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ORIGINAL ARTICLE

Hotspots and hotmoments of wildlife roadkills along a main highway in a high biodiversity area in Brazilian Amazonia

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ABSTRACT

Wildlife roadkills have become a concern in the Amazon biome due to the opening of major roads in recent decades. In this study, we aimed to describe wildlife roadkills in a 100-km stretch of the BR-163 highway, in western Pará state, determining which vertebrate groups are most affected and whether there are spatial (*hotspots*) and temporal (*hotmoments*) aggregations of roadkills. From July 2019 to June 2020, we carried out 25 surveys at 15-day intervals, from a vehicle at a maximum speed of 40 km h^{-1}. We recorded 351 individuals at an observed rate of 0.14 ind km^{-1} day^{-1}. Despite their lower detectability and faster carcass removal rate from the road due to small size, most recorded roadkills were amphibians (0.066 ind km^{-1} day^{-1}). We mapped several *hotspots* along the study stretch considering the total number of animals recorded, and separately for amphibians and reptiles. Multiple linear regression analyses indicated that the number of roadkills of all vertebrates, amphibians and reptiles recorded are influenced by temperature and precipitation. Information on places with the highest incidence of roadkills can support actions such as the installation of underpasses and fences, aimed at reducing the impacts on wild vertebrates of this Amazonian highway.

KEYWORDS: fauna-vehicle collisions, road ecology, conservation, mortality

Hotspots e *hotmoments* de atropelamentos de animais silvestres ao longo de uma rodovia em uma área de alta biodiversidade na Amazônia brasileira

RESUMO

Atropelamentos de fauna silvestre tornaram-se preocupantes no bioma amazônico devido à abertura de grandes rodovias nas últimas décadas. Neste estudo objetivamos caracterizar a fauna silvestre atropelada em um trecho de 100 km da rodovia BR-163, no oeste do estado do Pará, determinando quais grupos de vertebrados são mais afetados e se há agregações espaciais (*hotspots)* e temporais (*hotmoments*) de atropelamentos. De julho 2019 a junho 2020 realizamos 25 amostragens a intervalos de 15 dias, a partir de um veículo a uma velocidade máxima de 40 km h^{-1}. Registramos 351 indivíduos a uma taxa observada de 0.14 ind km^{-1} dia^{-1}. Apesar de sua menor detectabilidade e taxa de remoção mais acelerada da rodovia devido ao seu pequeno porte, a maioria dos atropelamentos foi de anfíbios (0.066 ind km^{-1} dia^{-1}). Mapeamos diversos *hotspots* ao longo do trecho estudado considerando o número total de animais registrados, e, separadamente, para anfíbios e répteis. Análise de regressão linear múltipla indicou que o número total de registros de vertebrados atropelados, e o de anfíbios e répteis é influenciado por temperatura e precipitação. Informação sobre locais com maior incidência de atropelamentos pode subsidiar ações como a instalação de estruturas de passagem de fauna e cercas de proteção, visando a diminuição dos impactos sobre vertebrados silvestres desta rodovia amazônica.

PALAVRAS-CHAVE: colisões fauna-veículo, ecologia de estradas, conservação, mortalidade

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INTRODUCTION

Roads are linear structures that connect locations and are fundamental to the social and economic development of modern society (Rezende and Coelho 2015). However, the dynamics of natural ecosystems is altered with the implementation, use and maintenance of this type of infrastructure, normally causing negative effects on biodiversity (Trombulak and Frissel 2000). The most direct and deleterious of these effects is the killing of wildlife on roads (Jackson and Fahrig 2011; Ree *et al*. 2015). In Brazil, about 44.8 million wild individuals are roadkilled each year on a road network of approximately 1.7 million kilometers (Dornas *et al*. 2012). Recent estimates indicate that more than 8 million birds and 2 million mammals are killed annually in Brazil as a result of vehicle collisions (González-Suárez *et al*. 2018).

Collisions between vehicles and animals often do not occur randomly, tending to cluster spatially or temporally (Gunson *et al*. 2011; Morelle *et al*. 2013). Spatial clusters generate roadkill *hotspots* that may differ among taxonomic groups (Teixeira *et al*. 2013). Generally, *hotspots* are related to the interaction between three groups of factors: 1) intrinsic characteristics of the road, such as the presence of curves, traffic flow intensity and vehicle speed (Klocker *et al*. 2006; Maschio *et al.* 2016); 2) landscape attributes, such as type and amount of native vegetation, presence of water bodies (Hengemühle and Cademartori 2008; Gumier-Costa and Sperber 2009) and crops (Gonçalves *et al*. 2018); and 3) aspects of the biology/ecology of the animals, such as body size, mobility and level of habitat specialization (Clevenger *et al*. 2003).

When collisions vary according to a temporal measure (e.g. daily, monthly, seasonal) they generate critical periods or roadkill *hotmoments* (Garrah *et al*. 2015; Gonçalves *et al*. 2018). Local climatic variations are among the main correlates of *hotmoments* (Coelho *et al*. 2012). The dynamics of weather variables, such as precipitation and temperature, is related to seasonal environmental and metabolic conditions that affect the activity and mobility schedules of animal species, which can make them more vulnerable to being roadkilled. This is the case of the formation of water puddles around the road that attract reproductive assemblages of amphibians, or the increased abundance of several species due to recruitment that occurs mainly in spring and summer (Clevenger *et al*. 2003).

