

Contents lists available at ScienceDirect

Journal for Nature Conservation



journal homepage: www.elsevier.com/locate/jnc

# Spatial and temporal patterns of road mortality in the Caspian whip snake (*Dolichophis caspius* Gmelin 1758) in Romania



Tiberiu Constantin Sahlean<sup>a,\*</sup>, Iulian Gherghel<sup>b,c</sup>, Răzvan Zaharia<sup>d,e</sup>, Viorel Dumitru Gavril<sup>a,d</sup>, Raluca Melenciuc<sup>c</sup>, Cătălin Răzvan Stanciu<sup>e</sup>, Alexandru Strugariu<sup>b</sup>

<sup>a</sup> Romanian Academy, Institute of Biology Bucharest, Bucharest, Romania

<sup>b</sup> Alexandru Ioan Cuza University of Iași, Institute of Interdisciplinary Research, Department of Exact and Natural Sciences, 700505 Iași, Romania

Ovidius University Constanța, Faculty of Natural and Agricultural Sciences, Aleea Universității nr. 1, corpul B, 900470 Constanța, Romania

<sup>d</sup> University of Bucharest, Faculty of Biology, 060031 Bucharest, Romania

<sup>e</sup> Oceanographic Research and Marine Environment Protection Society Oceanic-Club, 900674 Constanța, Romania

#### ARTICLE INFO

Keywords: Road mortality Road ecology Species distribution model Habitat fragmentation

# ABSTRACT

Roads significantly impact natural landscapes, posing threats to wildlife, particularly amphibians and reptiles. Among these, snakes are often overlooked, despite their vulnerability to vehicular collisions. This study investigates the road mortality patterns for the Caspian whip snake (*Dolichophis caspius*), an animal frequently killed by road traffic in Eastern Europe, including Romania. A database of 270 road-killed Caspian whip snakes, mostly adults, was compiled, showing road-kills were predominantly found on national and county roads. Our findings confirm the existence of "hot moments" when road-kills are more likely. The ensemble model emphasized high-risk road sections, with road density, terrain ruggedness and habitat configuration being the most important predictors. Such information can optimize management costs and guide targeted conservation efforts, holding implications for snake populations in Eastern Europe.

#### 1. Introduction

Roads are a key factor in the rapid expansion of the human population in all terrestrial habitats, allowing us to cross great distances. Still, they are also one of the most important disturbances for wildlife and a widespread ecological threat (Garriga et al., 2012; Lin, 2016; Trombulak & Frissell, 2000). Roads negative impact on wild animals have been well documented (e.g. reviewed in (Coffin, 2007; Fahrig & Rytwinski, 2009; Forman & Alexander, 1998; Trombulak & Frissell, 2000)), and include a wide range of effects, both direct and indirect, such as: (1) habitat loss during the construction phase and (2) habitat degradation during both construction and operational phase, through disturbance (noise), the presence of chemicals (e.g.: exhaust fumes, oil leaks), light pollution and secondary development (Forman & Alexander, 1998; Johnson & St-Laurent, 2011; Laurance, Goosem, & Laurance, 2009; Trombulak & Frissell, 2000), (3) creating a barrier effect that hampers the movement of species and impedes gene flow (Coffin, 2007; de Rivera et al., 2022; Forman & Alexander, 1998; Trombulak & Frissell, 2000), (4) creating an avoidance zone for species disturbed by noise, light or air pollution (Fahrig & Rytwinski, 2009), (5) is an attractant for species such as reptiles which use roads as a source of heat to regulate their body temperature, while necrophages are attracted to carcasses resulting from collisions with vehicles (Akrim, Mahmood, Andleeb, Hussain, & Collinson, 2019; McCardle & Fontenot, 2016; Sullivan, 1981), and, finally, (6) mortality caused by direct impact with vehicles (Coffin, 2007; Fahrig & Rytwinski, 2009; Forman & Alexander, 1998; Trombulak & Frissell, 2000). Road networks can thus lead to declines in population size and density (Fahrig, Pedlar, Pope, Taylor, & Wegner, 1995), reduced genetic diversity and reproductive output (Keevil et al., 2022; Noël, Ouellet, Galois, & Lapointe, 2007; Shine & Bonnet, 2009), shifts in sex ratios (Gibbs & Steen, 2005; Piczak, Markle, & Chow-Fraser, 2019) or modified behavior patterns (Ruiz-Capillas, Mata, Fernández, Fernandes, & Malo, 2021).

The most obvious and visible impact caused by road networks is wildlife-vehicle collisions (Coffin, 2007), which can occur as a result of multiple factors, some pertaining to each species' characteristics (e.g., movement speed, life history traits, behavior, physiology, habitat preferences, migration patterns) (Jochimsen, Peterson, & Harmon, 2014; McCardle & Fontenot, 2016; Piczak et al., 2019; Rytwinski & Fahrig, 2012; Schmidt, Lewison, & Swarts, 2020; Shepard, Dreslik, Jellen, &

\* Corresponding author. E-mail address: tiberiu.sahlean@ibiol.ro (T.C. Sahlean).

https://doi.org/10.1016/j.jnc.2023.126547

Received 5 October 2023; Received in revised form 15 December 2023; Accepted 16 December 2023 Available online 17 December 2023 1617-1381/© 2023 Elsevier GmbH. All rights reserved. Phillips, 2008; Shine, Lemaster, Wall, Langkilde, & Mason, 2004), driver behavior as well as road type and features (width, curvature, surface type) (Forman et al., 2003; Ramp, Wilson, & Croft, 2006; Wagner, Brune, & Popescu, 2021).

Amphibians and reptiles were among the earliest road casualties noted (Hodson, 1966; Savage, 1935). Still, snakes are rarely the subject of road ecology studies (Jochimsen et al., 2014), even though species in this group have a series of characteristics that make them prone to vehicle mortality (Andrews, Gibbons, & Jochimsen, 2008): relatively slow-moving, an elongate body form, thermoregulating on road surfaces or at the edges, usually too small to be visible on the road and even when they are observed they may be killed deliberately (Andrews & Gibbons, 2005; Bonnet, Naulleau, & Shine, 1999; Jochimsen et al., 2014; MacKinnon et al., 2005; McCardle & Fontenot, 2016; Sullivan, 1981).

The Caspian whip snake (Dolichophis caspius) is a large ( $\sim 2$  m), mobile species that inhabits south-eastern Europe, parts of Asia Minor, and a small part of western Asia, into Kazakhstan and southern Russia, including the area to the northwest of the Caspian Sea ((Ščerbak and Böhme, 1993, Sahlean et al., 2014, Fuhn and Vancea, 1961). D. caspius is a characteristic element of open steppe habitats, forest steppes, Mediterranean scrubland, and rocky outcrops at low to medium altitudes (0-1600 m A.S.L.) (Fuhn & Vancea, 1961; Sahlean, Gherghel, Papes, Strugariu, & Zamfirescu, 2014; Ščerbak & Böhme, 1993). Romania defines the northern distribution limit for the Caspian whip snake, the species being present along the Danube and its major tributaries, as well as the historical regions of Moldavia and Dobruja (Covaciu-Marcov & David, 2010; Ferenți, Cupșa, & Telcean, 2011; Sahlean et al., 2014; Sahlean et al., 2019). The species is known to be a frequent victim of road traffic within its range (Kambourova-Ivanova et al., 2012; Mollov, Kirov, Petrova, Georgiev, & Velcheva, 2013; Tok, Ayaz, & Cicek, 2011), although data is sparse and dispersed in different published papers. The same holds for Romania, where road-killed D. caspius specimens are a common sight, and it has not gone unnoticed, several publications having mentioned the alarming number of carcasses observed (Covaciu-Marcov, Cicort-Lucaciu, Pop, Lucaci, & Ferenți, 2020; Covaciu-Marcov & David, 2010; Covaciu-Marcov, Ferenți, Ghira, & István, 2012).

