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ROADKILLS OF MEDIUM AND LARGE-SIZED MAMMALS ON HIGHWAY BR-242, MIDWEST BRAZIL: A PROPOSAL OF NEW INDEXES FOR EVALUATING ANIMAL ROADKILL RATES

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Abstract: Habitat fragmentation due to transport infrastructure, such as highways, is receiving growing concern due to deleterious consequences of roadkill, a consequence of animal–vehicle collisions. This study was conducted on a 115 km section of the highway BR-242 between the Peixe Angical Hydroelectric Power Plant and Gurupi town, Tocantins state, Brazil. During twelve months, records of medium and large-sized mammals' roadkilled by vehicle collisions were weekly acquired. Photographic records, date and place of occurrence were obtained for each roadkilled animal. We also propose new indexes to evaluate roadkill rates. In total, 18 species, 17 genera, 10 families, and six orders were recorded. The species with the highest number of registers were: *Cerdocyon thous*, *Myrmecophaga tridactyla*, *Tamandua tetradactyla*, *Procyon cancrivorus*, *Nasua nasua* and *Dasyprocta leporina*, corresponding to 73.5% of roadkills. The curve of species accumulation showed no tendency for stabilization. The results showed that the roadkill indices were 0.003 species/km; 0.009 individuals/km; 0.375 species/monitoring campaign; 1,021 individuals/monitoring campaign; 306,667 kilometers traveled/hit species, and 115,00 kilometers traveled/animal. Seven species recorded are considered threatened: *Chrysocyon brachyurus*, *Leopardus pardalis*, *Leopardus wiedii*, *Lycalopex vetulus*, *Myrmecophaga tridactyla*, *Puma concolor*, and *Puma yagouaroundi*.

Keywords: Cerrado; Road Ecology; savanna; vehicle collision; wildlife.

INTRODUCTION

The Cerrado domain is represented by various physiognomic types of vegetation and occupies *ca.* 90% of the area of the state of Tocantins, Brazil. This biodiversity hotspot (Myers *et al.* 2000) has been progressively occupied by large enterprises, causing landscape modifications (Tabarelli *et al.* 2010). The impact of this occupation implies habitat losses, with the consequent reduction in species richness and abundance of medium and large-sized mammals (Grelle *et al.* 2006, Barthelmess & Brooks 2010, Torres *et al.* 2012).

The expansion of urban areas caused by economic development is generally associated with an expansion of the road network (Cunha *et*

al. 2010, Barni *et al.* 2012), which is generally assumed to negatively affect the wildlife. For example, road networks subdivide a natural landscape by removing portions of habitat and inhibiting the dispersion or attracting some species (Silva *et al.* 2007, Barthelmess 2014, Grilo *et al.* 2015).

The areas with the highest death rates of wild animals by animal-vehicle collisions have certain characteristics that favor the passage of these animals, such as proximity to water courses (Bueno *et al.* 2015, Freitas *et al.* 2015a), fragments of riparian forests (Ascensão *et al.* 2017), preserved gallery forests and ecological corridors (Cáceres *et al.* 2012, Barthelmess 2014, Niemi *et al.* 2014), being affected also by the seasonality (Machado *et al.* 2015). The identification of these areas is important to provide subsidies for the implementation of infrastructure aiming to prevent roadkill, such as wildlife underpass and fencing (standardized by van der Ree *et al.* 2015, as "wildlife crossings"), and to reduce the impacts on wild mammal populations (Cáceres *et al.* 2012, Machado *et al.* 2015, Rytwinski *et al.* 2016).

Brazilian highways usually do not include wildlife crossings, speed bumps, or efficient wildlife warning signs at critical sites in order to avoid accidents. This is the case of the Brazilian highway BR-242 that extends through the states of Bahia, Mato Grosso and Tocantins, Brazil, being considered the main route for tourism and outflow of agricultural productions (*e.g.*, soybean, maize and cotton). Furthermore, this highway presents a high traffic volume and is surrounded by multiple vegetation types with rich and abundant animal biodiversity (Santiago 2016).

