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Male-biased road mortality of eastern green lizards (*Lacerta viridis*) in Northeast Hungary

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In sexually reproducing animals, different sex and age groups often experience different mortality rates throughout their life cycles (Owens 2002). One of the reasons behind this is the difference in dispersal tendency between juveniles and adults and as well as between adult males and females (Trochet et al. 2016). During dispersal, individuals must take the risks associated with leaving their familiar home range, stay without shelter and get entangled in uncertain circumstances for longer than usual periods (Bonnet et al. 1999). In reptiles, dispersal is often associated with the explorative movement of juveniles from their place of birth (Léna et al. 1998) and additionally, with parental care and mate search in adults (Trochet et al. 2016).

The main cause of reptile mortality during dispersal is usually predation (Molnár et al. 2016): however, novel anthropogenic factors might also have an influence, such as road mortality (Bonnet et al. 1999). Roads can have a variety of effects on local wildlife despite their small size in the landscape. For example, roads can reduce population connectivity and fragment habitats (Trombulak & Frissell 2000), but most obviously they lead to the direct mortality of animals via vehicular collisions (Fahrig & Rytwinski 2009). Beyond these intrinsic factors, extrinsic elements might also influence road mortality. Land use near the road usually has a significant effect on spatial mortality patterns (Sillero et al. 2019), while traffic volume may also increase mortality, although its effect is ambiguous (Erritzoe et al. 2003).

In this study we monitored roadkilled lizards around Tokaj, Northeast Hungary (Fig. 1A) to better understand the differences in mortality between species, age and sex groups, and we investigated the effects of time, roadside land use types and traffic volume in relation to mortality. During the monitoring, we also counted roadkilled snakes which are presented in another study (Szabolcs et al. 2024).

We monitored a total of 58 km of roads. Roads here run through a diverse landscape predominantly with hillside dry meadows, oak forests, and vineyards on one side and wet meadows, floodplain forests and arable land on the other (Fig. 1A). We monitored the road in 2013 and 2020 in two-week periods in the activity season of reptiles in Hungary from April to October. Surveys were conducted for a one-day duration in one direction of the road. Road sections were of a similar width with two lanes and two-way traffic. Monitoring was performed by one of the authors (MS) who travelled by bicycle at a steady speed (ca. 20 km/h). He recorded the location of every lizard carcass observed on the road with a GPS device (Garmin eTrex 10) identified them at species level and determined their sex if this was possible. Most of the lizards living in the area show sexual dimorphism, thus it was usually easy to distinguish between adult females and males, although some roadkills were found in such a bad shape that it was not possible to determine sex. Carcasses were removed from the road to avoid multiple counting.

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We used QGIS 3.26 for spatial analyses and visualisation and R 4.0.2 (R Core Team 2021) for statistical analyses. We downloaded the Ecosystem Map of Hungary (Ministry of Agriculture 2019, Tanács et al. 2019) in raster format to investigate the effects of landscape structure on road mortality. We calculated the amount of the different land use types in 500 m buffers. We chose this size to adequately cover lizard home ranges as they usually move a couple of hundred meters during a year (Sound & Veith 2000, Molnár et al. 2016). We set the buffers to 1 km long road sections and then to the point localities of lizard roadkills to compare the two groups. Originally, we acquired 39 land use types which we reduced to 11 by merging similar ones (Fig. 1A). We also downloaded the most recent traffic volume data from the website of Hungarian Public Roads (2021) in vehicle-per-day format and assumed no change in volumes between the study years. Traffic data was given for 5–10 km long road sections. If a 1 km road section used in our analyses overlapped with the confluence of two sections with different traffic data, we averaged the values there. Other factors, such as the type of roads or the distance to larger towns might also influence road mortality (Wagner et al. 2021), but our study area and study design only enabled us to test the above detailed factors effectively.

We investigated the effect of time with χ^2 test comparing observed monthly patterns in mortality to an expected equal distribution. We compared the amount of land use types in road section buffers and lizard point buffers with a Kruskal-Wallis test. We also compared traffic volume between all the sections and the sections with roadkilled lizards with a Mann-Whitney test. To further investigate underlying factors, we also built a generalised linear mixed-effects model (GLMM) with the 'lme4' package (Bates et al. 2015). Before this we reduced land use types using principal component analysis (PCA). In the GLMM, months, PCA variables and traffic volume were the independent variables, while the number of roadkills was the dependent variable. Survey year and road section ID were used as random effects to control for temporal and spatial non-independence.

We found 41 roadkilled lizards of which 37 were eastern green lizard (*Lacerta viridis*), three were sand lizard (*L. agilis*) and one was eastern slow worm (*Anguis colchica*). These three and no other lizard species are known to inhabit this region. All of them were identified as adults with one exception. It was a *L. viridis* which was either a subadult animal or an adult female, the specimen being too damaged for better determination. In *L. viridis* 29 were males, seven were unidentified and one was the aforementioned specimen. There were two males and one female in *L. agilis* while the sex of the *A. colchica* remained unidentified. We excluded the latter two species from further analyses due to their low sample sizes.

