How do Temporal and Spatial Features Affect Anteater Roadkill in Brazil?

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Abstract

Movement, dispersion, ecology and animal behavior are possible determining factors for the mortality rate of species on roads. Understanding the magnitude of roadkill and the possible features that affect the specie mortality via vehicular collision are essential to propose conservation measures. Medium-large mammals are highly vulnerable to collision given their general high mobility, low reproductive rates, specialist diets and low population densities. Among them, the superorder Xenarthra are in the top 10 of species most affected by the roadkill collision in Brazil. However, there is no research containing information about the influence of temporal and spatial features on Xenartha's mortality rate. Therefore, we evaluated the patterns of roadkill of two species, the giant anteater (*Myrmecophaga tridactyla*) and the southern tamandua (*Tamandua tetradactyla*) across a temporal and spatial gradient in the Brazilian Center-Western region. The surveys were conducted along four different road transects (1,259 km) between April 2017 and March 2018. A total of 303 individuals (1.2 individuals/100 km/year) were recorded, including 174 giant anteater and 129 southern tamanduas. We tested whether roadkills vary seasonally, and additionally, we identified the hotspots for each species across each highway using the modified 2D-Ripley K test and the 2D-Hotspot identification analysis. We used regression analyses and generalized linear models to test the influence of temporal (e.g. temperature and humidity), and spatial (e.g. forest coverage %) features on roadkill rates, respectively. Males of the southern tamandua were killed at a proportion of 3:1, while the roadkill rate for male giant anteaters was 1.5:1, revealing an equal tendency in the roadkills' genders, if we consider the natural sexual ratio is 1:1 for both species. No influence of temperature and humidity were registered in any evaluated roadkill pattern. However, the female roadkill of both species peaked in the rainy season which can reveal the influences of seasonal factors on female's movement, contributing to an increased collision rate. Males were killed on roads at similar rates throughout the year. We found in total ten roadkill hotspots for both species on all roads. In general, the numbers of giant anteater roadkill were negatively related to traffic and the proportion of vegetation and positively related to density of fragment, with these variables contributing together to the roadkill patterns. Thus, the spatial aggregation of roadkills is explained by both proportion of vegetation and traffic characteristics of roads, which may influence the anteaters' behavior of crossing this barrier, and landscape structures around roads, which seems to affect their movement patterns. This study reinforces the value of using specific traits to analyze roadkill rates and the need for the integration of areas to provide efficient mitigation measures.

Keywords: Mammals, Xenarthra, Seasonality, Conservation, Road Ecology, traffic, landscape structure

Introduction

Mortality of wild animals due to roads is one of the most important threats to biodiversity in the world (Assis et al., 2019; Beraldi et al., 2019; Grilo et al., 2009). Although considered connected to the development of human action, roads cause landscape fragmentation and habitat loss, which, summed with the roadkill of animals, may result in a loss of biodiversity (Jackson and Fahrig, 2011; Jochimsen, 2006). Billions of roadkill records in the world are registered every year (Bishop and Brogan, 2013; Erickson et al., 2005; Grilo et al., 2020; Seiler et al., 2004), affecting not only individuals that survive, but also population plasticity and persistence.

Mortality due to roads can affect the dynamic of populations by the reduction of the size of natural populations (Duffett et al., 2020; Forman and Alexander, 1998; Pinto et al., 2018). Additionally, the populations can be divided by this anthropogenic barrier. The combined effect of reductions in population size and changes of individuals' flux dynamics may lead to the loss of genetic diversity and local extirpation (Reed and Frankham, 2003). Therefore, local extinctions may be possible when the loss of individuals affects the immigration and/or exceeds the number of individuals born in the population (Forman and Alexander, 1998).

Generally, roadkill does not happen randomly, but at certain points on roads and during some periods of the year, with certain seasonality in some species (Ascensão et al., 2017; Clevenger et al., 2003; Ferreguetti et al., 2020). Extrinsic factors such as temporal and spatial features (e.g. density of vegetation, proportion of water and traffic) has proven to have a direct relationship with roadkill rates, explaining the patterns of roadkill in some taxa (Caceres, 2011; Clevenger et al., 2003; Coelho et al., 2008a; Ferreguetti et al., 2020; Garriga et al., 2017). For example, the temporal variation in roadkill may be related to the phenology of biological events, such as mate searching, dispersal and migration periods, and these activities can lead to a gender- and life stage-specific differential mortality (Ferreguetti et al., 2020;

Grilo et al., 2013). On the other hand, variations in: vehicle traffic between road stretches, highway design (which influences vehicle speed and the driver's visibility of road), and surrounding landscape composition and arrangements (which influence fauna abundance and mobility) are some factors than may be responsible for spatial aggregations of roadkills (Clevenger et al., 2003; Duffett et al., 2020; Seiler et al., 2004).

Most studies about road mortality only provide check lists of killed taxa, with no attached information on the patterns and consequences of mortality. Particularly, few studies have focused on the relationships between spatiotemporal variables and sex ratio roadkill patterns in Xenarthras, one of the top groups in mortality by collisions (Ascensão et al., 2017; Cáceres et al., 2010; Zimbres et al., 2013). Here, we aimed to evaluate the impact of roads on the mortality of two species of anteater along a large extension of a heterogeneous urban-rural landscape. We evaluated the spatial and temporal roadkill pattern of the southern tamandua (*Tamandua tetradactyla*) and the giant anteater (*Myrmecophaga tridactyla*) for one year along 1,259 km of pavemented one-way and two-way roads.

Xenarthra are one of the ancient placental group (Gibb et al., 2016) with two species of anteaters belonging to the Myrmecophagidae family inhabiting almost the whole territory of Brazilian (90 %) and are considered particularly vulnerable due to habitat loss and fragmentation, due to wildfires and roadkill in some Brazilian states (Bertassoni et al., 2019; Diniz and Brito, 2013; Miranda et al., 2014; Silveira et al., 1999; Superina and Loughry, 2015). The anteaters are in the top 10 of species with high mortality by roadkill in Brazil (Ascensão et al., 2019; Cáceres et al., 2010; Diniz and Brito, 2013; Garriga et al., 2017) and thus, they are especially vulnerable to roadkill because they have large spatial requirements, small populations, tend to live at low densities, and occupy small geographic ranges or exhibit migratory behaviors (Caceres, 2011; Ferreguetti et al., 2020; Grilo et al., 2020).