According to the National Institute of Statistics, at the end of 2021, Romania had 86199 km of public roads used by almost 9 million vehicles, a 3.8 % increase compared to the previous year (ACEA, 2023). The mean road density was 0.46 km/km<sup>2</sup>, and the mean distance to a road was 1.4 km (based on data from the INIS Geoportal, https://geoportal. gov.ro). The traffic also increased by 34 % between 2013 and 2021, from 46,345 to 62,161 million vehicle-kilometers (V.K.M.) (Eurostat https://ec.europa.eu).

Despite being relatively abundant within its distribution range in Romania, the species is classified as vulnerable (V.U.) by the Romanian Red Book of Vertebrates (Iftime, 2005), and it is protected through European and national legislation (Habitats Directive 92/43/E.E.C., Law 49/2011). Moreover, common species and taxa with increased vagility are more likely to suffer high vehicular mortality rates, which can lead to population declines (Carr & Fahrig, 2001; Ford & Fahrig, 2007; Grilo, Bissonette, & Santos-Reis, 2009).

Species distribution modeling has become a staple tool for biologists and there are now over 6000 studies published over a 20-year period that have used species distribution models (S.D.M.s) in some form (Araújo et al., 2019) with applications ranging from conservation of endangered species, climate change, evolutionary biology, paleobiology or the study of invasive taxa to name just a few (Elith et al., 2011; Sahlean et al., 2014). It is therefore surprising that S.D.M.s have rarely been used in road ecology to infer locations with high likelihood of mortality. Whilst there is a large body of work describing casualty patterns in terms of local road features, our search revealed only a handful of studies (Garrote, Fernández-López, López, Ruiz, & Simón, 2018; Ha & Shilling, 2018; Kantola, Tracy, Baum, Quinn, & Coulson, 2019; Schmidt et al., 2020) which used MaxEnt to predict road-mortality for a few animals, including butterflies, mammals, and birds. Our study had five main objectives: (1) create a database of roadkilled Caspian whip snakes, (2) analyze the resulting information to detect spatio-temporal patterns of road mortality, (3) test whether ensemble S.D.M.s can lead to the creation of risk maps, identifying locations where there is an increased likelihood of vehicular collisions, (4) identify the factors which create favorable conditions for increased likelihood of collisions, and (5) identify relevant management recommendations.

# 2. Methods

# 2.1. Road mortality database

We compiled a database of road-killed *D*. caspius records based on (i) our own observations, collected during 2010–2022, from March to November, during routing and occasional field surveys within the species' range in Romania, (ii) previously published studies (Covaciu-Marcov et al., 2020; Covaciu-Marcov & David, 2010; Covaciu-Marcov et al., 2012; Sahlean et al., 2019), and (iii) online citizen science recordings (iNaturalist, Ornitodata, Observation.org, and Facebook groups dedicated to records of road-killed animals – RoadKill and D.O.R. (Dead on Road)). For the citizen science data to be included, it needed to satisfy the following conditions: (1) observations included a clear picture of the animal for precise identification and (2) events contained G. P.S. positioning for precise location.

Besides location, we extracted, where possible, information regarding the number of killed individuals found, the precise or approximate event date, and the specimen's reproductive status (in the form of adult/subadult/juvenile). Juvenile Caspian whip snakes have a conspicuous model and are easily identifiable; we treated individuals with obscured markings as subadults, and adults were defined as individuals with uniform coloring and no juvenile pattern. Subsequently, we extracted more information based on the location of the kill (Table 1).

For the modeling phase, the road-kill database was aligned to the resolution of the spatial variables (500 m) using SDMToolbox (Brown, Bennett, & French, 2017), and each point was centered on the road axis using the function *Near* from the ArcGIS 10.7.1 ((ESRI), 2019) toolbox; observations from non-public roads or outside the environmental predictors were removed. In total, 202 road-kill events were used for predictions.

#### 2.2. Environmental variables

We generated a set of eight environmental variables (Table 2), which we used to predict road sectors with increased likelihood of road-kills; all variables were based on the Romanian road network, which we downloaded from the INIS geoportal (https://geoportal.gov.ro), and then processed by eliminating all non-roads (like hiking trails or paths). Next, we used ArcGIS to create evenly spaced points along roads every 500 m, and the points were used to split the road network into segments. We generated a 500-meter square grid in ArcGIS and retained only the squares along the road network, based on which we obtained zonal statistics. Points were then used to extract mean values from the zonal statistics rasters and then merge back to the segmented road network. Finally, we converted the road network from vector to raster to obtain

#### Table 1

Variables extracted based on the location of the road-kills and included in the database.

Variable	Description/Levels
Road type	A – highway, D.N. – national road, D.J. – county road, D.C. –
	communal road, OTHER – other types of roads
Landcover	Agriculture, Forest, Grassland, Mix natural-agriculture, Shrub,
class	Urban, Other
Class	UIDall, Olliel

#### Table 2

Predictors used for modeling and the relative importance (based on A.U.C.).

Code	Name and description	Relative importance (%)
agri_perc	Percentage of agricultural landcover	4.7
dist_nat	Distance to natural features (in meters)	2.9
hli	Heat Load Index	3.4
nat_perc	Percentage of natural landcover	1.6
rddens	Road density (in km/km <sup>2</sup> )	23.7
rugged	Terrain ruggedness index	6.1
sinuosity	Road sinuosity, a value of 1 means a straight	2.4
	line	
tcover	Tree cover density (in %)	4.5

the final predictors.

The 500-meter length of road segments (and, inherently, modeling resolution) was chosen since shorter sections can be justified more easily for mitigation measures and have greater chances of implementation due to smaller costs (Langen et al., 2007; Lima Santos et al., 2017).

The variables pertaining to landcover were created based on Corine Landcover 2018, which we downloaded from the Copernicus website (https://land.copernicus.eu). To obtain the percentage of agricultural and natural landcovers we collapsed all relevant categories for that landcover. For agriculture, we merged all categories from 2-Agricultural areas, except 231-Pastures and 243-Land principally occupied by agriculture, with significant areas of natural vegetation, which we included with the natural categories. For natural landcovers we included all codes from 3-Forest and seminatural areas and 4-Wetlands and the two categories from 2-Agricultural areas. We used *Tabulate intersection* combined with the 500-meter grid to calculate the percentage of agricultural or natural landcover, then used the points to extract the values, introduce them to the vector road network, and create the predictor raster.

The distance to natural features was calculated using the *Near* function in ArcGIS based on the natural landcover categories mentioned above. We generated a heat load index (H.L.I.) using the Geomorphometry and Gradient Metrics v2.0 toolbox (Evans et al., 2014), accounting for different solar radiation levels received by slopes facing a specific orientation. The terrain ruggedness index measures how flat or rugged each cell is compared to the ones in the vicinity and was calculated using WhiteboxTools v2.2.0 (Lindsay, 2014). The heat load index and the ruggedness index were based on the 30-meter elevation model downloaded from the Copernicus website (https://land.copernicus.eu), and were resampled to the desired 500-meter resolution.

