

# *Assessing Brazilian reptiles' road-kill risks using trait-based models*

Article

Accepted Version

Rahhal, N. D. F. ORCID: <https://orcid.org/0000-0002-9652-5754>, Pinto, F. A. S. ORCID: <https://orcid.org/0000-0002-8814-3182>, Medrano-Vizcaíno, P. ORCID: <https://orcid.org/0000-0003-3122-048X>, Francisco, C. N. ORCID: <https://orcid.org/0000-0002-8688-9810> and Bruno, S. F. ORCID: <https://orcid.org/0000-0002-7267-9310> (2023) Assessing Brazilian reptiles' road-kill risks using trait-based models. *Austral Ecology*, 48 (7). pp. 1361-1382. ISSN 1442-9993 doi: 10.1111/aec.13383 Available at <https://centaur.reading.ac.uk/112558/>

It is advisable to refer to the publisher's version if you intend to cite from the work. See [Guidance on citing](#).

To link to this article DOI: <http://dx.doi.org/10.1111/aec.13383>

Publisher: Wiley

All outputs in CentAUR are protected by Intellectual Property Rights law, including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in the [End User Agreement](#).

[www.reading.ac.uk/centaur](http://www.reading.ac.uk/centaur)

## **CentAUR**

Central Archive at the University of Reading

Reading's research outputs online

# ASSESSING BRAZILIAN REPTILES' ROAD-KILL RISKS USING TRAIT-BASED MODELS

Natã Dutra Fernandes Rahhal<sup>1,\*</sup>, Fernando Antonio da Silva Pinto<sup>2</sup>, Pablo Medrano-Vizcaíno<sup>3,4</sup>, Cristiane Nunes Francisco<sup>1</sup>, Sávio Freire Bruno<sup>5</sup>.

<sup>1</sup> Universidade Federal Fluminense, Escola de Engenharia, Programa de Pós-Graduação em Engenharia de Biosistemas, Niterói, Rio de Janeiro, Brazil.

<sup>2</sup> Instituto Nacional da Mata Atlântica, Santa Teresa, Espírito Santo, Brazil.

<sup>3</sup> University of Reading, School of Biological Sciences, Ecology and Evolutionary Biology, Reading, United Kingdom.

<sup>4</sup> Red Ecuatoriana para el Monitoreo de Fauna Atropellada (REMFA), Quito, Ecuador.

<sup>5</sup> Universidade Federal Fluminense, Departamento de Patologia e Clínica Veterinária, Setor de Animais Selvagens/Silvestres, Niterói, Rio de Janeiro, Brazil.

\* Correspondent author: Rua Passo da Pátria, 156 bloco D sala 236 - São Domingos, Niterói, Rio de Janeiro, 24210-240, Brazil. [natarahhal@id.uff.br](mailto:natarahhal@id.uff.br). +55 22 98827-2904.

**Abstract:** Reptiles are an understudied group in road ecology, despite evidence of their high vulnerability to road mortality. Recently, trait-based models have been demonstrated to be valuable tools for explaining and predicting road mortality risks for birds and mammals. The present study aimed to apply such models for reptiles for the first time. We fitted eight random forest regression models, controlling for different survey design variables, to explain 782 empirical road-kill rates for Brazilian reptiles and selected the best-performing model to predict road mortality risks for 572 continental species. The results showed that species that are habitat generalists, omnivorous, viviparous, cathemeral, and have intermediate clutch/litter sizes are at a higher risk of being road-killed. The relationships for other traits included in our models were uncertain, but our findings suggest that population density and species-specific behavioural responses to roads and traffic may play an important role in road mortality risks. Geographical location and survey design variables (especially sampling speed and sampling time) were more important in explaining the variance of the empirical road-kill rates than any of the tested ecological and functional traits. Besides adding evidence of the vulnerability of the Amazon region to vertebrate road-kills, this study highlights some similarities between the relationships identified here and those found for birds and mammals (such as with body mass and habitat breadth). We also corroborate that trait-based models are useful tools to aid in conservation efforts but indicate that they can be biased by the methodologies used to collect empirical data. Future road-kill surveys should therefore use methods specifically designed for reptiles and estimate both observer's efficiency and carcass removal rates.

**Key-words:** conservation; herpetofauna; life-history traits; road ecology; random forest.

## INTRODUCTION

The first three decades of the 21st century have seen a surge in infrastructure projects, including roads (Ascensão *et al.*, 2018; Meijer *et al.*, 2018; Elhacham *et al.*, 2020). Despite being undeniably relevant for human societies, roads are a cause of many ecological impacts, such as habitat fragmentation and degradation, logging, and direct mortality (Forman *et al.*, 2003; Coffin, 2007; Laurance, Goosem & Laurance, 2009). However, many of these infrastructure projects lack proper risk assessment and cost-benefit analysis, and thus fail to consider impacts on biodiversity (Flyvbjerg, 2009; Laurance *et al.*, 2014; Ibisch *et al.*, 2016).

At least 21.1% of reptile species are threatened with extinction (Cox *et al.*, 2022), and roads seem to be a significant threat to their populations (Fahrig & Rytwinski, 2009; Rytwinski & Fahrig, 2012; Gonçalves *et al.*, 2018). In particular, roads have already been singled out as the cause of population depression in tortoises (Boarman & Sazaki, 2006), and even low road mortality rates can increase the risk of extinction for some snake populations (Row, Blouin-Demers & Weatherhead, 2007).

Nevertheless, whereas road-kill survey studies are well represented in the currently growing Latin American road ecology, little is known about the effects of roads on reptiles, especially when compared to other terrestrial vertebrates (Colino-Rabanal & Lizana, 2012; Oliveira *et al.*, 2020; Pinto, Clevenger & Grilo, 2020). This disparity could be explained by issues such as lower carcass detectability and higher removal rates (*e.g.*, by predators and scavengers), combined with sampling designs focused on medium to large-sized vertebrates (Santos, Carvalho & Mira, 2011; Teixeira *et al.*, 2013b; Barrientos *et al.*, 2018; Silva, Crane & Savini, 2021), and the fact that collisions with larger animals present greater risks to the economy and human health (Abra *et al.*, 2019). It is known, however, that not all species are affected equally (Rytwinski & Fahrig, 2015), with mortality depending on extrinsic factors

such as road design, landscape composition and configuration, and availability of resources (Clevenger, Chruzc & Gunson, 2003; Coelho, Kindel & Coelho, 2008; Teixeira *et al.*, 2013a; Bueno, Sousa & Freitas, 2015), and on intrinsic factors such as the species' movement patterns, abundance, and ecological and functional traits.

Reptiles' responses to roads have been scarcely investigated, and most studies have only considered local scales that evidently reflect the reality of those surveyed areas and of a few selected species (*e.g.*, see Jochimsen *et al.*, 2004; Andrews & Gibbons, 2005; Lima *et al.*, 2015; Jacobson *et al.*, 2016). On the other hand, large-scale analyses can provide more comprehensive and reliable information about the general patterns of wildlife mortality. This has already been carried out for birds and mammals in Brazil (González-Suárez, Ferreira & Grilo, 2018), Europe (Grilo *et al.*, 2020), and Latin America (Medrano-Vizcaíno *et al.* 2022), but such information remains unknown for reptiles (but see Rytwinski & Fahrig, 2012).

Given this, Brazil serves as an appropriate case study. It encompasses two global biodiversity hotspots (*i.e.*, Atlantic Forest and Cerrado) (Myers *et al.*, 2000) and is the third most reptile-diverse country in the world (Costa, Guedes & Bérnils, 2021), harbouring important areas for the conservation of these animals (Böhm *et al.*, 2013). Brazil also contains the largest roadless area in the world (Ibisch *et al.*, 2016), but projections indicate major expansions in road infrastructure (Meijer *et al.*, 2018). Therefore, there is a need for knowledge to enable a better management of the current road network, as well as several sustainable planning opportunities for the near future. In this study, we present a machine learning trait-based model aiming to provide the first assessment of reptile road-kill risks at a national level. For this, we related a set of life-history traits to the magnitude of Brazilian reptiles' road-kill rates and spatially predicted the risk of these occurrences throughout the country. We also tested different data subsets of road-kill rates in order to assess the role of

distinct survey designs, thus providing a valuable tool for planning both research at more refined scales and conservation actions for the most adversely affected species.

## MATERIALS AND METHODS

### *Data Collection*

We developed a dataset of reptile road-kill rates across Brazilian roads using a compilation of the data provided by the data paper of Grilo *et al.* (2018), the literature review of Pinto, Cleverger & Grilo (2020), and the literature dataset of the IUCN Latin American and Caribbean Transport Working Group (<https://latinamericatransportationecology.org/publications/>). Additionally, we conducted a systematic search for studies published between 2018 and February 2022. This search was performed in the ‘Science Direct’, ‘Scopus’ and ‘Web of Science’ databases using the following keywords in Portuguese and English: (“*atropelamento*” OR “*roadkill*” OR “*road mortality*”) AND (“*vertebrados*” OR “*vertebrates*” OR “*répteis*” OR “*reptiles*”). We collected, from each study, the number of individual carcasses of each species reported, data on survey design (sampling intervals, total number of inspections, total sampling period, length of the sampled road stretch, sampling speed and sampling methods), and the geographic coordinates of the approximate midpoint of each studied area. Taxonomic information was updated following the names available at Uetz *et al.* (2021), and road-kill rates were calculated by dividing the number of carcasses reported by the length of the sampled road stretch (in kilometres) and by the total sampling period (in years).