Both anteaters, the giant anteater and the southern tamandua, are also largely found in different landscape, from open grassland savanna in the Cerrado, wetlands in the Pantanal, up to transitional forests and mountain tropical regions (Ascensão et al., 2017; Clozato et al., 2015; Desbiez and Medri, 2010). Nevertheless, although southern tamandua have a preference for forested areas for feeding and resting (Desbiez and Medri, 2010), giant anteaters are habitat generalist with preferences for heterogeneous habitats (Quiroga et al., 2016; Vynne et al., 2011). Both species are myrmecophagous and termitophagous with lower body temperatures and lower basal metabolic rates than others mammals (McNab, 1984). Their daily activities are diurnal, nocturnal to diurnal (crepuscular) or nocturnal, with habitat use widely related to ambient temperature (Camilo-Alves and Mourão, 2006; Rodrigues et al., 2008). Therefore, all the above features and difference of biological characteristics contribute to the increase of impacts by roads and possibly to differences in spatial and temporal patterns of mortality.

Considering the background, we expected that: (i) the majority of roadkill data would be represented by species with larger body masses, because of their high dispersion capacity and larger home range; (ii) we expected that males and females would have the same number of deaths, since we adopted that the proportion of births for the two species is 1:1; (iii) the anteater's mortality rates caused by roadkill would be influenced by climatic variables with increased number of events in the drier and hotter months, as temperatures encourage animals to reduce their activity patterns due to thermoregulation costs; (iv) mortality would be different between seasons, due to the possible effect of seasonality on species movement, related to the time of greatest movement in the reproductive period or the availability of resources. Spatially, we expected that (v) the roadkill events would aggregate at different specific points along the roads, especially near vegetation fragments and water, and far from urban areas and intense traffic. Additionally, we expected that (vi) landscape features at larger scales would influence roadkill patterns due to their influence on anteater movement dynamics. We expected that roadkills would be positively related to the amount of habitat and water, as well as traffic, and negatively related to density of habitat patches, and urban areas. This expected pattern was based on the behavior of each species and gender, with more roadkill events in landscapes where the anteater has to move more intensely to supply their resources requirements. We tested and discussed each environmental variable and the possible implication for conservation and mitigation measures of each species.

Material and methods

Ethics Statements

The biological sampling authorization was obtained through the SISBIO-ICMBio (Authorization System and Biodiversity Information, Chico Mendes Institute for Biodiversity Conservation, Ministry of Environment, Brazil), under the number 53798-4. The research was approved by the Ethics Committee on the Animal Experimentation (CEUA/UFSCar) protocol number 1584280817, and the genetic resource access was registered under SisGen A9F8717.

Study Area and Data Collection

The study was carried out in the Brazilian Center-Western region across four main roads in the state of Mato Grosso do Sul (MS). The Center-Western vegetation is predominantly Savanna, but also includes the Pantanal and flooded areas, and the region's economy is based on livestock, soybeans, and tourism, with a large highway system present mostly in the central region. The study area presents subtropical climate (Aw in the Köppen classification) characterized by a dry winter, with temperatures below 18 ºC, and a wet summer with temperatures above 22 ºC. During the study period the mean temperatures and humidity were 23.94 ± 2.19 °C and 56.77 ± 10.67 % in the dry (from April to September) and 25.33 ± 2.14 °C and 63.17 ± 10.35 % in the rainy (from October to March) seasons (t-test =-5.48, df = 29.09, $p < 0.001$).

We conducted the surveys along four different one- and two-way roads, defined as our transects and referred hereafter as ‘T1’, ‘T2’, ‘T3’ and ‘T4’ (Figure 1). The municipality of Campo Grande (20°28'15” S - 54º37'15” W) was the starting point of each transect: T1, along BR-262 to the bridge over the Paraguay River (total extension: 397 km); T2, along BR-262 to Três Lagoas (305 km); T3, along BR-163 and BR-267 to Nova Andrandina (275 km) and T4, along MS-040 to Bataguassu (300 km; see Figure 1). All transects covered 1,259 km of paved road subjected to different land cover and climatic variability along their extensions. The transects were surrounded by portions of Pantanal and Savanna.

We monitored the four roads fortnightly, maintaining regular intervals, from April 2017 to March 2018, resulting in 25 surveys. We performed the surveys by car maintaining a regular speed of maximum 60 km h^{-1} in the daytime period from 6:00 am until the daytime necessary to cover the entire stretch. We surveyed a total of 5,036 km every month, considering the four sampled transects. Each road was surveyed in two-directions on the same day, and the positions of all anteater carcasses were annotated with a GPS information receiver. Since the methodology used and the effort was the same for all transects, the results of the surveys are comparable. We removed the animal carcasses from the road once they had been recorded and so we collected tissue samples for further molecular sex identification (Chapter I).

Sampling of Environmental Variables

For all transects we recorded two temporal variables i) temperature (ºC), ii) relative humidity (%). The climate variables data were obtained from the National Institute of Meteorology (INMET, www.inmet.gov.br). The highways were sectioned in 20 km-long segments, based on the home range registered for species (Bertassoni et al., 2017; Desbiez and Medri, 2010; Medri and Mourão, 2005; Shaw et al., 1987). To obtain more accuracy in the variable values for each segment, we measured the Euclidean distance (m) between the central point of each segment and all nearest climatological stations and chose the station with the shortest distance to obtain climatic data. In total, we collected information from ten climatological stations located in ten municipalities from the state of Mato Grosso do Sul (Agua Clara, Aquiduana, Bataguassu, Campo Grande, Corumbá, Ivinhema, Miranda, Rio Brilhante, Sindrolândia, Três Lagoas).

Land cover and sampling of spatial variables

We obtained the land use and cover maps from the 2018 MapBiomas Collection 4.1 of Brazil, with 30 m of spatial resolution. First, using Quantum GIS v. 3.4.10-Madeira software (QGIS Development Team, 2020), we summed up the categories of land cover to four classes: native vegetation (Forest formation, Savanna formation, Wetland and Grassland formation), urban (Urban infrastructure, other non-vegetated and mining areas), water (Rivers and Lakes) and agriculture (Forest plantations, pastures, annual and perennial crops, semi perennial crops). Then, around each segment, within a 10 km-radius buffer (the largest possible until overlap between contiguous segments), we recorded five landscape variables: 1) proportion of vegetation, 2) proportion of urban area, 3) proportion of agriculture, 4) proportion of water, and 5) density of vegetation fragments (Table 1). For extracting the proportional values of each category for each segment, we used the LecoS-Landscape Ecology Statistics plug-in implemented in QGIS (QGIS Development Team, 2020). Additionally, for each segment we collected data on traffic intensity (daily traffic volumes in 2018; http://servicos.dnit.gov.br/) for each transect. No recent traffic counts were available for T4. For this reason, the T4 was not included in the traffic analyses.