Road density was calculated in ArcGIS using the line density function and represents the number of kilometers of road network in a square kilometer cell. Sinuosity is measured as the degree of road curvature along each 500-meter road segment and it was calculated using the Stream Gradient & Sinuosity Toolbox for ArcGISv10 (Dilts, 2015).

The tree cover density raster was acquired from the Copernicus website (https://www.copernicus.eu), and data were extracted based on the grid we created and then used to generate the predictor raster.

#### 2.3. Model development

The road-kill locations database was rarefied at the resolution of the environmental predictors (500 m) before being used for modeling using the SDMToolbox (Brown et al., 2017), and we used a 70 %-30 % database split for model training and testing.

Before the modeling phase, the rasters were split into two groups of predictors: (1) the first one was used for training the model, and consisted of layers clipped to the convex hull of the distribution records, using a 1 km buffer, and (2) a second set encompassing the entire study area, which was composed of all counties where the species' presence has been confirmed, based on the road-kill records from this database, as well as published occurrence data from Sahlean et al. (2019). The operation was conducted in ArcGIS with the SDMToolbox add-on.

Next, using the Variance Inflation Factor (V.I.F.) index implemented in the USDM package (Naimi, Hamm, Groen, Skidmore, & Toxopeus, 2014) in R version 4.2.2 (R Core Team, 2022), we tested for spatial autocorrelation, but no variables were excluded as V.I.F. value was less than 10 (Chatterjee & Hadi, 2006).

We opted for ensemble modeling using the sdm package in the R software environment (Naimi and Araújo, 2016) to emphasize road sectors with a high risk of Caspian whip snake mortality. Models were trained using the predictors clipped to the study area and six methods: G. L.M. (Generalized Linear Models), G.A.M. (Generalized Additive Models), R.F. (Random Forests), Maxent (through the java implementation, v. 3.4.4), B.R.T. (Boosted Regression Trees) and MARS (Multivariate Adaptive Regression Spline). Afterwards, the models were projected to the entirety of the study area (as defined above). The settings used for model training consisted of 3-fold cross-validation with 6 repetitions, and 10.000 random background points, generated only along the road lines.

We evaluated all the 108 generated models using two metrics: A.U.C. (Area Under the Receiver Operating Characteristic (R.O.C.) Curve) and the T.S.S. (True Skill Statistic). The ensemble did not include all generated models, instead we applied a selection algorithm which included only models where A.U.C. or T.S.S. values were greater than mean plus half a standard deviation (Eustace, Esser, Mremi, Malonza, & Mwaya, 2021).

# 2.4. Post-modeling analysis

The ensemble model was thresholded to obtain only high ( $\geq 0.5$ ) mortality risk sectors. Next, we created 500-meter buffers around the road sectors with high mortality risk and used the landcover map to extract relevant information regarding the area occupied by the different types of habitats around those road sectors.

# 2.5. Roadkill data analysis

We calculated the number of kills per month (kpm) by dividing the number of road-killed individuals found during a month by the number of field days and multiplying by the total number of days for that specific month. Next, we performed a chi-square ( $\chi^2$ ) goodness of fit test to explore significant differences in the number of observed kills per month during the active season (April – November), and used post-hoc pairwise comparisons with Holm correction to examine which months were significantly different to each other.

Statistical analyses were performed in R version 4.2.2 (R Core Team, 2022) and the graphs were created using the package *ggplot2* (Wickham, 2016). Maps were generated using ArcGIS 10.7.1.

#### 3. Results

# 3.1. Road mortality - Spatial and temporal patterns

The final database contains 251 events recorded between 2003 and 2022, corresponding to 270 road-killed Caspian whip snake individuals. Most of the road-kills were recorded in the Dobruja, and the southwestern part of Romania, along the Danube, and between Craiova and Bechet (Fig. 1).

For the individuals where a reproductive state was assigned (N = 150), most of the road-kills were composed of adults (N = 130) and very few juveniles (N = 11) and subadults (N = 9).

Road-kills were found primarily on national (N = 148) and county roads (N = 81), the rest being found on highways (N = 9), communal roads (N = 18) or on other roads (N = 14).

According to our database, the first road-killed Caspian whip snakes were found at the beginning of April. Mortality continued until mid-November when the last individual was found (Fig. 2). The largest number of casualties were registered during the months of May (N =



Fig. 1. Location and density of road-kill events collected in the current database.

54), June (N = 96), September (N = 28) and October (N = 44) and the highest values of kills per month were calculated for the months of June (111 kpm), September (74 kpm) and October (95 kpm). There was a significant difference in the number of kills per month ( $\chi^2 = 98.2$ , df = 7, p < 0.01), and significant differences between the months with peak mortality (June, September, October) and the months from the beginning (April) and end (November) of the activity season, as well as the months of summer (July, August) (Table 3).

The main type of habitat in a 500-meter radius around each casualty was represented by agricultural land (N = 169), followed by urbanized habitats (N = 50), and only 38 individuals were found in locations where roads cross forests or grasslands.

#### 3.2. Road mortality model

The ensemble mortality models obtained moderate performance metrics, with A.U.C. values between 0.73 and 0.79 and T.S.S. ranging from 0.38 to 0.46 (Table 4, Supplementary Material Figures S1 – S3). The highest contributing variables (based on A.U.C.) were road density (23.7 %), ruggedness (6.1 %) and percentage of agricultural land (4.5 %), followed by tree cover (4.5 %), heat load index (3.4 %), distance to natural features (2.9 %), road sinuosity (2.4 %), and percentage of natural areas (1.6 %) (Table 2, Supplementary Material Figure S4).

The response curves for the predictors used and the "maximum mortality risk space" defined by the first two most important variables (Fig. 4) show that, according to this model, road-kills are likely to occur at high road densities (above 10 km/km<sup>2</sup>), in areas with primarily flat terrain, an increased percentage of agricultural land, and reduced

percentage of tree cover (Fig. 5). Other conditions with a reduced of influence are a high degree of solar radiation (large heat load index value), roads very close or very far from natural features, straight roads (sinuosity closer to 1), and a high percentage of natural areas surrounding the roads (Fig. 5).

The ensemble model identified roads with high risk of Caspian whip snake road-kill incidents in areas where these events are known to occur frequently, such as Dobruja, especially the southern part of the region, on the roads south of Craiova, the south-western tip of the country, but also highlighted new areas where *D. caspius* road-kills have not been reported until now, such as the center part of the study area, the main road from Giurgiu to Bucharest, as well as roads from eastern Romania, in Galați, Brăila and Ialomița counties (Fig. 3).

In areas where the ensemble model showed an increased risk of road mortality ( $\geq$ 0.5), the primary type of habitat was represented by agricultural land (65 %), followed by artificial surfaces (19 %) and grasslands (8 %).

# 4. Discussions

Road impacts on amphibians and reptiles in Romania are largely unknown, as there have been only a few studies in this direction (Cicort-Lucaciu, Sas-Kovács, & Covaciu-Marcov, 2016; Ciolan, Cicort-Lucaciu, Sas-Kovács, Ferenți, & Covaciu-Marcov, 2017; Covaciu-Marcov et al., 2020; Covaciu-Marcov et al., 2012; Hartel, Moga, Oellerer, & Puky, 2009; Iosif, Rozylowicz, & Popescu, 2013).

It is difficult to determine whether the 270 road-killed *D. caspius* individuals are a significant number relative to the entire population,



Fig. 2. Road mortality of Caspian whip snakes during the active season – April to November; the size of the circles represents the quantity of individuals killed on the road.