Therefore, this study aimed (1) to describe the species richness and composition, (2) to analyze the effect of the vegetation type of the road edge on roadkill, and (3) to evaluate the effect of seasonality on the frequency of medium and large-sized mammals on highway BR-242, in the state of Tocantins, Brazil. Additionally, standardization formulas to calculate roadkill rates are proposed. Herein, we hypothesize that mammal species richness and composition are in accordance to inventories of the Cerrado domain and that number of roadkilled mammals will be higher in sites with higher presence and/or heterogeneity of the vegetation type of the road edge.

MATERIAL AND METHODS

Study area

This study was carried out in a 115 km perimeter of the highway BR-242, between the Peixe Angical Hydroelectric Power Plant and Gurupí town, located in state of Tocantins, Brazil (Figure 1). The two-way paved road is surrounded by fragments of native vegetation, constituted by Cerrado *stricto sensu*, riparian forest and gallery forest, which are inserted in a matrix predominantly occupied by pastures (Figure 1). The Cerrado *stricto sensu* is a vegetation type of the Brazilian savannah (Cerrado biome), which presents xeromorphic aspect consisting of a subtype of Cerrado *lato sensu* (Ribeiro & Walter 1998). Riparian forest is a forest formation that borders medium and large rivers whose canopies on both sides of the watercourse are separated and therefore do not form galleries (Ribeiro & Walter 1998). Instead, in the gallery forest the canopies form galleries along a watercourse (Ribeiro & Walter 1998). The pastures are the predominant type of vegetation in the study area, constituted by grasses of the genus *Brachiaria* (Poales, Poaceae). On both sides of the highway BR 242, the predominant landscape matrix consisted of pastures with fragments of Cerrado stricto sensu, riparian forests, gallery forests and ecological corridors.

According to the Köppen classification, the climate of the region is type Aw, presenting summer rains and a dry winter. The rainy period occurs from December to March, with average rainfall of 228 mm, and the dry period from May to September, with average rainfall of 14.8 mm. April and October are considered mid-season (average rainfall of 135 mm) (data for 2005 - Climate-Data.Org 2014, http://pt.climate-data.org/).

Data collection

Samplings were carried out four times a month, between January and December 2005, totalizing 48 monitoring campaigns. Each monitoring campaign lasted one day. A car with a driver and an observer at an average speed of 40 to 60 km/h (Santos *et al.* 2012) was used. The road monitoring was conducted in the morning and consisted of 115 km round trips during each monitoring campaign totalizing 5,520 km monitored during the entire study.

