Original Article

Vertebrates' roadkill in the southern region of the Atlantic Forest, Paraná coast – Brazil

Padrões de atropelamentos de vertebrados na região sul da Mata Atlântica, litoral do Paraná – Brasil

I. C. R. Cavallet^{a,b*} , L. M. Diele-Viegas^c , P. B. Mariotto^b and R. R. Lange^a

^aUniversidade Federal do Paraná, Programa de Pós-graduação em Ciências Veterinárias, Curitiba, PR, Brasil ^bInstituto Federal do Paraná, Eixo Tecnológico de Meio Ambiente e Saúde, Paranaguá, PR, Brasil ^cUniversidade Federal da Bahia, Instituto de Biologia, Laboratório de Biodiversidade no Antropoceno, Salvador, BA, Brasil

Abstract

Being a significant global biodiversity hotspot, the Atlantic Forest has been drastically reduced by human activities. Among the anthropic activities that most affect the biodiversity of this biome is the construction and operation of roads and highways. Between harmful effects of these infrastructures, wildlife roadkill is currently considered one of the biggest causes of mortality of wild vertebrates. This study evaluated patterns of vertebrates' roadkill on two roads in the coastal region of the largest continuous remnant of the Brazilian Atlantic Forest. For twelve months, we carry out weekly samples with a motor vehicle at a constant speed of 40 km/h to search for carcasses along the roads. All carcasses found were georeferenced and identified to the lowest possible taxonomic level. Then, using Siriema v.2.0 software, we analyzed roadkill aggregation and the spatial distribution of hotspots of wildlife roadkill. In 43 sampling days, 209 road-killed animals were registered (average roadkill rate of 0.105 and 0.111 animals/kilometer/day for PR-407 e PR-508, respectively). Extrapolating the rates found, we estimate that about 1,773 animals can be roadkill every year on these roads. The most affected groups were birds (33.01%) and amphibians (30.62%), followed by reptiles (19.13%) and mammals (17.33%). Warmer months had the highest roadkill rates. We found two critical roadkill hotspots for the PR-407 (kilometer 11.7 to 12.5 and kilometer 14.7 to 16.7). For the PR-508, we found a critical point of 5.2 km (kilometer 5 to 10.2). As a short-term measure, we recommend installing speed reducers in the identified stretches and implementing environmental education campaigns with residents and tourists, especially during the summer months, aiming to mitigate the roadkill on both roads. However, due to the importance and environmental fragility of the area, we emphasize the need for running periodic road ecology and local wildlife population viability studies in the medium and long term.

Keywords: road ecology, wildlife-vehicle collision hotspots, roadkill rates, Paraná coast.

Resumo

Sendo um importante hotspot de biodiversidade global, a Mata Atlântica tem sido drasticamente reduzida pelas atividades humanas. Entre as atividades antrópicas que mais afetam a biodiversidade desse ecossistema está a construção e operação de estradas e rodovias. Considerado um dos efeitos mais nocivos desses empreendimentos, o atropelamento de animais silvestres é atualmente uma das maiores causas de mortalidade de vertebrados silvestres. Neste estudo, avaliamos os padrões de atropelamentos de pequenos vertebrados em duas estradas na região litorânea do maior remanescente contínuo de Mata Atlântica brasileira. Durante doze meses realizamos coletas semanais com um veículo motorizado a uma velocidade constante de 40 km/h para busca de carcaças ao longo das estradas. Todas as carcaças encontradas foram georreferenciadas e identificadas até o menor nível taxonômico possível. Em seguida, usando o software Siriema v.2.0, analisamos a distribuição e os padrões espaciais dos eventos para identificar pontos de atropelamento de animais selvagens. Em um total de 43 dias de amostragem, foram registrados 209 animais atropelados (taxa média de atropelamentos de 0,105 e 0,111 animais/guilômetro/ dia para PR-407 e PR-508 respectivamente). Extrapolando os índices encontrados, podemos estimar que cerca de 1.773 animais podem ser atropelados todos os anos nessas estradas. Os grupos mais afetados foram aves (33,01%) e anfíbios (30,62%), seguidos de répteis (19,13%) e mamíferos (17,33%). Os meses mais quentes tiveram as maiores taxas de atropelamentos. Durante o período encontramos dois pontos críticos de atropelamentos para a rodovia PR-407 (quilômetro 11,7 a 12,5 e quilômetro 14,7 a 16,7). Para a rodovia PR-508, encontramos um ponto crítico de 5,2 km (quilômetro 5 a 10,2). Como medida de curto prazo, recomendamos a instalação de redutores de velocidade nos trechos identificados e a implementação de campanhas de educação ambiental com moradores e turistas, principalmente nos meses de verão, visando mitigar o atropelamento em ambas as vias. No entanto,

