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Research Letters

Use of unfenced highway underpasses by lowland tapirs and other medium and large mammals in central-western Brazil

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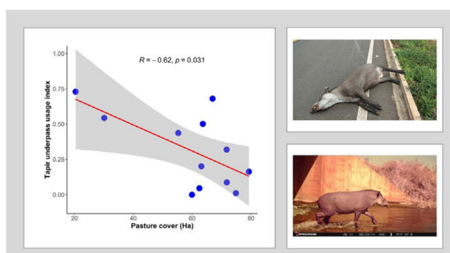
HIGHLIGHTS

- The lowland tapir was the most recorded species using the underpasses.
- Tapir underpass use summed more than 180 tons of biomass that crossed safely under the highway.
- Mammal species assemblages differed among use in cattle boxes and drainage culverts.
- Traffic activity on the highway peaked opposite to the activity peak of the mammals in the crossings.
- Countries with limited financial resources should consider retrofitting of existing highway underpasses.

GRAPHICAL ABSTRACT



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ABSTRACT

Wildlife road mortality is a worldwide problem. In tropical developing countries, it is urgent to implement and test mitigation measures to reduce wildlife-vehicle collisions, especially because financial resources are limited and alternatives should be considered such as retrofitting of existing underpasses. We assessed herein the use of 12 unfenced crossing structures, including drainage culverts and cattle boxes, by medium and large mammals along a highway in the Brazilian Cerrado. Underpass usage was monitored for 3682 trapping nights from September 2017 to May 2018. We recorded 20 species of medium and large wild mammals in a total of 4212 events. The most frequent mammals were lowland tapirs (*Tapirus terrestris*, $n = 1154$), followed by capybaras (*Hydrochoerus hydrochaeris*, $n = 910$), and crab-eating foxes (*Cerdocyon thous*, $n = 271$). Underpass usage did not differ between drainage culverts and cattle boxes, but species composition differed among the structures, suggesting that terrestrial mammals prefer cattle boxes while some semiaquatic species used only culverts. We identified 28 different individual tapirs using the underpasses and we estimated over 180 tons of tapir biomass crossing under the highway. Tapir underpass

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usage did not differ between cattle boxes and culverts, but it was negatively associated with the presence of pasture area around the structures. Our study suggests that, although underpasses show high mammal usage suggesting a satisfactory level of connectivity, combining this method with fencing would be critical to substantially decrease wildlife road mortality and increase human safety.

Introduction

Many tropical areas have been extensively modified by human activities, including the expansion of the transportation network (Gibbs et al., 2010; Foley et al., 2011; Laurance et al., 2014). At least 25 million kilometers of roads are planned to be built by 2050, of which 90% are expected to occur in developing countries, including tropical regions such as the Brazilian Amazon and Cerrado, that sustain exceptional biodiversity and provide vital ecosystem services (Dulac, 2013; Laurance et al., 2014).

For wildlife, the effects of roads and traffic include habitat loss (Forman et al., 2003; Eigenbrod et al., 2008), reduction in habitat quality in areas adjacent to the road (e.g. noise, lights, pollution, visual disturbance; Forman et al., 2003; Eigenbrod et al., 2009; Parris et al., 2009), barrier effect, including potential interruption of migration and dispersion (Nellemann et al., 2001; Vistnes et al., 2004; Lesbarrères and Fahrig, 2012), and direct mortality by collision with motorized vehicles (Forman and Alexander, 1998; Fahrig and Rytwinski, 2009).

The unnatural mortality of animals due to vehicle collisions is a primary, measurable, and obvious impact that can affect the demographic structure of populations (Mumme et al., 2000; Steen and Gibbs, 2004), reduce species abundance (Arévalo and Newhard, 2011), and create local population sinks (Nielsen et al., 2006). Most road ecology studies worldwide focus on medium and large mammals, here defined as members of eight orders that have species ≥1 kg, i.e., Artiodactyla, Carnivora, Cingulata, Didelphimorphia (family Didelphidae, tribe Didelphini), Perissodactyla, Pilosa, Lagomorpha, and large Rodentia (families Caviidae, Cuniculidae, Dasyproctidae, and Erethizontidae) (Van Niekerk and Eloff, 2005; Klöcker et al., 2006; Vidya and Thuppil, 2010; Huijser et al., 2013). Besides their important ecological roles (Cuarón, 2000; O'Farrill et al., 2013), medium and large mammals can also cause serious vehicle collisions, risking human safety and generating economic losses for drivers, passengers, and road managers (Conover et al., 1995; Bruinderink and Hazebroek, 1996; Huijser et al., 2008; Abra et al., 2019).

Collisions between mammals and vehicles have increased over the last decades. In North America, for example, wildlife-vehicle collisions increased 4-fold in the last 50 years. In the Brazilian state of São Paulo, animal-vehicle collisions jumped from about 138,000 in 2003 to 150,000 in 2012 and wild mammal road mortality increased 65% from 2009 to 2014 (Abra, 2019; Abra et al., 2019; Hill et al., 2020). In this scenario, different efforts have been carried out to justify, plan, design, and fund effective mitigation measures.