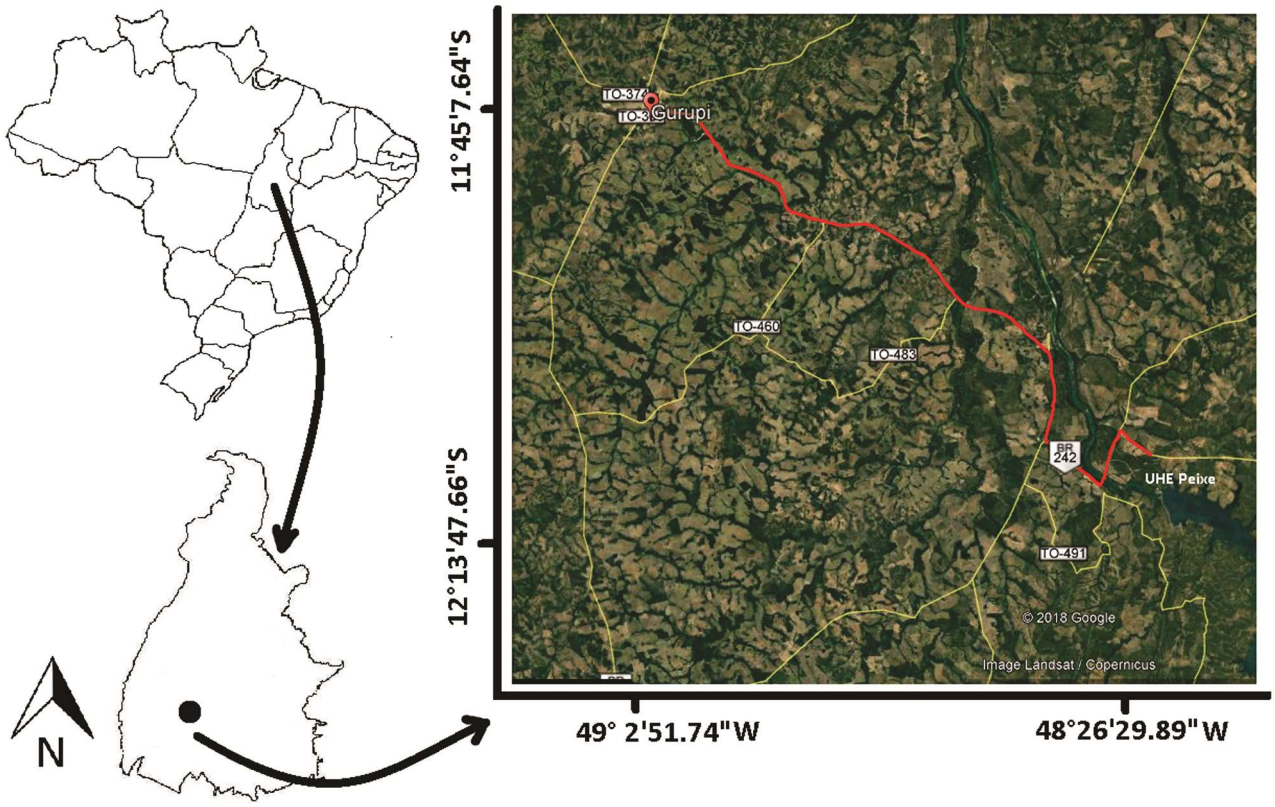

Figure 1. Location of the study area: highway BR-242 (red line), state of Tocantins, Midwest Brazil (Source: Adapted from Google Earth).

The date and a detailed description of the predominant type of vegetation in the road edge were recorded for each medium or large-sized mammal found dead within an area of up to one meter from the road. Photographs of the dead animal and the geographical coordinates were also taken. After these procedures, each registered animal was removed from the site in order to avoid duplicate counts of the same individual. The identification of species were based on their current geographical distributions and followed the descriptions in Reis *et al.* (2006), Emmons & Feer (1990), Auricchio (1995), Oliveira & Cassaro (1997), and Patton *et al.* (2015). Recent taxonomic reviews were adopted, including information on *Leopardus* (Trigo *et al.* 2008), and information in Gutiérrez & Marinho-Filho (2017). The species nomenclature was expressed according to Paglia *et al.* (2012).

Data analysis

Sampling efficiency was evaluated using species accumulation curves using the first-order Jackknife nonparametric richness estimator. The curves were obtained for 100 randomizations using the EstimateS software version 9.0 (Colwell 2013) and the Statistica Release software version 7 (Statsoft 2005).

Six roadkill evaluation indices were developed in this study as follows: IEAs expresses the number of species per traveled kilometer; ITAs expresses the number of roadkilled species in each monitoring campaign; IEAn expresses the number of roadkilled individuals by traveled kilometer; ITAn expresses the number of roadkilled individuals in each monitoring campaign; IKAs expresses the distance traveled to register a roadkilled species; and IKAn expresses the distance traveled to register a roadkilled animal (Table 1).

The non-parametric chi-squared (χ_2) test (Triola 1999) was used to evaluate the frequency of roadkills observed between dry and rainy seasons and between sites with native (*i.e.*, Cerrado, riparian forest and gallery forest) and anthropogenic vegetation (*i.e.*, pasture), considering an α of 0.05.

The classification of species into threatened categories was carried out according to the national list (MMA 2014) and the International Union for Conservation of Nature Red List of Threatened Species (IUCN 2017).

Table 1. Indexes used in the analysis of the records of medium and large-sized mammals roadkilled on the highway BR-242, state of Tocantins, Midwest Brazil.