Data Analysis

Roadkill Description

We evaluated the number of roadkill for both species (giant anteater and southern tamandua) separately and compared roadkill rate results between sexes, according to sex identification information obtained previously (Chapter I). To characterize the roadkill patterns, we evaluated whether the number of road-killed individuals were different between sex per species than the expected value, using Chi-square tests. We compared our sexual proportion of roadkill with sex ratio values previously obtained for giant anteaters: 1:1 (Desbiez et al., 2020), 2:1 (Camilo-Alves, 2003), and 3:1 (Mourão and Medri, 2002). Since no data on sex ratios for southern tamandua are available, we considered the expected ratio of roadkills between species 1:1, but we felt it necessary to conduct our analysis with the same sexual ratio described for the giant anteater.

Temporal and climatic patterns on roadkills

We grouped months by season to test whether roadkills vary seasonally, performing a two-tail unpaired t-tested analysis. When the data did not fit the requirements of the parametric tests, even after log transformation, we performed the non-parametric Mann-Withney test. We performed all analyses in R software v. 3.2.5 (R Development Core Team, 2019).Additionally, to test the influence of temperature and humidity on roadkill rates (individuals/ 100 km) we used multiple regression analysis with the months as the replicas.

Spatial pattern of roadkills

To investigate the spatial aggregation patterns of roadkills, initially we used the function “mortality rate estimate” in SIRIEMA v. 2.0 (Coelho et al., 2011) to estimate the road mortality rate for specie and transect. More details about this approach can be found in Teixeira et al. (2013). Additionally, we multiplied the roadkill rate (roadkill/km/day) per 100 to standardize all results. To evaluate the non-randomness of the spatial distribution of events over multiple scales (Coelho et al., 2008), we analyzed the roadkill events in each transect separately using SIRIEMA v. 2.0 program (Coelho et al., 2011). We analyzed by specie: giant anteater and southern tamandua and by sex per species: males and females. We used the modified 2D-Ripley K test (Coelho et al., 2008; 2012) and the 2D-Hotspot identification analysis for identification of the highest roadkill aggregation points (hotspots) in each transect. The first test was done with an initial radius of 500 meters, radius increments of 1000 meters, 1,000 simulations, and a confidence limit of 95 %. The initial radius choice was the used for mitigation measures, such as speed reducers (Teixeira et al., 2013). The second test was used to identify the segment with highest roadkill aggregations (hotspots). This test was done by dividing each transect into segments with the same length (100 meters). The radius used was 500 meters, 1,000 simulations, a confidence limit of 95 %. More details of analysis can be search in Coelho et al. (2011).

After identifying the roadkill hotspots per species and sex, we investigated the characteristics of such points. To generate a comparison dataset, we used QGIS v. 3.4.10-Madeira to generate random points in the same number of detected hotspot points along each transect. To analyze the land cover around each hotspot, we designed a buffer with a radius of 500 m around the hotspots and the random points to identify what features surround each heat point. Then we extracted the following variables from the landcover map: 1) proportion of vegetation, 2) distance to urban area, 3) proportion of agriculture, and 4) traffic. These analyses were processed using QGIS v. 3.4.10-Madeira software and the size and percentage of the classes for each buffer were calculated using LecoS-Landscape Ecology Statistics plug-in (Jung, 2016). Finally, to test whether the evaluated variables differed between random points and hotspots, we performed Chi-square tests to the proportion data and two-tailed unpaired t-tests to traffic data.

At a landscape level, to understand which spatial features influence roadkill rates, we used generalized linear models (GLMs) in a multi-model averaging approach to test if the predictor variables (proportion of urban area, density of vegetation, proportion of water, proportion of vegetation and traffic) explained the number of roadkills of each species or the presence of roadkill for each sex of giant anteater. The low presence of southern tamandua females in our roadkill data did not permit the gender analysis to be carried out for this species. We considered the negative binomial distribution of number of roadkills analysis since it best fits the data, correcting the overdispersion issue; and binomial distribution to presence/absence of roadkill data. We previously checked for the absence of multicollinearity among predictors by calculating the variance inflation factor (VIF), using package "car" in R software v. 3.2.5 (R Development Core Team, 2019). We excluded the agriculture proportion variable, since they were inflating the variance, due to its strong correlation with proportion of native vegetation cover (r = -0.96). After this exclusion, all VIF values were lower than 2, suggesting independence among predictors (Neter et al., 1996).

The number of roadkills of each species, and the presence or absence of giant anteater females and males were obtained for each segment. For each model, we calculated Akaike Information Criterion (AIC) and the difference of each model and the best model (ΔAIC). Models with $\Delta\text{AIC} \leq 2$ were considered as equally plausible (Zuur et al., 2010), and the Akaike's weight of evidence (w_i, ranging from 0 to 1, with larger numbers indicating greater support) values for all possible combinations. We ranked the models based on the w_i from the higher ranked model until the total of sum be > 0.95, which represents the set of models that best explain the numbers or gender of the roadkill. Then, we evaluated the relative importance of each predictor variable by the $\sum w_i$ of each candidate model in which this variable appeared and of all equally plausible models. Furthermore, we calculated for each predictor their unconditional variance from 95 % confidence set of models to assess the association between each predictor and the response variable. Thus, we considered important the predictor variables that had: a high sum of w_i and the model-averaged unconditional variance was lower than the model-averaged parameter estimates. We performed all analyses with the "glmulti" package in R software v. 3.2.5 (R Development Core Team, 2020). The same methods were used to select the best models to explain the factors for species and sex.

Results

We recorded 320 killed individuals (1.28 individuals/100 km/day), including 191 giant anteaters (60 females, 85 males, and 46 without gender) and 129 (17 females, 75 males, and 37 without gender) southern tamandua for one year. The roadkill number was the same for both species (X^2 = 3.073, df = 1, p=value = 0.080). Transect T1 had the largest number of killed individuals (1.68 individuals/100 km/day), followed by T3 (1.27 individuals/100km/year), T2 (1.03 individuals/100 km/day), and T4 (0.96 individuals/100 km/day) with considered all roadkill events. The roadkill number for both evaluated species were higher in the T1 than the others transects ($F_{3,44}$ = 0.864, R^2 = 0.019, p = 0.025); Figure 2a). The giant anteater roadkills were equal among the transects ($F_{3,44}$ = 0.036, R^2 = 0.510, p = 0.829; Figure 2b). The southern roadkills were higher in the T1 than the other transects ($F_{3,44}$ = 0.456, R^2 = 0.190, p < 0.001; Figure 2c), which is the same pattern found for all roadkills.

For giant anteaters, the roadkill of females and males happened every month, while no female roadkill was reported for the southern tamandua in five months (May, July, August, September and January; Figure 3). The highest roadkill rate was in February, likely due to the highest number of male southern tamandua roadkills. The roadkill rate found by male giant anteaters was significantly different than the expected sexual ratio of 3:1 (males and females, respectively), while the mortality of southern tamandua males was significantly higher than expected in a sexual ratio of 1:1 and 2:1 (Table 2).