Worldwide, there are more than 40 different types of mitigation measures available, but the most common and robust measures are the combination of fences and safe crossing opportunities, such as underpasses and overpasses, which are widely used in North America and Europe (Gloyne and Clevenger, 2001; Cain et al., 2003; Clevenger and Waltho, 2005; Mata et al., 2005; Huijser et al., 2016; Gonzalez-Gallina et al., 2018). When implemented and maintained correctly, these fenced passages were found to reduce up to 97% of the large mammal–vehicle collisions, especially North-American and European ungulates (Sawyer et al., 2012; Gagnon et al., 2015; Huijser et al., 2016). Wildlife passage systems have two distinct roles: first, the fences block the animals' access to the road and thus, guide them to a safe crossing structure; second, the crossing structure has the potential to reestablish the structural and functional connectivity between habitats bisected by roads.

It is known that merely fencing the road or specific road sections can be highly effective, even reducing large mammal mortality by up to 86% (see Rytwinski et al., 2016 and references therein). However, when long sections of fences are not combined with safe crossing opportunities, they may increase the barrier effect (Jaeger and Fahrig, 2004; Rytwinski et al., 2016). It is also known that under or overpasses without adequate fences do not reduce road mortality effectively even if some individual animals may eventually learn to use them instead of crossing the highway (Rytwinski et al., 2016; Banhos et al., 2020).

Currently, knowledge, implementation such as the studies on the efficiency of mitigation measures are geographically biased toward developed countries in temperate regions. In tropical developing countries such as in Latin America, this knowledge is still under construction, considering the intrinsic aspects of the ecology of tropical wildlife and their habitats (Abra, 2012; Gonzalez-Gallina et al., 2018). Therefore, it is urgent that tropical developing countries implement and test the efficiency of mitigation measures to effectively protect their high biodiversity. Nevertheless, because developing countries have limited financial resources and trials of many measures used elsewhere can be costly, mitigation alternatives should be considered and explored, such as the use and retrofitting of existing drainage culverts, bridges, and cattle boxes (Grilo et al., 2008).

In this study, we first assessed the use of pre-existing unfenced underpasses by medium and large wild mammals in a recently paved highway (MS-040) in the Cerrado biome of central-western Brazil. In addition, we undertook specific analyzes for lowland tapirs (*Tapirus terrestris*), the most recorded species of this study. Tapirs have a double appeal in the context of reduction of mammal-vehicle collision. First, they provide important ecosystem services through seed dispersal and maintenance of biodiversity and ecosystem services (Paolucci et al., 2019). Second, in the context of road ecology, tapirs can weigh up to 300 kg, with females being larger than males (average, male = 180 kg, female = 200 kg), and have the potential to cause severe road accidents (Medici, 2011; Medici et al., 2016; Medici and Abra, 2019). A recent accident involving a tapir in the studied highway, resulted in three human deaths and one severe injury due to a collision between a van and a truck (July 8, 2020; G1, 2020). Considering the landscape configuration, we asked if differences in passage use by tapirs were related to measurable environmental variables. Ultimately, the study design allowed us to address current and basic questions related to wildlife using crossing structures in Brazil.

Methods

Study area

The study was carried out along a ca. 50 km stretch of the MS-040 highway in Mato Grosso do Sul State in central-western Brazil, between September 2017 and May 2018 (Fig. 1). This State encompasses three different biomes, the Atlantic Forest, Cerrado and Pantanal (wetland), with 166 mammal species recorded (Tomas et al., 2017). We selected this specific site based on preliminary reports categorizing this stretch of highway as a hotspot for lowland tapir roadkill (Medici et al., 2016). The MS-040 highway is 220 km long and connects the capital city of the state, Campo Grande, to

Fig. 1. Underpasses along Highway MS-040 in Mato Grosso do Sul State, Brazil.

a smaller county, Santa Rita do Pardo. The highway has two-lanes with no shoulders. The average speed limit is 80 km/h. Along its entire length, there are 52 underpasses of which 12 were monitored during this study including: seven cattle boxes, four culverts, and one bridge. All 12 underpasses are inside the main roadkill hotspot for lowland tapirs. The region crossed by the highway is dominated by cattle pastures and agriculture, as well as small patches of Cerrado vegetation and riparian forests. The climate is seasonally characterized by a rainy season from October to March, with an average annual rainfall of 1,250 mm (range 1,000–1,500 mm) and a drier period from April to September (Koppen's As or Aw).

Some studies on roadkill of medium- and large-sized mammals have been carried out on this highway, mainly by Brazilian conservation projects Lowland Tapir Conservation Initiative (LTCI) – Institute for Ecological Research (IPÊ) and "Anteaters & Highways Project" (Medici et al., 2016; Ascensão et al., 2019; Medici and Abra, 2019).

The upgrade of the MS-040 highway was completed in November 2015, turning a dirt road into a two-lane paved highway. New cattle underpasses were built to reconnect cattle herds from ranches bisected by the highway. In the environmental licensing process required for this upgrade, mitigation measures were recommended to reduce wildlife road mortality and increase human safety but have yet to be implemented.

