







## Research Letters

## Increase in the use of crossing structures does not mean a decrease in roadkill numbers



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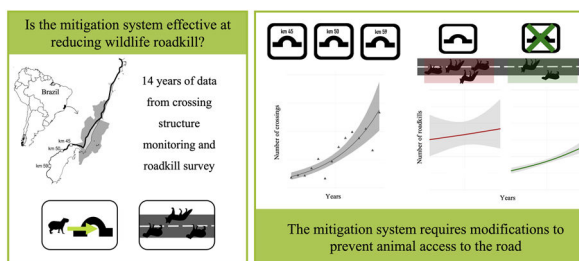
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## HIGHLIGHTS

- Roads threaten wildlife by causing roadkill and reducing connectivity.
- We analyzed data on roadkills and wildlife crossings usage from 14 years of daily monitoring.
- The number of wildlife crossings at mitigation structures increased over time.
- Roadkill numbers have increased over the years, even in mitigated segments.
- Wildlife crossings structures are likely to fail to reduce roadkill due to absence of proper fencing.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Roads significantly impact wildlife by increasing mortality and reducing connectivity. Wildlife crossing structures and associated fencing are used worldwide to increase habitat connectivity and reduce roadkill. We assess the effectiveness of mitigation systems composed of clusters of crossing structures and fences in southeastern Brazil, based on 14 years of data for ground-dwelling species (including medium-to-large mammals, large reptiles and a bird). We analyzed whether crossing and roadkill records changed over the years and if roadkill hotspots were eliminated on segments where mitigation measures were installed. Results showed a significant increase in crossing structure usage over time in all mitigation clusters. However, roadkill persisted in mitigated segments, remained higher than in non-mitigated segments, and roadkill hotspots overlapped all mitigation clusters. These results indicate that crossing structures alone do not sufficiently reduce road mortality without appropriate fencing to prevent road access. Our findings highlight the need for well-designed mitigation strategies, reinforcing that fencing quality and extent are critical for reducing roadkill.

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**Introduction**

Roads impact wildlife by increasing mortality and reducing connectivity and habitat quality (Teixeira et al., 2020). Wildlife-vehicle collisions and the barrier effect of roads can limit or impede animal movement between roadsides, affecting population dynamics (Ceia-Hasse et al., 2018; Jackson and Fahrig, 2011). To minimize these impacts, different mitigation measures are being implemented around the world. For example, wildlife crossing structures have been shown to allow animal movement across roads (Soanes et al., 2024) and fences have been reported to reduce roadkill and guide individuals to these structures (Rytwinski et al., 2016).

The evaluation of mitigation effectiveness is critical to assess if measures are fulfilling their purpose and, when ineffective, to understand the reason and explore adaptations (Smith et al., 2015). For example, the function of culverts as wildlife crossing structures could be improved by adding a dry ledge inside them (Villalva et al., 2013), while longer fence extensions are more effective than short sections to reduce roadkill (Huijser et al., 2016). Also, evaluating effectiveness requires aligning appropriate measures with the specific objectives of the adopted mitigation structures. If the goal is to reduce mortality, then roadkill numbers should be used to assess mitigation effectiveness. Relying solely on crossing records will not reflect the system's effectiveness for mortality (van der Grift et al., 2013). Therefore, assessing mitigation effectiveness requires a goal-oriented approach, utilizing the best available study designs and measures.

Study designs that have been used to assess effectiveness of mitigation efforts include control-impact (CI), before-after (BA), and before-after-control-impact (BACI) (Christie et al., 2019). BACI is the preferable design as it allows for control of confounding factors that could influence the response variable beyond the treatment of interest (Christie et al., 2019; Roedenbeck et al., 2007). However, CI designs are the alternative when data for the before mitigation stage are unavailable, whether due to poor planning or logistic restrictions. In these situations, using multiple temporal replicates helps counteract the