*e-mail: izabel.cavallet@ifpr.edu.br Received: April 28, 2022 – Accepted: February 23, 2023

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devido à importância e fragilidade ambiental da área, ressaltamos a necessidade de estudos de ecologia viária e de viabilidade populacional da fauna local a médio e longo prazo.

Palavras-chave: ecologia de estradas, hotspots de atropelamento de fauna, taxas de atropelamento, litoral do Paraná.

1. Introduction

The Atlantic Forest, considered one of the top five global biodiversity hotspots, is the second-largest rainforest in Latin America, covering much of the Brazilian Coast (Ribeiro et al., 2011; Marques et al., 2021). It is mainly formed by 245,173 small fragments of secondary forests, most (83.4%) with less than fifty hectares (Ribeiro et al., 2011). The largest of these fragments is in Serra do Mar Mountain range and covers the states of São Paulo, Paraná, and Santa Catarina. With over one million hectares, this fragment still conserves about 36.5% of its original cover and is vital for maintaining the Atlantic Forest's southern portion. Such importance led the area to be recognized as a Biosphere Reserve of the Atlantic Forest by UNESCO in 1994 (Marques et al., 2021).

Despite this importance, agriculture, urban development, and the expansion of linear infrastructures such as roads and highways have been a significant threat to the Atlantic Forest (Ribeiro et al., 2011). The implementation and operation of roads and highways have complex and varied impacts on biodiversity and the ecosystem (Forman et al., 2003; Coffin, 2007; Bager, 2018). Among these impacts, the following stand out: loss of habitat and environmental quality (noise and light disturbances, pollution, and microclimate changes), barrier effect and interruption of migration and dispersion, and direct mortality from vehicle collisions (Forman et al., 2003; Coffin, 2007). The last is categorized as some of the biggest causes of vertebrate mortality globally (Forman et al., 2003; Dornas et al., 2012; Hill et al., 2019; Schwartz et al., 2020). In addition to the direct impact on the individual, vehicle collisions can cause a faster population decline than other indirect impacts of humans on biodiversity, such as habitat fragmentation (Forman et al., 2003; Moore et al., 2021). Roadkill incidence can often exceed the natural mortality rate from the species, affecting their population density and sexual structure, causing an imbalance in ecosystems and their environmental services (Coelho et al., 2008; Santos and Ascensão, 2019).

Defining measures to reduce wildlife roadkill is challenging since each area has specific characteristics and different collision patterns (Forman et al., 2003; Coffin, 2007; Bueno et al., 2015; Schwartz et al., 2020). Even though there is a legal obligation for highway concession holders to submit reports on the animal-vehicle collisions both at the federal level (Normative Instruction 13/2013 IBAMA) and the state level (Resolution 98/2016 CEMA-PR and State Law 19939/2019), the problems of sampling and underreporting official data are reported by literature (Dornas et al., 2012; Balčiauskas et al., 2020).