The identification of *hotspots* and *hotmoments* of wildlife roadkills can help to direct actions and optimize the cost/benefit of mitigation measures aimed at reducing roadkills (Beaudry *et al*. 2010; Barthelmess 2014). In Brazil, several studies have identified landscape attributes, such as proximity to flooded areas or greater forest cover, associated with road factors, such as traffic intensity, that are often related to the formation of *hotspots* (Coelho *et al*. 2008; Cáceres *et al*. 2012), while seasonal aspects, such as temperature, and precipitation are commonly associated with the formation of *hotmoments* (Coelho *et al*. 2012).

In the Amazon region, where biodiversity is high and there are usually two distinct seasons determined by rainfall, relatively few studies have assessed the occurrence of *hotspots* and *hotmoments*. Considering the environmental importance of Amazonian ecosystems, and the steady increase in road opening, mainly in the south and eastern part of the biome, the effect of roadkills is still poorly known in the Amazon (Paraguassu-Chaves *et al*. 2020). It is plausible to assume that, as in other regions, the patterns of wildlife roadkill in the Amazon vary depending on the environmental heterogeneity patterns along roads and highways.

We surveyed wildlife roadkills along 100 km of the BR-163 highway in the state of Pará, in the eastern Brazilian Amazon. In most of the studied section, the road separates the protected area of the Tapajós National Forest, with a high diversity of vertebrates (Henriques *et al*. 2003; Sampaio *et al*. 2010; Rosa *et al*. 2021), from an area with intense changes in the landscape due to agricultural expansion. We aimed to identify points of concentration of wildlife roadkills (*hotspots*) and to understand the relationship between collisions and seasonality in precipitation and temperature to identify possible temporal aggregations (*hotmoments*) in roadkills.

MATERIAL AND METHODS

Study area

We collected data on a 100-km segment, between km 40 and 140 of the BR-163 highway (between 02°40’47,17”S; 54°50’54,90”W and 03°32’08,09”S; 54°52’13,90”W), located in Belterra county, Pará state, Brazil. This road is 1,780 km long, connecting the cities of Cuiabá, in Mato Grosso state, to Santarém, in Pará state. In the studied section, the road has little topographic variation (Gonçalves and Santos 2008), with a predominance of straight segments. The stretch is paved and has a single lane in each direction, with a precarious shoulder, which, in some places, is partially covered by vegetation, mainly grasses. Including the shoulders, the road is 11 m wide.

The climate of the region is humid tropical, of type Am in the Köppen system (Ruschel 2008), and the average annual temperature varies between 25 °C and 26 °C, with average annual precipitation oscillating around 2.000 mm (Oliveira Junior *et al*. 2010). There are two distinct seasonal periods, one more rainy (January to June) and one drier (July and December) (Espírito-Santo *et al*. 2005). There is little seasonal variation in temperature, which is mainly due to the cloud cover in periods of higher rainfall, allowing for a milder climate in the rainy season, which coincides with the summer in the southern hemisphere.

The predominant original landscape along the sampled stretch was dense rainforest (Espírito-Santo *et al*. 2005).

Common tree species in this vegetation include Brazil nut, *Bertholletia excelsa* H. B. K., angelim, *Hymenolobium excelsum* Ducke, and ipe, *Tabebuia* spp. (Silva *et al.* 1985; Andrade *et al.* 2015). Of the 100 km covered in the study, 77 km border the conservation area of Tapajós National Forest (Figure 1). In this section, the road is bordered on one side by forests and on the other by private areas that form a mosaic landscape composed of forest remnants, pastures and crops, mainly soybean and corn. From km 0 to 10, the human population density is higher due to the proximity to the urban area of Belterra. Between km 48 and 61 the landscape is pasture and crops on both sides of the road. The Tapajós National Forest has a high richness of mammals (Sampaio *et al.* 2010), birds (Less *et al.* 2013; Henriques *et al.* 2003) and lizards (Oliveira 2015; Oliveira *et al.* 2016).

Data collection

We sampled the 100-km section every fifteen days between July 2019 and June 2020, totaling 2,500 km cumulative distance in 25 surveys. We used a motor vehicle that traveled the study section at a speed of up to 40 km h^{-1}, always with an observer and the driver (three observers throughout the 25 samples). Since the sampled section is relatively long, the beginning of the surveys was alternated between the ends of the section, with a survey starting at km 40 and ending at km 140, and the next, after 15 days, starting at km 140 and and ending at km 40, always starting at 7:00 am and ending around 11:00 am. During the sampling, we considered all animals that were roadkilled on both sides of the road, including the shoulder, resulting in a surveyed area of approximately 11 m wide by 100 km long.

For each roadkilled animal identified on the road, we recorded the coordinates with a GPS device (Garmin 62sc), photographed the specimen, identified it to the lowest possible taxonomic level and removed the carcass from the road. Whenever possible, we identified the animal in the field, but, when this was not possible, we used taxonomic identification guides and/or support from specialist researchers on each taxonomic group.

To assess the factors that may be related to roadkill, we obtained information on the following climatic variables: 1) precipitation from the previous day of each survey; 2) accumulated precipitation from the seven days before each survey; 3) average temperature of the previous day; and 4) average temperature for the previous seven days (Supplementary Material, Table S1). The data were from meteorological station # 82246 of the National Institute of Meteorology (INMET) located in Belterra county, approximately 10 km from the beginning of the study area (https://tempo.inmet.gov.br/TabelaEstacoes/82246).

