sempre apresenta desafios consideráveis para os pesquisadores e tomadores de decisões. Sendo assim, uma alternativa é utilizar uma abordagem conservadora, ou seja, utilizar vários anos de monitoramento e escalas espaço-temporais maiores com a finalidade de diminuir o erro na identificação dos padrões de atropelamento ante possíveis variações anuais. Em relação ao número de atropelamentos, destaca-se a quantidade estimada de atropelamentos da espécie na região e que são claramente subestimadas quando considerados apenas os casos observados. Ademais, os dados observados também se apresentam inadequados como uma medida de ordem na magnitude de atropelamentos, uma vez que características específicas da rodovia, tráfego e metodologia afetam a probabilidade de registro de carcaças. Portanto, as estimativas de mortalidade devem incluir em suas abordagens correções de eficiência de detecção e persistência de carcaças. Finalmente, perante os altos níveis de atropelamentos, é necessária a mitigação de atropelamentos das espécies na região de estudo, especialmente na Várzea do Canal São Gonçalo.

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