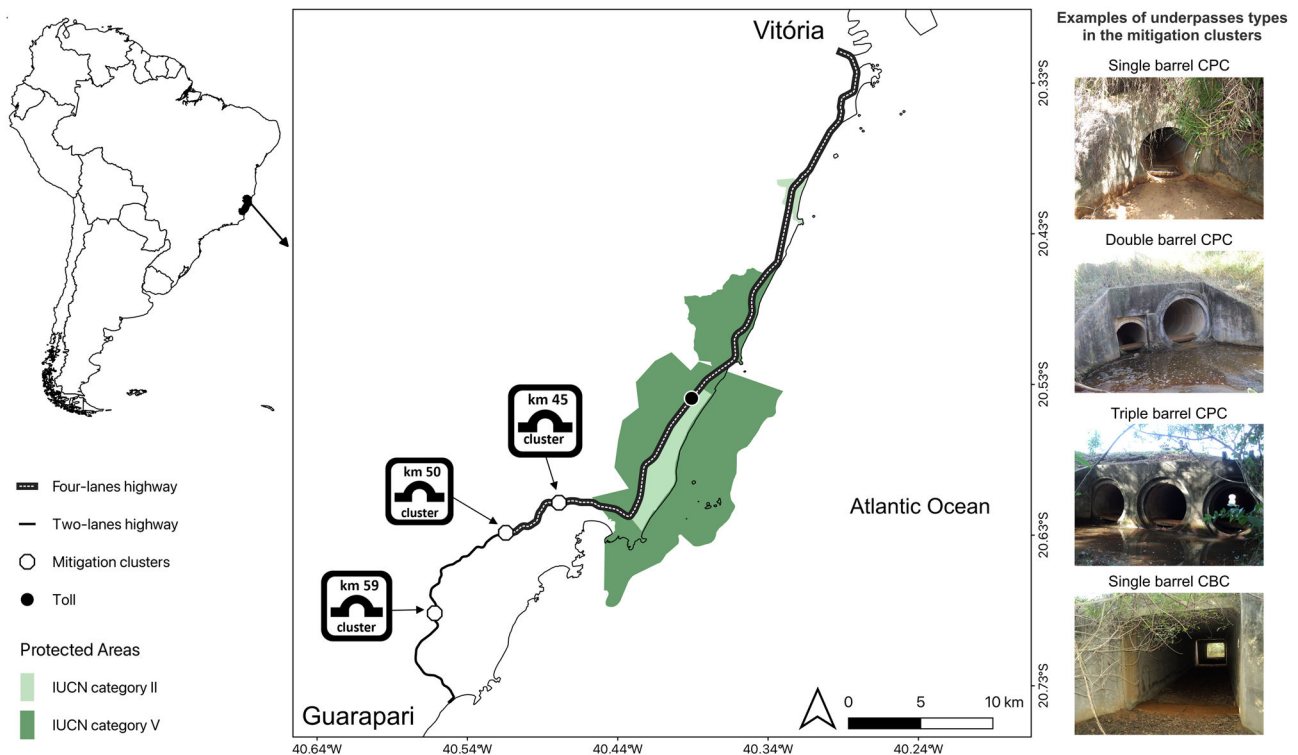
limitations of the CI design, controlling fluctuations and stochastic factors (after-control-impact cf. De Palma et al., 2018).

We evaluate a mitigation system composed of three clusters of multiple wildlife crossing structures and fences installed during improvements to a coastal toll highway in southeastern Brazil to reduce wildlife road mortality. We analyze the use of wildlife crossing structures and roadkill data of ground-dwelling species weighing more than 1 kg, including medium-to-large mammals, reptiles (boid snakes, tegu lizard and broad-snouted caiman), and red-legged seriemas, collected daily over 14 years. Our aim was to assess whether the crossing and roadkill records (number and hotspot locations) changed over the years. We expected a gradual increase in the number of crossings, given the known time lag in habituation behavior for newly mitigation measures (Bond and Jones, 2008; Clevenger and Waltho, 2003; van der Grift et al., 2013). Furthermore, if fences were effective in blocking animal access to the road, we should expect a significantly lower number of roadkill records on mitigated road segments compared to those on non-mitigated road segments and the disappearance of roadkill hotspots on mitigated segments (Rytwinski et al., 2016).