Identifying and analyzing patterns of wildlife roadkill in each region is essential to support mitigating proposals, such as underpasses, overpasses, fences, and speed reducers. Even without demographic knowledge of local populations, understanding which species are affected, how many animals die, and where they are dying on the road can be the first step in predicting the potential effects of vehicle collisions on wildlife populations (Forman et al., 2003; Dornas et al., 2012). Monitoring and intensive data collection can also provide valuable and relevant information regarding the species' behavior, population trends, life habits, and even biogeography (Dornas et al., 2012; Schwartz et al., 2020).

Given the growing number of proposals for installations and expansion of road structures on the Paraná coast and their potential impacts on fauna, mitigation measures must be taken and improved based on studies considering the aspects of the animal-vehicle collisions where such roads will be implemented and expanded (Coelho et al., 2008). Thus, this study aimed to analyze aspects of vertebrate roadkill, including main species affected and possible aggregations, in a one-year time span on two state roads located in the southern region of the Atlantic Forest Biosphere Reserve.

2. Materials and Methods

2.1. Study area

The Paraná coast comprises seven municipalities located at Atlantic Forest, in Serra do Mar Mountain range region. As it shelters a large part of the Atlantic Forest remnants, the Paraná coast has about 80% of its territory covered by environmental protected areas (Paula et al., 2018). Our study focused on two state roads between the Serra do Mar and the municipalities of Matinhos and Pontal do Paraná (PR-407 and PR-508), which are the primary access to the Paraná coastal region in southern Brazil. The roads are located at sea level, and local vegetation is characterized as an Ombrophilous Dense Forest. Despite having distinct structural characteristics, the roads are close and parallel to each other, presenting a similar flow of vehicles, which increases during the summer season because of beach tourism.

The 'Engenheiro Argus Thá Heyn' road, PR-407, covers the municipalities of Paranaguá and Pontal do Paraná and is 18.85 km long (beginning at -25°33'38.65″ -48°34'55.73″ and ending at -25°41'50.17″ -48° 28'33.00″), consisting of two lanes with shoulders on both sides. The maximum speed allowed is 90 km/h. Its topography is flat, with urbanized areas at the beginning and end of Paranaguá and Praia de Leste's beach resort in Pontal do Paraná. Its layout is around two fully environmental protected areas: the Palmito State Park (1,788.9ha) and the Guaraguaçu Ecological Station (1,188.8ha).

The 'Elísio Pereira Alves Filho' road, PR-508, covers the municipalities of Paranaguá and Matinhos and is 31.21km long (beginning at -25°34'5.52" -48°36'54.91" and ending at -25°49'0.75" -48°32'17.87"), presenting four lanes with no center bed or shoulders. The maximum speed allowed

is 100 km/h. It borders the sustainable use protected area called Guaratuba Environmental Protection Area (total area: 199,447ha) and the Saint-Hilaire/Lange National Park, a fully protected conservation unit (total area: 25,126.5ha).

2.2. Data collection

Sampling efforts were carried out weekly between April 2016 and March 2017, starting at dawn as soon as the light allowed the carcasses to be seen on the track. The team consisted of a driver and two observers, with no variation in the observers and their functions in the sampling period to reduce sources of variation in observers' capacity. The carcasses found on the carriageway and shoulder were georeferenced with GPS, identified at the lowest possible taxonomic level, and photographed together with their surroundings. Then, the carcasses were removed to the vegetation region beyond the shoulder to avoid recounting. Monitoring was made by car at a constant speed of 40 km/h. We monitored 15 km of PR-407 road (towards beaches/BR-277) and 29.5 km of PR-508 road (towards BR-277/beaches). Kilometers one to three of PR-407 and the final kilometer of PR-508 were excluded from our analysis since they are inserted in the urban area of Paranaguá and Matinhos, respectively. Besides, this strech of the PR-407 features a Jersey-type structure (concrete safety barrier dividing the lanes), which sets it apart from the rest of the road.

2.3. Data analysis

Observed roadkill rates were obtained for each of the evaluated roads by calculating the total number of carcasses divided by the number of kilometers travelled and by days of effort. These rates were used to compare roads with different mileage or between months with varying sampling efforts.