Data analysis

To determine the number of roadkills per kilometer as a function of the days sampled, we calculated the roadkill rate for all vertebrate groups and separately for each class (mammals, birds, amphibians and reptiles). For this, we divided the number of individuals roadkilled by the total number of kilometers of the stretch (100 km) and then by the total number of days sampled (25 days), generating the observed rate of roadkilled individuals per kilometer per day (ind km^{-1} day^{-1}). We considered only the rates of roadkills

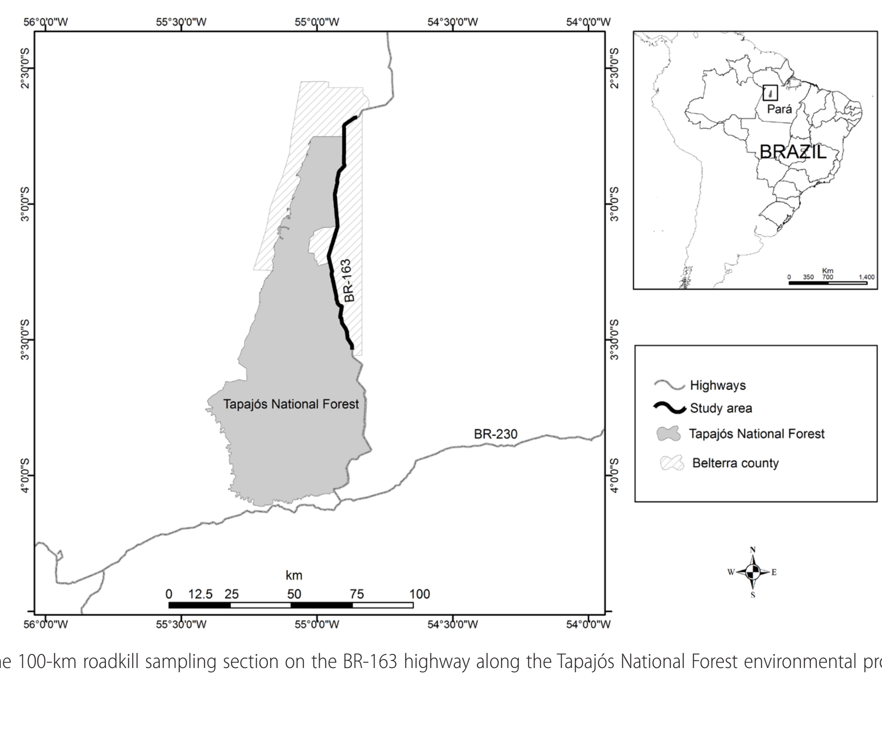

Figure 1. Location of the 100-km roadkill sampling section on the BR-163 highway along the Tapajós National Forest environmental protection area, in Belterra county, Pará state, Brazil.

as observed, as we did not analyze carcass removal rates, nor detectability of different animal types or among observers.

To investigate the spatial distribution of roadkills and identify possible *hotspots*, we performed two analyzes in the SIRIEMA 2.0 software (Coelho *et al.* 2014). The first was the Ripley-2D K-Statistic (Ripley 1981; Cressie 1993; Levine 2004; Coelho *et al.* 2014), which evaluated the non-randomness of the spatial distribution of roadkills, considering the total number of animals recorded and each class separately, with 95% confidence interval ($\alpha = 0.05$). In this test, a circle of radius predefined by the authors is centered on the location of the first roadkill recorded from the beginning of the survey area, and all other roadkills within this circle are summed. The 2D analysis maintains the two-dimensionality of the road, which can cause variation in its extension within the different circles. To adjust this detail, the sum of pedestrian accidents within each area is multiplied by a correction factor based on the length of the road within the circle. This process is repeated until all roadkills have been evaluated. Finally, a general sum is obtained that provides the aggregation value for the stipulated radius. After the analysis with the initial radius, the test proceeds with increasing radii (radius increment) until reaching the total length of the road. We used an initial radius of 100 m, corresponding to the scale at which most measures to mitigate fauna trampling can be efficient, depending on the target species (Teixeira *et al.* 2013). Subsequent radius increments of 400 m were used for each stage, with 1000 simulations, according to pre-defined parameters of Ripley's K test – 2D.

Next, we performed the 2D *hotspot* analysis to identify the stretches with the highest aggregation of roadkills (*hotspots*). As we detected significant clusters at the 100-m scale in Ripley's K analysis – 2D, this was also the radius value adopted for the analysis of *hotspots,* with the same number of simulations. The road is initially divided into segments of equal size, which, in our analysis were 740 segments of 136 m each. The size of the segments is defined by the author of the analysis and cannot be greater than twice the scale used in the test, which, in our case, was 100 m, to prevent sections of the highway from being excluded from the analysis. Then, a circle centered on the central area of the first segment is inserted and all roadkills within this area are summed and subsequently multiplied by a correction factor for the same reasons as in the first analysis. The procedure is performed for all segments, resulting in an aggregation intensity factor of roadkills at each location of the highway.

For the identification of *hotmoments*, we evaluated the effect of climatic variables on the number of animals roadkilled by means of multiple linear regression analysis for all vertebrates combined and for each class separately. The response variable was the number of roadkilled animals, and the explanatory variables were the precipitation of the previous day, accumulated precipitation of the previous seven days, average temperature of the previous day and average temperature of the previous seven days (Supplementary Material, Table S1). The assumptions for parametric analysis were confirmed using the Shapiro-Wilk (normality), Durbin Watson (residual independence) and Breusch-Pagan (homoscedasticity) tests. The variables did not show multicollinearity (> 0.7) and therefore different models were tested with all possible combinations of the explanatory variables. In all analyzes we used the R Studio software version 3.6.0 (R Core Team, 2019).