The transportation agency responsible for maintaining the highway (AGESUL – *Agência Estadual de Gestão de Empreendimentos*) did not implement the mitigation measures and the Mato Grosso do Sul State environmental agency (IMASUL – *Instituto de Meio Ambiente de Mato Grosso do Sul*) did not investigate the non-compliance with such measures. Both agencies are currently the target of a public civil lawsuit filed by the Brazilian Attorney General's Office of Mato Grosso do Sul State in 10 million Brazilian Reais (~US$ 2.63 million) (Medici and Abra, 2019).

Underpass wildlife monitoring

A total of 12 underpasses (seven cattle boxes, four drainage culverts and one bridge) were monitored with camera-traps (Stealth Cam STC-G34, motion sensor) (Fig. 2, Table 1). We used two cameras per passage, one in each entrance on both sides of the highway, totaling 24 cameras. The cameras were placed at an approximate height of 50 cm above the ground and installed outside of the underpasses but directed towards the entrances of the structures, to allow the recording of the animals' acceptance and rejection crossings rates (Fig. 2d). The cameras were programmed to capture a sequence of nine photos and/or videos of 30 s from the detection of the animal by the sensor, with an interval of five seconds between sequences, and were kept in full-time operation for 263 days from September 2017 to May 2018. Memory cards and rechargeable batteries were replaced every 15 days.

Table 1
Characteristics of monitored underpasses along Highway MS-040 in Mato Grosso do Sul State, Brazil.

ID	Structure	WP	Width × height (m)
1	CB	No	2 × 2.5
2	DC	Yes	2.5 × 2.5
3	CB	No	3 × 3
4	Bridge	Yes	30 × 6
5	CB	No	2 × 2
6	DC	Yes	2 × 2
7	CB	No	2 × 2
8	DC	Yes	2 × 2
9	CB	No	2 × 2
10	CB	No	2 × 2
11	DC	Yes	2.5 × 2.5
12	CB	No	2 × 2

CB = cattle box, DC = drainage culvert, WP = water presence.

Tapir data collection

Camera-trap records of tapirs were carefully screened by two tapir specialists and authors of this study (EPM and ACC) for sexing, age class estimation and social organization of the animals (reproductive pairs, females with young). Whenever possible, individual tapirs were identified through unique morphological patterns in each individual (e.g. natural marks, scars and fissures on the ear, shape of the tail and pelvic girdle, shape of the head, extension, and height of the mane). During the monitoring of the underpasses, we also recorded the number of opportunistic sightings of live tapirs and road killed tapir carcasses along highway MS-040.

Fig. 2. Types of underpasses monitored along Highway MS-040 in Mato Grosso do Sul State, Brazil: (a) drainage culvert, (b) cattle box, (c) bridge and (d) view of drainage culvert, red circle shows the installation of the camera-trap outside of the structure.

Traffic volume counts

As there are no official traffic volume counts for highway MS-040, we carried out vehicle counting following the Federal Transportation Agency methodology (DNIT, 2020). We undertook 15 sessions of data collection at three different points along the highway (km 28, km 100 and km 150), including five 24-hour periods in each point. Data were collected on weekdays, weekends, and holidays. The averaged traffic volume data was compared to the usage time of wildlife in the underpasses.

Data analyses

We quantified the use of crossing structures by mammals using descriptive analyses. The usage index was calculated by summing the number of complete and unknown crossings by a given mammal species divided by the number of days the passage was monitored. We considered independent events to be consecutive photographs or videos of the same species separated by at least 1 h (Tobler et al., 2008).

We classified the events in the underpasses in three categories: (i) complete crossing, when it was possible to confirm the entrance and exit of the same individual by the two camera traps; (ii) unknown crossing, when animals were recorded by at least one of the cameras with a clear behavior of entering and leaving the underpass; (iii) no-crossing, when the presence of individuals was recorded but with a clear behavior that the animal did not cross the underpass.

Alpha diversity indices were calculated using the independent events. We calculated the species richness (Sobs) ^{0}D, Shannon's exponential index (e^H) ^{1}D, and the Inverse Simpson's index (1/D) ^{2}D for all camera trap stations and compared the indexes between the cattle boxes and culverts.

For tapir-specific analyzes, including complete crossings and unknown crossings, the 12 sampled passages were divided in three categories: culverts, cattle boxes, and bridge. Culverts and cattle boxes were compared in relation to the tapir usage index using Wilcoxon rank sum tests ($P < 0.05$).

We calculated the estimated tapir mass that used the underpasses, including complete and unknown crossings, during the study period, using the mean body mass for adults (male = 180 kg, female = 200 kg), subadults (100–180 kg), and juveniles (70 kg) based on the dataset of the Lowland Tapir Conservation Initiative.

The relationship between complete and unknown crossings, and the detection by camera traps were also used to determine the presence and diversity of user species, as well as the anthropic influence.

To verify the influence of landscape attributes on the usage index of tapirs, we used every structure ($n = 11$) except for the bridge, which we excluded because it had significantly different dimensions. We measured the following landscape attributes (in hectares) within a 1 km radius of each underpass: pasture area, native forest area, and grassland area (MapBiomas Project Collection 4.1; 30 m resolution; using ArcGIS ESRI, 2015). To verify the influence of domestic livestock on the passage of tapirs, we also quantified the usage index of cattle.