$IEA_S = \frac{S}{L.C}$	$ITA_S = \frac{S}{C}$
$IEA_N = \frac{N}{L.C}$	$ITA_N = \frac{N}{C}$
$IKA_S = \frac{1}{S.\frac{1}{L.C}}$	$IKA_N = \frac{1}{N.\frac{1}{L.C}}$

IEA_S = Spatial Index of Roadkill of Species; IEA_N = Individual Roadkill Index; ITA_S = Temporal Index of Roadkill of Species; ITA_N = Temporal Index of Individual Roadkill; IKA_S = Linear Index of Roadkill of Species; IKA_N = Linear Index of Individual Roadkill; S = total number of roadkilled species recorded in the study; N = total number of individuals roadkilled in the study; L = length of the highway monitored in the study; C = total number of monitoring campaigns conducted in the study.

RESULTS

The species accumulation curve did not stabilized, since the observed species richness (S obs [Mao Tau] = 18 species) was lower than the estimated richness (S Jack 1 Mean = 29.75 species), indicating a sample efficiency of 60.5% (Figure 2).

Forty-eight medium and large-sized mammals, belonging to 18 species, six orders, ten families, and 17 genera, were recorded (Table 2). The roadkill indices obtained were 0.003 species/km; 0.009 roadkilled individual/km; 0.375 species/monitoring campaign; 1,00 individual/monitoring campaign; 306,667 km/roadkilled species, and 115,000 traveled km/roadkilled individual.

Among the species found, *Cerdocyon thous* (Carnivora, Canidae) presented the highest number of individuals (N = 14), followed by *Myrmecophaga tridactyla* (Pilosa, Myrmecophagidae; N = 7), *Tamandua tetradactyla* (Pilosa, Myrmecophagidae; N = 6), *Procyon cancrivorus* (Carnivora, Procyonidae; N = 4), *Nasua nasua* (Carnivora, Procyonidae; N = 3) and *Dasyprocta leporina* (Rodentia, Dasyproctidae; N = 2), totaling 73.5% of all individuals reported in this study. The other 12 species presented only one register (Table 2 and Supplementary material 1).

The largest numbers of roadkilled individuals of medium and large-sized mammals were found in patch of the road bordered by pastures (N = 14), followed by Cerrado *stricto sensu* (N = 12), riparian forests (N = 9), gallery forests (N = 7), and ecological corridors (N = 6) (Figure 3). Roadkill occurred more frequently in areas of native vegetation (Cerrado *stricto sensu*, riparian forests, and gallery forests; N = 34) when compared to pastures (N = 14; $\chi 2 = 9.000$, df = 1.000, p = 0.002).

Twenty-six individuals were recorded in the dry season and 14 in the rainy season (Figure 4), but the number of registers did not differ between seasons ($\chi_2 = 3.600$, gl = 1.000, p = 0.058).

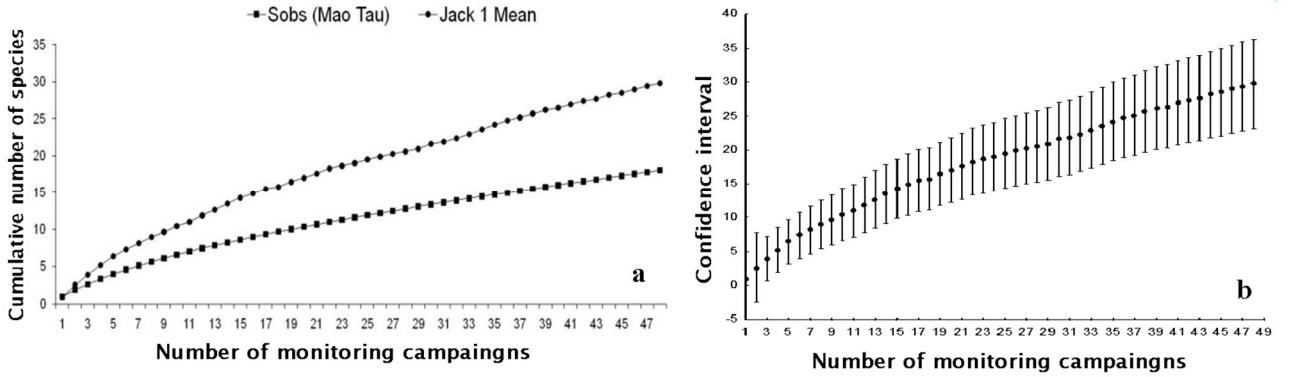

Figure 2. Species accumulation curve (a) and confidence interval for the estimated number of roadkilled mammal species (b) recorded on highway BR-242, state of Tocantins, Brazil. S obs = observed species richness; Jack 1 Mean = estimated species richness using the Jackknife 1 estimator.