We did not find a relation between the variation of temperature and humidity per month that would explain the temporal pattern of run over for both species (Table 3, Figure 3). Estimated total roadkill rates did not vary significantly between seasons, but the test of female roadkill rates from both species were significantly higher during colder months (roadkill rates from giant female anteaters, w = 185, p = 0.031 and roadkill rate from southern tamandua of females, w = 216, p = 0.037, Table 4).

Ripley's statistical analyses showed that clustering of roadkills occurred at scales up to 200 meters, indicating that roadkills are not randomly distributed along the roads. The 2D-Hotspot analysis showed several places along the road where there are noticeable higher frequency of roadkills and that may be the focus for further efforts to determine priorities in conservation management. For both species we identified ten sections that were classified as hotspots and only one section coincided for the two anteaters (Figure 4). For giant anteater hotspot analyses we found five hotspots for all transects. Three for T1 and one for T2 and one for T3, while for southern tamandua hotspots we found two for T1, and one for T2, T3 and T4 (Figure 4). All hotspots occurred in the agricultural areas for giant anteaters and southern tamanduas, with a mean proportion of 0.19 and 0.35, respectively. However, the comparison of composition spatial features between hotspots and random points (with absence of roadkill aggregations) do not identify significant differences (Table 5). The distance of each hotspots from urban areas never had any effect on roadkills.

Giant anteaters and southern tamandua roadkills had a reasonable association with certain landscape attributes (explained deviance = 27 % and 39 % for giant anteaters and southern tamanduas respectively). At landscape level, we found support for three spatial variables (e.g. proportion of vegetation, proportion of water, and proportion of traffic) that can explain roadkill occurrences for giant anteaters and southern tamanduas. Anteater roadkills were positively related to density of fragment and negatively related to the proportion of vegetation and traffic (Table 6, Figure 5). Overall, the models could explain a small fraction of the spatial patterns for roadkills of each gender (Table 7) but had a reasonable power for each species. The importance of models was lower when we try explaining the gender differences in roadkills.

Discussion

Roadkills show a relevant impact on anteater populations, because of the intense mortality rate registered for both southern tamanduas and giant anteaters and the influence of roads on anteaters' population dynamics revealed by the spatiotemporal pattern of roadkills. Roadkill numbers are similar for both species, contradicting our first prediction (i) that the tendency for mortality should be higher for species with larger home range, dispersion capacity, and higher body mass. However, the roadkills by sex ratios are different between species (prediction ii). The roadkills seem to affect mainly the males of southern tamanduas and may vary from equal proportion by sex ratio to mainly effecting females according to some scenarios of natural sex ratio. Additionally, there is no relation between roadkill rate and climate variables, contradicting our prediction (iii) on thermoregulatory influences on movement dynamics. But we show that the patterns of female roadkills are related to seasonality, revealing some influence of seasonal factors on species' movement dynamics, as expected (prediction iv) (Ascensão et al., 2017). Spatially, as we expected (prediction v) the roadkill patterns are nonrandom, but the evaluated local environmental characteristics of hotspots do not explain the aggregation of roadkills in such points. However, we highlight the importance of both local (traffic) and landscape features (fragmentation degree and habitat amount) in roadkill mortality, although not all variables had the expected relationship (prediction vi).

The larger number of anteater roadkills may be considered high when we compared our results with other studies with similar analyses effort, as found by Ascensão et al. (2017) who found 124 giant anteaters and 116 southern tamanduas in the same region of our study, in a period of 13 months in an analysis effort of 23,000 km. Anteaters are usually found in all list of taxa killed on roads in Brazil (Araujo et al., 2020; Ascensão et al., 2019, 2017; Garriga et al., 2017; Ribeiro et al., 2017) probably reflecting an overall high abundance of anteaters in some Brazilian regions (Cáceres et al., 2010). Additionally, several biological features of

Xenarthras may contribute to the high incidence of roadkills (e.g. poor vision) compared with other groups (Freitas et al., 2014; Meri Medri et al., 2010; Redford, 1985). In contrast with others studies, where species with larger body mass require large areas for their survival (Anacleto and Marinho-Filho, 2001; Medri and Mourão, 2003), which increases the number of casualties in some species, and despite the fact that the giant anteater can exceed 35 kg in weight, about seven times the body mass of the southern tamandua (approximately 5 kg; Rodrigues and Marinho-Filho, 2003), we found that the total of number of roadkills in both species was similar. All surveys were done following similar protocols as other studies, considering the persistence time of the southern tamandua carcasses (Santos et al., 2011) and our results can be explained basically by the biology of the species. For this reason we raise the possibility that species may deal with the roads in their habitat by altering their activity patterns and other behaviors (de Jong, 1995; Jepsen and Topping, 2004; Komers, 1997).

The roadkill mortality by sex was the same in males and females of giant anteaters. As expected, there was no difference between expected and observed roadkill sex ratios in giant anteaters, in the scenario for natural populations with 1:1 sex ratio. The tolerance for overlap of territory between both sexes is high for giant anteaters (Bertassoni et al., 2017; Miranda, 2004; Anteaters & Highways *pers. comm)* resulting in the same area probably having the same number of males and females. Thus, this could also partially explain the absence of sex bias mortality in giant anteaters. However, our results indicate a different overview from that found by Mourão and Medri (2002).Nevertheless, whether the sex ratio indicated by these is the current giant anteater population, this would represent a high impact on females of the species and would therefore have a greater impact for populations located near highways.

Yet, males of southern tamanduas were killed three times more than females, which may represent an intense removal of males from the population or populations naturally biased to males in the sex ratio. Currently there is no data on sex rate or the mating systems of southern tamanduas, so it is difficult to advance in the explanation the impact of roadkills on population balance. In ideal natural populations of mammals, it would be expected that there would be the same proportion of males and females in the population (1:1) (Bulmer and Taylor, 1980), however, in some cases it is possible to find two males for one female (2:1) which happens in some vertebrates (Aresco, 2005; Sillero-Zubiri et al., 1996). Therefore, whether we consider these characteristics in natural populations of southern tamanduas, the biases of which animals suffered roadkills would become more evident. Thus, whether considering the same mating system of two species with an absence of differential dispersion between sexes, in any panorama previously shown, the loss of individuals in populations represents a loss in the genetic diversity of the southern tamandua species and even possible changes in their effective population size in a given time (Forman and Alexander, 1998; Reed and Frankham, 2003). In the same line, population viability analyses in giant anteaters suggest that mortality due to road kill may not necessarily lead to the extinction of local populations, yet point to a possible reduction in the population's resilience and ability to withstand or recover from other anthropogenic threats on species (Desbiez et al., 2020)

Our results found no evidence for the relationship between roadkill and temperature but suggest the relation between female anteater roadkills and the rainy season (from October to March). Some studies in mammals have shown that during the dry season mortality levels are higher because the demand for food forces individuals to move across heterogeneous landscapes and thus crossing roads several times (Bueno and Almeida, 2010; Grilo et al., 2009). Although some studies did not find seasonal influences on the roadkill of mammals (Ferreira et al., 2014; Orlandin et al., 2015). To others, the number of road accidents in the dry season is significantly lower than in the rainy season (Caceres, 2011; Ferreguetti et al., 2020). This variation in the results found probably depends on biological and ecological characteristics of the species studied, such as dispersion, mating systems, foraging, dispersion, and the food habits of species. In our case, the increase of female mortality in the rainy season may be related to the reproductive period of females or the greater availability of resources during this season, however, there is no information on dispersal or reproductive periods for anteaters.