To analyze the usage index as a function of the covariates, we conducted four pairwise linear regression analyses, as our response variable (usage index) had a Gaussian distribution: 1) tapir usage index on forest cover (continuous), (2) tapir usage index on pasture cover (continuous), (3) tapir usage index on grassland cover (continuous), (4) tapir usage index on cattle usage index (rate).

Results

Underpass wildlife monitoring

From September 2017 to May 2018, we carried out 3,682 trapping nights and recorded 20 species of medium- and large-sized wild mammals visiting the underpasses (Table 2, Appendix 1).

Considering only independent events, a total of 4,212 individuals were recorded, for a rate of 114.39 events/100 trap nights.

Table 2
Mammal use of underpasses along Highway MS-040 in Mato Grosso do Sul State, Brazil. The crossing rate was calculated for both complete and unknown crossings.

Mammal species	Crossings						
	Threat category (trend)	Usage index	Type of passages used	NC_C (*n*, %)	U_C (*n*, %)	C_C (*n*, %)	N° Ind. (*n*, %)
Lowland tapir *(Tapirus terrestris)*	VU (▼)	0.27	CB, DC, B	146; 5.67%	510; 19.80%	498; 10.33%	1,154; 44.80%
Capybara *(Hydrochoerus hydrochaeris)*	LC (—)	0.20	DC, B	155; 6.02%	538; 20.89%	217; 8.42%	910; 35.33%
Crab-eating fox *(Cerdocyon thous)*	LC (—)	0.06	CB	34; 1.32%	158; 6.13%	79; 3.07%	271; 10.52%
Giant anteater *(Myrmecophaga tridactyla)*	VU (▼)	0.02	CB, B	6; 0.23%	57; 2.21%	35; 1.36%	98; 3.80%
Yellow armadillo *(Euphractus sexcinctus)*	LC (—)	0.0081	CB	6; 0.23%	27; 1.05%	3; 0.12%	36; 1.40%
Azara's agouti *(Dasyprocta azarae)*	DD (▼)	0.0065	CB,	4; 0.16%	24; 0.93%	0; 0%	28; 1.09%
Crab-eating racoon *(Procyon cancrivorus)*	LC (▼)	0.0057	CB, DC, B	4; 0.16%	9; 0.35%	12; 0.47%	25; 0.97%
Ocelot *(Leopardus pardalis)*	LC (▼)	0.0030	CB	1; 0.04%	9; 0.35%	2; 0.08%	12; 0.47%
Maned wolf *(Chrysocyon brachyurus)*	NT (?)	0.0011	CB	2; 0.08%	2; 0.08%	2; 0.08%	6; 0.23%
Water opossum *(Chironectes minimus)*	LC (▼)	0.0011	DC	1; 0.04%	4; 0.16%	0; 0%	5; 0.19%
Nine-banded armadillo *(Dasypus novemcinctus)*	LC (—)	0.0014	CB	0; 0%	5; 0.19%	0; 0%	5; 0.19%
Southern naked-tail armadillo *(Cabassous unicinctus)*	LC (?)	0.0011	CB	0; 0%	4; 0.16%	0; 0%	4; 0.16%
Pantanal cat *(Leopardus braccatus)*	NA(NE)	0.0011	CB	0; 0%	4; 0.16%	0; 0%	4; 0.16%
Giant armadillo *(Priodontes maximus)*	VU (▼)	0.008	CB, B	1; 0.04%	2; 0.08%	1; 0.04%	4; 0.16%
Tayra *(Eira barbara)*	LC (▼)	0.003	CB	2; 0.08%	0; 0%	1	3; 0.12%
Neotropical otter *(Lontra longicaudis)*	NT (▼)	0.008	DC	0; 0%	3; 0.12%	0; 0%	3; 0.12%
Gray brocket deer *(Mazama gouazoubira)*	LC (▼)	0	-	3; 0.12%	0; 0%	0; 0%	3; 0.12%
Southern tamandua *(Tamandua tetradactyla)*	LC (?)	**0.008**	CB	0; 0%	3; 0.12%	0; 0%	3; 0.12%
Southern American Coati *(Nasua nasua)*	LC (▼)	0.003	CB	0; 0%	1; 0.04%	0; 0%	1; 0.04%
Collared peccary *(Pecari tajacu)*	LC (—)	0	-	1; 0.04%	0; 0%	0; 0%	1; 0.04%
Total				366; 14.21%	1,360; 52.80%	850; 33%	2,576; 100%

NC_C = not completed crossing, U_C = unknown crossing, C_C = completed crossing, N° Ind. = number of individuals, VU = vulnerable, LC = least concern, DD = data deficient, NT = near threatened, NA = not assessed. Population trend: ▼= decreasing, — = stable and **?** = unknown, NE = not evaluated. Type of passages used: CB = cattle box, DC = drainage culvert, B = bridge.

Records included wild mammal species (n = 2,666; 63.3%), domestic species (n = 883; 20.97%), and humans (n = 663; 15.7%). The most frequent wild mammal was the lowland tapir (n = 1,154; 44.8%), followed by the capybara (n = 910; 35.33%), and the crab-eating fox (n = 271; 10.52%) (Fig. 3). Two species, the coati (*Nasua nasua*) and collared peccary (*Pecari tajacu*), were recorded only once during the study period (Table 2). Three of the recorded species, i.e., lowland tapir, giant armadillo (*Priodontes maximus*), and giant anteater (*Myrmecophaga tridactyla*), are classified as "Vulnerable" to extinction in both the Brazilian Red List and the IUCN Red List of Threatened Species (Medici et al., 2018; IUCN, 2019). Our survey recorded 33% of the threatened mammals that potentially occur in the study area (Tomas et al., 2017).