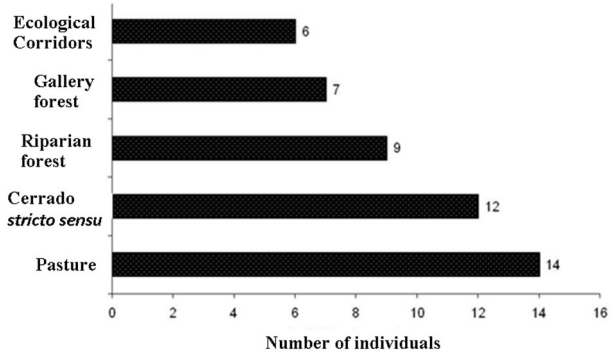

Figure 3. Number of medium and large-sized roadkilled mammals recorded among the physiognomic types of vegetation on highway BR-242, state of Tocantins, Midwest Brazil.

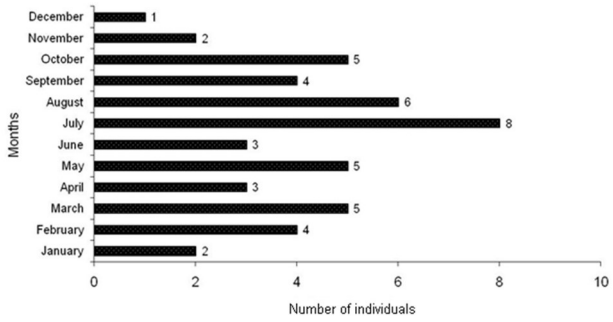

Figure 4. Number of medium and large-sized roadkilled mammals recorded during 2005 on highway BR-242, state of Tocantins, Midwest Brazil.

DISCUSSION

The non-stabilization of the rarefaction curve of species richness indicates that there are other species occurring in the region, which were not registered. As roadkills occur at random and the sampling lasted only one year, the mammal species richness was probably underestimated in the present study, demanding long-term studies. However, important information was reported in this study related to roadkilled mammals in the Cerrado biome.

Comparisons of the mortality rate of medium and large-sized mammals due to roadkill with other studies are difficult to carry out since the studies are conducted in different locations and at different seasons. In addition, they do not use indexes or standardized methodologies that allow adequate comparisons for each taxonomic group. Only one study that used a similar index to could be found (Bager & Rosa 2010). However, comparisons with the present study were not possible because Bager & Rosa (2010) also included reptiles and birds and their index was part of a priority ranking. Here, we created and presented roadkill rates in order to standardize and compare with the results of other studies. We found values, using our indexes, higher than the obtained by Cherem *et al.* (2007), Gumier-Costa & Sperber (2009), Cunha *et al.* (2010) and Cáceres *et al.* (2012), and lower than the values of Prado *et al.* (2006) and Santos *et al.* (2012). Considering the number of roadkilled individuals, our study presented higher indices than those observed by Cherem *et al.* (2007) and Gumier-Costa & Sperber (2009) and lower than those reported by Cunha *et al.* (2010), Cáceres *et al.* (2012) and Santos *et al.* (2012). In the present study, a new species record was found every 306,667 km traveled, which is a lower rate than those recorded in other studies conducted in the states of Santa Catarina, Pará, Goiás, and Mato Grosso do Sul, Brazil (Cherem *et al.* 2007, Gumier-Costa & Sperber 2009, Cunha *et al.* 2010, Cáceres *et al.* 2012). It is important to note that variations in the results may be due to detectability rate (Teixeira *et al.* 2013), sample effort, duration of studies, type and location of roads, and seasonality. In the present study, except for *Dasyprocta*, the recorded species were also found in studies conducted in the states of Santa Catarina (Cherem *et al.* 2007), Mato Grosso (Melo & Santos-Filho 2007), Rondônia (Turci & Bernardes 2009), Goiás (Cunha *et al.* 2010), and Mato Grosso do Sul (Cáceres *et al.* 2012).