We used the Siriema v.2.0 software (Coelho et al., 2014) to identify roadkill spatial aggregates (hotspots) on each road. Firstly, the modified Ripley K-2D statistical test was used, which maintains the road tracing's twodimensionality to assess the non-randomness of the spatial distribution of events along different scales (Coelho et al., 2008). We identified potential significant groupings in animal roadkill and on what scale they occur using an initial radius of 300 meters with a radius increase of 500 meters and 1000 simulations (95% confidence interval). Then, we implemented the 2D HotSpot Identification analysis to identify the main roadkill stretches (hotspots). We based the variation between the radius for calculating hotspots on each road on previous Ripley K analyses showing the smallest radius at which the roadkill aggregations were significant for each road [for details of the methodology and analysis Siriema v2.0 software, see Coelho et al. (2014)]. This procedure results in a roadkill aggregation intensity value for each road segment (100 meters). In this sense, we calculated hotspots with a 95% confidence interval, considering 1000 repetitions and a radius of 13.8 km for the PR-407 and 2 km for the PR-508. We considered that values for aggregation intensity above the upper confidence level of 95% indicate a significant roadkill hotspot.

3. Results

During the 12 months of road monitoring, we have found 209 roadkilled animals, 68 of which were on PR-407 road (average observed roadkill rate of 0.105 animals/ kilometer/day) and 141 on the PR-508 road (average observed roadkill rate of 0.111 animals/kilometer/day). With these calculated rates, we can estimate a minimum number of 1773 animal-vehicle collisions on the roads studied per year, with about 577 animals on PR-407 and 1196 on PR-508. The observed roadkill rates varied on both roads throughout the year, with the highest rates found in warmer months.

In this work, vertebrates of four classes, 15 orders, 25 families, 33 genera, and 35 species were identified as being affected by roadkill on the studied roads (Table 1). Of the 209 animals found, most were birds (33.01%) and amphibians (30.62%), followed by reptiles (19.13%) and mammals (17.22%). However, this composition varied over the months of the year, as shown in Figure 1. In addition to the variation in the groups of vertebrates most affected, we also observed variation when comparing the two roads (Table 2).

We identified significant aggregations on a 13.8 km scale in the roadkill distribution at PR-407. From this radius, we found two significant hotspots (95% confidence) along the 15 km analyzed: the stretch between kilometers 11.7 and 12.5 (800 meters) and the stretch between kilometers 14.7 to 15.7 (1000 meters). For PR-508, we identified significant aggregations on a 2 km scale in the distribution of roadkill. We found a single significant hotspot (95% confidence) from kilometer 5 to 10.2, involving 5.2 km of the 29.5 km analyzed. The stretches with the vertebrate's

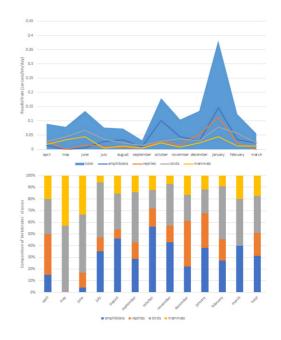


Figure 1. Variation of roadkill rates (carcass/km/day) of different vertebrates' classes through time (A) and monthly composition of vertebrates classes (B).

