Road mortality of water snakes in active versus protected floodplains in light of landscape structure and traffic volume in north-eastern Hungary

Short title: Road mortality of water snakes

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Abstract
Flood protection measures can have large impacts on ecosystems and their biodiversity, yet direct comparisons of active and inactive floodplains are rare. We compared the number of roadkilled individuals of two semiaquatic water snakes (*Natrix natrix* and *N. tessellata*) on 58 km of road sections bordered either by an active floodplain or a flood-protected former floodplain in NE Hungary based on surveys conducted once every two weeks in three years. We found unexpectedly high road mortality of snakes, which was rather similar across years. Mortality had a spring and an autumn peak, corresponding to the times when snakes emerge from and return to hibernating sites. Road mortality was more severe and more predictable in the flooded than in the flood-protected area, even though traffic was more intense in the latter. Our results show that small-scale spatial differences in road mortality are mediated by landscape structure along the road, while the effects of traffic intensity and the age and sex of the individuals were negligible. For conservation implications, our study suggests that establishing culvert passages under the road and artificial hibernating sites on the floodplain-side of the roads in critical sections to reduce the road-related mortality.

Keywords flood control, hibernation, inundation, landscape composition, roadkill, wetland, *Natrix*, life history

Introduction

Flood control measures can have a large impact on ecosystems, habitats and species (Poff et al. 1997). Typically, wetland ecosystems in flood-protected floodplains are first separated from the river and its active floodplain by systems of levees (or in other words dykes or embankments). Later the former floodplain areas are often drained and land use is converted for agriculture, forestry or urbanisation (Guida et al. 2015). While remaining wetlands in lower-lying areas such as oxbow lakes and marshes can provide habitat to floodplain species, these water bodies and wetlands are often isolated and/or managed differently, e.g. for angling/fishing/recreation than active floodplain wetlands, which can further impact ecosystems, habitats and species. The impact of flood control measures on ecosystems and biodiversity are rarely known because many flood control measures in Asia, Europe and North America were implemented long before concerns over biodiversity emerged and direct comparisons of active vs. flood-protected floodplains are rare (Haugaasen and Peres 2006). Although it is well-known that flood control and river regulation can lead to the impoverishment of ecosystems and habitats (Strayer and Dudgeon 2010), flood-controlled areas are often more extensive and more converted from the natural status than the remaining active floodplains restricted along the regulated rivers, which often limits the possibility of meaningful comparisons.

Comentado [BT1]: I suggest considering to divide the sentence to make it shorter and easier to follow.

Despite their relatively small area, linear structures such as roads can have a high impact on wildlife living in surrounding areas. Roads can disrupt landscape connectivity and lead to habitat fragmentation (Trombulak and Frissell 2000), reduce gene flow between populations (Riley et al. 2006), and, most visibly, can lead to the direct mortality of animals through collisions with vehicles, which can result in reduced population sizes (Fahrig and Rytwinski 2009). Road-related mortality usually occurs non-randomly along the road and are often confined to specific time and space intervals related to biological and environmental factors (Sillero et al. 2019; Medinas et al. 2021; Russo-Petrick and Root 2023). Biological factors, such as reproductive features of animals, can explain the seasonality of increased road mortality, for example, when traffic co-occurs with the dispersal of juveniles from their places of birth or~~s~~ hatching (Seo et al. 2013) or with the migration of adults to breeding sites (Elzanowski et al. 2008). Environmental factors such as landscape features and roadside habitats can also have a major impact on the spatial patterns of road mortality (Matos et al. 2012). Finally, the overall increase in the intensity of vehicle traffic may also increase road mortality, however, its effect is ambiguous and can be limited to certain animals (Mazerolle 2004).

Most snake species are highly mobile and are frequent victims of road mortality, which can be responsible for eliminating as much as 10-20% of individuals of a population per year (Roe et al. 2006) and may even lead to increased risk of population extinction (Row et al. 2007). Snakes are often hit by cars when they move between different habitat types, for example, when they leave hibernation sites for foraging areas in their active season (Shine and Mason 2004), when they disperse from hatching sites as juveniles, when they search for conspecifics for mating, or when females look for nest sites for egg-laying (Bonnet et al. 1999). Because snakes often use different habitats for different life activities (feeding, resting, mating, egg-laying, hibernating), they are highly suitable for monitoring the impact of road mortality on biodiversity.