Mean underpass usage index for all wild mammal species lumped together was 0.47, varying from 0.26 for tapirs to zero for gray brocket deer (*Mazama gouazoubira*) and collared peccary, which were recorded by the cameras but did not enter the culvert (Table 2). The Wilcoxon rank sum test did not recover a statistically significant difference (W = 7, P = 0.2, r = 0.39) between the number of individuals using culverts and cattle boxes (Fig. 4). However, overall species diversity was significantly higher at cattle boxes, considering both species richness (W = 27, P = 0.017, r = 0.75) and proportional abundances, as inferred by Shannon's exponential (W = 28, P = 0.006, r = 0.79) and Inverse Simpson' index (W = 27, P = 0.01, r = 0.74; Fig. 5).

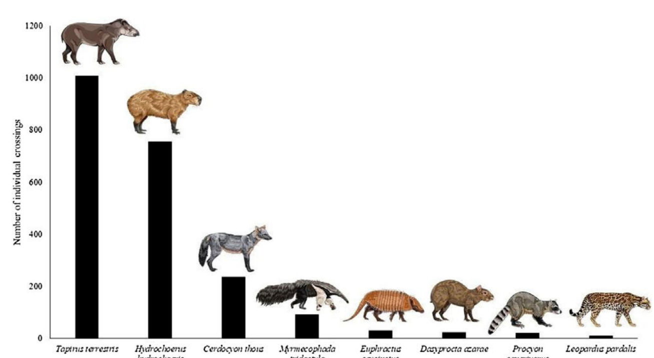

Fig. 3. Relative frequency of medium- and large-sized mammals sampled at the 12 monitored underpasses along Highway MS-040 in Mato Grosso do Sul State, Brazil. Only species with ≥10 independent records are shown.

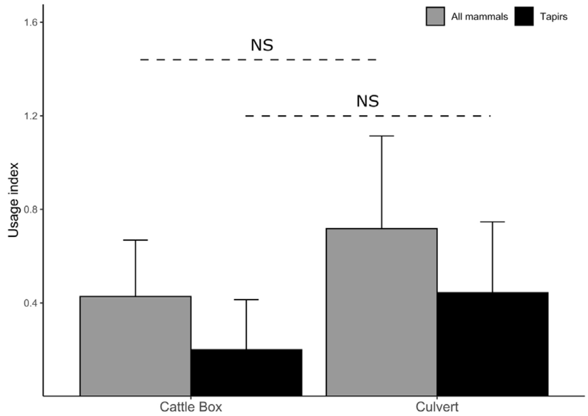

Fig. 4. Usage index of all 18 species of wild mammals and of only tapirs at both crossing structures analyzed along Highway MS-040 in Mato Grosso do Sul State, Brazil. Bars indicate mean values for each structure and lines are the mean + standard deviations. NS = non-significant ($P > 0.05$).

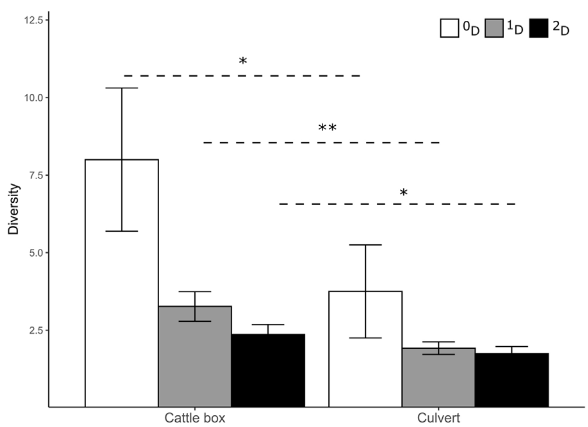

Fig. 5. Medium- and large-sized mammal diversity profile for two types of crossing structures analyzed along Highway MS-040 in Mato Grosso do Sul State, Brazil. ^0D = species richness, ^1D = Shannon's exponential index, ^2D = Inverse Simpson. * = significant for $P < 0.05$; ** = significant for $P < 0.01$.

Despite the differences in diversity of species between cattle boxes and culverts, some species were recorded only in culverts and vice-versa. The semi-aquatic river otter (*Lontra longicaudis*) and water opossum (*Chironectes minimus*), for example, were recorded only in culverts, while terrestrial wild canids and felids, were recorded only in cattle boxes (Table 2).

Traffic volume × mammal crossing time

Vehicle traffic was high between 6 am and 6 pm with peaks at 8 am and 5 pm, being the activity during this period amounting to 82.62% of the total traffic volume in MS-040 (Fig. 6). The activity period of medium- and large-sized mammals, including tapirs, peaked in opposite times of traffic volume. For mammals in general, the peak was at 4 am and 6 pm, while for tapirs it was between 3–5 am and 7–8 pm.