| Class | Order | Family | Genera | Specie | PR-408 | PR-407 | Total |
|----------|--------------------|--|--------------------------------------|---|--------|--------|-------|
| Amphibia | Anura | No family identi | 27 | 7 | 34 | | |
| | | Bufonidae No genera identification | | | | - | 1 |
| | | | Rhinella | No specie identification | 4 | 1 | 5 |
| | | Hylidae | No genera identifica | ation | 2 | - | 2 |
| | | | Boana | No specie identification | 2 | - | 2 |
| | | | | B. faber (Wied-Neuwied, 1821) | 2 | - | 2 |
| | | | Trachycephalus | T. mesophaeus (Hensel, 1867) | 1 | - | 1 |
| | | Leptodactylidae | Leptodactylus | No specie identification | 6 | 1 | 7 |
| | | | | L. latrans (Steffen, 1815) | 7 | 3 | 10 |
| Reptiles | Squamata | No family identi | fication | | 2 | 4 | 6 |
| | | Colubridae | Chironius | C. fuscus (Linnaeus, 1758) | - | 2 | 2 |
| | | | | C. laevicollis (Wied-Neuwied, 1824) | - | 1 | 1 |
| | | | Spilotes | S. pullatus (Linnaeus, 1758) | 3 | - | 3 |
| | | Dipsadidae No genera identification | | | | 1 | 1 |
| | | | Erythrolamprus | E. asculapii (Linnaeus, 1758) | - | 2 | 2 |
| | | | | E. miliaris (Linnaeus, 1758) | 5 | 7 | 12 |
| | | | Helicops | <i>H. carinicaudus</i> (Wied- Nieuwied, 1825) | 1 | - | 1 |
| | | | Oxyrhopus | <i>O. clathratus</i> (Duméril, Bibron & Duméril, 1854 <i>)</i> | 4 | - | 4 |
| | | Teiidae | Salvator | <i>S. merianae</i> (Duméril & Bibron, 1839) | 3 | 2 | 5 |
| | | Viperidae | Botrops | <i>B. jararaca</i> (Wied-Neuwied, 1824) | 2 | - | 2 |
| | | | | B. jararacussu (Lacerda, 1884) | 1 | - | 1 |
| Birds | No order identific | ation | | | 17 | 5 | 22 |
| | Accipitriformes | Accipitridae | Rupornis | R. magnirostris (Gmelin, 1788) | - | 1 | 1 |
| | Apodiformes | Trochilidae | Ramphodon | R. naevius (Dumont, 1818) | 2 | - | 2 |
| | Caprimulgiformes | Caprimulgidae | No genera identifica | ation | - | 1 | 1 |
| | | | Nyctidromus | N. albicollis (Gmelin, 1789) | 1 | - | 1 |
| | Charadriiformes | Charadriidae | Vanellus | V. chilensis (Molina, 1782) | - | 2 | 2 |
| | Gruiformes | Rallidae | Aramides | A. saracura (Spix, 1825) | 1 | 3 | 4 |
| | | | Gallinula | G. galeata (Lichtenstein, 1818) | - | 1 | 1 |
| | Strigiformes | Strigidae | Megascops | M. choliba (Vieillot, 1817) | 6 | 3 | 9 |
| | Passeriformes | Passeriformes No family identification | | | 9 | 5 | 14 |
| | | Thraupidae | Tachyphonus | T. coronatus (Vieillot, 1822) | 1 | - | 1 |
| | | | Tangara | T. cyanocephala (Müller, 1776) | - | 1 | 1 |
| | | | | T. seledon (Müller, 1776) | - | 1 | 1 |
| | | | Sicalis | S. flaveola (Linnaeus, 1766) | 1 | 1 | 2 |
| | | Turdidae | Turdus | T. albicollis (Vieillot, 1818) | 1 | - | 1 |
| | | | | <i>T. amaurochalinus</i> (Cabanis, 1850) | 1 | - | 1 |
| | | Tyrannidae | Pitangus | P. sulphuratus (Linnaeus, 1766) | - | 1 | 1 |
| | | | Tyrannus | T. melancholicus (Vieillot, 1819) | 1 | - | 1 |
| | | Tytiridae | No genera identification | | 1 | - | 1 |
| | | - | Pachyrampus No specie identification | | | 1 | 1 |
| | Trogoniformes | Trogonidae | Trogon T. rufus (Gmelin, 1788) | | - | 1 | 1 |

Table 1. List of species roadkilled from April 2016 to March 2017 on the PR-407 and PR-508 roads.