# Table 3 Results of the chi-square post-hoc pairwise comparisons for kills per month.

	Мау	June	July	August	September	October	November
April May June July August September October	n.s.	p < 0.01 p < 0.01	n.s. n.s. p < 0.01	n.s. n.s. p < 0.01 n.s.	p = 0.01 n.s. n.s. p < 0.01 p < 0.01	p < 0.01 p = 0.02 n.s. p < 0.01 p < 0.01 n.s.	n.s. n.s. p < 0.01 n.s. n.s. n.s. p < 0.01

Table 4

Performance metrics fo	r the	five	modeling	methods	used
------------------------	-------	------	----------	---------	------

Method	AUC	TSS
GLM	0.74	0.42
GAM	0.74	0.41
RF	0.79	0.46
Maxent	0.75	0.42
BRT	0.76	0.41
MARS	0.73	0.38

but studies have shown that observations made by car (as most of the records are) are most likely to underreport the number of victims, as smaller animals are harder to spot and are less likely to persist long enough to allow detection (Elzanowski, Ciesiołkiewicz, Kaczor,

Radwańska, & Urban, 2009; Langen et al., 2007; Santos et al., 2015; Slater, 2002; Teixeira, Coelho, Esperandio, & Kindel, 2013) and the number of road-kills was high enough to warrant several publications on the subject in Romania (Covaciu-Marcov et al., 2020; Covaciu-Marcov et al., 2012; Ferenți et al., 2011).

*D. caspius* is one of the fastest and largest snakes in Europe and has high vagility, characteristics which have often been associated with an increased risk of road traffic mortality (Kimberly M. Andrews & Gibbons, 2005; Bonnet et al., 1999; Carr & Fahrig, 2001). Evidence from Europe (the whip snake, *Hierophis viridiflavus*) and North America (the black racer, *Coluber constrictor*) indicate that the species that are ecologically similar to *D. caspius* appear to cross roads more frequently compared to smaller and more sedentary snakes (Andrews & Gibbons, 2005; Bonnet et al., 1999). In the Iberian Peninsula *Malpolon monspessulanus*, a snake similar in size and ecology to *D. caspius*, was the



Fig. 3. Geographic position of the study area in Romania (left inset) and the map of the ensemble model showing areas with high risk of road-kills occurring (warmer colors used for increased mortality risk), as well as road-kill locations from our database.

most frequent road-killed reptile (Garriga et al., 2012). Moreover, experiments on *Coluber constrictor* have shown that, contrary to expectations, individuals generally have a tendency to remain still instead of fleeing when they are approached by moving vehicles (Andrews & Gibbons, 2005).

The ensemble model and the risk map we developed showed that the likelihood of road-kills increases with road density, probably because it increases the chances of individuals crossing the roads (Supplementary Material Figures S4, Fig. 5). In fact, other authors have found a very strong relationship between road density and the number of road crossings in turtle populations ( $R^2$  between 0.73 and 0.91) (Gibbs & Shriver, 2002), an increased risk of mortality or wildlife-vehicle collisions as a result of increasing road densities (Bastianelli et al., 2021; Frair, Merrill, Beyer, & Morales, 2008; González-Suárez, Zanchetta Ferreira, & Grilo, 2018; Patrick & Gibbs, 2010; Philcox, Grogan, & Macdonald, 1999), inverse correlations between species abundance and road density (Apps, McLellan, Woods, & Proctor, 2004; Reijnen, Foppen, & Veenbaas, 1997), as well as reduced species richness (Findlay & Bourdages, 2000) while still others identified an increased risk of extinction with increasing road density (Anderson, Farmer, Ferretti, Houde, & Hutchings, 2011). The species' large size is also a characteristic that makes D. caspius vulnerable to vehicles, as it increases the chance that an individual is run over by a car. Moreover, this is highly related to driver behavior, as some will intentionally run snakes over (Andrews & Gibbons, 2005; Bonnet et al., 1999; Jochimsen et al., 2014; MacKinnon et al., 2005; McCardle & Fontenot, 2016), but also the authors' personal experience) which is easier to do with a larger animal. Even our data is composed overwhelmingly of adults, which can be biased because adults are easier to observe and remain on the road for

longer (Elzanowski et al., 2009; Garriga et al., 2012). Another possible cause is the fact that adults are much more exposed to road traffic, as they possess a larger body size, but also travel greater distances: males in search of a mate and females in order to find a nesting site (Bonnet et al., 1999).

Our data shows that the road-kill rate for the Caspian whip snake is not constant over the entirety of the active season. There is a significant difference in the mortality rate for each month: the number of road-kills is reduced in April and November, and two peaks are evident, one in May-June and another towards the end of the species' activity period, in September-October. Other studies have noted this seasonality in roadkills, both in snakes as well as other reptiles (Andelković & Bogdanović, 2022; Beaudry, Demaynadier, & Hunter JR, 2010; Bonnet, Naulleau, & Shine, 1999; Garriga et al., 2012; Ile, Maier, Cadar, Covaciu-Marcov, & Ferenți, 2020; Jochimsen, Peterson, & Harmon, 2014; Wagner, Brune, & Popescu, 2021). This seasonal tendency in reptile road-kills has been explained by the increased activity of adults in spring, as males are in search of mates and females look for nesting spots, while autumn has typically been associated with a rise in the number of road-killed neonates (Bonnet, Naulleau, & Shine, 1999; Ile, Maier, Cadar, Covaciu-Marcov, & Ferenți, 2020; Jochimsen, Peterson, & Harmon, 2014; Keevil et al., 2022; MacKinnon et al., 2005). Our data lacks the necessary depth to separate age categories for the entirety of the active season, but from the existing observations, the number of road-killed neonates was low. This is likely because the bulk of observations were made by car, but other authors (Covaciu-Marcov et al., 2012) have also argued that the second peak is related to increased levels of activity after the hot and dry summer characteristic for southern Romania, when reptiles are mostly inactive. A future study is



Fig. 4. "Maximum mortality risk space" described by the two most important predictors used for generating the model (road density and terrain ruggedness).



Fig. 5. Response curves for the eight predictors used for creating the ensemble model (see Table 2 for complete names and description of each variable).

necessary to investigate whether there are more road-killed neonates during this second peak.

Another defining factor related to the presence of road-kills is the type of habitat through which the road passes, and studies have found that the number of victims is greater in areas with natural habitats, as opposed to artificial surfaces (like agricultural fields) (Anđelković & Bogdanović, 2022; MacKinnon et al., 2005; Malo, Suárez, & Díez, 2004). Our results differ in this regard, as one of the most important

contributing variables for our ensemble model was the percentage of agricultural land, and the response curves (Fig. 5) showed an increased risk of road-kill in areas with where agricultural fields are prevalent, while distance to natural features and the percentage of natural features were not as prominent in creating the model. Moreover, road sections with high risk of mortality in our model traverse mainly agricultural fields and artificial areas. We believe this is, at least in part, determined by each species' biology and ecology, as *D. caspius* successfully survives

in arable land, especially field where there is some degree of natural vegetation (Covaciu-Marcov et al., 2020).