Although we found roadkill hotspots for both species, the characteristics described and tested for them were no different from the rest of the road. This means that other factors may be responsible for the high aggregation found at specific points on the road. For example, variations in vehicle traffic between road stretches, highway design which influences vehicle speed, and the driver's visibility of the road, are all factors that can and should be included in future analyses.

Despite our efforts to collect the largest number of variables to explain the pattern of anteater roadkills, using the most common variables in landscape studies, the power of explanation was low. We suggest that, more than proportion of vegetation for that species, it is the traffic that is causing the pattern of mortality found, a discovery that needs more attention from mitigation measures. Although some studies show a higher number of roadkills in areas with a higher volume of traffic (Coelho et al., 2008b; Jackson and Fahrig, 2011; Row et al., 2007), our results indicated the opposite. This result may be partially explained by the fear of the animals to the landscape with the most traffic volume (Mendes et al., 2020). For example, some studies showed the least occurrence of roadkill for birds in fragments near highways with high noise pollution and low habitat quality (Brotons and Herrando, 2001; Peris and Pescador, 2004). On the other hand, a study on giant anteaters highlighted the negative effect of habitat fragmentation by the road as well as the proportion of vegetation surrounding the road, indicating that many of the areas used in this study are below the minimum needed to sustain a viable population (Pinto et al., 2018). Therefore, we suggest that additional, finer resolution, field-derived habitat and road variables (road width, presence of curves, floor type, and topography) should be included in future road ecology analyses.

Conclusion

In this study, we have shown the importance of using information such as sex in the mortality analysis to better understand the patterns of roadkill by species. Understanding the seasonality and spatial variations in species roadkill patterns are directly proportional to the impact of the conservation measures. In conclusion, according to our results, road surveys and mitigation measures for anteaters in the Brazilian Center-Western region should be prioritized in the rainy season and monitoring should be enhanced for landscapes with low density and low proportion of vegetation, i.e. the most degraded areas, near the roads with low volume of vehicles.

Finally, we suggest that more studies using more specific traits be used for future road ecology analyses and mitigation measures, especially for large body mass and charismatic species. This may be a more effective measure with greater social impact.

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Tables and Figures

Table 1. Predictor variables tested for explaining the roadkill events of *Myrmecophaga tridactyla* and *Tamandua tetradactyla* in Brazilian Center-Western

	Variables name, unit (type)	**Symbol**	**Definition**
	Environmental variables		
i	Mean Temperature (ºC)	T ºC	Bioclimatic variables derived from the monthly temperature and humidity values. Data were generated from INMET (www.inmet.gov.br).
ii	Humidity (%)	RH	
	Landscape variables		
1	Proportion of vegetation	Pv	Proportion of primary or secondary succession stages of remnants of forest and savanna in the sampled area
2	Density of vegetation	Dv	Number of patches of native vegetation per sampled area (N/ km²)
3	Proportion of urban area	Pu	Proportion of urban areas and human buildings in the sampled area
4	Proportion of agriculture	Pa	Proportion of crops and pasture mosaics
5	Proportion of water	Pw	Proportion of rivers, lake and pounds
6	Distance to urban area	Du	Euclidean distance (m) between the nearest urban center and hotspot point
7	Traffic	T	Daily traffic volumes in 2018. Data were generated to Plano Nacional de Contagem de Tráfego – PNCT (http://servicos.dnit.gov.br/)

Table 2. Results of the Chi-square test (X^2) for mortality of *Myrmecophaga tridactyla* and *Tamandua tetradactyla* according to three expected scenarios of local population sexual ratio (see the main text for references about expected sexual ratio). Significant values are indicated in bold ($p \leq 0.05$).

Specie	Expected Sexual Ratio								
	1:1			2:1			3:1		
	X^2	df	p	X^2	df	p	X^2	df	p
Myrmecophaga tridactyla	2.54	1	0.111	1.03	1	0.309	**6.45**	**1**	**0.011**
Tamandua tetradactyla	**21.53**	**1**	**3.47 E 10-6**	**4.67**	**1**	**0.035**	1.48	1	0.223

Table 3. Climatic variables relationship with mortality rate of giant anteater (*Myrmecophaga tridactyla*) and southern tamandua (*Tamandua tetradactyla*). T ºC: monthly mean temperature; RH: monthly mean relative humidity.

	β	SE	t	p
All				
T ºC	0.089	0.098	0.906	0.370
RH	0.038	0.044	0.869	0.390
T ºC: RH	-0.001	0.002	-0.759	0.452
Giant anteater				
T ºC	0.224	0.128	1.753	0.087
RH	0.111	0.058	1.919	0.062
T ºC: RH	-0.004	0.002	-1.855	0.070
Males giant anteater				
T ºC	0.104	0.072	1.447	0.155
RH	0.065	0.032	2.003	0.051
T ºC: RH	-0.002	0.001	-1.918	0.062
Females giant anteater				
T ºC	0.028	0.056	0.500	0.619
RH	0.007	0.025	0.260	0.796
T ºC: RH	0.000	0.001	-0.196	0.846
Southern tamandua				
T ºC	-0.015	0.066	-0.229	0.820
RH	-0.012	0.030	-0.391	0.698
T ºC: RH	0.001	0.001	0.458	0.649
Males Southern tamandua				
T ºC	0.031	0.063	0.486	0.629
RH	0.018	0.029	0.644	0.523
T ºC: RH	-0.001	0.001	-0.508	0.614
Females Southern tamandua				
T ºC	-0.021	0.035	-0.593	0.556
RH	-0.012	0.016	-0.778	0.441
T ºC: RH	0.001	0.001	0.870	0.389

Table 4. Difference between rainy and dry season on mortality rates of giant anteater (*Myrmecophaga tridactyla*) and southern tamandua (*Tamandua tetradactyla*). In bold the significant relationships ($p \leq 0.05$).