**Materials and methods**

*Study area*

This study was carried out on the ES-060, a 67.5 km highway that connects the municipalities of Vitória (−20.3138°, −40.2904°) and Guarapari (−20.7394°, −40.5515°) in the state of Espírito Santo, Southeastern Brazil (Fig. 1). ES-060 crosses four protected areas; two of IUCN category II – National Park (Jacarenema and Paulo Cesar Vinha Parks, World Database of Protected Areas IDs: 555576222 and 555682160) and two of IUCN category V – Protected landscape/seascape (Lagoa Grande and Setiba Environmental Protection Areas, World Database of Protected Areas IDs: 555576215 and 555636625) (Dudley, 2008; IUCN, 2024). The measured average traffic at a toll located halfway between Vitória and Guarapari (Fig. 1) was around 10,



**Fig. 1.** Location of highway attributes (toll, lane number, and mitigation clusters) and protected areas along the ES-060 highway, including examples of the underpasses. CPC means “concrete pipe culvert” and CBC means “concrete box culvert”.

700 vehicles/day from 2009 to 2017 (ES, 2023).

ES-060 was under concession between 2000 and 2023, meaning that the responsibility for management, operation and maintenance was transferred from the government to a private company, the Rodovia do Sol S/A concessionaire. Between 2000 and 2003 two highway improvements were implemented: the highway was widened from two to four lanes over 38 km (km 12–50), and a two-lane 17.5 km extension was added expanding the highway length from 50 km to 67.5 km. During these constructions, culverts and bridges, expected to also function as wildlife crossing structures (Taylor and Goldingay, 2003; Young et al., 2023), were installed in three locations hereafter identified as km 45 cluster, km 50 cluster, and km 59 cluster (Fig. 1). As there were no previous roadkill data, the installation of wildlife crossing structures was carried out based on technical opinion, prioritizing road sections between vegetation remnants also considering engineering constraints. Each mitigation cluster has a different number of structures with different attributes (detailed information on each mitigation cluster is available in the Supplementary material (SM) – Table 1) and fences installed to guide animals to use the crossing structures. Variations on fence types and extensions in each cluster were due to local terrain characteristics. The km 45 cluster is composed of a bridge and seven structures (four single barrel, one double barrel, and two triple barrel culverts) installed from km 44.2 to km 46.4 to allow wildlife crossings and to connect remnants of restinga (coastal sandy soil vegetation) and mangroves. It contains two types of fences implemented at both sides of the road with height of 1.2 m: a galvanized welded fence with 15 cm horizontal wire opening, 6 lower wires with 10 cm between wires and 3 upper wires with 20 cm between wires (1880 m on southbound roadside and 969 m on northbound roadside), and a chain link fence with 7.6 cm and 14-gauge wire (length of 460 m on southbound roadside and 464 m on northbound roadside). The km 50 cluster is composed of two single barrel concrete pipe culverts installed primarily for drainage purposes. It contains a galvanized welded fence of 1.2 m in height, covering 400 m on the north-south roadside. The km 59 cluster has three structures (one single barrel and two double barrel concrete pipe culvert) installed from km 59.7 to km 60.2 as wildlife crossings, and it contains galvanized welded fence (also 1.2 m in height), covering 800 m on both roadsides. Both km 50 and km 59 clusters connect restinga remnants on both sides of the road.

#### Data collection

We used a dataset collected by the private concessionaire Rodovia do Sol S/A after highway improvements. Data were collected in an environmental monitoring program created as part of the conditions required during the environmental permitting process of the ES-060 toll road.

The data used include ground-dwelling species larger than 1 kg, aiming to reduce detection errors in both wildlife crossing structures and roadkill surveys (Barrientos et al., 2018) and reduce weakness of the assumption of spatial and temporal homogeneity in detection. These are also the species expected to be directly benefited by the fence and culvert types adopted as mitigation structures in this highway. We considered ground-dwelling species any species that predominantly or frequently moves on the ground, including medium-to-large mammals, tegu lizards (*Salvator merianae*), broad-snouted caiman (*Caiman latirostris*), and red-legged seriema (*Cariama cristata*), the only included bird. We grouped the records of the large snakes *Boa constrictor*, *Epicrates cenchrus* and *Corallus hortulanus* species as Boidae (or boids), and of *Dasytus novemcinctus*, *D. septemcinctus* and *Euphractus sexcinctus* species as Dasypodidae (or armadillos), due to the possibility of misidentification of sympatric species from each family based on sand bed tracks.