Apart from road density, other factors that contributed to the ensemble predicting the likelihood of road mortality in D. caspius were terrain ruggedness, tree cover, and road sinuosity. The model showed an increased response to areas with reduced ruggedness (flat areas) (Supplementary Material Figure S4, Fig. 5). Such areas are likely favored by snakes for crossing, as it allows faster movement rates (Harvey & Larsen, 2020), or for basking, although snakes can be mistakenly identified as basking on roads Andrews and Gibbons (2005). Reduced tree cover increases the chances of vehicle incidents as snakes are more exposed to predators, but also accounts for high levels of solar radiation (Jochimsen et al., 2014; Shine et al., 2004). In both cases, snakes tend to seek cover as fast as possible, increasing the chances of vehicle collision. The influence of road sinuosity is explained in terms of the speed of the approaching vehicle, as travel speeds will be higher in a straight line, which is precisely what the response curve shows, as the response increases as sinuosity approaches 1 (straight line); the contrary has been reported for mammals such as stone marten (Martes foina), genet (Viverra genetta) or the Egyptian mongoose (Herpestes ichneumon) where collisions increase with road sinuosity (Grilo, Ascensão, Santos-Reis, & Bissonette, 2011; Grilo et al., 2009). The different results could be attributed to different behaviors - snakes tend to freeze when approached by vehicles (Andrews & Gibbons, 2005), and are even known to thermoregulate on roads (Sullivan, 1981), while some species of mammals such as weasels (Mustela nivalis) and Western polecats (Mustela putorius) showed strong signs of road avoidance (Grilo, Bissonette, & Santos-Reis, 2008).

Road-kills are the primary type of ecological data resulting from the study of roads (Chyn, Lin, Chen, Chen, & Fitzgerald, 2019) and citizen science is of paramount importance to filling gaps in temporal and spatial observations, both of which would require significant time and financial effort (Heigl, Horvath, Laaha, & Zaller, 2017) if not for the contribution of many volunteers, including people with intimate knowledge of certain surroundings (Fraisl et al., 2022). While there are some inherent problems associated with these types of data, such as their "presence-only" and ad-hoc nature and certain underlying biases (Heigl et al., 2017; Périquet, Roxburgh, le Roux, & Collinson, 2018; Petrovan, Vale, & Sillero, 2020), citizen science is relevant to developing road mortality hotspots maps and conveying road-kill patterns (Périquet et al., 2018; Petrovan et al., 2020). Our own results show that citizen science data can be successfully combined with standardized surveys from scientists in order to enhance our knowledge of road mortality patterns and develop risk models. Numerous tentative biodiversity or road-kill initiatives have begun to take shape in Romania in recent years, which report data as part of international databases, in groups on social networks or as part of local databases; besides the common problems plaguing all citizen science databases, these initiatives suffer greatly from the lack of centralized aggregation, expert verification and possibility of adding standardized data. A concentrated initiative to aggregate existing data and standardize future observations collected would be of great benefit to the scientific knowledge in Romania as well as for conservation as a science and practice.

## 5. Conclusions and conservation implications

Road mortality is known to significantly impact reptile populations (Andrews, Langen, & Struijk, 2015) and population declines of 50 % or more have been recorded for snake species living near roads (Rudolph, Burgdorf, Conner, & Schaefer, 1999). Furthermore, road-kills are a poor predictor of actual species abundance, as large, mobile snakes are significantly more at risk (Bonnet et al., 1999). As such, it is difficult to say whether a high number of road-kills is an indicator of large populations, a consequence of the species' characteristics, an increase in interest among amateur or professional herpetologists, or simply an artefact of sampling bias.

The findings highlight the possibility of a significant threat from road mortality for the Caspian whip snakes in Romania. The species' large size and increased vagility, coupled with its ecological characteristics, make it more susceptible to road traffic incidents (Andrews and Gibbons, 2005; Bonnet et al., 1999; Carr and Fahrig, 2001). The study emphasizes the importance of considering road density and habitat type in understanding and addressing road mortality risks (Andelković & Bogdanović, 2022; MacKinnon et al., 2005; Malo et al., 2004).

As of August 2023, the Conservation Evidence website (https:// www.conservationevidence.com) lists 29 actions related to reptile conservation for mitigating of threats related to transportation, but none of them have been evaluated for effectiveness so far. With respect to our study and Romania, we believe management measures which could be applied successfully relate to cutting and mowing of roadside vegetation, education and/or awareness campaigns to improve behavior towards reptiles, and the usage of signage to warn motorists about wildlife presence. As our study highlights the existence of "hot moments", periods during the year with increased frequency of road-kills, temporary warning signs could be erected during the corresponding months to increase driver awareness, therefore also avoiding the usage of permanent signs which have been shown to lead to driver habituation (Tanner & Perry, 2007). This action can be combined with temporary speed reductions and public outreach in the form of seasonal press releases (Beaudry et al., 2010), and be applied only to select road sections, for example by combining the 500-meter segments in our risk maps with data regarding previous road-kill locations, as application to hundreds or thousands of kilometers in the Caspian whip snake's range (Covaciu-Marcov et al., 2020) would imply significant costs. Removal of vegetation has been suggested as a management practice which can reduce the number of snakes (Durner & Gates, 1993), and if applied to habitat directly adjacent to roads can lead to a reduction in the number of roadkilled snakes (Wagner et al., 2021). However, as the Caspian whip snake is not a priority species and in no immediate threat of extinction (Iftime, 2005) it is unlikely that single-species measures will be taken, furthermore stressing the need for subsequent road ecology studies on other species of amphibians and reptiles (Iosif et al., 2013), as hotspots of road mortality usually overlap across species (Langen et al., 2007) and multispecies measures have greater chances of being applied. For the moment we believe that the knowledge and the risk map developed as part of our study can be evaluated individually by protected areas managers and, in conjunction with local information regarding the species' populations, assess whether management measures are appropriate.

While the ensemble model correctly identified areas with increased risk of mortality in Dobruja and to the south of Craiova, it also highlighted new areas where there are no reports of Caspian whip snake mortality yet. This can be a result of limited sampling, as studies on amphibian and reptile mortality are severely lacking in Romania (see Introduction), or an artefact of the heterogenous nature of our database, leading to only moderate performance metrics for the ensemble model. Therefore recalibration of the risk model is of utmost importance, as is conducting systematic walking and driving surveys with careful attention to timing, resulting in standardized data (Langen et al., 2007), especially in areas where there is a high degree of uncertainty in our ensemble model.

# Funding

This research was supported by the Romanian Academy through grant RO1567-IBB09/2024, and the Romanian Ministry of Research, Innovation, and Digitization, CNCS – UEFISCDI, PNCDI III, by the following grants: PN-III-P1-1.1-PD-2021-0591 and PN-III-P1-1.1-TE-2021-1452.

#### CRediT authorship contribution statement

Tiberiu Constantin Sahlean: . Iulian Gherghel: Conceptualization,

Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing. **Răzvan Zaharia:** Conceptualization, Investigation, Resources, Validation, Visualization, Writing – review & editing. **Viorel Dumitru Gavril:** . **Raluca Melenciuc:** Conceptualization, Investigation, Resources, Validation, Visualization, Writing – review & editing. **Cătălin Răzvan Stanciu:** . **Alexandru Strugariu:** Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Validation, Visualization, Writing – review & editing.

# Declaration of competing interest

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

## Data availability

Data will be made available on request.

# Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jnc.2023.126547.

