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

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

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

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

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 & Bérnils, 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 taxonspecific 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.

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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 *et al*. (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 *et al*., 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 *et al*., 2021), they may be intentionally road-killed (see Secco *et al*., 2014;

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

	“terrestrial”, “semiaquatic” or “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 *et al*., 2019). Alternatively, however, ambush foraging species often have cryptic coloration and are more likely to not exhibit escape behaviours when facing traffic (see Lima *et al*., 2015; Jacobson *et al*., 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 *et al*., 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 (*e.g.*, see Shine *et al*., 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 (*e.g.*, 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 *et al*., 2015), and considering the importance of reproductive behaviours for herpetofaunal road-kill patterns (see Jochimsen *et al*., 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 squared 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

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

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