	Test	df	p
Giant anteater	t = 0.901	42	0.373
Males	W = 369.5	46	0.093
Females	**W = 185**	**44**	**0.032**
Southern tamandua	t = 1.664	43	0.103
Males	t = 0.947	43	0.349
Females	**W = 216**	**38**	**0.038**
All	t = -0.097	42	0.923

Table 5. Analysis of spatial variables obtained in a buffer with a radius of 500 m around hotspots and random points of giant anteater (*Myrmecophaga tridactyla*) and southern tamandua (*Tamandua tetradactyla*) across all roads of Mato Grosso do Sul.

	X^2	df	p
Giant anteater			
Proportion of vegetation	10	9.0	0.351
Proportion of agriculture	10	9.0	0.351
Proportion of Urban area	4.6	4.0	0.323
Distance to urban area	t = -0.639	4.4	0.554
Traffic	t = 1.131	7.7	0.292
Male of giant anteater			
Proportion of vegetation	26.7	26.0	0.427
Proportion of agriculture	32	30.0	0.368
Proportion of Urban area	16.3	16.0	0.436
Distance to urban area	t = -0.895	21.3	0.380
Traffic	t = -0.851	14.3	0.408
Female of giant anteater			
Proportion of vegetation	10	7.0	0.189
Proportion of agriculture	10	8.0	0.265
Proportion of Urban area	5.2	5.0	0.392
Distance to urban area	t = -0.488	7.1	0.640
Traffic	t = -1.659	6.7	0.142
Southern tamandua			
Proportion of vegetation	10	9.0	0.351
Proportion of agriculture	10	9.0	0.351

Proportion of Urban area	5.2	5.0	0.392
Distance to urban area	t = 0.981	4.0	0.381
Traffic	t = 0.258	5.9	0.804

Table 6. Results of generalized linear models (GLM) that best explained giant anteater (*Myrmecophaga tridactyla*) and southern tamandua (*Tamandua tetradactyla*) roadkill events. We ranked, according to AIC, the set of models for which Akaike weights (*w*i) summed > 0.95. The values of model-averaged parameter estimate (β) and unconditional variance (UV) are also indicated. The equally parsimonious models are indicated in bold. The variables included in each model are indicated with X.

Model	Proportion of vegetation	Proportion of urban	Proportion of water	Density of vegetation	Traffic	AIC	ΔAIC	w_i
Giant anteater								
Model 1	**X**		**X**		**X**	**231.27**	**0.00**	**0.317**
Model 2	**X**		**X**	**X**	**X**	**232.99**	**1.72**	**0.134**
Model 3	**X**	**X**	**X**		**X**	**233.05**	**1.78**	**0.130**
Model 4	X				X	233.28	2.01	0.116
Model 5	X	X	X	X	X	234.90	3.63	0.052
Model 6	X			X	X	234.97	3.70	0.050
Model 7			X		X	234.99	3.72	0.049
Model 8	X	X			X	235.24	3.97	0.044
Model 9		X	X		X	236.24	4.97	0.026
Model 10			X	X	X	236.46	5.19	0.024
Model 11	X	X		X	X	236.96	5.69	0.018
β	-1.953*	0.376	-44.332	0.013*	-2.1 E-04*			$\Sigma w_i = 0.96$
UV	1.256	1.086	1.256 E +03	3.0 E-03	2.21 E-08			
Southern tamandua								
Model 1		**X**	**X**		**X**	**222.10**	**0.00**	**0.213**
Model 2		**X**	**X**	**X**	**X**	**223.21**	**1.11**	**0.122**
Model 3		**X**			**X**	**223.69**	**1.59**	**0.096**
Model 4			**X**		**X**	**223.85**	**1.75**	**0.089**
Model 5	**X**	**X**	**X**		**X**	**223.90**	**1.80**	**0.087**
Model 6	**X**	**X**	**X**	**X**	**X**	**224.07**	**1.97**	**0.080**
Model 7	X	X		X	X	224.47	2.37	0.065
Model 8		X		X	X	224.52	2.42	0.064
Model 9	X	X			X	225.18	3.08	0.046
Model 10			X	X	X	225.21	3.11	0.045
Model 11	X		X		X	225.84	3.74	0.032
Model 12	X		X	X	X	226.98	4.88	0.018
β	-0.2498*	-81.767	-0.02743	0.0567*	-1.749 E-04*			$\Sigma w_i = 0.95$
UV	0.1359	51.458	1.673 E +03	1.018 E-02	2.066 E-08			

* Values with an asterisk indicate cases in which the unconditional variance was smaller than the model-averaged parameter estimates, suggesting safe interpretation of β.

Table 7. Results of generalized linear models (GLM) that best explained gender's roadkill events in *Myrmecophaga tridactyla*. We ranked, according to AIC, the set of models for which Akaike weights (*w*i) summed ≥ 0.95. The values of model-averaged parameter estimate (β) and unconditional variance (UV) are also indicated. The equally parsimonious models are indicated in bold. The variables included in each model are indicated with X.

Model	Proportion of vegetation	Proportion of urban	Proportion of water	Density of vegetation	Traffic	AIC	ΔAIC	w_i
Males								
Model 1	**X**		**X**		**X**	**73.71**	**0.00**	**0.212**
Model 2	**X**				**X**	**73.72**	**0.01**	**0.211**
Model 3	**X**	**X**	**X**		**X**	**74.17**	**0.46**	**0.169**
Model 4	**X**	**X**			**X**	**75.35**	**1.64**	**0.093**
Model 5	**X**			**X**	**X**	**75.49**	**1.78**	**0.087**
Model 6	**X**		**X**	**X**	**X**	**75.59**	**1.88**	**0.083**
Model 7	X	X	X	X	X	76.17	2.46	0.062
Model 8	X	X		X	X	77.23	3.52	0.037
β	-4.692*	5.170	-0.289	-0.018*	-5.643 E-04		$\Sigma\ w_i = 0.95$	
UV	4.318	1.19 E +02	1.730 E +03	8.84 E-03	8.63 E-08			
Females								
Model 1		**X**	**X**		**X**	**80.09**	**0.00**	**0.144**
Model 2	**X**	**X**	**X**		**X**	**80.39**	**0.30**	**0.124**
Model 3	**X**				**X**	**80.61**	**0.52**	**0.111**
Model 4	**X**	**X**			**X**	**80.96**	**0.87**	**0.093**
Model 5	**X**			**X**	**X**	**81.42**	**1.33**	**0.074**
Model 6	**X**		**X**		**X**	**81.63**	**1.54**	**0.067**
Model 7		**X**			**X**	**81.66**	**1.57**	**0.066**
Model 8		**X**	**X**	**X**	**X**	**82.07**	**1.98**	**0.054**
Model 9	X	X	X	X	X	82.11	2.02	0.052
Model 10	X	X		X	X	82.29	2.20	0.048
Model 11					X	82.57	2.48	0.042
Model 12	X		X	X	X	82.62	2.53	0.041
Model 13			X		X	83.00	2.91	0.034
β	-1.697*	1.209	-2.553	0.046*	-2,94E-01*		$\Sigma\ w_i = 0.95$	
UV	3.645	3.292 E+ 02	1.380 E+ 03	1.487 E-02	7.650 E-08			

* Values with an asterisk indicate cases in which the unconditional variance was smaller than the model-averaged parameter estimates, suggesting safe interpretation of β.