#### References

- (ESRI), E. S. R. I. (2019). ArcGIS Release 10.7.1. In. Redlands, CA. ACEA (2023). European Automobile Manufacturers' Association. Vehicles in use Europe 2023. In.
- Akrim, F., Mahmood, T., Andleeb, S., Hussain, R., & Collinson, W. J. (2019).
- Spatiotemporal patterns of wildlife road mortality in the Pothwar Plateau, Pakistan. *Mammalia*, *83*, 487–495.
- Andelković, M., & Bogdanović, N. (2022). Amphibian and Reptile Road Mortality in Special Nature Reserve Obedska Bara, Serbia. *Animals*, 12, 561.
- Anderson, S. C., Farmer, R. G., Ferretti, F., Houde, A. L. S., & Hutchings, J. A. (2011). Correlates of Vertebrate Extinction Risk in Canada. *BioScience*, *61*, 538–549.
- Andrews, K. M., & Gibbons, J. W. (2005). How do Highways Influence Snake Movement? Behavioral Responses to Roads and Vehicles. *Copeia*, 2005, 772–782.
- Andrews, K. M., Gibbons, J. W., & Jochimsen, D. M. (2008). Ecological effects of roads on amphibians and reptiles: A literature review. In J. C. Mitchell, R. E. J. Brown, & B. Bartholomew (Eds.), *Urban Herpetology* (pp. 121–143). Salt Lake City, UT: Society for the Study of Amphibians & Reptiles.
- Andrews, K. M., Langen, T. A., & Struijk, R. P. (2015). Reptiles: Overlooked but often at risk from roads. *Handbook of road ecology*, 271–280.
- Apps, C. D., McLellan, B. N., Woods, J. G., & Proctor, M. F. (2004). Estimating Grizzly Bear Distribution and Abundance Relative to Habitat and Human Influence. *The Journal of Wildlife Management*, 68, 138–152.
- Araújo, M. B., Anderson, R. P., Márcia Barbosa, A., Beale, C. M., Dormann, C. F., Early, R., ... Rahbek, C. (2019). Standards for distribution models in biodiversity assessments. *Science Advances*, 5, eaat4858.
- Bastianelli, M. L., Premier, J., Herrmann, M., Anile, S., Monterroso, P., Kuemmerle, T., ... Heurich, M. (2021). Survival and cause-specific mortality of European wildcat (*Felis silvestris*) across Europe. *Biological Conservation*, 261, Article 109239.
- Beaudry, F., Demaynadier, P. G., & Hunter JR, M.I. (2010). Identifying Hot Moments in Road-Mortality Risk for Freshwater Turtles. *The Journal of Wildlife Management*, 74, 152–159.
- Bonnet, X., Naulleau, G., & Shine, R. (1999). The dangers of leaving home: Dispersal and mortality in snakes. *Biological Conservation*, 89, 39–50.
- Brown, J. L., Bennett, J. R., & French, C. M. (2017). SDMtoolbox 2.0: The next generation Python-based GIS toolkit for landscape genetic, biogeographic and species distribution model analyses. *PeerJ*, 5, e4095.
- Carr, L. W., & Fahrig, L. (2001). Effect of Road Traffic on Two Amphibian Species of Differing Vagility. Conservation Biology, 15, 1071–1078.
- Chatterjee, S., & Hadi, A. S. (2006). Regression analysis by example. John Wiley & Sons. Chyn, K., Lin, T.-E., Chen, Y.-K., Chen, C.-Y., & Fitzgerald, L. A. (2019). The magnitude of roadkill in Taiwan: Patterns and consequences revealed by citizen science. *Biological Conservation*, 237, 317–326.
- Cicort-Lucaciu, A., Sas-Kovács, I., & Covaciu-Marcov, S.-D. (2016). Non road human influence upon road mortality on three secondary roads in the Vâlsan River protected area, Romania. Oltenia, Studii și Comunicări, Științele Naturii, 32, 99–106.
- Ciolan, E., Cicort-Lucaciu, A.-Ş., Sas-Kovács, I., Ferenți, S., & Covaciu-Marcov, S.-D. (2017). Wooded area, forest road-killed animals: Intensity and seasonal differences of road mortality on a small, newly upgraded road in western Romania. *Transportation Research Part D: Transport and Environment*, 55, 12–20.
- Coffin, A. W. (2007). From roadkill to road ecology: A review of the ecological effects of roads. Journal of Transport Geography, 15, 396–406.