To assess the use of crossing structures by wildlife, sand track beds were installed at each entrance with sandy clay soil to record animal tracks, dimensioned to fit the width of the crossing structure (SM – Fig. 1) (Hardy et al., 2003; Mystajek et al., 2020). Sand beds were

checked every working day by a specialized technician between January 2004 and December 2017. Eight structures (out of a total of 13 structures across the three clusters) were monitored on the same days, while the other five crossing structures had a constant presence of water and could not be surveyed (SM – Table 1). Even structures primarily set up for drainage were surveyed with sand beds, as they could also be used as a crossing structure by wildlife (Taylor and Goldingay, 2003; Young et al., 2023). For each track, we recorded the structure ID, date, species and movement trajectory (if inwards or outwards; SM – Fig. 1). After recording the tracks, the sand beds were raked to clear previous marks. Crossings were considered successful only when tracks of the same species were registered at both entrances of the structure on the same occasion, indicating the same movement direction and a complete crossing (inwards at one side and outwards at the other).

Roadkill data were obtained by car surveys carried out by one road inspector, due to a condition imposed by the environmental permitting process (operation license). Surveys covered the entire road extension (67.5 km) every 3 h, at a speed of 60–80 km/h, between January 2004 and December 2017. The road was divided into segments of 500 m, and for each carcass, the inspector recorded the date and segment ID. All carcasses were collected and sent to the Rodovia do Sol Fauna Laboratory, stored in freezers, and then identified by specialists to the lowest possible taxonomic level.

#### Data analysis

We used Generalized Linear Models (GLMs), using Negative Binomial Distribution to deal with overdispersion, to analyze changes in crossing and roadkill numbers over time as described below. We performed the GLM analyses using the “glm.nb” function from the “MASS” package (Ripley et al., 2013) and checked the residuals using the “checkResiduals” function from the “performance” package (Lüdtke et al., 2020). All analyses were performed in the R environment version 4.3.1 (R Core Team, 2023).

We analyzed the number of wildlife crossings over time to assess whether the number of crossings varied since the mitigation structures were installed. We analyzed each cluster separately (km 45, km 50, and km 59) due to differences in the number and type of mitigation structures in each one. We used the total number of crossings recorded annually in each cluster as the response variable, considering all structures that make up each mitigation cluster.

We analyzed if roadkill numbers varied over time for mitigated and non-mitigated segments using the after-control-impact design (De Palma et al., 2018). We used the number of roadkill recorded on each segment of 500 m of mitigated (impact) and non-mitigated (control) sites. Mitigated sites ( $n = 9$  segments) encompass any segments that overlap the three mitigation clusters, including wildlife crossing structures and/or fences. In contrast, non-mitigated sites ( $n = 98$  segments) are all other segments of the road except those located within 500 m on either side of the mitigation clusters and the first 11 km of the road, as it crosses an urban center of a city (SM – Fig. 2). We used the annual number of roadkill per segment as the response variable and the year as the predictor. Before this analysis, we performed a Moran I test and found no spatial autocorrelation for the number of roadkill in each segment (Moran I statistic = 0.041,  $p = 0.29$ ).

Furthermore, we evaluated whether roadkill hotspots spatially overlapped with mitigation clusters. First, we evaluated whether fencing resulted in an overall disappearance of roadkill hotspots across all species over the entire survey period. Second, we subdivided the study period into three timeframes (T1: 2004–2007; T2: 2008–2012; and T3: 2013–2017) to assess whether hotspot patterns were influenced by a potential decline in fence effectiveness over time. Under effective mitigation, we expected hotspots to be absent from fenced road sections regardless of the time frame considered. We also individually analyzed the ten most recorded species in the roadkill and crossing surveys, which comprise 13 species in total, as some species are the most recorded in

both surveys (SM – Table 2). To describe hotspot patterns, we used a two-step approach using the Siriema v.2.0 software (Coelho et al., 2014). First, we used the modified Ripley's K statistic to identify significant aggregation of roadkills and at which spatial scales (Coelho et al., 2012). We used an initial radius of 500 m, with successive 500 m increments (500 m, 1000 m, 1500 m, etc., until the full extent of the road was reached) 100 simulations of random distribution of the same number of carcasses, and a 95% confidence interval. Then, we used the 2D HotSpot Identification to locate the hotspots along the road (Coelho et al., 2012) for datasets that exhibited aggregation on the lowest scale (500 m radius) in the K statistics. For the hotspot location analysis, we used a radius of 500 m, 100 subdivisions of the highway (each segment with 675 m), 1000 simulations of the random roadkill distribution of the same number of carcasses, and a 95% confidence interval. We considered as hotspots all segments with a roadkill intensity value (number of observed carcasses - mean number of carcasses from simulations) higher than the upper confidence limit.