As reptile carcasses’ removal rates are relatively high, which could influence any road-kill estimates (see Bager & Rosa, 2011; Santos, Carvalho & Mira, 2011; Teixeira *et al.*, 2013b; Santos *et al.*, 2015), we also corrected the road-kill rates included in two of our

generated models (see the following sections). This process of correction, performed as proposed by González-Suárez, Ferreira & Grilo (2018), accounted only for carcass persistence probability, as extrapolating removal rates from different regions is easier than determining detectability, which is case-specific (see Santos *et al.*, 2016; Barrientos *et al.*, 2018). Since Santos, Carvalho & Mira (2011) did not make any general estimate for terrestrial turtles and crocodilians, we assumed that all Testudines have the same carcass persistence probability and Crocodylia has the same persistence as mammals in the order Carnivora (Table S1).

In the present work, we defined the total reptile richness in Brazil as 572 continental species from 32 families. To compile our species list, we used information from: (1) the road-kill rates dataset generated in the previously described phase of data collection, and (2) the IUCN Red List of Threatened Species™ (IUCN, 2021), later using (3) the geographical distributions dataset of Roll *et al.* (2017) as a filter for inclusion or exclusion of individual species. We then validated all taxonomic information based on *The Reptile Database* (Uetz *et al.*, 2021) and excluded marine turtles (five species), two exclusively insular viperids (*Bothrops alcatraz* and *B. insularis*) and two *species affinis*. The ecological and functional traits data for all these species were obtained from the primary literature, field guides, and datasets such as those from Meiri (2018) and Meiri *et al.* (2021).

Based on specialized literature (*e.g.*, Jochimsen *et al.*, 2004; McCardle & Fontenot, 2016; Rincón-Aranguri *et al.*, 2019) and data availability, we selected ten potentially relevant traits to explain species-specific susceptibility of reptiles to road-kills. Of these, three (body mass, leg development and body temperature) are related to morphology or physiology, five (main activity substrate, activity time, foraging strategy, trophic level, and habitat breadth) to behaviour and/or habitat use, and two (clutch size and reproductive mode) are reproductive traits (see Table 1 for rationale and more details). All our data is available in a public repository: <https://doi.org/10.6084/m9.figshare.c.6079788>.



## *Data Analysis*

We used R v.4.1.2 (R Core Team, 2022) and RStudio v.2021.09.2+382.pro (RStudio Team, 2022) to develop predictive models through random forest regression algorithms, following the methods described by González-Suárez, Ferreira & Grilo (2018). In brief, random forests are robust classification and regression algorithms that generate a model with multiple decision trees of controlled variance and merge these results to determine more accurate predictions (Breiman, 2001; Cutler *et al.*, 2007).

In order to assess how survey design could bias our results, we fitted eight models with 2,000 trees using the “randomForest” R package (Liaw & Wiener, 2002) functions. Their performance was assessed by checking the total variance explained. In all models, we included the approximate coordinates of the midpoint of the original studied roads, one taxonomic variable (family), the 10 selected traits, and survey intervals (time between samplings, in days) as predictors. Each one ran with a different data input and comprised different sets of predictors (see Table 2 for details). The first model used our complete road-kills dataset, the second used a data subset filtered by the availability of information on sampling speed (km/h), sampling method (by bicycle, with a motorized vehicle, on foot, and on foot and by bike or car) and sampling time (morning, morning and afternoon, evening, morning and evening, throughout all day, and morning, afternoon and evening) – which are variables known to influence carcass persistence and detectability (see Santos, Carvalho & Mira, 2011; Teixeira *et al.*, 2013b). The other six model used data subsets filtered by survey periods (the time that the survey lasted, in years) and survey intervals that are known to minimize sampling biases (see Bager & Rosa, 2011; Santos, Carvalho & Mira, 2011).

As trait data is not available for all species (see Table 1), missing were estimated with an imputation method, also based on random forests, available in the “missForest” R package (Stekhoven & Bühlmann, 2012). Although there are more powerful methodologies to fill gaps in traits datasets, the chosen procedure presents itself as a proper option due to its capability of dealing both with continuous and categorical variables simultaneously (see Johnson *et al.*, 2021). To capture the uncertainty of this process, 15 imputed datasets were generated and utilized to run each predictive model.

Once the models were generated, we chose the one with the lowest mean squared error to develop spatial predictions. For this process, we considered a hypothetical survey interval of six days (median value of the empirical data included in the selected model) throughout Brazil and superimposed a grid of 50 km x 50 km cells on the country map. The centroid of each cell was used as the coordinate source for the hypothetical surveys, and the final predicted road-kill rate was the median of the predictions from the 15 imputed datasets. The road length (in kilometres) present in each of the cells was calculated based on the data made available by the National Department of Transport Infrastructure (<http://servicos.dnit.gov.br/vgeo/>), which includes official planned and existing federal and state roads, both paved and unpaved. Despite the importance of variables such as road avoidance behaviour and differences in crossing probabilities between species and in relation to road pavement (see Andrews & Gibbons, 2005; Robson & Blouin-Demers, 2013; Proulx, Fortin & Blouin-Demers, 2014), information about these factors for reptiles is still scarce; therefore, we assumed the absence of both.

Finally, the resulting spatial values were used to calculate road-kill risks for each species (through the median of the estimated rates for all cells where the species is distributed according to Roll *et al.*, 2017) and road-kill risks per cell (through the summation of the risks

of all species with occurrence in each cell), both in individuals/km/year and in individuals/year.

Other R packages used during the analyses were “cowplot” (Wilke, 2020), “ggplot2” (Wickham, 2016), “forestFloor” (Welling et al., 2016), “pdp” (Greenwell, 2017) and “plotmo” (Milborrow, 2021) for visualization and plotting of results, and “data.table” (Dowle & Srinivasan, 2021), “dplyr” (Wickham *et al.*, 2021), “foreign” (R Core Team, 2020), “plyr” (Wickham, 2011) and “reshape2” (Wickham, 2007) for data reading and manipulation.

## RESULTS

We screened 43 studies, from which we extracted 782 road-kill rates representing 175 reptile species and comprising 22 families (17 from Squamata, four from Testudines and one from Crocodylia). The most frequently reported species were the black-and-white tegu (*Salvator merianae*), the red-tailed boa (*Boa constrictor*) and the Lichtenstein’s green racer (*Philodryas olfersii*), with 41, 33 and 30 road-kill rates, respectively. Nearly 40% ( $n = 65$ ) of the species registered have only one road-kill rate reported. Most of the species in our road-kills dataset ( $n = 146$ ) are defined by the IUCN as Least Concern, while two others (the black spiny-necked swamp turtle, *Acanthochelys spixii*, and the Vanderhaege’s toad-headed turtle, *Mesoclemmys vanderhaegei*) are defined as Lower Risk/Near Threatened, one (the Pantanal swamp turtle, *Acanthochelys macrocephala*) is defined as Near Threatened, one (the Caatinga coral snake, *Micrurus ibiboboca*) as Data Deficient and 25 as Not Evaluated. The observed road mortality rates (median = 0.021 ind./km/year; SD = 0.992 ind./km/year) ranged from 0.001 ind./km/year (reported for the cascabel rattlesnake, *Crotalus durissus*) to 13.75 ind./km/year (reported for the Patagonia green racer, *Pseudablabes patagoniensis*). For threatened (*i.e.*, Critically Endangered, Endangered and Vulnerable), Near Threatened and

Data Deficient species only, these values (median = 0.052 ind./km/year; SD = 0.754 ind./km/year) ranged from 0.014 ind./km/year (reported for *A. macrocephala*) to 2.5 ind./km/year (reported for *A. spixii*).

Our models explained from 47.06% to 72.37% of the variance of the observed road-kill rates. However, when accounting only for taxonomic family and life-history traits, values ranged from -7.2% to 12.08% (see Table 2). The seventh model – which included as predictors the approximate coordinates, survey interval, taxonomic family and the traits – performed the best (*i.e.*, was the one with the lowest mean squared error), and resulted in a variance explained of 61.4% (merged value for all 15 imputed datasets, which ranged from 61.4% to 62.51% with a standard deviation of 0.002). Although the imputation process had a relatively high error value for continuous variables (normalized root mean squared error, NMRSE = 0.487), it performed well for categorical variables (proportion of falsely classified, PFC = 0.081). The road-kill rates predicted later by the model matched the observed road-kill rates well, but with a slight tendency to underestimate those values (see Figure 1).