- Covaciu-Marcov, S. D., Cicort-Lucaciu, A.Ş., Pop, D. R., Lucaci, B. I., & Ferenți, S. (2020). More road-killed Caspian Whipsnakes (*Dolichophis caspius*): An update on the species distribution along the Danube, in Romania. *Amphibian & Reptile Conservation*, 14, 183–189.
- Covaciu-Marcov, S. D., & David, A. (2010). Dolichophis caspius (Serpentes: Colubridae) in Romania: New distribution records from the northern limit of its range. Turkish Journal of Zoology, 34, 119–121.
- Covaciu-Marcov, S. D., Ferenți, S., Ghira, I., & István, S. (2012). High road mortality of Dolichophis caspius in southern Romania. Is this a problem? What can we do? North-Western Journal of Zoology, 8, 370–373.
- de Rivera, C. E., Bliss-Ketchum, L., Lafrenz, M., Hanson, A., McKinney-Wise, L., Rodriguez, A., ... Temple, A. (2022). Visualizing Connectivity for Wildlife in a World Without Roads. Frontiers in Environmental. Science, 71.
- Dilts, T. E. (2015). Stream Gradient and Sinuosity Toolbox for ArcGIS 10.1. University of Nevada Reno. Available at: http://www.arcgis.com/home/item.html?id=c8eb4ce 1384e45258ccba1b33cd4e3cb. Accessed: 2022 Dec 22nd.
- Durner, G. M., & Gates, J. E. (1993). Spatial Ecology of Black Rat Snakes on Remington Farms, Maryland. The Journal of Wildlife Management, 57, 812–826.
- Elith, J., Phillips, S. J., Hastie, T., Dudík, M., Chee, Y. E., & Yates, C. J. (2011). A statistical explanation of MaxEnt for ecologists. *Diversity and Distributions*, 17, 43–57.
- Elzanowski, A., Ciesiołkiewicz, J., Kaczor, M., Radwańska, J., & Urban, R. (2009). Amphibian road mortality in Europe: A meta-analysis with new data from Poland. *European Journal of Wildlife Research*, 55, 33–43.
- Eustace, A., Esser, L. F., Mremi, R., Malonza, P. K., & Mwaya, R. T. (2021). Protected areas network is not adequate to protect a critically endangered East Africa Chelonian: Modelling distribution of pancake tortoise, *Malacochersus tornieri* under current and future climates. *PloS one*, 16, e0238669.
- Evans, J. S., Oakleaf, J., Cushman, S. A., & Theobald, D. (2014). An ArcGIS Toolbox for Surface Gradient and Geomorphometric Modeling, version 2.0-0. Available: http://evansmurphy.wix.com/evansspatial. Accessed: 2022 Dec 22nd.
- Fahrig, L., Pedlar, J. H., Pope, S. E., Taylor, P. D., & Wegner, J. F. (1995). Effect of road traffic on amphibian density. *Biological Conservation*, 73, 177–182.
- Fahrig, L., & Rytwinski, T. (2009). Effects of roads on animal abundance: An empirical review and synthesis. Ecology and society, 14.
- Ferenți, S., Cupşa, D., & Telcean, I. C. (2011). Dolichophis caspius (Gmelin, 1789) is indeed continuously distributed in Southern Romania: Zoological and conservational implications of identifying new populations. Carpathian Journal of Earth and Environmental Sciences, 6, 273–276.
- Findlay, C. S., & Bourdages, J. (2000). Response Time of Wetland Biodiversity to Road Construction on Adjacent Lands. *Conservation Biology*, 14, 86–94.
- Ford, A. T., & Fahrig, L. (2007). Diet and body size of North American mammal road mortalities. *Transportation Research Part D: Transport and Environment*, 12, 498–505.
- Forman, R. T., Sperling, D., Bissnette, J. A., Clevenger, A. P., Cutshall, C. D., Dale, V. H.,
- ... Heanue, K. (2003). Road ecology: Science and solutions. Island press. Forman, R. T. T., & Alexander, L. E. (1998). Roads and their major ecological effects. Annual Review of Ecology, Evolution, and Systematics, 29, 207–231.
- Frair, J. L., Merrill, E. H., Beyer, H. L., & Morales, J. M. (2008). Thresholds in landscape connectivity and mortality risks in response to growing road networks. *Journal of Applied Ecology*, 45, 1504–1513.
- Fraisl, D., Hager, G., Bedessem, B., Gold, M., Hsing, P.-Y., Danielsen, F., ... Haklay, M. (2022). Citizen science in environmental and ecological sciences. *Nature Reviews Methods Primers*, 2, 64
- Fuhn, I. E., & Vancea, Ş. (1961). Reptilia (Testoase, Sopîrle, Serpi) (Vol. 14): Editura Academiei.
- Garriga, N., Santos, X., Montori, A., Richter-Boix, A., Franch, M., & Llorente, G. A. (2012). Are protected areas truly protected? The impact of road traffic on vertebrate fauna. *Biodiversity and Conservation*, 21, 2761–2774.
- Garrote, G., Fernández-López, J., López, G., Ruiz, G., & Simón, M. A. (2018). Prediction of Iberian lynx road–mortality in southern Spain: A new approach using the MaxEnt algorithm. *Animal Biodiversity and Conservation*, 41.
- Gibbs, J. P., & Shriver, W. G. (2002). Estimating the Effects of Road Mortality on Turtle Populations. *Conservation Biology*, 16, 1647–1652.
- Gibbs, J. P., & Steen, D. A. (2005). Trends in Sex Ratios of Turtles in the United States: Implications of Road Mortality. *Conservation Biology*, 19, 552–556.
- González-Suárez, M., Zanchetta Ferreira, F., & Grilo, C. (2018). Spatial and species-level predictions of road mortality risk using trait data. *Global Ecology and Biogeography*, 27, 1093–1105.
- Grilo, C., Ascensão, F., Santos-Reis, M., & Bissonette, J. A. (2011). Do well-connected landscapes promote road-related mortality? *European Journal of Wildlife Research*, 57, 707–716.
- Grilo, C., Bissonette, J. A., & Santos-Reis, M. (2008). Response of carnivores to existing highway culverts and underpasses: Implications for road planning and mitigation. *Biodiversity and Conservation*, 17, 1685–1699.
- Grilo, C., Bissonette, J. A., & Santos-Reis, M. (2009). Spatial-temporal patterns in Mediterranean carnivore road casualties: Consequences for mitigation. *Biological Conservation*, 142, 301–313.
- Ha, H., & Shilling, F. (2018). Modelling potential wildlife-vehicle collisions (WVC) locations using environmental factors and human population density: A case-study from 3 state highways in Central California. *Ecological Informatics*, 43, 212–221.
- Hartel, T., Moga, C. I., Oellerer, K., & Puky, M. (2009). Spatial and temporal distribution of amphibian road mortality with a *Rana dalmatina* and *Bufo bufo* predominance along the middle section of the Târnava Mare basin, Romania. *North-Western. Journal* of Zoology, 5.
- Harvey, J. A., & Larsen, K. W. (2020). Rattlesnake migrations and the implications of thermal landscapes. *Movement Ecology*, 8, 21.

Heigl, F., Horvath, K., Laaha, G., & Zaller, J. G. (2017). Amphibian and reptile road-kills on tertiary roads in relation to landscape structure: Using a citizen science approach with open-access land cover data. *BMC Ecology*, 17, 24.

Hodson, N. (1966). A survey of road mortality in mammals (and including data for the grass snake and common frog). Journal of Zoology, 148, 576–579.

Iftime, A. (2005). Reptiles. In N. Botnariuc & V. Tatole (Eds.), The Red Book of Vertebrates in Romania (in Romanian) (pp. 173-196). Bucharest: Curtea Veche.

Ile, G.-A., Maier, A.-R.-M., Cadar, A.-M., Covaciu-Marcov, S.-D., & Ferenți, S. (2020). Dead snakes and their stories: morphological anomalies, asymmetries and scars of road killed *Dolichophis caspius* (Serpentes, Colubridae) from Romania. *Herpetozoa*, 33, 77–85.

Iosif, R., Rozylowicz, L., & Popescu, V. D. (2013). Modeling road mortality hotspots of Eastern Hermann's tortoise in Romania. *Amphibia-Reptilia*, 34, 163–172.

Jochimsen, D. M., Peterson, C. R., & Harmon, L. J. (2014). Influence of ecology and landscape on snake road mortality in a sagebrush-steppe ecosystem. *Animal Conservation*, 17, 583–592.

Johnson, C. J., & St-Laurent, M.-H. (2011). Unifying Framework for Understanding Impacts of Human Developments on Wildlife. In D. E. Naugle (Ed.), Energy Development and Wildlife Conservation in Western North America (pp. 27–54). Washington, DC: Island Press/Center for Resource Economics.

Kambourova-Ivanova, N., Koshev, Y., Popgeorgiev, G., Ragyov, D., Pavlova, M., Mollov, I., & Nedialkov, N. (2012). Effect of traffic on mortality of amphibians, reptiles, birds and mammals on two types of roads between Pazardzhik and Plovdiv region (Bulgaria)–Preliminary Results. Acta Zoologica Bulgarica, 64, 57–67.

Kantola, T., Tracy, J. L., Baum, K. A., Quinn, M. A., & Coulson, R. N. (2019). Spatial risk assessment of eastern monarch butterfly road mortality during autumn migration within the southern corridor. *Biological Conservation*, 231, 150–160.

Keevil, M. G., Noble, N., Boyle, S. P., Lesbarrères, D., Brooks, R. J., & Litzgus, J. D. (2022). Lost reproductive value reveals a high burden of juvenile road mortality in a long-lived species. *Ecological Applications*, n/a, e2789.

Langen, T. A., Machniak, A., Crowe, E. K., Mangan, C., Marker, D. F., Liddle, N., & Roden, B. (2007). Methodologies for Surveying Herpetofauna Mortality on Rural Highways. Journal of Wildlife Management, 71, 1361–1368.

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

Lima Santos, R. A., Ascensão, F., Ribeiro, M. L., Bager, A., Santos-Reis, M., & Aguiar, L. M. S. (2017). Assessing the consistency of hotspot and hot-moment patterns of wildlife road mortality over time. *Perspectives in Ecology and Conservation*, 15, 56–60.

Lin, S.-C. (2016). Landscape and traffic factors affecting animal road mortality. Journal of Environmental Engineering and Landscape Management, 24, 10–20.

Lindsay, J. B. (2014). The Whitebox Geospatial Analysis Tools project and open-access GIS. Proceedings of the GIS Research UK 22nd Annual Conference, The University of Glasgow, 16-18 April, DOI: 10.13140/RG.2.1.1010.8962. Accessed: 2022 Dec 22nd.