Among the 18 species, six are listed as threatened according to the national list (MMA 2014) and three according to the IUCN list (IUCN 2017), total of seven species: *Myrmecophaga tridactyla*, *Chrysocyon brachyurus*, *Lycalopex vetulus*, *Leopardus pardalis*, *L. wiedii*, *Puma concolor*, and *P. yagouaroundi*. This number is higher than those found by Cherem *et al.* (2007), Cunha *et al.* (2010), and Cáceres *et al.* (2012). There is an increased number of species classified as threatened due to anthropic actions (see Tabarelli *et al.* 2010) and due to the conservation status of the Cerrado domain (Myers *et al.* 2000)

Table 2. Species list of large and medium-sized mammal species roadkilled on the highway BR-242, state of Tocantins, Midwest Brazil. N = number of individuals, P = pasture, S = Cerrado *stricto sensu*, F = riparian forest, G = gallery forest, C = ecological corridor, MMA = threat category according to national list (MMA 2014), IUCN = threat category according to global list (IUCN 2015), VU = Vulnerable, NT = Near Threatened.

Taxon	Common name	N	Local	MMA	IUCN
Didelphimorphia					
Didelphidae					
Didelphis albiventris Lund, 1840	White-eared Opossum	1	P	-	-
Pilosa					
Myrmecophagidae					
Myrmecophaga tridactyla Linnaeus, 1758	Giant-anteater	7	P,S	VU	VU
Tamandua tetradactyla (Linnaeus, 1758)	Lesser-anteater	6	F,G,C	-	-
Cingulata					
Dasypodidae					
Dasypus septemcinctus Linnaeus, 1758	Seven-banded-armadillo	1	P	-	-
Euphractus sexcinctus (Linnaeus, 1758)	Six-banded-armadillo	1	P	-	-
Artiodactyla					
Cervidae					
Mazama americana (Erxleben, 1777)	Red-brocket-deer	1	P	-	-
Primates					
Aotidae					
Alouatta caraya (Humboldt, 1812)	Black Howler monkey	1	F		
Carnivora					
Canidae					
Cerdocyon thous (Linnaeus, 1766)	Crab-eating-fox	14	P,S,F,G,C		
Chrysocyon brachyurus (Illiger, 1815)	Maned-wolf	1	P	VU	NT
Lycalopex vetulus Lund, 1842	Hoary-fox	1	P	VU	
Felidae					
Leopardus pardalis (Linnaeus, 1758)	Ocelot	1	P		
Leopardus wiedii (Schinz, 1821)	Margay-wild-cat	1	P	VU	NT
Puma concolor (Linnaeus, 1771)	Puma	1	P	VU	
Puma yagouaroundi (É. Geoffroy, 1803)	Jaguarundi	1	G	VU	
Mustelidae					
Eira barbara (Linnaeus, 1758)	Tayra	1	P		
Procyonidae					
Nasua nasua (Linnaeus, 1766)	Coati	3	F,G,C		
Procyon cancrivorus (G. Cuvier, 1798)	Crab-eating-racoon	4	P,F,G,C		
Rodentia					
Dasyproctidae					
Dasyprocta leporina (Linnaeus, 1758)	Agouti	2	P,F		

Therefore, there is an urgent need to reduce roadkill, increasing species conservation.