Road mortality of *L. viridis* was confined to four months in the spring and summer with a peak from May to June (Fig. 1B), which significantly differed from an equal distribution across the months (χ^2 = 28.395, df = 6, p < 0.0001).

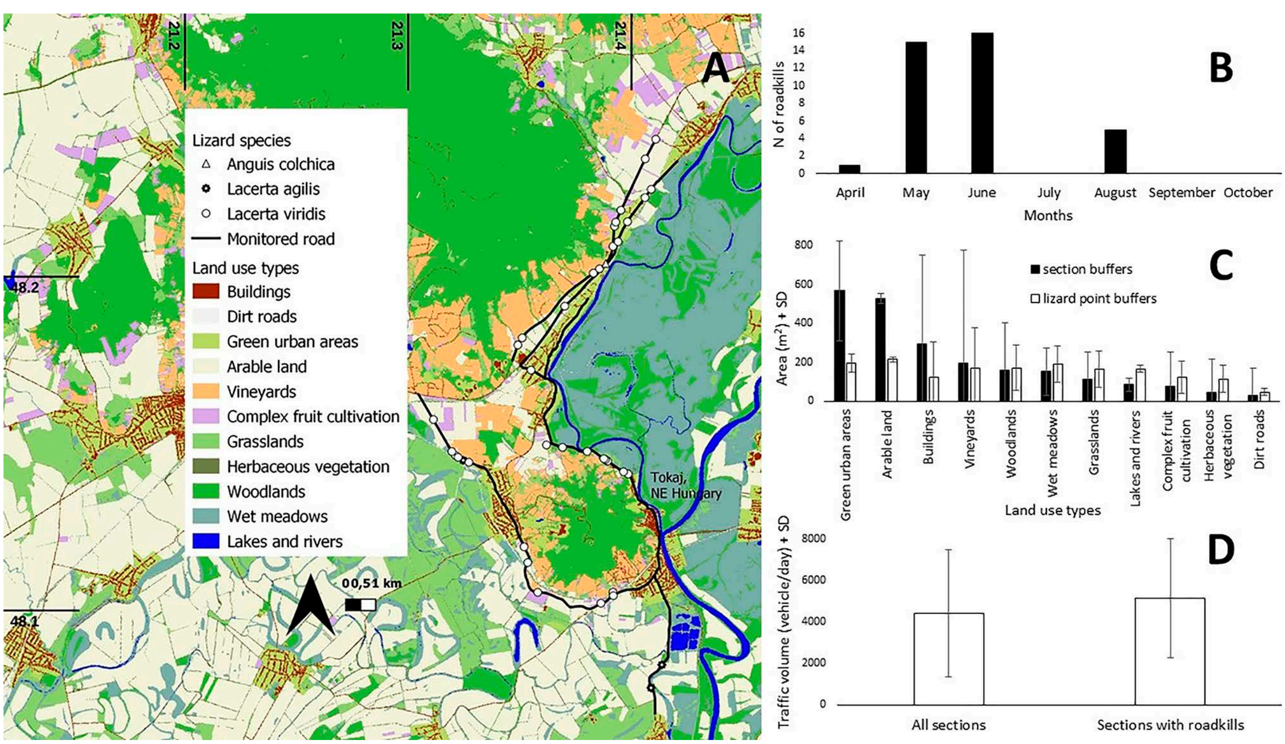

Figure 1. Study area around Tokaj, NE Hungary, where the different colours of the map correspond to the different land use types and the dots indicating roadkilled lizards (A), monthly frequency of roadkilled eastern green lizards (*Lacerta viridis*) (B), proportion of different land use types in the section and the roadkill buffers (C), and traffic volume in all the sections and the sections with roadkills (D).

Table 1. Principal Component Analysis of land use types. Only significant factor loadings are shown.

	PC1	PC2	PC3	PC4
Eigenvalues	3.3120	2.0230	1.5120	1.005
% of variance	30.113	18.395	13.749	9.133
Cumulative % of variance	30.113	48.508	62.257	71.39
Green urban areas	-0.838	-0.374		
Buildings	-0.781			
Arable land	0.779		-0.301	-0.376
Herbaceous vegetation	0.765			0.318
Complex fruit cultivation	0.581		-0.447	
Lakes and rivers	-0.498	0.452		
Wet meadows		0.804		
Woodlands		0.675		0.499
Dirt roads		-0.579		0.424
Vineyards	0.300		0.763	0.304
Grasslands		0.311	0.749	-0.325

Table 2. Parameter estimates of models. Significant estimates are highlighted with bold letters.