Tapir crossing analyses

Through photo-identification, it was possible to recognize 28 individual tapirs, of which 25 were adults, two juveniles and one calf. Among these, 12 females and 16 males were identified. Tapirs are primarily solitary, although it is common to sight reproductive pairs and females with offspring. Tapirs usually produce a single offspring after a lengthy gestation period of 13–14 months (390–410 days) and the calf stays with its mother for 12–18 months (Medici, 2011). Both male and female tapirs reach sexual maturity at four years of age. The tapir life span in the wild is estimated to be 20–25 years (Medici and Desbiez, 2012). During this study, five reproductive pairs and four females with calves were detected. Tapirs are wide-ranging species, and this methodology has allowed us to observe that at least seven individuals used more than one underpass, in which cases the structures were close or adjacent to each other (ca. 2 km). Moreover, the bridge was used by at least eight different tapir individuals followed by underpasses ID-7 and ID-8 which were used by seven different tapir individuals each.

In 11 of the 12 structures, tapirs crossed to the opposite side of the highway. Although there were no differences between the usage of cattle boxes and culverts ($W = 14$, $P > 0.1$, $r = 0.45$; Fig. 4), some passages were more intensely used than others (Fig. 7). The 2,210 tapir crossing events represent a total of 186,010 kg of tapirs using the structures ($\bar{x} = 15{,}500$ kg, $\pm 13{,}129$), ranging from 36,480 kg in a single underpass to zero. Tapir usage was most intense between km 120 and km 140 of the MS-040 highway.

Univariate regressions suggest that the usage index of the passages by tapirs was negatively correlated with the area of pasture around the underpass which represents 79% of the land use (Fig. 8). The index of underpass usage by tapirs also had a weak negative correlation with the cattle usage index, and a weak positive association with the area of forest and native grasslands around the structures which correspond to 19% of land use (Fig. 8).

During the study period, we recorded seven direct tapir sightings along the monitored highway section. In most cases, the animals crossed the highway using the pavement (Fig. 9a). In addition, we recorded 21 road-killed tapirs in the same period along highway MS-040, of which nine were found within the 50 km of the monitored highway section (Fig. 9b).

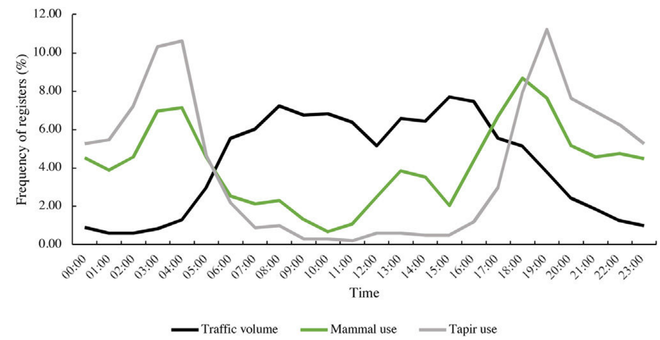

Fig. 6. Frequency of records for vehicle counting and complete and incomplete crossing of wild mammals and tapirs based on traffic counts made between September and December 2017 along Highway MS-040 in Mato Grosso do Sul State, Brazil.

Discussion

This study shows that unfenced underpasses are suitable for crossings by large and medium-sized mammals, especially tapirs, from the point of view of the maintenance of connectivity. Our results suggest the possibility of retrofitting the existing underpasses, which were originally built for other purposes than wildlife crossings, i.e., for water drainage and for cattle passage (Grilo et al., 2008; Huijser et al., 2013). The main advantage of using these passages is that they are cost-effective for highway managers, as the structures have already been built. Drainage culverts are necessary to route water under the road, cattle boxes are required by local landowners to provide connectivity for domestic animals and people. Primarily for developing countries, the retrofitting of existing underpass structures can be valuable in encouraging highway