RESULTS

In the 25 surveys, we recorded 351 vertebrates roadkilled, which represents an observed roadkill rate of 0.14 ind km^{-1} day^{-1}, or an average of one animal roadkilled every 7 km per day (Table 1). The class with the highest observed roadkill rate was amphibians (n = 164, TA = 0.066 ind km^{-1} day^{-1}), followed by mammals (n = 66, TA = 0.026 ind km^{-1} day^{-1}), reptiles (n = 62, TA = 0.025 ind km^{-1} day^{-1}) and, finally, birds (n = 59, TA = 0.024 ind km^{-1} day^{-1}).

In total, we identified 47 species, however, 54% of the animals found could not be identified to species level, given the degree of decomposition of the carcass. All amphibians recorded were anurans and could only be identified to order level due to the small size and the state of deterioration of the carcasses. We identified 77% of individual mammals to species level (nine species) and the remaining 23% could only be identified to higher taxonomic levels. *Tamandua tetradactyla* (Linnaeus, 1758), was the most recorded mammal (n = 20), followed by *Cerdocyon thous* (Linnaeus, 1766) and *Didelphis marsupialis* Linnaeus, 1758 (n = 8 each). Of the 62 individual reptiles recorded, we identified 95% to species level (17 species). The most common species were the snakes *Boa constrictor* Linnaeus, 1758 (n = 17) and *Epicrates cenchria* (Linnaeus, 1758) (n = 9). Of the 59 bird records, we identified 86% to species level (21 species). *Coragyps atratus* (Bechstein, 1793) was the most recorded species (n = 24), while all the others had three or less records (Table 1).

Considering all the records of roadkilled animals, we observed aggregations of roadkill points at almost all analyzed scales. Amphibians and reptiles showed aggregation from the 100-m scale radius. We found no evidence for aggregation of mammals and birds (Figure 2).

We identified several roadkill *hotspots*, 21 for vertebrates, 9 for amphibians, and 7 for reptiles (Figure 3). At km 122 of the highway (km 82 of the 100-km sampling section), an amphibian *hotspot* overlapped with a reptile *hotspot*. As there were no roadkill aggregations for the other classes, possibly the *hotspots* for all vertebrates were influenced by amphibians and reptiles.

Table 1. Number of records of vertebrate roadkills (N) recorded along 100 km of the BR-163 highway (Pará, Brazil) between July 2019 and June 2020. Taxonomy follows the Brazilian Committee of Ornithological Records (CBRO) for birds (Piacentini et al. 2015), the species list of the Brazilian Society of Herpetology (Costa and Bérnils 2018) for reptiles, and the most recently updated and commented survey of mammals occurring in Brazil (Quintela *et al.* 2020). None of the identified species is listed as endangered by the International Union for Conservation of Nature (IUCN). TA = roadkill rate (ind km^{-1} day^{-1}).

Taxon	Common name	N	TA
AMPHIBIANS			
Anura	frog/treefrog	164	0.0656
Total		**164**	**0.0656**
REPTILES			
Testudines			
Testudinidae			
*Chelonoidis carbonarius (*Spix, 1824*)*	red-footed tortoise	3	0.0012
Squamata			
Amphisbaenidae			
Amphisbaena fuliginosa Linnaeus, 1758	blind snake/two headed snake	6	0.0024
Boidae			
Boa constrictor Linnaeus, 1758	boa constrictor	17	0.0068
Epicrates cenchria (Linnaeus, 1758)	rainbow boa	9	0.0036
Colubridae			
Chironius carinatus (Linnaeus, 1758)	vine snake	1	0.0004
Drymarchon corais (Boie, 1827)	indigo snake	1	0.0004
Phrynonax polylepis (Peters, 1867)	northeastern puffing snake	1	0.0004
Spilotes *pullatus* (Linnaeus, 1758)	tropical chicken snake	1	0.0004
Dipsadidae			
Dipsas indica Laurenti, 1768	neotropical snail- eater	1	0.0004
Erythrolamprus aesculapii (Linnaeus, 1758)	South American false coralsnake	1	0.0004
Erythrolamprus reginae (Wagler, 1824 *in* Spix, 1824)	royal ground sneke	1	0.0004
Imantodes cenchoa (Linnaeus, 1758)	blunthead tree snake	2	0.0008
Oxyrhopus petolarius (Reuss, 1834)	forest flamesnake	5	0.0020
Pseudoboa neuwiedii (Duméril, Bibron e Duméril, 1854)	Neuwied's false boa	1	0.0004
Pseudoboa sp.		2	0.0008
Viperidae			
Bothrops atrox (Linnaeus, 1758)	common lancehead	6	0.0024
Bothrops taeniatus Wagler, 1824 *in* Spix, 1824	speckled forest pit viper	1	0.0004
Lachesis muta (Linnaeus, 1766)	South American bushmaster	2	0.0008
Unidentified squamate		1	0.0012
Total		**62**	**0.0248**
BIRDS			
Tinamiformes			
Tinamidae			
Crypturellus soui (Herman, 1783)	little tinamou	2	0.0008
Podicipediformes			
Podicipedidae			
Tachybaptus dominicus (Linnaeus, 1766)	least grebe	1	0.0004
Cathartiformes			
Cathartidae			
Coragyps atratus (Bechstein, 1793)	black vulture	24	0.0096
Cathartes melambrotus Wetmore, 1964	greater yellow-headed vulture	1	0.0004
Gruiformes			
Rallidae			
Neocrex erythrops (Sclater, 1867)	paint-billed crake	1	0.0004
Columbiformes			
Columbidae			
Columbina talpacoti (Temmink, 1810)	ruddy ground-dove	3	0.0012
Patagioenas cayennensis (Bonnaterre, 1792)	pale-vented pigeon	1	0.0004
Opisthocomiformes			
Opisthocomidae			
Opisthocomus hoazin (Müller, 1776)	hoatzin	1	0.0004

Table 1. Continued.