## Results

During 14 years of surveys, 9080 crossings by 17 taxa were recorded in all mitigation clusters (detailed information about species in SM – Table 3). The cluster at km 45 presented 6026 crossings, while the cluster at km 50 and 59 presented 643 and 2,411, respectively. As expected, we observed an increase in the number of crossings over time in each mitigation cluster ( $p < 0.001$ ,  $df = 12$ ; Fig. 2, SM – Table 4).

During the roadkill surveys, 1493 carcasses of 20 taxa were recorded on the entire highway (detailed information about species in SM – Table 3). A total of 210 roadkills were recorded at mitigated sites ( $1.67 \pm 1.53$  records per segment,  $n = 9$ ; SM – Table 5) and 1204 at non-mitigated sites ( $0.88 \pm 1.33$  records per segment,  $n = 98$ ; SM – Table 5). We found a significant effect of year ( $p < 0.001$ , estimate = 0.0618, model  $df = 1494$ ) and a non-significant effect of segment type (mitigated vs. non-mitigated;  $p = 0.097$ ) on the number of roadkills, with the interaction of these variables also being non-significant ( $p = 0.099$ ) (SM – Table 6). Although the roadkill growth rate was steeper in non-mitigated segments, mitigated segments maintained a higher overall number of roadkill throughout the study period (Fig. 3). This persistence of high and increasing roadkill numbers demonstrates that fencing had low effectiveness in preventing roadkill.

This ineffectiveness of fences was additionally supported by the observed roadkill hotspots overlap at all the mitigation clusters,

considering the full survey time. This general pattern holds when we look at each timeframe, except for the cluster at km 50 in T3 (Fig. 4; complementary results on SM – Figs. 3 and 4). Roadkill hotspots were also present at other locations along the road, with the spatial distribution of the hotspots remaining stable irrespective of the timeframe analyzed.

Roadkill aggregations on the 500-m scale were observed for eight of the 13 recorded taxa (including anteaters, armadillos, canids, opossums, porcupines, raccoons, rodents, and snakes), for which we performed the 2D HotSpot Identification analysis (Fig. 5; complementary results in SM – Figs. 3 and 4). Six taxa showed hotspot zones that overlapped the cluster locations: Boidae, *Cerdocyon thous*, *Coendou insidiosus*, Dasypodidae, *Didelphis aurita*, and *Procyon cancrivorus* (Fig. 5). *Salvator merrianae* and *Sylvilagus brasiliensis* did not show aggregation on the 500-m scale, and *Cuniculus paca*, *Eira barbara*, and *Lontra longicaudis* had only a few roadkill records; therefore, we did not perform the hotspot analysis for these species. Although we could not perform the hotspot analysis for *Cuniculus paca*, we highlight that its roadkill records overlap the cluster at km 45.

## Discussion

We found an increase in the number of crossings and roadkills over time (2004–2017). The number of crossings indicates that all mitigation clusters were used more over the years. However, our results illustrate that the increase in the use of wildlife crossing structures does not mean a decrease in roadkill in segments with mitigation measures. Roadkill hotspots were still found in mitigated segments whether we look at the entire survey duration or the more restricted timeframes and using a multi-species or single-species dataset.

The use of wildlife crossing structures is expected to increase over the years after their installation, as individuals become more habituated to the presence of these structures (van der Grift et al., 2013). An increase in use over time has been shown for different species at different locations, such as bears, wolves, and ungulates in the United States and Canada (Clevenger and Waltho, 2003; Gagnon et al., 2011), and wild boars, deer, hares, and foxes in Poland (Mysłajek et al., 2020). Our results also showed this pattern for all mitigation clusters. This indicates that wildlife crossing structures are functional to facilitate safe road crossings for animals (Soanes et al., 2024).