| Class | Order | Family | Genera | Specie | PR-408 | PR-407 | Total |
|---------|-------------------------|------------------|-------------------------------------|--------------------------------|--------|--------|-------|
| Mammals | No order identification | | | | | 4 | 5 |
| | Carnivora | Canidae | Cerdocyon C. thous (Linnaeus, 1766) | | 1 | 1 | 2 |
| | | Mustelidae | Galictis | <i>G. cuja</i> (Molina, 1782) | 1 | - | 1 |
| | Chiroptera | No family identi | ification | | | - | 1 |
| | | Molossidae | sidae No genera identification | | | | 3 |
| | | Phyllostomidae | No genera identifica | 2 | - | 2 | |
| | Cingulata | Dasypodidae | Dasypus | No specie identification | 2 | - | 2 |
| | Didelphimorphia | Didelphidae | No genera identification | | | - | 1 |
| | | | Didelphis | No specie identification | 1 | - | 1 |
| | | | | D. aurita (Wied-Neuwied, 1826) | 9 | 2 | 11 |
| | | | Marmosa | M.paragaryanus (Tate, 1931) | 1 | - | 1 |
| | | | Philander | P. frenatus (Olfers, 1818) | 1 | - | 1 |
| | Rodentia | No family identi | fication | | | 2 | 4 |
| | | Erethizontidae | Sphiggurus | S. villosus (F. Cuvier, 1823) | 1 | - | 1 |

Table 1. Continued...

Table 2. Variation in the roadkill rates (carcass/km/day) between classes of vertebrates on the PR-407 and PR 508 highways.

| Classes | Total | % | Roadkill rate | PR-407 | % | Roadkill rate | PR-508 | % | Roadkill rate |
|------------|-------|-------|------------------|--------|-------|------------------|--------|-------|------------------|
| Birds | 69 | 33.01 | 0.0360 | 27 | 39.70 | 0.0428 | 42 | 29.79 | 0.0338 |
| Amphibians | 64 | 30.62 | 0.0334 | 12 | 17.65 | 0.0190 | 52 | 36.88 | 0.0419 |
| Reptiles | 40 | 19.13 | 0.0209 | 19 | 27.95 | 0.0301 | 21 | 14.89 | 0.0169 |
| Mammals | 36 | 17.22 | 0.0188 | 10 | 14.70 | 0.0158 | 26 | 18.44 | 0.0209 |
| TOTAL | 209 | 100 | 0.1092 | 68 | 100 | 0.1079 | 141 | 100 | 0.1138 |

roadkill hotspots on both roads and their positions within the buffer zone of existing environmental protected areas in the region are shown in Figure 2.

4. Discussion

In the present study, the observed roadkill rates for the PR-508 road were higher than PR-407. The difference in the total roadkilled animals can be related to differences in the roads' speed and structural limits (number of lanes and the presence of shoulders). Two-lane roads with shoulders, such as the PR-407 road, are likely to present shorter carcass persistence time than on four-lane roads without a shoulder. Santos and Ascensão (2019) suggest that this can occur because narrow roads with shoulders tend to have less vehicle flow and speed, encouraging scavengers to seek food on the road. This may partly explain the lower observed roadkill rates on PR-407.

Although we acknowledge that factors beyond the number of carcasses, road mileage and days of effort can influence the persistence of carcasses and the efficiency of observers, potentially leading to biased roadkill rates (Franceschi et al., 2021), comparing the observed roadkill rates of two different roads can be informative to understand the roadkill dynamic in these roads, becoming a starting point for future studies to delve into analyzes that cover other characteristics not yet studied.

There was no variation in the team over the 43 field efforts in this study to reduce a possible variation in the observer's efficiency that could influence in their roadkill observations. Although this work could not consider the carcass persistence rate due to financial and logistical limitations, it is likely that not all roadkilled animals end up dying on the roadside. Some could be rescued and treated, and most of them could die off the road in forest environments where they cannot be found (Forman et al., 2003; Cirino and Freitas, 2018). For a more appropriate understanding of these patterns, we strongly recommend that future studies focus on carcass persistence analysis for these locations.