Predicted rates ranged from 0.006 ind./km/year (for the two-headed sipo, *Chironius bicarinatus*) to 0.293 ind./km/year (for the red worm lizard, *Amphisbaena alba*), and were predominantly higher for species classified by the IUCN as Least Concern (LC), although all other categories seem to have high overall predicted rates as well (Figure 2). For species without empirical road-kill rates, these values ranged from 0.007 ind./km/year (for the Brazilian sipo, *Chironius laevicollis*) to 0.170 ind./km/year (for the garden tree boa, *Corallus hortulana*).

Survey coordinates and taxonomic family were key predictors in all our models (Figures 3 and S1-S4), but as each road-kills dataset used a different data subset, the patterns identified in the partial dependence plots are not exactly the same (Figures 4 and S5-S11). Even though, in general, our models point to higher rates in areas located in southern and both

247 eastern and western regions of the country – which coincides with localities of higher species  
248 richness and/or presence of roads (Figure 5a,b). In model 7, the one used for our predictions,  
249 the highest mortality risks were associated with Brazil’s eastern portion, while the lowest  
250 were associated with southern territories (Figure 5c). Notably higher rates were found  
251 amongst Emydidae (Testudines, one species) consistently across most models, except for  
252 models 6 and 7, in which Amphisbaenidae (Squamata, 23 species), Boidae (Squamata, 12  
253 species) and Elapidae (Squamata, 24 species) were associated with the highest predicted road  
254 mortality risks.

255         Model 2 suggests that survey design variables are more important than any of our  
256 selected species’ traits (Figure S1), and higher predicted rates are related to sampling speeds  
257 of less than 20 km/h (although there is also a peak at 50 km/h), two samplings per day  
258 (especially one in the morning and one in the afternoon), survey periods of less than two  
259 years, survey intervals of less than five days, and on foot samplings (Figure S6).

260         As for the traits, there are general patterns across models (see Figures 3, 4 and S1-  
261 S11), with models 4 and 5 being the only ones to result in negative values of variance  
262 explained. In summary, the highest road-kill rates were associated with greater habitat  
263 breadth, intermediate or larger clutch sizes (generally 10-20 hatchlings or neonates per litter),  
264 viviparous reproductive mode, omnivorous diets, and cathemeral behaviour. Body masses  
265 around and bellow 50 kg seem to be linked to the highest predicted rates, while species with  
266 more than 100 kg and, especially, less than 10 kg are related to the lowest ones – but the  
267 models show contrasting patterns. Other traits also returned unclear relations: either sit-and-  
268 wait or mixed foraging strategy and aquatic or terrestrial habits were related to higher  
269 mortality rates – but the predicted values vary, and it is not possible to define a fair “pattern of  
270 importance”. For leg development, relations are also variable, but legless species (here  
271 recognized as snakes, limbless lizards, and lizards with a reduced or vestigial pair of limbs)

seem to have lesser associated risks. And for body temperature, models 1 to 3 indicate lower temperatures related to higher risks while models 4 to 8 indicate higher temperatures related to higher risks (but all models had a peak of predicted rates around 27 °C).

Our spatial predictions also revealed important patterns. Although they are expected to vary with different models due to the different relations returned, we can safely conclude that the cumulative maps of road mortality risk (Figure 5c-d) indicate a distribution pattern that is consistent using both median predicted rates for the cell (Figure S12a) and lower and upper confidence interval estimates (respectively 5% and 95%) (Figure S12c-d). The areas with higher predicted risks also had the highest standard deviation values (*i.e.*, highest variability amongst species) (Figure S12b). When we ran the same model with uncorrected road-kill rates (model 6), the spatial patterns remained qualitatively similar but quantitatively different (see Figure S13-S15).

Total aggregation (sum) indicates an amount of 21,317.060 ind./km/year (when excluding cells without roads, this value drops to 15,401.917 ind./km/year) and 2,513,040.927 ind./year for all the country. The Chaco lancehead (*Bothrops diporus*) was the species with the lowest predicted road-kill rate, while *Amphisbaena alba* was the one with the highest (median values: 0.009 ind./km/year and 0.206 ind./km/year, respectively). Upon exclusion of planned roads, we estimate 21,168.643 ind./km/year and 2,146,883.652 ind./year – which implies that the implementation of planned roads in Brazil could result in a 17.05% increase in the yearly reptile road-kill rates.

As expected, when considering the road network (Figure 5b,d), predicted road-kill rates predominantly indicate higher risks in areas with a higher presence of roads. However, the spatial distribution of the included road-kill rates in each model (Figure S16) greatly affected the results (see Figures 4 and S5-S11). For example, in model 7, southern Brazil had the lowest predicted risk of all country, in a pattern that should not be expected when

considering existing surveys for the region. ~~When summing all values per cell of each Brazilian biome (*sensu* IBGE, 2019), the Cerrado has the highest predicted road kills per year (~1 million), followed by the Atlantic Forest with 813,808.563 ind./year, the Caatinga with 703,065.815 ind./year, the Amazon with 507,393.503 ind./year, the Pantanal with 43,627.463 ind./year, and the Pampas with 21,688.838 ind./year.~~

When mapping predicted road mortality rates only for threatened, Near Threatened and Data Deficient species, the areas with higher predicted risks remained largely the same, even though the species richness distribution pattern changed considerably (which affected the spatial patterns of predicted risks) (see Figure S17). These predictions point to a total of 507.426 ind./km/year (when excluding cells without roads, this value drops to 350.753 ind./km/year) and 61,586.447 ind./year for threatened, Near Threatened and Data Deficient reptiles across Brazil. When excluding planned roads, we estimate an amount of 504.171 ind./km/year and 52,188.749 ind./year – *i.e.*, these species could have their yearly road-kills increased by almost 18% by the construction of planned roads across the country.

## DISCUSSION

Our results provided the first nationwide assessment of reptile road mortality, identifying ecological and functional traits associated with road-kill risks and areas more prone to reptile road-kills at the national level. Similar to previous Latin American studies focused on birds and mammals (González-Suárez *et al.* 2018; Medrano-Vizcaíno *et al.* 2022), our models show a non-random pattern of road mortality risk for reptiles in Brazil. In particular, we found higher road-kill rates associated with habitat generalism, greater body mass, larger clutch sizes, cathemerality, viviparity, and omnivorous diet. Additionally, the geographic location, taxonomic family, survey interval and other survey design variables can

influence road mortality magnitudes as well. We also predicted high road mortality risks in areas in the central-eastern and north-eastern portions of Brazil.

Although we have identified unique patterns for reptile road-kill risks, some of the traits analysed (for example, body mass and habitat breadth) indicate similar relationships to those previously identified for birds and mammals in South America (González-Suárez, Ferreira & Grilo, 2018; Medrano-Vizcaíno *et al.*, 2022). This highlights the general nature of these traits as sources of road-kill risk not only for endotherms but probably for all tetrapods. Previous studies have linked greater body mass to increased road-kill risks, especially in mammals (*e.g.*, Ford & Fahrig, 2007; Barthelmess & Brooks, 2010), and possible explanations for these results included large home range requirements (Rytwinski & Fahrig, 2015) and dispersal capacities (Barbosa *et al.*, 2020) and sampling biases in road-kill surveys (Santos, Carvalho & Mira, 2011). For reptiles, however, data availability on home range is very poor, and the available information is biased by inappropriate methodologies (see Passos, Galdino & Rocha, 2015; Crane *et al.*, 2021). In addition, body mass data are not usual in the herpetological literature, so we used maximum body masses estimated through allometric equations (Meiri *et al.*, 2021), which could be a relevant source of uncertainty and bias for our results. Experiments conducted in Brazil (*e.g.*, Teixeira *et al.*, 2013b) have shown that monitoring small-bodied species road-kills on foot leads to higher accuracy, reducing the bias towards large-bodied species in car-based surveys. Hence, our results on body mass could be strongly associated with these sampling biases, as at least 72% of our road-kills dataset was provided by surveys conducted using a motorized vehicle.

Our road-kills dataset comprises approximately 20.6% of the Brazilian reptile species (see Costa, Guedes & Bérnills, 2021), representing a diverse range of habits and species ecology. This proportion is similar to the one found by Medrano-Vizcaíno *et al.* (2023) in Ecuador, where approximately 21.2% of the species recognized for the country have been



recorded as road-kills – although they counted citizen science data as well, which could make their sample more diverse. No other studies have analysed reptile road-kill patterns at this large scale, limiting direct comparisons of our results with road-kill data from other countries. It highlights the need for further studies in different regions to facilitate cross-country comparisons and inform effective conservation strategies. Nevertheless, our findings reveal that some groups of reptiles are more vulnerable to road mortality than others.