Taxon	Common name	N	TA
Cuculiformes			
Cuculidae			
Coccyzus melacoryphus (Vieillot, 1817)	dark-billed cuckoo	1	0.0004
Crotophaga ani (Linnaeus, 1758)	smooth-billed ani	3	0.0012
Strigiformes			
Strigidae			
Megascops choliba (Vieillot, 1817)	tropical screech-owl	1	0.0004
Nyctibiiformes			
Nyctibiidae			
Nyctibius griseus (Gmelin, 1789)	common potoo	1	0.0004
Caprimulgiformes			
Caprimulgidae			
Hydropsalis sp		1	0.0004
Nyctidromus albicollis (Gmelin, 1789)	common pauraque	1	0.0004
Caprimulgidae	nightjar	1	0.0004
Falconiformes			
Falconidae			
Milvago chimachima (Vieillot, 1816)	yellow-headed caracara	2	0.0008
Passeriformes			
Icteridae			
Sturnella militaris (Linnaeus, 1758)	red-breasted meadowlark	1	0.0004
Thraupidae			
*Tachyphonus rufus (*Boddaert, 1783)	white-lined tanager	1	0.0004
Thraupis episcopus (Linnaeus, 1766)	blue-gray tanager	2	0.0008
Volatinia Jacarina (Linnaeus, 1766)	blue-black grassquit	1	0.0004
Tityridae			
Tityra semifasciata (Spix, 1825)	masked tityra	1	0.0004
Tyrannidae			
Myiophobus fasciatus (Statius Müller, 1776)	bran-colored flycatcher	1	0.0004
Pitangus sulphuratus (Linnaeus, 1766)	great kiskadee	1	0.0004
Unidentified Passeriformes		2	0.0008
Unidentified birds		4	0.0016
Total		**59**	**0.0236**
MAMMALS			
Didelphimorfia			
Didelphidae			
Didelphis marsupialis Linnaeus, 1758	common opossum	8	0.0032
Didelphis sp	opossum	1	0.0004
Cingulata			
Dasypodidae			
Dasypus (Dasypus) novemcinctus Linnaeus, 1758	nine-banded armadillo	3	0.0012
Cabassous unicinctus Linnaeus, 1758	southern naked-tailed armadillo	1	0.0004
Pilosa			
Myrmecophagidae			
Tamandua tetradactyla (Linnaeus, 1758)	collared anteater	20	0.0080
Primata			
Callitrichidae			
Mico argentatus (Linnaeus, 1771)	silvery marmoset	4	0.0016
Rodentia			
Echimyidae			
Proechimys sp.	Tome's spiny-rat	1	0.0004
Rodentia	rodent	2	0.0008
Chiroptera			
Phyllostomidae			
Artibeus (Artibeus) lituratus (Olfers, 1818)	great fruit-eating bat	1	0.0004
Carollia perspicillata (Linnaeus, 1758)	Seba's short-tailed bat	1	0.0004

Table 1. Continued.

Taxon	Common name	N	TA
Carnivora			
Canidea			
Cerdocyon thous (Linnaeus, 1766)	crab-eating fox	8	0.0032
Procyonidae			
Nasua nasua (Linnaeus, 1766)	South American coati	5	0.0020
Unidentified mammals		11	0.0044
Total taxa		**66**	**0.0264**
Total individuals		**351**	**0.1404**

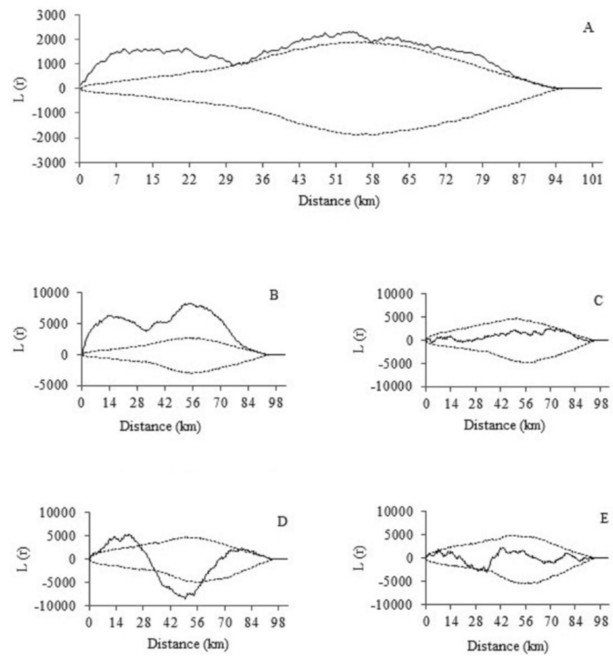

Figure 2. Clustering analysis of wildlife roadkills recorded along a 100-km section of BR-163 highway in Pará state, Brazil from July 2019 to June 2020. A – all vertebrates; B – amphibians; C – mammals; D – reptiles; E – birds. The solid line represents the distribution of observed roadkills and the dotted lines, the 95% confidence interval. Clustering occurs when the L(r) function (continuous line) is above the upper confidence limit. The parameters used were an initial radius of 100 m with a radius increment of 400 m in 1000 simulations.