In contrast, fences installed in the mitigation segments are clearly ineffective, not blocking target species from accessing roads. Roadkill

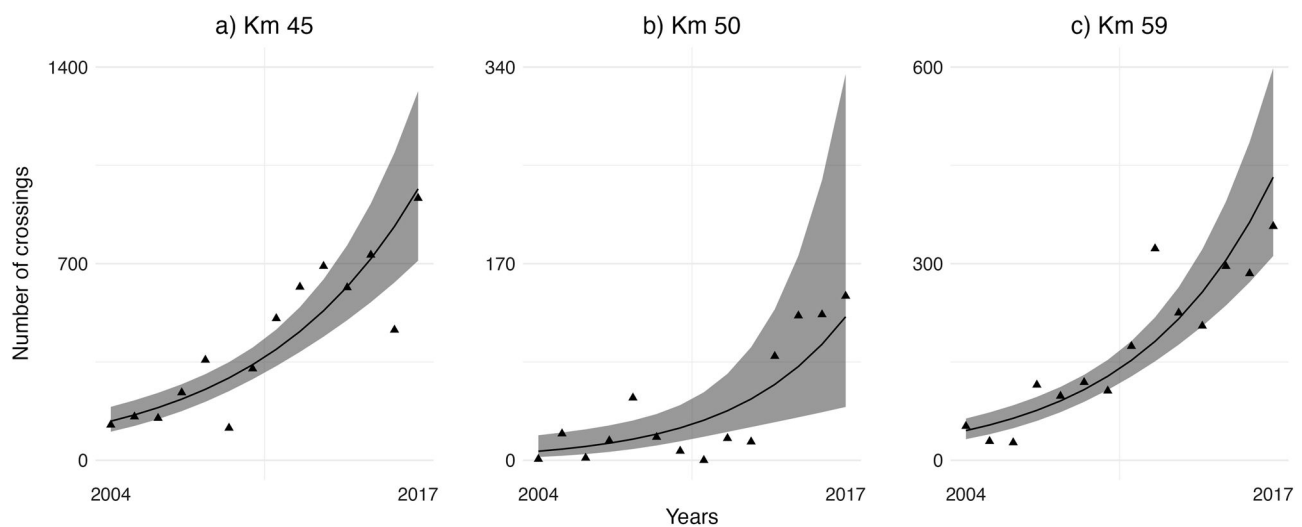
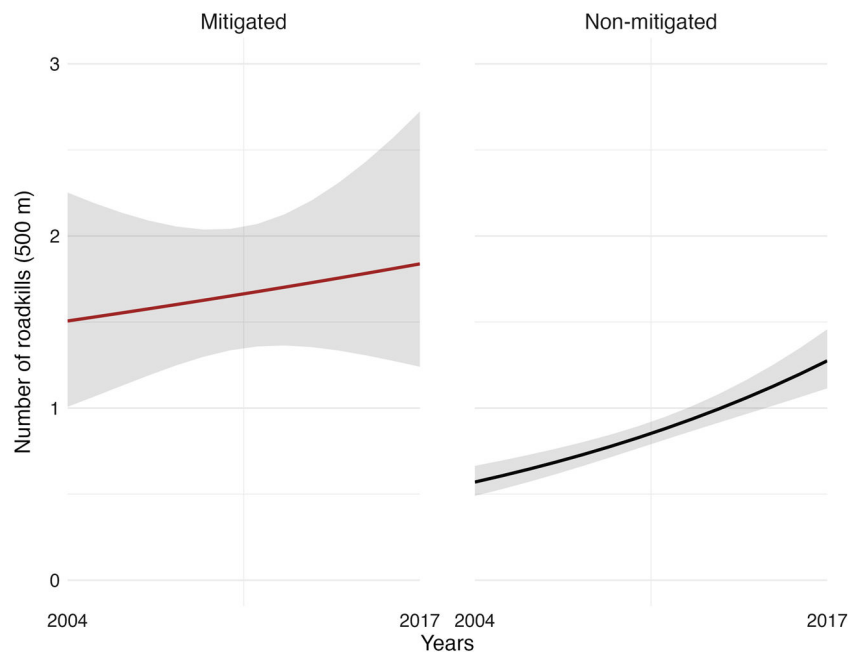
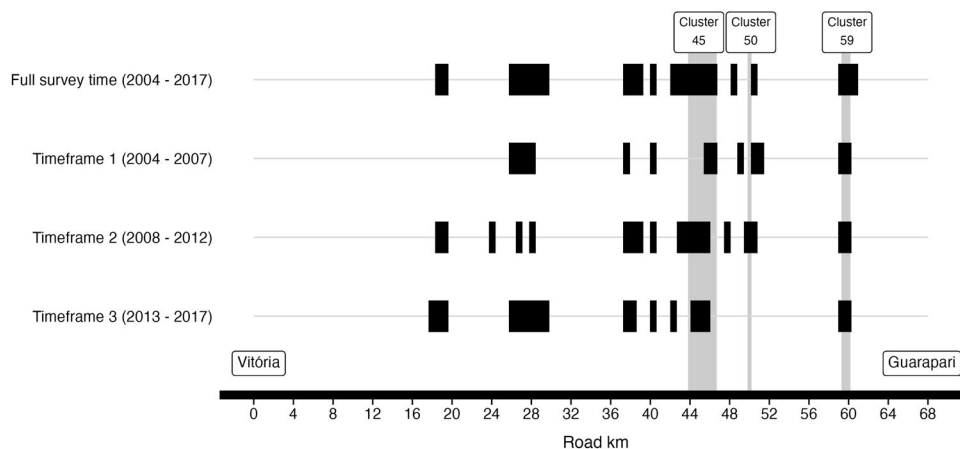