Figure 1: Map of the study area, Mato Grosso do Sul (MS) state, with the surveyed roads, hereafter, referred as ‘T1’, ‘T2’, ‘T3’ and ‘T4’. The city of Campo Grande was the central point for the begin of each transect: T1, along the BR-262 to the bridge over the Paraguay River (397km); T2, along the BR-262 to the Três Lagos (305km); T3, along the BR163 and BR-267 to Nova Andrandina (275km) and T4, along the MS040 to Bataguassu (300km). The flag symbol indicates the location of meteorological stations of MS.

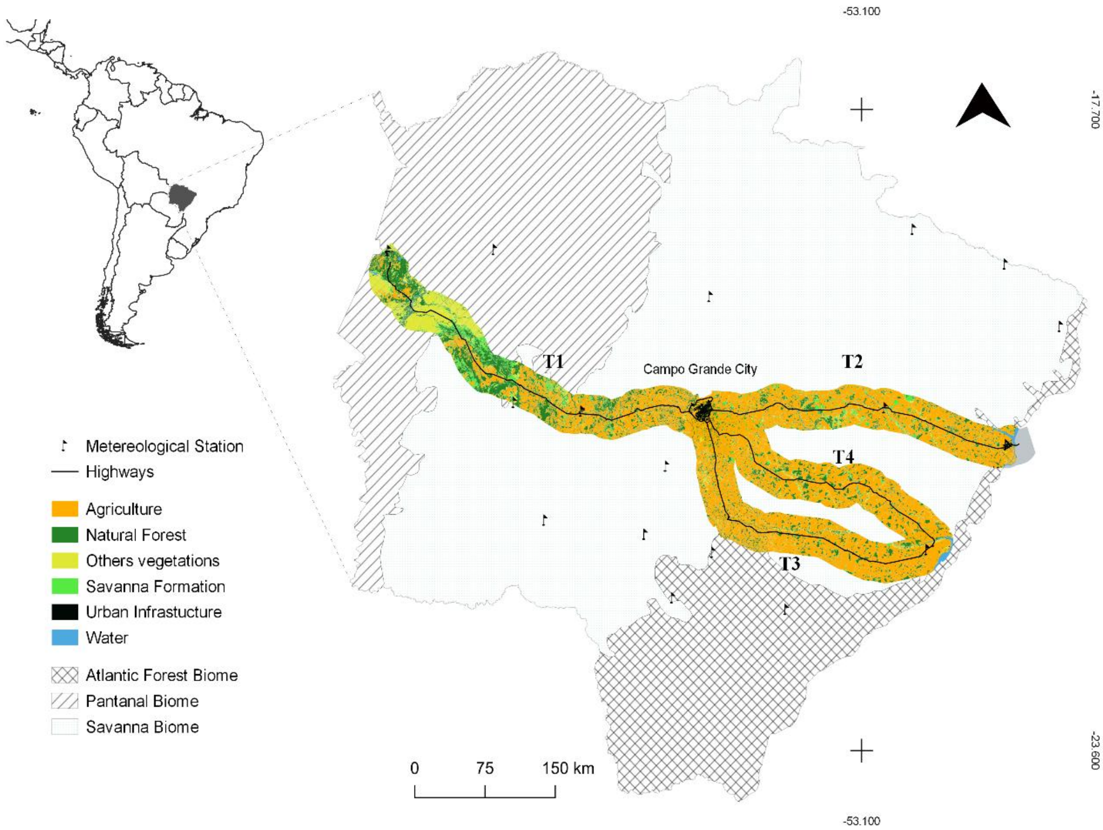

Figure 2. Number of roadkill events per evaluated transects. Numbers of roadkill (N) for summed species (A), *Myrmecophaga tridactyla* (B) and *Tamandua tetradactyla* (C). In each boxplot, the box encompasses the range of number of roadkill, the line is the median, and outliers are shown outside the white dots. The letters A and B above each bar are indicating the significant differences on roadkill between transects.

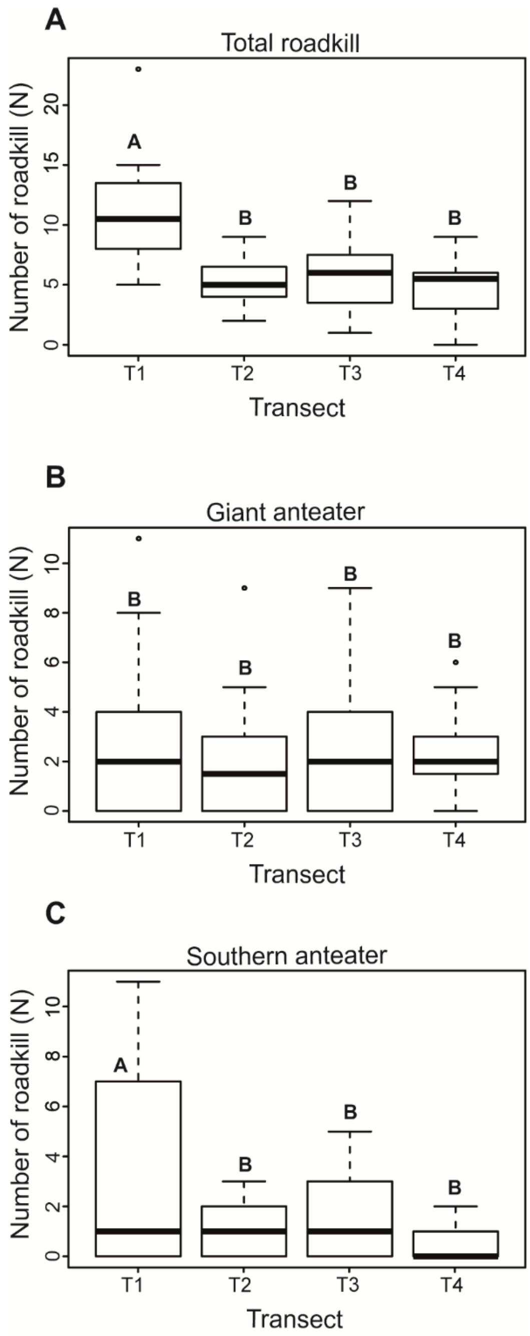

Figure 3. Monthly climatic (A) and roadkill rate for *Myrmecophaga tridactyla* (B) and *Tamandua tetradactyla* (C).