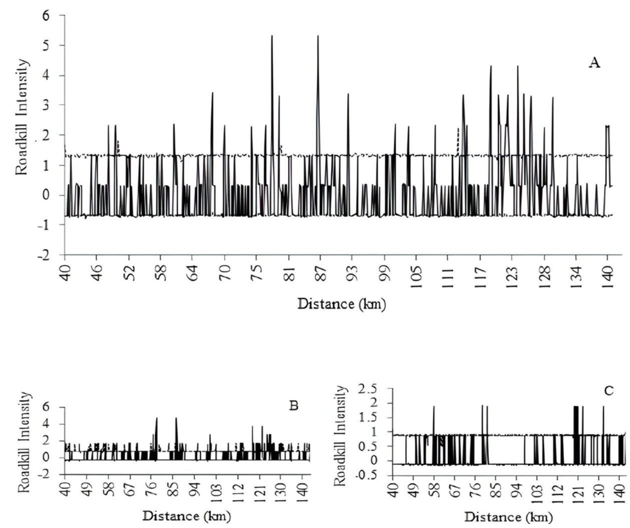

Figure 3. *Hotspots* of total vertebrate (A), amphibian (B) and reptile (C) roadkills along 100 km of the BR-163 highway in Pará, Brazil. The solid line represents the intensity of the roadkills, and the dotted lines, the 95% confidence interval. The points where the black line crosses the upper limit of the confidence interval indicate the occurrence of *hotspots*.

The overall number of vertebrate roadkills, as well as amphibian and reptile roadkills separately were significantly influenced by temperature and rainfall in the multiple regression models (Supplementary Material, Table S2), while bird and mammal roadkills were not influenced by temperature and precipitation.

Amphibian roadkills increased significantly with increased rainfall in the previous day and in the previous seven days (Supplementary Material, Table S2). Models 3 and 4 indicated an increase in roadkills with the increase in precipitation in the previous day combined with the decrease in the average temperature of the previous day and in the last seven days. Reptile roadkills increased significantly with the increase in precipitation in the previous seven days and the decrease in the average temperature in the previous day. Amphibian data influenced models for the overall data, as amphibians accounted for about 47% of all roadkills.

DISCUSSION

Our roadkill rate (0.14 ind km^{-1} day^{-1}) was similar to that obtained in other studies in the Amazon region (Pinheiro and Turci 2013) and in other regions in Brazil (Silva *et al.* 2013; Deffaci *et al.* 2016; Valadão *et al.* 2018). Studies close to protected areas do not always show a high roadkill rate, however, there are associated factors that can contribute to the increase of these rates, such as the type of road, spatial arrangement of landscape elements and preferential attractions that distinctly influence the home range of those species that may cross from one side to the other of the road (Chyn *et al.* 2020; Cerqueira *et al.* 2021). Better conserved areas tend to contribute to increased roadkill occurrence when vehicle traffic on adjacent roads is high, especially in protected areas (Garriga *et al.* 2012; Costa *et al.* 2022). However, the fact that roadkill studies use different methodologies frequently makes comparisons difficult (Dornas *et al.* 2012). It is very likely that both our roadkill rates and the observed number of roadkilled species were underestimated due to sampling insufficiency and the use of a vehicle instead of monitoring on foot (Costa *et al.* 2015; Santos *et al.* 2015).

Compared to our study, other surveys of wild vertebrate roadkills recorded a lower species richness with lower sampling effort (28 and 35 species, respectively, by Pracucci *et al*. 2012 and Deffaci *et al*. 2016), and higher richness with higher sampling effort (49 and 57 species, respectively, by Silva *et al*. 2013 and Carvalho *et al*. 2015), corroborating that recorded roadkill richness increases with monitoring effort to achieve sampling suffieciency (Bager and Rosa 2011). It is noteworthy in this context that Deffaci *et al*. (2016) and Carvalho *et al*. (2015) did not consider amphibians in their surveys. Other factors that sampling effort can affect the detection of roadkills, such as the speed of the vehicle used in the survey and the experience of the sampler (Collinson *et al*. 2014), so that comparisons among roadkill surveys should be done with caution. In addition, due to the degree of damage and/or decomposition of the carcasses, it is commonly not possible to identify 100% of the recorded roadkills to species level (only 191 of 351 individuals in our study). Considering the high and largely yet to be identified species richness in the Amazon, it is even possible that new species of vertebrates are found in roadkill monitorings in this region.

Our roadkill rate was mainly influenced by amphibians, since they represented almost half of the sample. Our results corroborate studies carried out in the Amazon biome and in the Atlantic Forest (Turci and Bernarde 2009; Castro *et al.* 2020; Batista *et al*. 2022). The latter authors worked on the BR-163 highway, but in a longer stretch than ours. We believe that the large number of amphibians recorded is related to the presence of water bodies in some places along the study stretch, and to reproductive behaviors associated with the period of greater rainfall (Ramos-Abrantes *et al*. 2018). We also emphasize that even though it is the most recorded vertebrate group in the present study, the rate of amphibian roadkills is certainly underestimated, considering that they are small animals easily removed by scavangers or even decomposed on the road within a short time afther being killed (Santos *et al*. 2011; Ratton *et al*. 2014).