MacKinnon, C. A., Moore, L. A., Brooks, R. J., Nelson, G., Nudds, T., Beveridge, M., & Dempster, B. (2005). Why did the reptile cross the road? Landscape factors associated with road mortality of snakes and turtles in the South Eastern Georgian Bay area. In Proceedings of the Parks Research Forum of Ontario (PRFO) and Carolinian Canada Coalition (CCC) Annual General Meeting (pp. 153-166).

Malo, J. E., Suárez, F., & Díez, A. (2004). Can we mitigate animal-vehicle accidents using predictive models? *Journal of Applied Ecology*, 41, 701–710.

McCardle, L. D., & Fontenot, C. L. (2016). The influence of thermal biology on road mortality risk in snakes. *Journal of Thermal Biology*, 56, 39–49.
Mollov, I. A., Kirov, K. H., Petrova, S. T., Georgiev, D. G., & Velcheva, I. G. (2013).

Mollov, I. A., Kirov, K. H., Petrova, S. T., Georgiev, D. G., & Velcheva, I. G. (2013). Assessing the Influence of the Automobile Traffic on the Amphibians and Reptiles in the Buffer Zone of Biosphere Reserve" Srebarna" (NE Bulgaria). *Ecologia Balkanica*, 5.

Naimi, B., Hamm, N. A., Groen, T. A., Skidmore, A. K., & Toxopeus, A. G. (2014). Where is positional uncertainty a problem for species distribution modelling? *Ecography*, 37, 191–203.

Noël, S., Ouellet, M., Galois, P., & Lapointe, F.-J. (2007). Impact of urban fragmentation on the genetic structure of the eastern red-backed salamander. *Conservation Genetics*, 8, 599–606.

Patrick, D. A., & Gibbs, J. P. (2010). Population structure and movements of freshwater turtles across a road-density gradient. *Landscape Ecology*, 25, 791–801.

Périquet, S., Roxburgh, L., le Roux, A., & Collinson, W. J. (2018). Testing the Value of Citizen Science for Roadkill Studies: A Case Study from South Africa. Frontiers in Ecology and Evolution, 6.

Petrovan, S. O., Vale, C. G., & Sillero, N. (2020). Using citizen science in road surveys for large-scale amphibian monitoring: Are biased data representative for species distribution? *Biodiversity and Conservation*, 29, 1767–1781. Philcox, C. K., Grogan, A. L., & Macdonald, D. W. (1999). Patterns of otter Lutra lutra road mortality in Britain. Journal of Applied Ecology, 36, 748–761.

Piczak, M. L., Markle, C. E., & Chow-Fraser, P. (2019). Decades of Road Mortality Cause Severe Decline in a Common Snapping Turtle (*Chelydra serpentina*) Population from an Urbanized Wetland. *Chelonian Conservation and Biology*, 18(231–240), 210.

R Core Team. (2022). 2022. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project. org/. In (R version 4.2.1 (2022-06-23) ed.).

Ramp, D., Wilson, V. K., & Croft, D. B. (2006). Assessing the impacts of roads in periurban reserves: Road-based fatalities and road usage by wildlife in the Royal National Park, New South Wales, Australia. *Biological Conservation*, 129, 348–359.

Reijnen, R., Foppen, R., & Veenbaas, G. (1997). Disturbance by traffic of breeding birds: Evaluation of the effect and considerations in planning and managing road corridors. *Biodiversity & Conservation*, 6, 567–581.

Rudolph, D. C., Burgdorf, S. J., Conner, R. N., & Schaefer, R. R. (1999). In Preliminary evaluation of the impact of roads and associated vehicular traffic on snake populations in eastern Texas (pp. 129–136). Florida: Florida Department of Transportation Tallahassee.

Ruiz-Capillas, P., Mata, C., Fernández, B., Fernandes, C., & Malo, J. E. (2021). Do Roads Alter the Trophic Behavior of the Mesocarnivore Community Living Close to Them? *Diversity*, 13, 173.

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

Sahlean, T. C., Gherghel, I., Papeş, M., Strugariu, A., & Zamfirescu, Ş. R. (2014). Refining climate change projections for organisms with low dispersal abilities: A case study of the Caspian whip snake. *PloS one*, 9, e91994.

Sahlean, T. C., Strugariu, A., Zamfirescu, Ş. R., Chişamera, G., Stanciu, C. R., Gavril, V. D., & Gherghel, I. (2019). Filling the Gaps: Updated Distribution of the Caspian Whip Snake (Dolichophis caspius, Reptilia: Colubridae) in Romania. Russian Journal of Herpetology, 26, 1–3.

Santos, S. M., Marques, J. T., Lourenço, A., Medinas, D., Barbosa, A. M., Beja, P., & Mira, A. (2015). Sampling effects on the identification of roadkill hotspots: Implications for survey design. *Journal of Environmental Management*, 162, 87–95.

Savage, R. M. (1935). 3. The Influence of External Factors on the Spawning Date and Migration of the Common Frog, Rana temporaria temporaria Linn. Proceedings of the Zoological Society of London, 105, 49–98.

Ščerbak, N. N., & Böhme, W. (1993). Coluber caspius - Kaspische Pfeilnatter oder Springnatter. In W. Böhme (Ed.), Handbuch der Reptilien und Amphibien Europas, Band 3/I., Schlangen (Serpentes) I (pp. 83-96): Aula-Verlag Wiesbaden.

Schmidt, G. M., Lewison, R. L., & Swarts, H. M. (2020). Identifying landscape predictors of ocelot road mortality. *Landscape Ecology*, 35, 1651–1666.

Shepard, D. B., Dreslik, M. J., Jellen, B. C., & Phillips, C. A. (2008). Reptile Road Mortality around an Oasis in the Illinois Corn Desert with Emphasis on the Endangered Eastern Massasauga. *Copeia*, 2008, 350–359.

Shine, R., & Bonnet, X. (2009). Reproductive biology, population viability, and options for field management. In S. J. Mullin, & R. A. Seigel (Eds.), Snakes: Ecology and Conservation (pp. 172–200). New York, USA: Cornell University Press.

Shine, R., Lemaster, M., Wall, M., Langkilde, T., & Mason, R. (2004). Why did the snake cross the road? Effects of roads on movement and location of mates by garter snakes (*Thannophis sirtalis parietalis*). Ecology and society, 9.

Slater, F. M. (2002). An assessment of wildlife road casulties – the potential discrepancy between numbers counted and numbers killed. Web Ecol., 3, 33–42.

Sullivan, B. K. (1981). Observed Differences in Body Temperature and Associated Behavior of Four Snake Species. *Journal of Herpetology*, *15*, 245–246.

Tanner, D., & Perry, J. (2007). Road effects on abundance and fitness of Galápagos lava lizards (Microlophus albemarlensis). Journal of Environmental Management, 85, 270–278.

Teixeira, F. Z., Coelho, A. V. P., Esperandio, I. B., & Kindel, A. (2013). Vertebrate road mortality estimates: Effects of sampling methods and carcass removal. *Biological Conservation*, 157, 317–323.

Tok, C. V., Ayaz, D., & Cicek, K. (2011). Road mortality of amphibians and reptiles in the Anatolian part of Turkey. *Turkish Journal of Zoology*, 35, 851–857.

Trombulak, S. C., & Frissell, C. A. (2000). Review of Ecological Effects of Roads on Terrestrial and Aquatic Communities. *Conservation Biology*, 14, 18–30.

Wagner, R. B., Brune, C. R., & Popescu, V. D. (2021). Snakes on a lane: Road type and edge habitat predict hotspots of snake road mortality. *Journal for Nature Conservation*, 61, Article 125978.

Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis. New York: Springer-Verlag.