In particular, our results support the idea that species that are habitat generalist and use terrestrial habitats are at a greater risk of being road-killed (Coffin, 2007; Hill, DeVault & Belant, 2020; Medrano-Vizcaíno *et al.*, 2022). The activity substrate is an important aspect of a species' ecology, and in reptiles is related to specific sensory organs – for instance, arboreal diurnal snakes have more developed vision than terrestrial nocturnal snakes, which rely on chemoreception or thermo-orientation (Bernarde, 2012; Marques *et al.*, 2017). In this context, *S. merianae*, *B. constrictor*, and *P. patagoniensis* stand out among the most frequently reported and road-killed species in our dataset. These species are both habitat and dietary generalists, the red-tailed boa and the black-and-white tegu being large species that play many ecological roles (Quintino & Bicca-Marques, 2013; Cabral *et al.*, 2019; Marques, Eterovic & Sazima, 2019; Diniz *et al.*, 2021). The Patagonia green racer also has one record of road-kill scavenging (Ucha & Santos, 2017), which could increase the species' risk of road mortality if it is a frequent behaviour. Aquatic and semi-aquatic species may also have higher road mortality risks because they often move between water bodies and migrate for resources or reproduction (see Southwood & Avens, 2010).

As ectotherms, reptiles are highly dependent on environmental conditions (Bernarde, 2012), and may sometimes use roads for thermoregulation – which makes these environments a potential ecological trap, especially for viviparous species of snakes (McCardle & Fontenot, 2016). However, our results did not show a consistent relationship between specific body

temperature ranges and road-kill risks. Additionally, more than 70% of the estimates for this variable were imputed, so any outcomes should be interpreted with caution. Nevertheless, as survey location was pointed out as an important predictor in all our models, we suggest that ambient temperature or road temperature could also be valuable indicators of reptile road mortality risks.

Except for clutch size, all the other traits included in our analyses were predictor variables of minor relative importance in most models (Figures 3, S1-S4). However, our partial dependence plots may suggest some important relationships. For activity time, for instance, our results support the hypothesis that nocturnal activity is associated with higher road-kill rates. And although the predominance of cathemerality was not expected, such an outcome is not particularly surprising, as cathemeral species may be exposed to traffic both day and night due to their more flexible activity patterns (see Lara Resendiz, 2020). The relationships returned for leg development and foraging strategy, also, may indicate that Brazilian reptiles are a good group to study wildlife road-crossing behaviours and reactions to oncoming vehicles (see Andrews & Gibbons, 2005; Lima *et al.*, 2015), similarly to recent studies conducted in other countries with birds (*e.g.*, DeVault *et al.*, 2015) and mammals (*e.g.*, Brieger *et al.*, 2022). Furthermore, although we did not consider diet breadth in our analyses, the fact that an omnivorous diet was related to higher road mortality rates in all our models suggests that such variable is somehow associated with road-kill risks as well. Because patterns of extinction risk seem to be trophically skewed (see Atwood *et al.*, 2020) and the traits that predict vulnerability to threats, at least for mammals, often depend on the threat process in question (González-Suárez, Gómez & Revilla, 2013), future studies would benefit from testing whether reptile road mortality could influence the trophic structure of ecological communities.

Clutch size is related to populational processes, which are expected as strong predictors of road mortality risks (González-Suárez, Ferreira & Grilo, 2018). Despite being probably a biased proxy for population abundance or density, clutch size was included in our models because there are not many populational estimates for reptiles in the literature (see Santini, Issac & Ficetola, 2018). Moreover, because it has a positive linear relationship with body size (Meiri *et al.*, 2021) and body size has a negative linear relationship with population density, it is expected that clutch size will have some relationship with population density as well (Santini *et al.*, 2018). In this sense, our models also pointed out that viviparity, rather than oviparity, is related to higher road-kill rates. However, as viviparous and oviparous species (at least among Squamata) do not have significant differences in clutch or offspring sizes (Meiri *et al.*, 2020), relations between these traits do not seem like a feasible hypothesis. This may, however, along with the importance of clutch size in most of our models, indicate the relevance of other reproductive traits for reptile road mortality risks, especially the ones related to reproductive speed (such as maturity age), which have already been related to road-kill risks for other vertebrates (González-Suárez, Ferreira & Grilo, 2018; Grilo *et al.*, 2020; Grilo *et al.*, 2021).

Our results regarding taxonomic family may also be important mostly because, even though legless and cryptozoic/fossorial species were associated with lower mortality rates in most models, Amphisbaenidae and Elapidae were among the four families with the highest predicted rates in most models as well. This implies that other important variables were not included in our analyses, and some likely relevant examples are home range and scavenger behaviour. More mobile reptiles are expected to face greater risks of mortality (see Bonnet, Naulleau & Shine, 1999; Paterson *et al.*, 2019) and some species are already known to use roads as an opportunistic food source (*e.g.*, see Sazima & Strüssman, 1990; Marques *et al.*, 2017; Ucha & Santos, 2017; Sales, Lima & França, 2019). However, data availability on both

these variables is limited, and reports of carrion-eating for some species are based on speculation (*e.g.*, Marioni *et al.*, 2019; Rosenblatt *et al.*, 2022) or anecdotes.

At last, survey coordinates were important predictors of road-kill risks in all our models; however, the predicted patterns are not solely driven by species richness or road density distributions. We believe that, in addition to different configuration and composition of landscapes (not tested here, but see Clevenger, Chruscz & Gunson, 2003 and Bueno, Sousa & Freitas, 2015 for related discussion), the spatial distribution of our species' traits may also play a role in shaping latitudinal and longitudinal patterns of road-kill risks – for example, see Rapaccioulo *et al.* (2017) for biogeographic patterns of reptile body mass.

Nevertheless, our study also has limitations that need to be acknowledged. Brazilian reptile road-kill surveys are geographically biased towards the South-Central socio-geographic region of the country, and some of our data subsets, such as the one used for our predictions, have low spatial coverage (see Figure S14) – which may explain the abruptly separated blocks in which the predictions are spatially organized (González-Suárez, Ferreira & Grilo, 2018). Additionally, our results are at a national scale, and most likely do not represent studies at smaller scales, especially because of how local ecological communities are composed and distributed. Rincón-Aranguri *et al.* (2019) in the Colombian Llanos, for example, could not separate ecological groups of the most road-killed snakes, and some of the traits identified were signalled as potentially biased by sampling methodology or by the species community composition. Also, Brazil's continental extension should lead to different carcass persistence times in different environments and climatic conditions (*e.g.*, see Ratton, Secco & Rosa, 2014; Santos *et al.*, 2016), and therefore the use of correction factors based on the estimates of Santos, Carvalho & Mira (2011) for southern Portugal is not the most appropriate approach. As our models lack sufficient data for validation, this should be interpreted as evidence that our results, particularly those generated from models with smaller

data subsets, are biased by the empirical samplings, and thus should be interpreted with caution (see Ascensão, D'Amico & Barrientos, 2019 and Grilo *et al.*, 2019 for a discussion on the importance of validation and risks in road ecology models).

In this sense, it is essential to emphasize that our predicted road-kill rates and road-kill risk maps are not an accurate representation of the actual magnitude of reptile road-kills. The data subset used to generate these values is the one with the lowest data coverage and the lowest maximum and mean values (respectively, 0.084 and 0.08 ind./km/year), leading to a significant underestimation in our predictions. Furthermore, smaller species' carcasses degrade faster and are less likely to be detected (Jochimsen *et al.*, 2004; Andrews, Gibbons & Jochimsen, 2006; Santos, Carvalho & Mira, 2011), and thus, our empirical road-kill rates could still be underestimated even after correction. This suggests that the actual number of road-killed reptiles on Brazilian roads is much higher than the 2.5 million individuals per year calculated in this study. Nevertheless, the predicted mortality rates are far from low in the Amazon region, thus highlighting the vulnerability of this area to future road expansion projects. This is especially true when we consider its high species richness (Figure 5a) and vulnerability to anthropic impacts (Harfoot *et al.*, 2021), the great relevance of keeping areas road-less (Tisler, Teixeira & Nóbrega, 2022), and our expected increase in yearly road-kill rates after the construction of planned roads.

Another key aspect of this study is demonstrating the role of survey design in explaining road-kill rates and its potential to cause bias in any analyses like the ones we performed. The results of model 2, which accounted for more survey design variables than only survey interval, showed that survey design is, together with survey location, more influential in the explained variance of the empirical road-kill rates than any of the species' traits included in our models. The addition of these variables also reduced the importance of the taxonomic family (compared to the other models), which could indicate that the taxon-

specific road-kill risks identified may be, at least partially, due to sampling biases. This idea is supported by the fact that only a small percentage (about 3%) of the species included in the study weighed over 10 kg. In this sense, the finding that sampling speed and sampling time were the two most important predictors in model 2 (Figure S3) is likely a reflection of differences in detectability and carcass removal rates, aligning with previous research (see Santos, Carvalho & Mira, 2011; Teixeira *et al.*, 2013b).