Although mammal roadkills did not predominate in this study, unlike in several others (Braz and França 2016; Ramos-Abrantes *et al*. 2018; Valadão *et al*. 2018), most species we recorded are relatively common in studies of wildlife roadkill in Brazil (e.g. Pinheiro and Turci 2013; Oliveira *et al*. 2017; Santos *et al.* 2022). *Cerdocyon thous* and *D. marsupialis* are generalist omnivores apparently benefiting from anthropic landscapes and likely to find food, shelter and ease of movement along roads (Gardner 2008). *Tamandua tetradactyla* is arboreal, but moves on the ground, mainly at night in search of food, and has a defense strategy of standing up when threatened (Reis *et al*. 2010), which can have an obviously deleterious effect on individuals who encounter moving vehicles on the road.

Among the reptile roadkills recorded in our study, most were snakes. Reptiles are ectothermic and sometimes seek the heat of the asphalt to thermoregulate (Mccardle and Fontenot 2016). However, lizards are usually smaller (Feldman *et al*. 2015) and move faster than snakes, and may be less affected than snakes by roadkilling, or even go more unnoticed by the observer than snakes during sampling. Snakes have limitations on locomotion imposed by the asphalt layer due to strong friction with the asphalt, compared to natural terrain, and some snake species have immobility as a defensive tactic (Andrews and Gibbons 2005). Another fact to be considered is the popular notion that snakes are harmful animals, which likely makes them more prone to intentional roadkilling (Secco *et al*. 2014). All these factors may have contributed to the higher representation of snakes in our reptile sample.

The bird roadkills that we recorded may be linked mainly to food habits, since the presence of seeds (e.g. corn and soybeans that fall from trucks) on the road is a strong attraction for some species (Novelli *et al*. 1988; Prada 2004). The heat of the asphalt and the clearing of the road also attract numerous insects, which may help explain the occurrence of nocturnal aerial insectivores such as the Caprimulgidae and Nyctibiidae that we found. *Crotophaga ani* (Linnaeus, 1758), on the other hand, is commonly recorded in studies on wildlife roadkills, due to its abundance, low and slow flight, and its habit of hunting insects in shrubby vegetation (Ramos *et al*. 2011). The most recorded bird, *C. atratus*, feeds exclusively on carcasses and was probably attracted to the road by the presence of roadkilled animals (Laurance *et al*. 2009). This is probably also the case for *Milvago chimachima* (Vieillot, 1816), which opportunistically feeds on carcasses (Silva *et al*. 2013). Another factor that can interfere with the mortality of birds on roads is the relatively low body mass of animals in this class of vertebrates, which leaves some species more vulnerable to air displacement caused by high-speed vehicles, without necessarily occurring a collision between bird and vehicle (Prada 2004).

It is relevant that the spatial distribution of amphibian and reptile roadkills did not occur randomly, with the detection of several *hotspots* along the sampled stretch, including a common *hotspots* at km 122 of the road. In this section, the BR-163 is bordered by forest on both sides, indicating a circulation corridor for the local fauna. This result is similar to other studies which recorded a positive relation between amphibian and reptile roadkills and the presence of forests in the vicinity of roads (Glista *et al*. 2008). Although neglected in most studies on wildlife roadkills, mainly due to sampling methods, amphibians may be the group with the highest mortality due to vehicular collisions (Coelho *et al*. 2012), as pointed out in some studies (Glista *et al*. 2008; Garriga *et al*. 2012) and corroborated by our results.

The increase in records of amphibians and reptiles killed by roadkill in periods of greater rainfall may have occurred due to the physiological characteristics of these animals. Increased rainfall can result in microclimates with lower temperatures within the forest (Artaxo *et al*. 2005; Souza *et al*. 2012), causing reptiles, which are ectothermic, to seek out open areas such as roads to thermoregulate (McCardle and Fontenot, 2016), resulting in higher exposition to vehicle impacts. As for anuran amphibians, which are also ectothermic, the relationship between roadkill and rainfall is likely due to their reproductive activity, moving in large numbers to water bodies formed by the roads in the rainy season, and the posterior dispersal of young individuals (Aichinger 1987; Ramos-Abrantes *et al*. 2018). In the case of reptiles, and especially amphibians, there seem to be *hotspots* influenced by *hotmoments*, as the spatial clusters were positively related to precipitation (Coelho *et al*. 2012), occurring with greater intensity and frequency in certain months during the year.

CONCLUSIONS

Our results show a higher roadkill rate for amphibians, indicating a high mortality of these animals along the studied stretch. We identified roadkill *hotspots* for vertebrates in general, as well as for amphibians and reptiles in particular, which indicates that protective measures can be effective in minimizing the impacts of roadkills at these points of the surveyed stretch of the BR-163 highway. There are several models of structures that allow the safe crossing of fauna from one side of the road to the other (Smith *et al*. 2015), such as underpasses (tunnels) and overpasses (wooded viaducts or canopy bridges). Thus, we suggest the installation of passage structures and protection fences with screens adapted to the focal faunal groups impacted in the *hotspots*. Amphibian roadkills were more intense in the wettest months and were also associated, like reptiles, with climatic variations in temperature and precipitation. In view of this, actions such as the installation of fauna crossing structures will prove to be more effective for amphibians and reptiles in the rainy season. Finally, it is important that roadkill monitoring on the BR-163 highway is continued, to assess the long-term dynamics of identified *hotspots*, especially for amphibians, and to monitor the effectiveness of mitigation measures that can be implemented.