The high roadkill rates for *C. thous*, *M. tridactyla*, *T. tetradactyla*, *P. cancrivorus*, and *N. nasua* can be attributed to the home range, diet, and behavioral aspects of these species (Cáceres 2011). *Cerdocyon thous* has a home range of approximately two to five square kilometers, moving long distances for foraging (Trovati *et al.* 2007), which diet consists of fruits and live prey, but also can feed on human wastes (Rocha *et al.* 2008). This foraging behavior suggests that this species can be attracted to urban areas and highways. Although they are not threatened with extinction, many populations of *C. thous* are reduced due to roadkill nearby the Brazilian highways, since it is one of the species with the highest frequency of roadkill (Cáceres *et al.* 2012, Dornas *et al.* 2012, Machado *et al.* 2015).

Myrmecophaga tridactyla and *T. tetradactyla* occupy large home ranges, consisting of approximately 2.5 and 1.0 km^2, respectively (Trovati & Brito 2009, Bertassoni *et al.* 2017). Furthermore, these species present low visual acuity, which also contribute to the high rate of roadkilled individuals. In addition, the native vegetation and road design increases the probability of anteater roadkill (Freitas *et al.* 2015b). *Procyon cancrivorus*

is among the least studied Brazilian carnivorous species; and although it is not considered threatened, it is susceptible to habitat fragmentation, being a common victim of roadkill (see Cáceres *et al.* 2010). Moreover, *P. cancrivorus* is often hunted due to popular beliefs regarding its body (Cheida *et al.* 2006). *Nasua nasua* is a non-threatened procionid, however, studies show records of roadkilled individuals, which is a threat for the conservation status of the species (Beisiegel & Campos 2013).

On both sides of the highway BR-242, the predominant matrix type in the landscape consists of pastures with fragments of Cerrado *stricto sensu*, riparian forests and gallery forests. In the present study, roadkill occurred more frequently in areas bordered by native vegetation. These results corroborate the studies conducted by Cunha *et al.* (2010) and Cáceres *et al.* (2012) on the Cerrado domain. The high incidence of roadkill on highways that present fragments of native vegetation is related to the higher volume of local traffic; and studies conducted in the Cerrado domain indicate that fragments of native vegetation may provide favorable habitats for movements, shelter, food and water supply for animals during periods of resource scarcity (Calaça *et al.* 2010).

The number of roadkilled individuals did not differ between seasons, which may be a consequence of resource scarcity during all seasons, leading animals to travel large distances year round (Prado *et al.* 2006). Such results corroborate the study conducted by Orlandin *et al.* (2015), and are in disagreement with the results found by Prado *et al.* (2006) and Cáceres *et al.* (2012), which observed influence of seasonality on roadkill frequencies. The occurrence of roadkill events of medium and large-sized mammals in the dry season seems to be associated with resource scarcity, which is a condition that implies higher needs of animal locomotion. In the rainy season, roadkill events of some species might be associated with different biological cycles, such as longer periods of reproductive activity and a subsequent increase in population size (Grilo *et al.* 2009, Caceres *et al.* 2012).

The Cerrado domain is considered a biodiversity hotspot (Myers *et al.* 2000). However, its area has been rapidly reduced by human activities such as agriculture or urban development (Klink & Machado 2005). The wild mammal populations of the Cerrado domain are divided by the roads, leading to high rates of defaunation, losses in ecosystem functionality (as mentioned by Ripple *et al.* 2015) and genetic re-structuring of the populations (McCall *et al.* 2010). Therefore, future studies should focus on elucidating the processes and patterns of roadkill in this high-threatened Brazilian domain, which would help the government and other interested parts in building conservation plans with arguments based on reliable collaborative data.

This study is a pioneer in road impacts of medium and large-sized mammals in the Cerrado domain, at the state of Tocantins region. Moreover, the record of species with restricts and endemic distribution emphasizes the importance of conducting more inventories of the fauna, pointing out the highways as a threat to wild animals. Herein, we conclude that roadkill rates are higher in road areas surrounded by native vegetation, and the record of six species in categories of vulnerability to extinction shows the importance of future studies. Regarding the mitigation measures of the impacts generated by roads on wildlife, we suggest the implantation of speed reducers in parts of the roads that are bordered by native vegetation, since this study indicated that these areas act as ecological corridors between the fragments of vegetation intercepted by the highway.

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Supplementary material 1. Photos of some species recorded at Tocantins state, Brazil.

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