Moreover, our results challenge the assumption made by González-Suárez, Ferreira & Grilo (2018) that including only surveys with a maximum interval of seven days between samplings is the reason for qualitatively similar results between models with corrected and uncorrected road-kill rates. If that was the case, survey interval would probably stay as a more important predictor than the traits even when we also filter the models' data subsets for survey periods. Instead, when we only considered road-kill rates from studies with survey intervals of seven days or less and survey periods of two years or more, survey interval was the fourth most important predictor when using uncorrected rates (model 6) but the seventh most important predictor when using corrected road-kill rates (model 7) (Figure 3 and S5). This suggests that what might really explain such an outcome is that there is too much noise from the survey design of the empirical road-kill data. The fact that the variance explained improved both after proper filtering of our road-kills dataset (models 3 to 8) and after controlling for other survey design variables (model 2) supports this hypothesis, but larger datasets and data subsets are essential to test such an assertion.

## CONSIDERATIONS

We highlight that our study aids in understanding how wildlife is affected by road mortality and adds evidence that trait-based models are a useful tool for understanding and

predicting road mortality risks for vertebrates. Unlike previous studies using the same methodology, we show that controlling the models for survey design leads to significantly different results, and even if some traits still exhibit similar patterns, the importance (variance explained) and error (mean squared error) of each model can vary greatly.

This is the first large-scale analysis associating reptiles' ecological and functional traits and road-kill rates, thus contributing to identifying groups of species that may be most affected by the direct negative impacts of roads. With this, we expect to provide valuable insights into how future works should be planned to properly assess which species really are the most road-killed, leading to better mitigation and conservation management projects, as well as predictions at smaller scales. Although we did not consider the importance of landscape features in explaining road-kill patterns, our work contributes to a better understanding of the impacts of planned roads on road mortality rates and the identification of areas of greater vulnerability to the expansion of the road network. This way, our results corroborate the existence of intra-regional differences in road-kill risks (González-Suárez, Ferreira & Grilo, 2018; Grilo *et al.*, 2020; Medrano-Vizcaíno *et al.*, 2022), highlighting that research, conservation measures and environmental licensing processes need to consider regionality during planning and implementation/execution.

In agreement with Grilo *et al.* (2020), the next step should be the evaluation of how and how much Brazilian reptile populations are being impacted by road-kills. However, this approach is not yet common even in other countries (see Barrientos *et al.*, 2021), and for this to be achievable, there must be appropriate efforts to reduce bias in both survey designs and geographical distribution. Our main recommendation, thereby, is to focus on poorly sampled areas and to use methodologies specifically planned for reptile road-kill samplings. Also, in order to understand and minimize local and regional biases, the observer's efficiency and carcass removal rates should be estimated whenever possible.

## ACKNOWLEDGEMENTS

We thank Bruno F. R. do Nascimento, Henrique M. Louback and Lucas de L. Paiva for the help in collecting trait data; Luisa Diele-Viegas for providing body temperature data of some Amazonian species; and the Brazilian Network of Transportation Ecology Specialists (REET Brasil) for providing contacts and discussions that certainly helped us in this research's development. We also acknowledge the Coordination for the Improvement of Higher Education Personnel (CAPES) for funding this work through NDFR's scholarship. F.A.S.P. was supported by the National Council for Scientific and Technological Development (CNPq, Programa de Capacitação Institucional – PCI/INMA) of the Brazilian Ministry of Science, Technology and Innovation (MCTI).

## REFERENCES

- Abra F. *et al.* (2019) Pay or prevent? Human safety, costs to society and legal perspectives on animal-vehicle collisions in São Paulo state, Brazil. *PLoS ONE*, **14**(4), 1-22.
- Andrews K. M. & Gibbons J. W. (2005) How do Highways Influence Snake Movement? Behavioral Responses to Roads and Vehicles. *Copeia*, **2005**(4), 772-752.
- Andrews K. M., Gibbons J. W. & Jochimsen D. M. (2006) Literature Synthesis of the Effects of Roads and Vehicles on Amphibians and Reptiles. Report No. FHWA-HEP-080-005. Washington, D.C.: Federal Highway Administration (FHWA), U.S. Department of Transportation.



546 Ascensão F., D'Amico M. & Barrientos R. (2018) Validation data is needed to support  
547 modelling in Road Ecology. *Biological Conservation*, **230**, 199-200.  
548

549 Ascensão, F. *et al.* (2018) Environmental challenges for the Belt and Road Initiative. *Nature*  
550 *Sustainability*, **1**, 206-209.  
551

552 Assis J. R. *et al.* (2020) Snakes on highways in the Cerrado biome: an intentional conduct?.  
553 *Studies on Neotropical Fauna and Environment*, 1-8.  
554

555 Atwood T. B. *et al.* (2020) Herbivores at the highest risk of extinction among mammals,  
556 birds, and reptiles. *Science Advances*, **6(32)**.  
557

558 Bager A. & Rosa C. A. (2011) Influence of Sampling Effort on the Estimated Richness of  
559 Road-Killed Vertebrate Wildlife. *Environmental Management*, **47**, 851-858.  
560

561 Barbosa P. *et al.* (2020) Simulating the consequences of roads for wildlife population  
562 dynamics. *Landscape and Urban Planning*, **193**, 103672.  
563

564 Barrientos R. *et al.* (2018) A review of searcher efficiency and carcass persistence in  
565 infrastructure-driven mortality assessment studies. *Biological Conservation*, **222**, 146-153.  
566

567 Barrientos R. *et al.* (2021) The lost road: Do transportation networks imperil wildlife  
568 population persistence?. *Perspectives in Ecology and Conservation*, **19(4)**, 411-416.  
569

570 Bernarde P. S. (2012) *Anfíbios e répteis: introdução ao estudo da herpetofauna brasileira*.  
 571 Curitiba: Anolisbooks.  
 572  
 573 Barthelmess E. L. & Brooks M. S. (2010) The influence of body-size and diet on road-kill  
 574 trends in mammals. *Biodiversity and Conservation*, **19**, 1611-1629.  
 575  
 576 Boarman W. I. & Sazaki M. (2006) A highway's road-effect zone for desert tortoises  
 577 (*Gopherus agassizii*). *Journal of Arid Environments*, **65(1)**, 94-101.  
 578  
 579 Böhm M. *et al.* (2013). The conservation status of the world's reptiles. *Biological*  
 580 *Conservation*, **157**, 372-385.  
 581  
 582 Bonnet X., Naulleau G. & Shine R. (1999) The dangers of leaving home: dispersal and  
 583 mortality in snakes. *Biological Conservation*, **89(1)**, 39-50.  
 584  
 585 Breiman L. (2001) Random Forests. *Machine Learning*, **45**, 5-32.  
 586  
 587 Brieger F. *et al.* (2022) Behavioural reactions to oncoming vehicles as a crucial aspect of  
 588 wildlife-vehicle collision risk in three common wildlife species. *Accident Analysis &*  
 589 *Prevention*, **168**.  
 590  
 591 Bueno C., Sousa C. O. M. & Freitas S. R. (2015). Habitat or matrix: which is more relevant to  
 592 predict road-kill of vertebrates?. *Brazilian Journal of Biology*, **75(4, supl.1)**, 228-238.  
 593

594 Cabral S. *et al.* (2019) Potential seed dispersers: a new facet of the ecological role of *Boa*  
595 *constrictor constrictor* Linnaeus 1758. *Biota Neotropica*, **19(4)**, e20180626.  
596

597 Clevenger A. P., Chuzcz B. & Gunson K. E. (2003) Spatial patterns and factors influencing  
598 small vertebrate fauna road-kill aggregations. *Biological Conservation*, **109(1)**, 15-26.  
599

600 Coelho I. P., Kindel A. & Coelho A. V. P. (2008) Roadkills of vertebrate species on two  
601 highways through the Atlantic Forest Biosphere Reserve, southern Brazil. *European Journal*  
602 *of Wildlife Research*, **54**, 689-699.  
603

604 Coffin A. W. (2007) From roadkill to road ecology: a review of the ecological effects of  
605 roads. *Journal of Transport Geography*, **15(5)**, 396-406.  
606

607 Colino-Rabanal V. & Lizana M. (2012) Herpetofauna and roads: a review. *Basic and Applied*  
608 *Herpetology*, **26**, 5-31.  
609

610 Costa H. C., Guedes T. B. & Bérnils R. S. (2018) Lista de répteis do Brasil: padrões e  
611 tendências. *Herpetologia Brasileira*, **10(3)**, 110-279.  
612

613 Cox, Neil *et al.* (2022) A global reptile assessment highlights shared conservation needs of  
614 tetrapods. *Nature*. **605**, 285-290.  
615

616 Crane M. *et al.* (2021) Lots of movement, little progress: a review of reptile home range  
617 literature. *PeerJ*, **9**.  
618

619 Cutler D. R. *et al.* (2007) Random forests for classification in ecology. *Ecology*, **88**(11), 2783-  
620 2792.

621

622 DeVault T. L. *et al.* (2015) Speed kills: ineffective avian escape responses to oncoming  
623 vehicles. *Proceedings of the Royal Society B: Biology Sciences*, **282**(1801).

624

625 Dinerstein E. *et al.* (2017) An Ecoregion-Based Approach to Protecting Half the Terrestrial  
626 Realm. *BioScience*, **67**(6), 534-545.