	Estimate	SE	z	p
(Intercept)	-3.33	0.758	-4.395	0.00001
Traffic volume	**0.0002**	**0.00008**	**2.614**	**0.009**
PC1	0.118	0.268	0.440	0.660
PC2	0.289	0.182	1.594	0.111
PC3	-0.188	0.227	-0.829	0.407
PC4	0.130	0.152	0.858	0.391
Month	**0.316**	**0.087**	**3.618**	**0.0003**

We found no statistical difference in the area of land use types between the road sections and the roadkill buffers (Kruskal-Wallis, $\chi^2 = 0.243$, df = 1, p = 0.623). Land use types in roadkill buffers showed a rather even distribution, while section buffers were more skewed with a larger share of green urban areas, arable land and buildings (Fig. 1C). Traffic volume significantly differed between all the sections and the sections with lizard roadkills (Mann-Whitney U = 803.5, p < 0.0001) where the average number of vehicles per day was higher in the sections with lizard roadkills (5140.5 ± 2882.99) than all the sections (4421.2 ± 3070.90) (Fig. 1D). In the GLMM we used the first four PCA variables, which explained 71.39% of the total variance (Table 1). The GLMM showed that the number of roadkilled *L. viridis* were significantly positively influenced by traffic volume and months, while the PCA variables derived from land use types had no effect (Table 2).

The temporal peak of road mortality was congruent with the reproductive season of *L. viridis* (Fig. 1B) (Molnár et al. 2016). In an analysis on the diet of the common kestrel (*Falco tinnunculus*) Costantini et al. (2007) found male-biased predation on the closely related and morphologically cryptic Western green lizard (*L. bilineata*), but they could not disentangle whether there was higher preference for the more vividly coloured males or simply that hunting kestrels were more likely to encounter males than females. We do not assume that vehicle drivers differentiate between sexes if they even notice lizards on the road. Intentional killing of reptiles with vehicles may occur but drivers usually target objects on roads not species or specific subcategories (Secco et al. 2014). Thus, it seems that leaving the typical home-range and perform mate-searching dispersal activity by male lizards can explain increased mortality alone. Our data suggests that the explorative dispersal of juveniles and the search for proper nesting sites by female lizards is not associated with high road mortality in the study area.

In road mortality studies, different land use types surrounding the road (Fig. 1A, 1C) are usually very important in explaining spatial patterns (Matos et al. 2012, Sillero et al. 2019). It was not the case in our investigation (Table 2). *Lacerta viridis* is a habitat generalist species, which usually lives in dry open places such as warm grasslands with bushes, forest edges, orchards, and stone walls (Puky et al. 2005, Heltai et al. 2015). It can also thrive in anthropogenically modified areas such as vineyards, fruit plantations and gardens and sometimes can be found in atypical places such as closed forests. Thus, it is not surprising that land use types showed no effect on road mortality.

Traffic volume significantly positively influenced lizard road mortality (Fig. 1D, Table 2). Our knowledge on the relation of vehicular traffic and road mortality is ambiguous and limited. For example, in a study carried out on seven amphibian species, Mazerolle (2004) found only one where mortality increased with increasing traffic volume, whereas the patterns were not clear for five species and one species showed increasing mortality with decreasing traffic volume. It appears logical that increasing traffic volume rather increases than decreases road mortality in birds, however studies investigating this factor are rare (Erritzoe et al. 2003). In a similar analysis on lizard road mortality involving *L. bilineata*, traffic had no effect while the proximity of preferred habitats increased the chance of road mortality (Lebboroni & Corti 2006). On the other hand, the latter study was carried out after the breeding season in August, which might explain the difference in the importance of factors.

Lizard road mortality remained low during our investigation, but our results should be considered as a minimum estimation that is influenced by carcass persistence and detection probability (Barrientos et al. 2016). In a study on snakes, Santos et al. (2011) found that most of the roadkills disappear after one day on average, and only a few remain for longer times with a maximum of 14 days.

Compared to a parallel analysis where we investigated snakes in the same road sections, we found 1655 roadkills in two species in three years (Szabolcs et al. 2024) which is 27 times higher annually. This pattern is congruent with the observations that lizards are the least threatened among amphibians and reptiles in relation to road mortality (van der Ree et al. 2015). Road mortality seems a negligible threat to lizards in these local populations and we assume it is not a serious conservation concern. *Anguis colchica* is a secretive species reaching its range margin around the monitored road thus little is known about its local abundance, although it is a common species in some areas in Hungary (Puky et al. 2005). *Lacerta agilis* and *L. viridis* are the most common lizard species in Hungary (Puky et al. 2005) and in the study area as well. We did not measure the abundance of lizards in nearby areas, but in our experience, it is comparable to other localities in Hungary where they can be numerous (Heltai et al. 2015) and can reach more than 30 individuals per hectare per species (Mizsei et al. 2020). Using these estimates, we assume that thousands of lizards can live next to the monitored road which suggests that a very little fraction of them fall victim to vehicles. Animal velocity, or in other words, the speed they use when they cross the road likely affects road mortality probability (Iosif et al. 2013), thus, it is not surprising that these highly agile lizards rarely get killed on the roads.

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