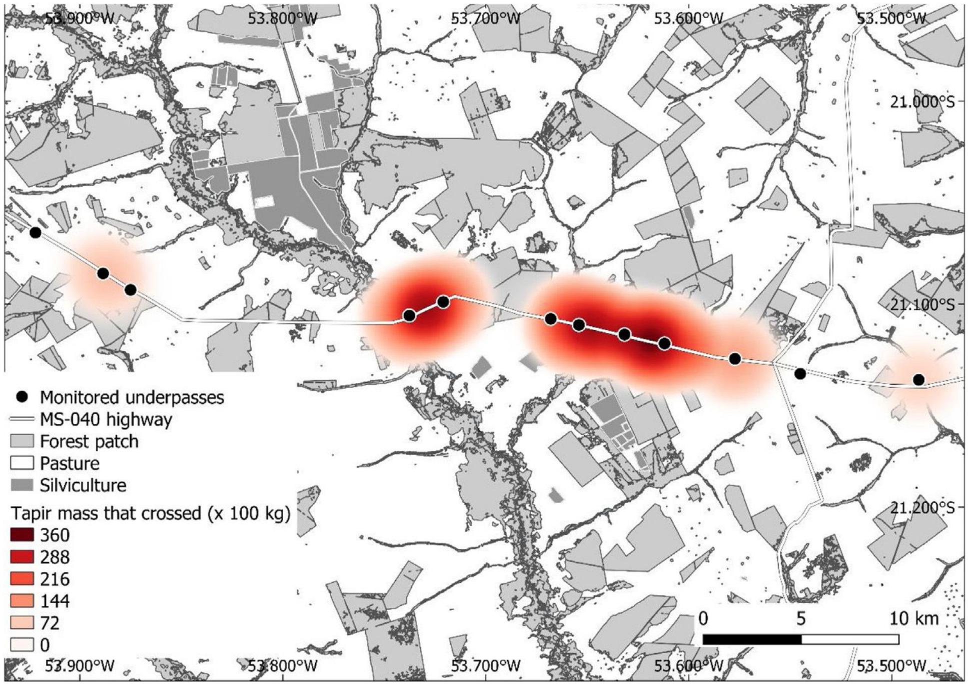

Fig. 7. Heatmap of the 12 monitored underpasses along Highway MS-040 in Mato Grosso do Sul State, Brazil, showing the amount of tapir mass (kilograms) that used the structures.

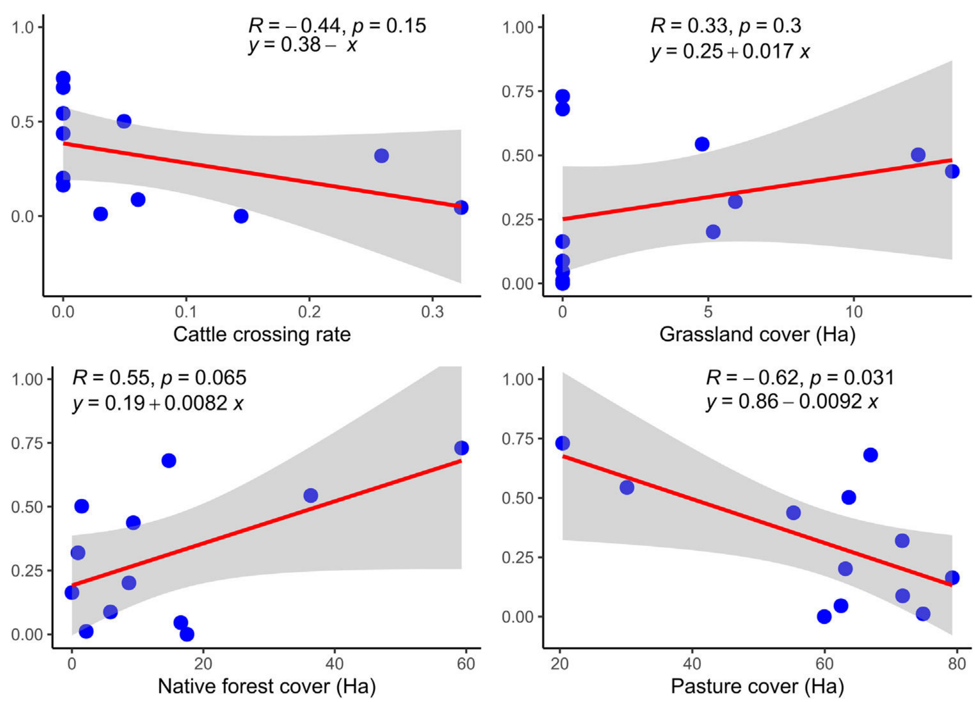

Fig. 8. Univariate regressions of tapir usage index (response variable), and four independent variables.

managers to implement measures aimed at reducing wildlife road mortality.

The lowland tapir was the most recorded species using the underpasses in our study. All tapir individuals registered using the underpasses summed more than 180 tons of biomass that crossed safely under the highway. This means that passages are already being used and have certainly reduced the number of tapirs on the lanes and increased traffic safety. However, because the highway is not fenced, it is not possible to verify if tapirs were using the underpasses more than crossing on the highway. In order to efficiently reduce road mortality, it is highly recommended to install fences in combination with underpasses, as studies suggest that this measure can reduce up to 86% of mammal-vehicle collisions as demonstrated in Highway 93 in Montana, United States (Huijser et al., 2016) and other regions in North America and Europe (see Rytwinski et al., 2016 and references therein).

Underpasses close to natural Cerrado fragments and bodies of water were more relevant for tapir crossings. These areas reflect a more appropriate tapir habitat (Medici, 2011), as the study site is largely dominated by agriculture and pasture. In Mato Grosso do

Fig. 9. (a) Lowland tapir crossing Highway MS-040, and (b) Tapir roadkill on the same highway. Source: Laurie Hedges, INCAB-IPÊ.

Sul State, cattle management demands human presence and both studied variables (pasture area and cattle usage index) were negatively associated to tapir crossings, corroborating previous studies (Land and Lotz, 1996; Clevenger and Waltho, 2000; Ng et al., 2004).