Fig. 2. The number of crossings in the mitigation clusters at km 45 (a), km 50 (b), and km 59 (c) over time. The black lines represent the predicted number of crossings, and the dark gray shaded areas represent confidence levels (95%). The black triangles represent the number of crossings recorded each year. Note the difference in the y-axis scales for each cluster.



**Fig. 3.** Predicted number of roadkills in 500 m segments of mitigated sites (red line) and non-mitigated sites (black line) along the study period. The gray-shaded areas represent confidence intervals (95%).

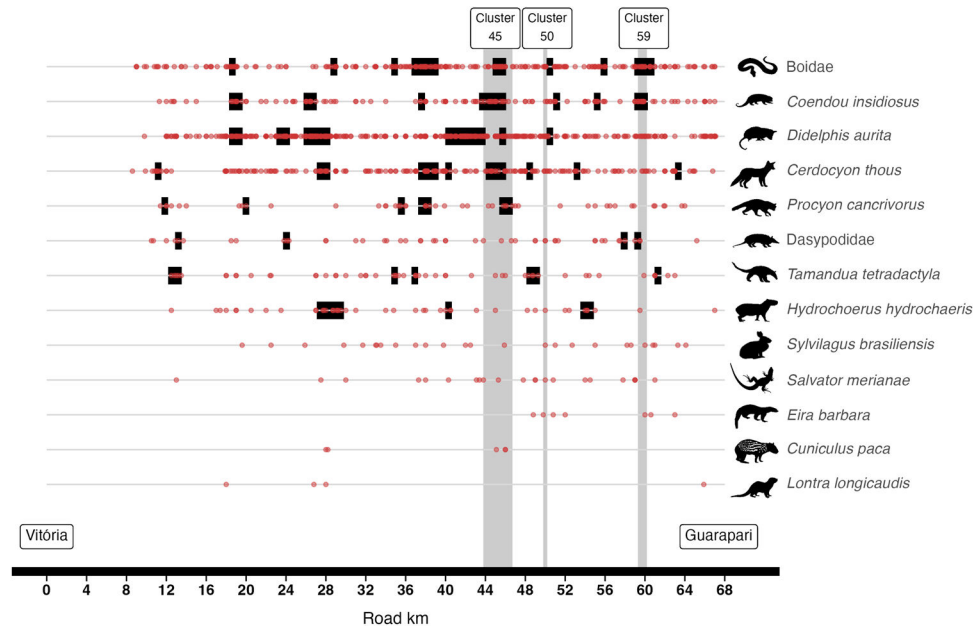


**Fig. 4.** Spatial distribution of hotspot zones considering all species together along the highway for the full survey time span (14 years) and three timeframes (T1, T2, and T3). The black stripes represent the hotspot zones identified in the 2D HotSpot Identification analyses. The gray shaded zones correspond to the location and extension of mitigation clusters.