We estimate that at least 1815 animals can be hit by vehicular collisions a year on these roads, most of which are small vertebrates. The roadkill of smaller animals is more challenging to detect and does not cause financial losses and human traumas or deaths, leading to underestimated official reports (Dornas et al., 2012; Balčiauskas et al., 2020). In this sense, scientific studies are often the largest data source for medium-sized vertebrates' roadkill and the only source for small vertebrates, which composes 90% of the country's roadkilled fauna (Bager, 2018). Thus, the regular collection of scientific data with appropriate

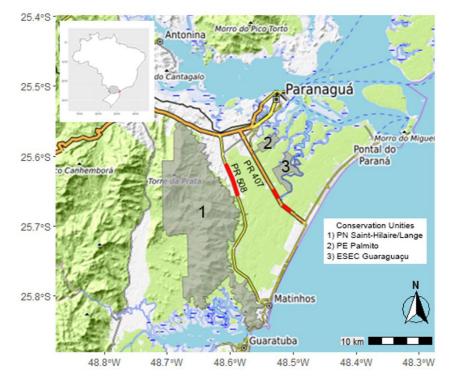


Figure 2. Hotspots of roadkill vertebrates on PR-508 and PR-407 roads and Conservation Units in their surroundings.

methodology is more valuable for identifying the road's negative impacts on local fauna and planning safety measures for both fauna and drivers.

Both roads showed similar patterns of observed roadkill rates along the year, with an increase in observed roadkill in the hottest season. Such increase is widely observed in the literature since the higher environmental temperature and rain incidence can increase animal activity (Hill et al., 2019; Schwartz et al., 2020). In addition, the studied roads are intensively used as main access roads to the beaches of Paraná in the summer season months, leading to an increase in road traffic and potentially increasing roadkill. Data such as these demonstrate that the temporary installation of speed reducers and the intensification of educational measures with residents and visitors during the summer season can be adopted as aids to reducing roadkill on the roads studied.

There was no variation in the team over the 43 field efforts in this study to reduce a possible variation in the observer's efficiency that could influence in their roadkill observations. Although this work could not consider the carcass persistence rate due to financial and logistical limitations, it is likely that not all roadkilled animals end up dying on the roadside. Some could be rescued and treated, and most of them could die off the road in forest environments where they cannot be found (Forman et al., 2003; Cirino and Freitas, 2018). For a more appropriate understanding of these patterns, we strongly recommend that future studies focus on carcass persistence analysis for these locations. In our study, amphibians were the most affected group in PR-508 and the third most affected group in PR-407. Amphibians are the most frequent roadkilled animals due to their activity patterns, population structure, and habitat preference (Glista et al., 2007). We found the highest observed roadkill rates in the hottest months for amphibians, with peaks in October and January, coinciding with the dispersion periods linked to these animals' reproduction (Silva et al., 2007).

Although no endangered species have been identified, it is essential to note that South America is the continent with the highest density and number of amphibian species, housing about a third of endangered species. The Atlantic Forest region located on the coast of Paraná is home to one of the richest Amphibian fauna in the state with many endemic species and a high potential for discovering new species (Leivas et al., 2018). Being roadkill on roads is one of the most important causes of the population decline of amphibians and one of the greatest threats to species (Coelho et al., 2012), and, considering the limits of observation of the methodology applied in this study, we suggest further studies with monitoring animal-vehicle collisions on foot to identify the impact in this group better.

About 87.5% of the reptiles found were snakes, which can be explained by the high diversity of this group in the region and their usual freezing behavior in vehicles' presence. The most significant factors that lead reptiles to roadkill are the use of asphalt for thermoregulation (rooming), the slowness and low reaction of animals to vehicles, and the intentional roadkill, especially in the case of snakes, the group most found in this study (Silva et al., 2007; Gonçalves et al., 2018).