627

628 Diniz H. S. *et al.* (2021) Diet of *Salvator merianae* (Squamata: Teiidae): New prey item and  
629 review of predation records. *North-Western Journal of Zoology*, **17**(2), 309-314.

630

631 Dowle M. & Srinivasan A. (2021) data.table: Extension of ‘data.frame’. R package version  
632 1.14.2.

633

634 Elhacham E. *et al.* (2020) Global human-made mass exceeds all living biomass. *Nature*, **588**,  
635 442-444.

636

637 Fahrig L. & Rytwinski T. (2009) Effects of Roads on Animal Abundance: an Empirical  
638 Review and Synthesis. *Ecology and Society*, **14**(1).

639

640 Feldman A. *et al.* (2015) The geography of snake reproductive mode: a global analysis of the  
641 evolution of snake viviparity. *Global Ecology and Biogeography*, **24**(12), 1433-1442.

642

Flyvbjerg B. (2009). Survival of the unfittest: why the worst infrastructure gets built - and what we can do about it. *Oxford Review of Economic Policy*, **25(3)**, 344-367.

Ford A. T. & Fahrig L. (2007) Diet and body size of North American mammal road mortalities. *Transportation Research Part D: Transport and Environment*, **12(7)**, 498-505.

Forman R. T. T. *et al.* (2003) *Road Ecology: science and solutions*. Covelo: Island Press.

Glaudas X. *et al.* (2019) Foraging mode, relative prey size and diet breadth: A phylogenetically explicit analysis of snake feeding ecology. *Journal of Animal Ecology*, **88(5)**, 757-767.

Gonçalves L. O. *et al.* (2018) Reptile road-kills in Southern Brazil: Composition, hot moments and hotspots. *Science of The Total Environment*, **615**, 1438-1445.

González-Suárez M., Ferreira F. Z. & Grilo C. (2018) Spatial and species-level predictions of road mortality risk using trait data. *Global Ecology and Biogeography*, **27(9)**, 1093-1105.

González-Suárez M., Gómez A. & Revilla E. (2013) Which intrinsic traits predict vulnerability to extinction depends on the actual threatening processes. *Ecosphere*, **4(6)**, 1-16.

Greenwell B. M. (2017) pdp: An R package for constructing partial dependence plots. *The R Journal*, **9(1)**, 421-436.

667 Grilo C. *et al.* (2018) BRAZIL ROAD-KILL: a data set of wildlife terrestrial vertebrate road-  
668 kills. *Ecology*, **99**(11), 2625-2625.  
669  
670 Grilo C. *et al.* (2021) Conservation threats from roadkill in the global road network. *Global*  
671 *Ecology and Biogeography*, **30**(11), 2200-2210.  
672  
673 Grilo C. *et al.* (2019) Reply: Modeling scenario of population response to roads as a  
674 conservation risk assessment strategy. *Biological Conservation*, **230**, 201-202.  
675  
676 Grilo C. *et al.* (2020) Roadkill risk and population vulnerability in European birds and  
677 mammals. *Frontiers in Ecology and the Environment*, **18**(6), 323-328.  
678  
679 Harfoot M. B. J. *et al.* (2021) Using the IUCN Red List to map threats to terrestrial  
680 vertebrates at global scale. *Nature Ecology & Evolution*, **5**, 1510-1519.  
681  
682 Hill J. E., DeVault T. L. & Belant, J. L. (2020) Research note: A 50-year increase in vehicle  
683 mortality of North American mammals. *Landscape and Urban Planning*, **197**, 103746.  
684  
685 IBGE (2019) *Biomass e sistema costeiro-marinho do Brasil: compatível com a escala*  
686 *1:250.000*. Rio de Janeiro: Instituto Brasileiro de Geografia e Estatística.  
687  
688 Ibisch P. *et al.* (2016) A global map of roadless areas and their conservation status. *Science*,  
689 **354**(6318), 1423-1427.  
690

IUCN (2021) *The IUCN Red List of Threatened Species. Version 2021-2*. Available from  
URL: <<https://iucnredlist.org>>.

Jacobson S. L. *et al.* (2016) A behavior-based framework for assessing barrier effects to  
wildlife from vehicle traffic volume. *Ecosphere*, **7**(4).

Jochimsen D. M. *et al.* (2004) *A literature review of the effects of roads on amphibians and  
reptiles and the measures used to minimize those effects*. Idaho: Idaho Fish and Game  
Department, USDA Forest Service.

Johnson T. F. *et al.* (2021) Handling missing values in trait data. *Global Ecology and  
Biogeography*, **30**(1), 51-62.

Lara Resendiz R. A. (2020) ¿Qué implicaciones ecofisiológicas tiene la actividad nocturna en  
reptiles “diurnos”? Una revisión. *Acta Biológica Colombiana*, **25**(2), 314-326.

Laurance W. F. *et al.* (2014) A global strategy for road building. *Nature*, **513**, 229-232.

Laurance W. F., Goosem M. & Laurance S. G. W. (2009) Impacts of roads and linear  
clearings on tropical forests. *Trends in Ecology & Evolution*, **24**(12), 659-669.

Liaw A. & Wiener M. (2002). Classification and regression by randomForest. *R News*, **2**(3),  
18-22.

715 Lima S. L. *et al.* (2015) Animal reactions to oncoming vehicles: a conceptual review.  
 716 *Biological Reviews*, **90**(1), 60-76.  
 717  
 718 Lima-Santos J., Costa H. C. & Molina F. B. (2020). The curse of being serpentiform:  
 719 Perceptions of snakelike animals in São Paulo, Brazil. *Ethnobiology and Conservation*, **9**.  
 720  
 721 Marioni B. *et al.* (2019). *Melanosuchus niger* (Black Caiman). Diet. *Herpetological Review*,  
 722 **50**(3), 565-566.  
 723  
 724 Marques O. A. V., Eterovic A. & Sazima I. (2019) *Serpente da Mata Atlântica: guia ilustrado*  
 725 *para as regiões costeiras do Brasil*. Cotia: Ponto A.  
 726  
 727 Marques O. A. V. *et al.* (2017) A rotten choice: feeding attempt by a coral snake (*Micrurus*  
 728 *frontalis*) on a dead pitviper (*Bothrops jararaca*) that had swallowed a bulky rodent.  
 729 *Herpetology Notes*, **10**, 137-139.  
 730  
 731 McCardle L. D. & Fontenot C. L. (2016) The influence of thermal biology on road mortality  
 732 risk in snakes. *Journal of Thermal Biology*, **56**, 39-49.  
 733  
 734 Medrano-Vizcaíno P. *et al.* (2022) Roadkill patterns in Latin American birds and mammals.  
 735 *Global Ecology and Biogeography*, **31**(9), 1756-1783.  
 736  
 737 Medrano-Vizcaíno P. *et al.* (2023) First national assessment of wildlife mortality in Ecuador:  
 738 An effort from citizens and academia to collect roadkill data at country scale. *Ecology and*  
 739 *Evolution*, **13**(3), e9916.



740

741 Meijer J. R. *et al.* (2018) Global patterns of current and future road infrastructure.

742 *Environmental Research Letters*, **13**(6).

743

744 Meiri S. (2018) Traits of lizards of the world: Variation around a successful evolutionary

745 design. *Global Ecology and Biogeography*, **27**(10), 1168-1172.

746

747 Meiri S. *et al.* (2021) Different solutions lead to similar life history traits across the great

748 divides of the amniote tree of life. *Journal of Biological Research-Thessaloniki*, **28**(3).

749

750 Meiri S. *et al.* (2020) Viviparity does not affect the numbers and sizes of reptile offspring.

751 *Journal of Animal Ecology*, **89**(2), 360-369.

752

753 Milborrow S. (2021) *plotmo: Plot a model's residuals, response, and partial dependence*

754 *plots. R package version 3.6.1.*

755

756 Myers N. *et al.* (2000) Biodiversity hotspots for conservation priorities. *Nature*, **403**, 853-858.

757

758 Oliveira S. L. *et al.* (2020) Ecologia de estradas: estado da arte no Brasil e no mundo.

759 *Brazilian Journal of Development*, **6**(12), 98516-98573.

760

761 Passos D. C., Galdino C. A. B. & Rocha C. F. D. (2015) Challenges and perspectives for

762 studies on home range of lizards from South America. *South American Journal of*

763 *Herpetology*, **10**(2), 82-89.