numbers presented an undesired increase over time, and after years of mitigation, they were still higher on mitigated segments than on non-mitigated sites. Notably, roadkill increased over time in both control and mitigation segments. This pattern may reflect rising traffic volume and road lethality (e.g., Denneboom et al., 2024; Saxena et al., 2020), and/or increased animal exposure to roads driven by changes in abundance or movement patterns in response to landscape dynamics. Distinguishing the relative contributions of these mechanisms warrants further investigation and the answer is expected to depend on target group (e.g., Denneboom et al., 2024). Additionally, roadkill hotspots still overlapped mitigation clusters over time. These findings reinforce the known conclusion that even functional wildlife crossing structures, when paired with fences that are not properly designed for the target species, tend not to be an effective mitigation measures against roadkill (Baxter-Gilbert et al., 2015; Ciocheti et al., 2017; Elliott and Stapp, 2007; Rytwinski et al., 2016).

Some recommendations can be made to improve the mitigation

system to increase safe crossings and reduce roadkill for all species. First, the current fencing system should be revised to ensure the installation of fences with extensions that account for the home range of the target species (Huijser et al., 2016). The effectiveness in reducing roadkill has been widely demonstrated if the right type of fence is being used to block animal access to the road (Huijser et al., 2016; Rytwinski et al., 2016; van der Ree et al., 2015). In addition to reducing roadkill, fencing also contributes to increasing wildlife use of crossing structures to cross the road safely (Denneboom et al., 2021). For example, we could expect that improvements to fences designed to block large and climbing snakes of the family Boidae (such as small mesh fencing with a lip and 100 cm in height; e.g., Macpherson et al., 2021) would not only reduce their roadkill rates but also encourage greater use of crossing structures. Second, more mitigation clusters (fences and wildlife crossing structures) should be implemented along the road, as the analyses indicated several hotspots on other road segments (e.g., km 28 and 38; Fig. 5). Finally, as some species that are seen as roadkill on the road are rarely



**Fig. 5.** Spatial distribution of roadkill records (red dots) and hotspot zones (black stripes) identified in the 2D HotSpot analysis along the highway for the most recorded taxa on crossings and roadkill surveys. The gray shaded zones correspond to the location and extension of mitigation clusters. The bottom five species did not have enough data to perform the hotspot analysis.

registered using crossing structures, this could indicate the need to improve the fencing and modify crossing structures to encourage their use. The use of wildlife crossing structures could increase with the appropriate type of fence to block animal access to the road and guide them to the crossing structures. The wildlife crossing structures could be assessed to identify factors affecting their use by different species, including structure type and dimensions, the presence of fencing, human activity, and the availability of vegetation and water within and near the structure (Denneboom et al., 2021).

A long-term survey provides an opportunity to assess the performance of mitigation measures over time (Gagnon et al., 2011). Our findings offer important information about the effectiveness of the mitigation system in a tropical and highly diverse region, adding to the literature on mitigation effectiveness, highly biased towards larger mammals and the Global North (Denneboom et al., 2021; Rytwinski et al., 2016; Soanes et al., 2024). Although we do not have data collected prior to mitigation implementation, 14 years of simultaneous monitoring of crossings and roadkill numbers gives us more confidence in evaluating the effectiveness of mitigation clusters. The trend of our results suggested that the three mitigation clusters are ineffective in avoiding roadkill, so adaptations in the mitigation system must be conducted, such as installing fences that in fact block animals from accessing the road.

## Conclusion

Our study highlights the importance of considering both crossing and roadkill indicators to improve understanding of mitigation effectiveness. Based on our findings, the mitigation clusters showed an increase in the number of crossings over time; however, the roadkill records also increased and were higher in the mitigation segments during the same period. The increased use of wildlife crossing structures suggests that the animals are habituated to them, and efforts must be made to implement effective fencing. Effective wildlife-exclusion fencing must act as a true physical barrier that prevents animals of different sizes and climbing and digging abilities from accessing the roadway and thereby reducing the risk of road mortality. Multispecies roadkill mitigation is not an easy challenge in megadiverse countries and new solutions need to be tested.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.pecon.2026.02.012>.

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