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SUPPLEMENTARY MATERIAL (only available in the electronic version)

Rocha *et al*. Hotspots and hotmoments of wildlife roadkill along a main highway in a high biodiversity area in Brazilian Amazonia

Table S1. Climatic data used in the multiple linear regression analyses of the effect of temperature and rainfall on wildlife roadkills sampled on 25 days along 100 km of the BR-163 highway from July 2019 to June 2020. Source: National Institute of Meteorology - INMET (2019/2020).

Month/Year	Sample	Precipitation previous day (mm)	Accumulated precipitation previous 7 days (mm)	Average temperature previous day (°C)	Average temperature previous 7 days (°C)
Jul/2019	1	0.00	14.70	26.90	28.00
Jul/2019	2	0.00	7.50	27.43	27.89
Aug/2019	3	3.50	37.50	27.06	27.28
Aug/2019	4	0.00	15.90	28.67	27.86
Sep/2019	5	0.00	0.00	28.77	27.47
Sep/2019	6	0.00	1.00	29.30	29.17
Oct/2019	7	0.00	27.90	28.93	27.99
Oct/2019	8	5.60	5.60	28.03	28.55
Oct/2019	9	0.00	0.00	28.27	27.05
Nov/2019	10	0.00	7.60	29.13	28.23
Nov/2019	11	0.00	1.60	28.03	27.64
Dec/2019	12	0.00	39.10	28.23	27.83
Dec/2019	13	0.00	8.00	27.63	27.91
Jan/2020	14	0.00	108.80	27.30	26.32
Jan/2020	15	0.00	12.20	26.53	27.07
Feb/2020	16	31.70	72.30	26.90	26.96
Feb/2020	17	10.30	69.40	27.10	26.72
Mar/2020	18	0.00	43.60	26.13	26.38
Mar/2020	19	0.00	107.40	26.97	26.96
Apr/2020	20	1.30	43.60	25.93	27.58
Apr/2020	21	8.40	142.80	24.97	26.13
Mai/2020	22	13.30	60.10	27.47	26.78
Mai/2020	23	0.00	73.50	26.73	26.42
Jun/2020	24	0.00	39.20	28.10	27.10
Jun/2020	25	7.80	29.40	27.20	27.68

Table S2. Multiple linear regression models for predicting the degree of dependence of vertebrate roadkill occurrence along 100 km of the BR-163 highway on 25 days between July 2019 and June 2020 on temperature and precipitation on the days prior to sampling. Models were constructed for the overall data (N-total) and for the data separated by class. The climatic variables considered in the models were (1) precipitation on the day before sampling; (2) accumulated precipitation from the seven days before sampling; (3) average temperature from the day before sampling; and (4) accumulated average temperature from the seven days prior to sampling. R2 = adjusted multiple correlation coefficient. Significance level (P-value) at 5% (α = 0.05). P-values in bold indicate statistical significance.

Variable	Coefficient	Standard error	Statistics *t*	P-value	R^2
N-total (model 1)					
Precipitation from the day before (mm)	0.40907	0.22638	1.807	0.08446	0.3917
Accumulated precipitation (mm)	0.11981	0.04125	2.905	**0.00822**	
N-total (model 2)					
Precipitation from the day before (mm)	0.4513	0.2140	2.109	**0.04654**	0.4274
Temperature from the day before (ºC)	-4.5989	1.4307	-3.214	0.00399	
N-total (model 3)					
Precipitation from the day before (mm)	0.4955	0.2272	2.181	**0.0402**	0.3462
Accumulated temperature (ºC)	-5.4225	2.1572	-2.514	**0.0198**	
N-total (model 4)					
Temperature from the day before (ºC)	-4.186	2.001	-2.092	**0.04820**	0.3368
Accumulated temperature (ºC)	-2.600	2.841	-0.915	0.36999	
Amphibian (model 1)					
Precipitation from the day before (mm)	0.44714	0.21990	2.033	**0.0548**	0.3438
Accumulated precipitation (mm)	0.06359	0.05744	1.107	0.2808	
Accumulated temperature (ºC)	-2.26010	2.89751	-0.780	0.4441	
Amphibian (model 2)					
Precipitation from the day before (mm)	0.44512	0.21792	2.043	**0.0533**	0.3555
Accumulated precipitation (mm)	0.09569	0.03970	2.410	**0.0248**	
Amphibian (model 3)					
Precipitation from the day before (mm)	0.4983	0.2159	2.308	0.0308	0.3334
Temperature from the day before (ºC)	-3.1911	1.4436	-2.211	**0.0378**	
Amphibian (model 4)					
Precipitation from the day before (mm)	0.5082	0.2140	2.375	**0.0267**	0.3371
Accumulated temperature (ºC)	-4.5587	2.0313	- -2.244	**0.0352**	
Reptile (model 1)					
Precipitation from the day before (mm)	0.05595	0.05816	0.962	0.3465	0.211
Accumulated precipitation (mm)	0.02366	0.01060	2.233	**0.0361**	
Reptile (model 2)					
Precipitation from the day before (mm)	0.06285	0.05506	1.141	0.2660	0.2547
Temperature from the day before (ºC)	-0.94370	0.36820	-2.563	**0.0177**	