Even though our findings did not detect differences in the number of individuals using drainage culverts or cattle boxes, species composition differed among the structures, suggesting that, in general, terrestrial mammals prefer cattle boxes while some semi-aquatic species such as capybara, water opossum and river otter rarely venture in dry passages. Both types of underpasses have the same approximate dimensions (~2 x 2 m) and based in our results their size seems suitable for species registered more frequently in the structures as lowland tapir, capybara, crab-eating fox, giant anteater, and yellow armadillo, among others. Some species, namely collared peccary, gray brocket, coati, neotropical otter and southern tamandua, had only a few records but are not considered rare in the study area, and some of them are even recorded in roadkill reports (Ascensão et al., 2017, 2019). Moreover, some of these least common species in our study area were frequently reported in other underpasses studies in central-western São Paulo state, Brazil (Abra, 2012). The coati, for example, accounted for 8.8% of all mammal crossings in the São Paulo study site (Abra unpublished data). Because we had data for one bridge only, it was not statistically possible to include it in the analysis, but the number of individuals and species richness recorded suggest that the bridge is a very important structure for wildlife use. As bridges cross over large water bodies, they are usually associated with riparian vegetation, a type of habitat widely used as ecological corridors in fragmented landscapes for wildlife dispersal, foraging and shelter (Lees and Peres, 2008). Riparian forests are one of the most important habitats for tapirs (Medici, 2010).

The peak of traffic activity on the MS-040 highway was between 6 am and 6 pm, quite the opposite of the activity peak of the mammals (including tapirs) recorded in the crossings. Specifically, for tapirs, the time of use in the underpasses matches the activity patterns previously recorded for the species in Brazil, Bolivia, and Peru (Noss et al., 2003; Tobler, 2008; Medici, 2010; Medici, 2011). In general, medium and large mammal species show nocturnal and crepuscular activity but it is tempting to suggest that some species may be avoiding the peak traffic activity along the highway due to noise, motion, and visual disturbance (Gaynor et al., 2018).

Although the underpasses seem to be suitable to maintain wild mammal permeability and safeguard a satisfactory habitat connectivity, we suggest that the use of fences would be critical to further decrease mortality of both humans and wildlife. The need to implement fences becomes more urgent when medium and large mammals routinely use areas adjacent to highways, as is the case in our study area. To increase the diversity of medium and large mammals using the underpasses, we recommend implementing distinct types of underpasses along the roads, as some semiaquatic species depend on water bodies to disperse and some terrestrial mammals prefer dry structures. Evidently, it is highly recommended that such measures are monitored to ensure accurate information about their efficiency in reducing road mortality and increasing connectivity for these species.

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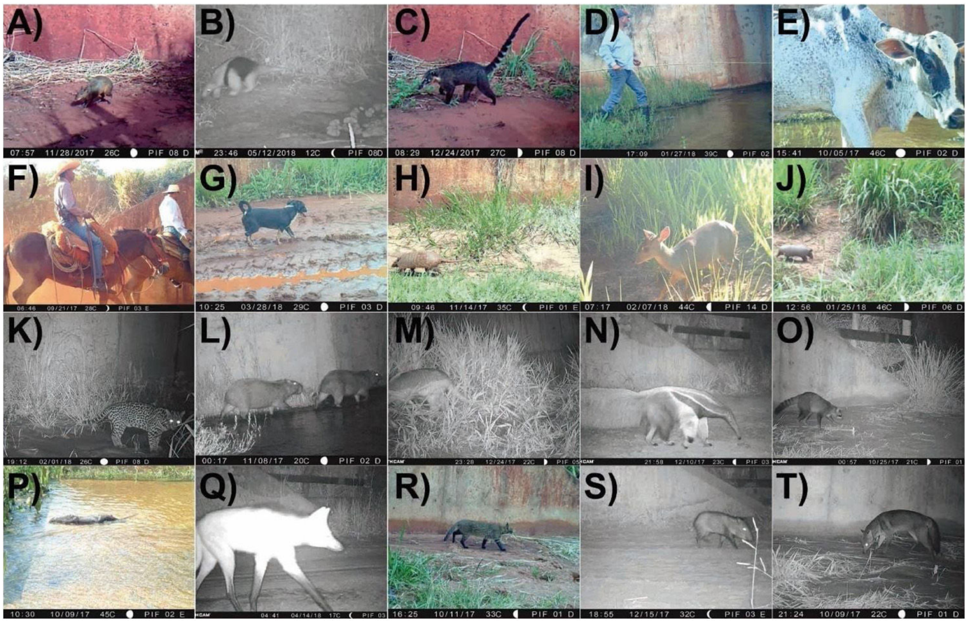
Appendix 1.

Mammal species recorded in the underpasses along Highway MS-040 in Mato Grosso do Sul State, Brazil: a. Azara's agouti (*Dasyprocta azarae*), b. Southern tamandua (*Tamandua tetradactyla*), c. South American coati (*Nasua nasua*), d. Human use, e. Cattle (*Bos indicus*), f. Horse (*Equus caballus*), g. Domestic dog (*Canis lupus familiaris*), h. Yellow armadillo (*Euphractus sexcinctus*), i. Gray brocket deer (*Mazama gouaboubira*), j. Southern naked-tail armadillo (*Cabassous unicinctus*), k. Ocelot (*Leopardus pardalis*), l. Capybara (*Hydrochoerus hydrochaeris*), m. Giant armadillo (*Priodontes maximus*), n. Giant anteater (*Myrmecophada tridactyla*), o. Crab-eating racoon (*Procyon cancrivorus*), p. Neotropical otter (*Lontra longicaudis*), q. Maned wolf (*Chrysocyon brachyurus*), r. Pampas cat (*Leopardus braccatus*), s. Collared peccary (*Pecari tajacu*), t. Crab-eating fox (*Cerdocyon thous*).



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