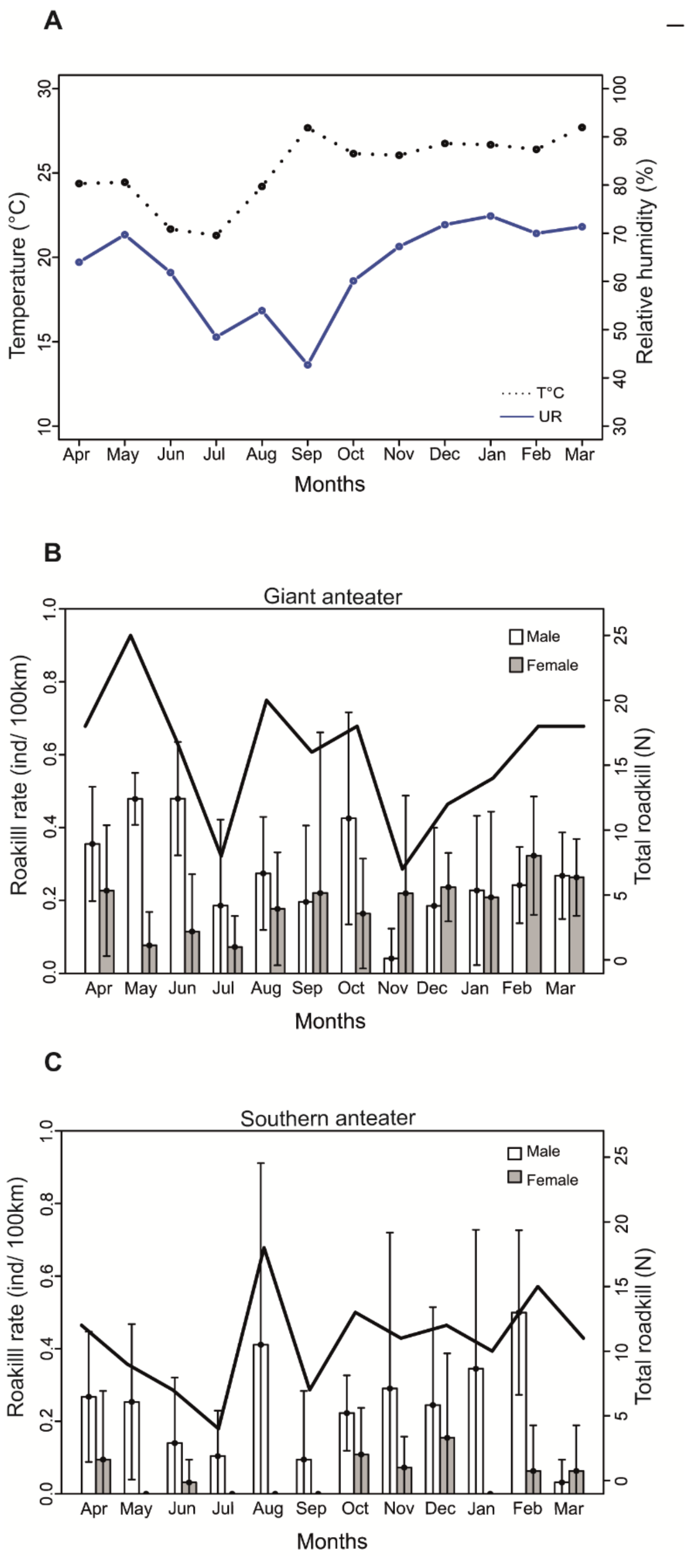

Figure 4. Roadkill hotspots along the roads studied in Mato Grosso do Sul state for giant (black) and southern (grey) anteaters.

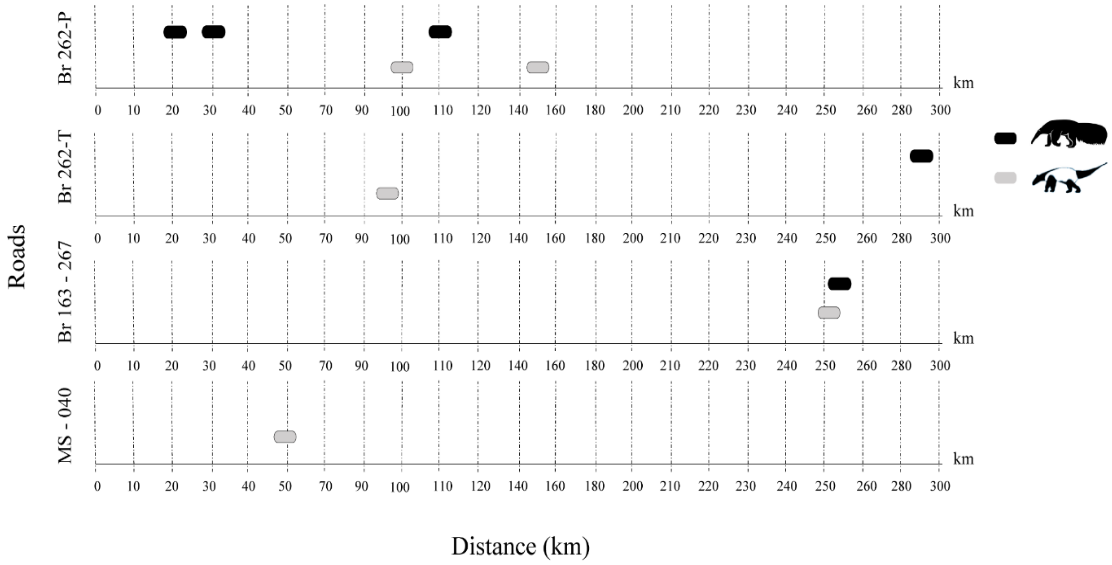

Figure 5. Relative importance of each predictor variable on the roadkill of *Myrmecophaga tridactyla* and *Tamandua tetradactyla* in Brazilian Center-Western. The predictor variables included the ΔAIC < 2 set of models (black bars) and in 95 % set of models (white bars). The importance of each variable is shown by the sum of Akaike weights (Σw_i). The sign (+/-) of Akaike weights (*w*AIC) represents the effect (positive or negative) of each predictor based on the model averaged parameters (β). Pu: Urban proportion; Dv: Density of vegetation; Pw: Proportion of water; Pv: Proportion of vegetation; T: Traffic. Bars with asterisk indicate cases in which the unconditional variance was smaller than the model-average parameter estimates.