Birds were the group with the largest variety of species identified [which was also seen by Coelho et al. (2008)] and the largest number of roadkilled individuals in both roads. Roads and their accessory structures, such as poles and signs, can provide access to food resources such as grains, prey, and carcasses by offering landing points that facilitate prey viewing and reduce foraging efforts (Ramos et al., 2011). Larger and carnivorous birds are especially susceptible roadkill than smaller, omnivorous birds (Hill et al., 2019), which is corroborated in our study, where the most observed species was *Megascops Choliba*, probably due to its eating habits since it may be using carcasses on the road as a food source.

The observed roadkill peaks for birds occurred in May-June and December-January. This result was also found by Rosa and Bager (2012), who obtained rates in summer and autumn that were twice as in spring and winter. The authors linked these events to the dispersion of juveniles (summer) and increased vehicle flow (summer and autumn).

The mammalian roadkill rate was 0.018 roadkill animals per kilometer per day, which is similar to the rate previously found for the same roads (0.017; Leite et al., 2012). The group presented the lowest and most constant observed roadkill rates throughout the study, which can be partially justified by removing the carcasses by the road concessionaire, as they have a larger size and can cause new accidents. Although no endangered mammals were found in this study, a roadkill of Puma concolor (Linnaeus, 1771) at the PR-407 road was observed in personal communication and news media. We must also consider that the absence of evidence of species on roads is not evidence of the regions' lack of species (Schwartz et al., 2020). Animals may not be seen on the roads for several factors, and we suggest studies with different methodologies to analyze this variation when compared with current studies. We recommend expanding and deepening studies about the size of the population and viability of the species found in the region providing better information about the potential impact of roads on certain species/populations than just the roadkill data alone (Schwartz et al., 2020).

The identified hotspots on both roads indicate the existence of environmental or human factors that affect the spatial distribution of these events. At PR-508, a hotspot was found, about 5km long, covering kilometers 5 to 10.2. In addition to coinciding with the largest urban agglomeration along the road, this area also has a straight path, which results in a higher vehicle speed. Also, during the monitoring period, it can be seen that this region had small bodies of water along the road. Variables related to the presence or distance of water bodies are recognized as important determinants of the spatial distribution of the running over of reptiles and amphibians (Gonçalves et al., 2018). These groups together totaled almost half of the roadkill during the study period. This road stretch is ideal for installing mitigation measures, mainly focused on the herpetofauna. As short-term measures, we indicate the intensification of environmental education campaigns for summer tourists and residents of the region and

speed controllers and increased Road Police activity in the kilometers identified as hotspots, especially during the summer months.

Although the radius found by the PR-407 highway hotspot analysis is too large for us to consider using the analysis to propose mitigation measures, the roadkill clusters found indicate a stretch of the highway that should be a priority for future monitoring. Two hotspots of about 1 km each were found on the PR-407, the first between kilometers 11.7 and 12.5 and the second between kilometers 14.7 to 15.7. When observing the hotspots found over satellite images of PR-407, we can observe that they are in regions close to the Guaraguaçu River. The Guaraguaçu River basin interconnects two integral protection areas (Saint-Hilaire-Lange National Park and the Guaraguaçu Ecological Station) to Paranaguá Bay, being an important biodiversity corridor between the Atlantic Forest and the estuary (Elste et al., 2019). This connectivity is fundamental to the gene flow and health of the diverse aquatic animal and plant communities (Bueno et al., 2015). The existence of two roadkill hotspots in this river's vicinity is quite worrying and signals the region's environmental sensitivity.

This study intends to be a starting point and alert to occurrence and relevance of fauna mortality due to roadkill in a region of significant ecological importance. Given the data obtained and the predictions for road expansion, we recommend deepening ecological and population studies and constant monitoring of fauna roadkill.

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