764

765 Paterson J. E. *et al.* (2019) Road avoidance and its energetic consequences for reptiles.  
 766 *Ecology and Evolution*, **9(17)**, 9794-9803.  
 767  
 768 Pinto F. A. S., Clevenger A. P. & Grilo C. (2020) Effects of roads on terrestrial vertebrate  
 769 species in Latin America. *Environmental Impact Assessment Review*, **81**.  
 770  
 771 Proulx C. L., Fortin G. & Blouin-Demers G. (2014) Blanding's turtles (*Emydoidea*  
 772 *blandingii*) avoid crossing unpaved and paved roads. *Journal of Herpetology* **48(2)**, 267-271.  
 773  
 774 Quintino E. P & Bicca-Marques J. C. (2013) Predation of *Alouatta puruensis* by *Boa*  
 775 *constrictor*. *Primates*, **54(4)**, 325-330.  
 776  
 777 R Core Team. (2020) *foreign: Read data stored by 'Minitab', 'S', 'SAS', 'SPSS', 'Stata',*  
 778 *'Systat', 'Weka', 'dBase', ... R package version 0.8-81*. R Foundation of Statistical  
 779 Computing.  
 780  
 781 R Core Team (2022) *R: A language and environment for statistical computing*. R Foundation  
 782 of Statistical Computing.  
 783  
 784 Rapacciuolo G. *et al.* (2017) The signature of human pressure history on the biogeography of  
 785 body mass in tetrapods. *Global Ecology and Biogeography*, **26(9)**, 1022-1034.  
 786  
 787 Ratton P., Secco H. & Rosa C. A. (2014) Carcass permanency time and its implications to the  
 788 roadkill data. *European Journal of Wildlife Research*, **60(3)**, 543-546.  
 789

790 Rincón-Aranguri M. *et al.* (2019) Road kill of snakes on a highway in an Orinoco ecosystem:  
791 landscape factors and species traits related to their mortality. *Tropical Conservation Science*,  
792 **12**, 1-18.

793

794 Robson L.E. & Blouin-Demers G. (2013) Eastern hognose snakes (*Heterodon platirhinos*)  
795 avoid crossing paved roads, but not unpaved roads. *Copeia*, **2013(3)**, 507-511.

796

797 Roll U. *et al.* (2017) The global distribution of tetrapods reveals a need for targeted reptile  
798 conservation. *Nature Ecology & Evolution*, **1**, 1677-1682.

799

800 Rosenblatt A. E. *et al.* (2022) What do adult black caiman (*Melanosuchus niger*) actually  
801 eat?. *Biotropica*, **54(1)**, 275-278.

802

803 Row J. R., Blouin-Demers G. & Weatherhead P. J. (2007) Demographic effects of road  
804 mortality in black ratsnakes (*Elaphe obsoleta*). *Biological Conservation*, **137(1)**, 117-124.

805

806 RStudio Team (2022). *RStudio: Integrated Development Environment for R*. RStudio, PBC.

807

808 Rytwinski T. & Fahrig L. (2012) Do species life history traits explain population responses to  
809 roads? A meta-analysis. *Biological Conservation*, **147(1)**, 87-98.

810

811 Rytwinski T. & Fahrig L. (2015) The Impacts of Roads and Traffic on Terrestrial Animal  
812 Populations. In: *Handbook of Road Ecology* (eds. R. van der Ree, D. J. Smith & C. Grilo)  
813 pp.407-413. Chichester: John Wiley & Sons.

814

815 Sales R. F. D., Lima M. L. S. & França B. R. A. (2019) Dead but delicious: an unusual  
816 feeding event by the Sertão Muçurana snake (*Boiruna sertaneja*) on a bird carcass.  
817 *Herpetology Notes*, **12**, 941-943.

818

819 Santini L. *et al.* (2018) Global drivers of population density in terrestrial vertebrates. *Global*  
820 *Ecology and Biogeography*, **27(8)**, 968-979.

821

822 Santini L., Isaac N. J. B. & Ficetola G. F. (2018) TetraDENSITY: A database of population  
823 density estimates in terrestrial vertebrates. *Global Ecology and Biogeography*, **27(7)**, 787-  
824 791.

825

826 Santos R. L. *et al.* (2016) Carcass persistence and detectability: reducing the uncertainty  
827 surrounding wildlife-vehicle collision surveys. *PLoS ONE*, **11(11)**.

828

829 Santos S. M. *et al.* (2015) Sampling effects on the identification of road-kill hotspots:  
830 Implications for survey design. *Journal of Environmental Management*, **162**, 87-98.

831

832 Santos S. M., Carvalho F. & Mira A. (2011) How Long Do the Dead Survive on the Road?  
833 Carcass Persistence Probability and Implications for Road-Kill Monitoring Surveys. *PLoS*  
834 *ONE*, **6(9)**.

835

836 Sazima I. & Strüssman C. (1990) Necrofagia em serpentes brasileiras: exemplos e previsões.  
837 *Revista Brasileira de Biologia*, **50(2)**, 461-468.

838

839 Secco H. *et al.* (2014) Intentional snake road-kill: a case study using fake snakes on a  
840 Brazilian road. *Tropical Conservation Science*, **7(3)**, 561-571.  
841

842 Shine R. *et al.* (2004) Why Did the Snake Cross the Road? Effects of Roads on Movement  
843 and Location of Mates by Garter Snakes (*Thamnophis sirtalis parietalis*). *Ecology and*  
844 *Society*, **9(1)**.  
845

846 Silva I., Crane M. & Savini T. (2021) The road less travelled: Addressing reproducibility and  
847 conservation priorities of wildlife-vehicle collision studies in tropical and subtropical regions.  
848 *Global Ecology and Conservation*, **27**.  
849

850 Silva M. X. G. *et al.* (2021) What are the factors influencing the aversion of students towards  
851 reptiles?. *Journal of Ethnobiology and Ethnomedicine*, **17(35)**.  
852

853 Southwood A. & Avens L. (2010) Physiological, behavioral, and ecological aspects of  
854 migration in reptiles. *Journal of Comparative Physiology B*, **180**, 1-23.  
855

856 Stekhoven S. J. & Bühlmann P. (2012) MissForest – non-parametric missing value imputation  
857 for mixed-type data. *Bioinformatics*, **28(1)**, 112-118.  
858

859 Teixeira F. Z. *et al.* (2013a) Are road-kill hotspots coincident among different vertebrate  
860 groups?. *Oecologia Australis*, **17(1)**, 36-47.  
861

862 Teixeira F. Z. *et al.* (2013b) Vertebrate road mortality estimates: Effects of sampling methods  
863 and carcass removal. *Biological Conservation*, **157**, 317-323.

864

865 Tisler T. R., Teixeira F. Z. & Nóbrega R. A. (2022) Conservation opportunities and  
866 challenges in Brazil's roadless and railroad-less areas. *Science Advances*, **8**(9).

867

868 Ucha J. & Santos T. G. (2017) Death and life on the roadway: scavenging behaviour of the  
869 green racer snake *Philodryas patagoniensis* (Girard, 1858) (Dipsadidae). *Herpetology Notes*,  
870 **10**, 439-441.

871

872 Uetz P. *et al.* (eds.) (2021). *The Reptile Database (23 May 2021 release)*. Available from  
873 URL: <<http://www.reptile-database.org>>.

874

875 Welling S. *et al.* (2016) Forest Floor visualizations of random forests. *ArXiv e-prints*.

876

877 Wickham H. (2016) *ggplot2: Elegant graphics for data analysis*. Springer-Verlag New York.

878

879 Wickham H. (2007) Reshaping data with the reshape package. *Journal of Statistical Software*,  
880 **21**(12), 1-20.

881

882 Wickham H. (2011) The split-apply-combine strategy for data analysis. *Journal of Statistical*  
883 *Software*, **40**(1), 1-29.

884

885 Wickham H. *et al.* (2021) *dplyr: A grammar of data manipulation*. R package version 1.0.7.

886

887 Wilke C. O. (2020) *cowplot: Streamlined plot theme and plot annotations for 'ggplot2'*. R  
888 package version 1.1.1.

**TABLES**

**Table 1:** Selected ecological and functional traits, and their respective descriptions and rationale. Data completeness is represented in parentheses in the first column.

| Trait (data completeness) | Description of the variable   | Rationale  |
|---------------------------|---|--|
| Habitat breadth (99.31%)  | number of Brazilian ecoregions, as defined by Dinerstein <i>et al.</i> (2017), included in the geographic distribution of each species. | since generalist species are more likely to forage and move in unknown and/or in a greater variety of habitats (Forman <i>et al.</i> , 2003; Coffin, 2007), a higher number of inhabited ecoregions is expected to be related to higher road-kill rates.   |
| Leg development (100%)    | development of the members of the species (“legless”, “one reduced pair”, “leg-reduced” or “four-legged”).                              | whereas serpentiform species (which have no limbs or have only reduced or vestigial limbs) are generally seen by society as aversive, frightening and disgusting (see Lima-Santos, Costa & Molina, 2020; Silva <i>et al.</i> , 2021), they may be intentionally road-killed (see Secco <i>et al.</i> , 2014; |

|                                  |   |  |
|----------------------------------|---|--|
|                                  |   | <p>Assis <i>et al.</i>, 2020) more often than other reptiles. Also, are usually faster, more agile and have greater site-fidelity than snakes (Andrews, Gibbons &amp; Jochimsen, 2006).</p> <p>Thus, it is expected that legless species present higher road-kill rates than species with well-developed members.</p>                  |
| Body mass (99.65%)               | Log <sub>10</sub> maximum body mass of adult individuals of the species, in kilograms (kg). | <p>larger reptile species tend to move more slowly, which may hinder or prevent them from adopting escape behaviours from oncoming vehicles (Jochimsen <i>et al.</i>, 2004; Lima <i>et al.</i>, 2015). They also tend to be more easily spotted, possibly inducing intentional road-kills, thus presenting higher road-kill rates.</p> |
| Main activity substrate (93.08%) | main activity substrate of the species (“arboreal/semiarboreal”, “cryptozoic/fossorial”,    | <p>ground-dwelling species are expected to present higher road-kill rates than species that are more active in the water or on vegetation, as they frequently</p>  |



|                            |  |   |
|----------------------------|--|---|
|                            | “terrestrial”,<br>“semiaquatic” or<br>“aquatic”).                                | move across surfaces that may include roads.  |
| Foraging strategy (79.06%) | foraging strategy of the species (“active foraging”, “mixed” or “sit-and-wait”). | because active foraging species actively search for prey and move more frequently than ambush or mixed foraging species, they are more likely to cross roads – thus, they are expected to present higher road-kill rates (see Bonnet, Naulleau & Shine, 1999; Glaudas <i>et al.</i> , 2019). Alternatively, however, ambush foraging species often have cryptic coloration and are more likely to not exhibit escape behaviours when facing traffic (see Lima <i>et al.</i> , 2015; Jacobson <i>et al.</i> , 2016). |
| Clutch size (61.94%)       | average (mean or midpoint) of hatchlings or neonates per litter.                 | since road-kills are usually concentrated on locally abundant species (Forman <i>et al.</i> , 2003), it is expected that species with larger litter sizes (used here as a proxy for abundance) are more affected  |

|                           |   |   |
|---------------------------|---|---|
|                           |   | by road-kills.  |
| Activity time (82.52%)    | main period of activity of the species (“diurnal”, “nocturnal” or “cathemeral”).          | species with nocturnal activity are expected to present higher road-kill rates, as their activity peaks coincide with times when both traffic and visibility are generally lower (thus decreasing the chances both of animals to avoid the road and of drivers to evade unintentionally road-killing the animals). Also, during the night paved roads are more likely to retain more heat than adjacent areas, thus creating potential ecological traps for reptiles seeking to use them for thermoregulation ( <i>e.g.</i> , see Shine <i>et al.</i> , 2004; McCardle & Fontenot, 2016). |
| Body temperature (20.41%) | average (mean or midpoint) body temperature of individuals of the species found in nature | considering the influence of thermal biology on reptiles’ road-kill risk ( <i>e.g.</i> , McCardle & Fontenot, 2016), it is expected that species that require higher  |

|                          |  |   |
|--------------------------|--|---|
|                          | (or, when not available, of captive specimens), in degrees Celsius (°C). | temperatures present higher road-kill rates, as they would be more likely to use roads to thermoregulate (see Andrews, Gibbons & Jochimsen, 2006).  |
| Reproductive mode (100%) | if the species is oviparous or viviparous.                               | since viviparity directly influences the ability of females to feed, move and escape predators, in addition to being a potentially negative adaptation in warmer regions (see Feldman <i>et al.</i> , 2015), and considering the importance of reproductive behaviours for herpetofaunal road-kill patterns (see Jochimsen <i>et al.</i> , 2004), it is expected that viviparous species present higher road-kill rates than oviparous species. |
| Trophic level (91.00%)   | if the species has a carnivorous, herbivorous or omnivorous diet.        | omnivorous species are expected to present higher road-kill rates, as they tend to be more generalist, which makes them more prone to forage on road edges.   |

**Table 2:** Models summary table, pointing out each model, its corresponding dataset, the number of road-kill rates and predictors included, and its results (variance explained and mean squared error).

| Model | Dataset   | road-kill rates included  | Predictors  | Mean square error | Variance explained (%) |
|-------|---|---|---|-------------------|------------------------|
| 1     | unfiltered  | 782 (uncorrected) road-kill rates comprising 175 species, from 43 studies | latitude + longitude + survey interval + family + traits  | 1.217             | 64.52                  |
|       |   |   | family + traits   | 3.367             | 1.92                   |
| 2     | filtered for data availability on sampling method, sampling speed and sampling time | 187 (uncorrected) road-kill rates comprising 104 species, from 19 studies | latitude + longitude + survey interval + survey period + sampling method + sampling speed + sampling time + family + traits | 1.127             | 72.37                  |
|       |   |   | family + traits   | 3.737             | 8.43                   |

|          |   |  |  |       |       |
|----------|---|--|--|-------|-------|
| <b>3</b> | filtered for<br>survey<br>periods of at<br>least two<br>years       | 371 (uncorrected)<br>road-kill rates<br>comprising 134<br>species, from 12<br>studies  | latitude + longitude +<br>survey interval +<br>family + traits | 1.342 | 47.06 |
|          |   |  | family + traits  | 2.278 | 10.16 |
| <b>4</b> | filtered for<br>survey<br>intervals of<br>seven days<br>or less     | 367 (uncorrected)<br>road-kill rates<br>comprising 104<br>species, from 23<br>studies  | latitude + longitude +<br>survey interval +<br>family + traits | 1.168 | 52.86 |
|          |   |  | family + traits  | 2.569 | -3.63 |
| <b>5</b> |   | 367 (corrected) road-<br>kill rates comprising<br>104 species, from 23<br>studies      | latitude + longitude +<br>survey interval +<br>family + traits | 1.216 | 49.73 |
|          |   |  | family + traits  | 2.594 | -7.2  |
| <b>6</b> | filtered for<br>survey<br>intervals of<br>seven days<br>or less and | 166 (uncorrected)<br>road-kill rates<br>comprising 51<br>species, from five<br>studies | latitude + longitude +<br>survey interval +<br>family + traits | 0.881 | 62.59 |
|          |   |  | family + traits  | 2.183 | 7.36  |
| <b>7</b> | survey<br>periods of at<br>least two                                | 166 (corrected) road-<br>kill rates comprising<br>51 species, from five                | latitude + longitude +<br>survey interval +<br>family + traits | 0.869 | 61.4  |

|   |  |   |  |       |       |
|---|--|---|--|-------|-------|
|   | years  | studies   | family + traits  | 2.050 | 8.94  |
| 8 | filtered for<br>survey<br>intervals of<br>15 days or<br>less and<br>survey<br>periods of at<br>least one<br>year | 384 (uncorrected)<br>road-kill rates<br>comprising 113<br>species, from 19<br>studies | latitude + longitude +<br>survey interval +<br>family + traits | 1.109 | 55.63 |
|   |  |   | family + traits  | 2.167 | 12.08 |

## FIGURE LEGENDS

**Figure 1:** Predicted and observed road-kill rates for 51 reptile species, axes in  $\log_{10}$  scale. Circles represent the median from all collected and predicted data for each species, error bars represent upper and lower confidence intervals (respectively, 95% and 5%) and the diagonal line represents a 1:1 relationship between observed and predicted rates.

**Figure 2:** Predicted and observed road-kill rates for each The IUCN Red List Category. Abbreviations are as follows: CR = Critically Endangered; DD = Data Deficient; EN = Endangered; LC = Least Concern; cd = Lower Risk/Conservation Dependent; nt = Lower Risk/Near Threatened; NT = Near Threatened; NE = Not Evaluated; VU = Vulnerable.

**Figure 3:** Relative variable importance of each predictor in model 7 across all imputed datasets, according to the mean decrease in accuracy defined by percentage increase in mean squared error when removing the variable (%IncMSE).

**Figure 4:** Partial dependence plots for each predictor variable in model 7 in relation to the predicted road mortality rates across all imputed datasets, in order of relative variable importance. Abbreviations are as follows: All = Alligatoridae; Alp = Alopoglossidae; Amp = Amphisbaenidae; Anl = Aniliidae; Anm = Anomalepididae; Bod = Boidae; Chl = Chelidae; Clb = Colubridae; Dct = Dactyloidae; Dpl = Diploglossidae; Dps = Dipsadidae; Elp = Elapidae; Emy = Emydidae; Gkk = Gekkonidae; Gym = Gymnophthalmidae; Hpl = Hoplocercidae; Ign = Iguanidae; Lsr = Leiosauridae; Lpt = Leptotyphlopidae; Llm = Liolaemidae; Phy = Phyllodactylidae; Pdc = Podocnemididae; Ply = Polychrotidae; Scn = Scincidae; Sph = Sphaerodactylidae; Ted = Teiidae; Tst = Testudinidae; Trpdp = Tropidophiidae; Trpdr = Tropiduridae; Typ = Typhlopidae; Vpr = Viperidae; aqt = aquatic; ar/ = arboreal/semiarboreal; cr/ = cryptozoic/fossorial; smq = semiaquatic; trr = terrestrial.

**Figure 5:** Maps of (a) richness of reptile species across Brazil, (b) road network density (km), and predicted road-kill rates in (c) ind./km/year and in (d) ind./year. Made with the free and open-source QGIS.