The aim of this study was to compare road mortality of two semi-aquatic snake species (grass snake *Natrix natrix*, dice snake *Natrix tessellata*) on roads running next to an active floodplain and a flood-protected former floodplain. To do this w~~W~~e assessed road mortality of snakes from spring to autumn in three years in the Tokaj area in NE Hungary. This area offered a unique setting for this study because the centrally located, mostly N to S running Tokaj-Hegyalja region is bordered by the active Bodrogzug floodplain on the north/east and by the flood-protected Taktaköz floodplain on the south/west (Fig. 1). We asked five specific questions: (i) What is the species composition of roadkills? (ii) What are the temporal and spatial patterns of road mortality? (iii) Do road mortality patterns differ between active and flood-protected floodplains? (iv) Which habitat types affect road mortality? (v) Does traffic intensity play a role in road mortality? Along with a general assessment of the potential of active vs. flood-protected floodplains and terrestrial and aquatic habitat types in maintaining snake populations, we also provide conservation recommendations to reduce the road mortality of snakes and other animal species in floodplain areas.

Comentado [BT2]: I suggest keeping this information only in the methods section, where the study area is described in detail.

Materials and Methods

Study area and species. The study area (Fig. 1) includes the southern part of the wine-growing Tokaj-Hegyalja region which ends at Tokaj town (E 21.4124, N 48.1215). This part of the Tokaj-Hegyalja region is bordered by two floodplain areas that share most of the habitat types of the region but differ in hydrological regime. Both lowland floodplain areas are dominated by floodplain meadows, forests and arable lands, whereas the hillside areas are dominated by meadows, forests, and vineyards. Both lowlands were active floodplains of rivers Bodrog (Bodrogzug) and Takta (Taktaköz) and were subject to regular inundation by large floods arriving on river Tisza until the mid-1850s (Fig. 1).

Then, construction of a system of levees and drainage canals led to the cessation of floods in the Taktaköz floodplain. Even though this former floodplain is now protected from floods by levees and canals, there are still many wetlands close to natural state, mostly in the form of oxbow lakes and marshes. Inland excess water also regularly occurs, usually after snowmelt, and is diverted from croplands by drainage canals to the rivers. The levees in the area are 6-8 m high longitudinal piles of soil with a mild slope covered by grasslands thus they do not represent barriers for snake movement.

In contrast, the Bodrogzug floodplain has remained an active floodplain since the 1850s. It is less canalized, and water level is mostly regulated by the operating needs of the hydroelectric power plant at Tiszalök, 25 km downstream from Tokaj. This floodplain is used today as flood-water storage basin, and water stored here often covers large areas in any time of the year, effectively mimicking the floods of the Bodrog and Tisza rivers that used to occur before river regulation (Boldogh et al. 2016).

We focused our study on two semi-aquatic snake species, the grass snake *Natrix natrix* and the dice snake *Natrix tessellata*. Three other snake species (Aesculapian snake *Zamenis longissimus*, smooth snake *Coronella austriaca*, adder *Vipera berus*) were also found in the survey but we excluded them from the statistical analyses due to low sample sizes. In the case of the most abundant snake, *N. natrix*, genetic evidence suggests that the two areas form one population (Kindler et al. 2017), with no physical barrier between them.

Field methods. We selected a total of 58 km of road neighbouring both floodplains for surveys. The surveyed road sections run through areas where the habitat types characteristic to the region are well represented (Fig. 1). Roads north from the confluence of rivers Bodrog and Tisza (in Tokaj city) and east from the Hegyalja hills belonged to the active, flooded Bodrogzug region, whereas roads south of the Bodrog-Tisza confluence and west of Hegyalja belonged to the flood-protected Taktaköz region (Fig. 1). All roads surveyed were similar in width with two lanes and two-way traffic.

Snake road mortality was monitored in surveys conducted once every two weeks throughout the entire activity period (late March to early November) of the target snake species in 2012, 2013 and 2020. One of the authors (MS) surveyed all road sections in one direction in a one-day duration using a bicycle with a steady speed (c. 20 km/h). Upon finding a snake on the road, the surveyor recorded the specimen's location with a GPS device (Garmin eTrex 10), identified the species and measured its size (snout-vent length, SVL), and determined its sex if it was possible. Snakes were also removed from the road to avoid multiply counting. Using literature sources on *N. natrix* and our SVL measurements, we classified snakes into three age categories as juveniles (SVL < 20 cm), subadults (20 cm < SVL < 30 cm) or adults (SVL > 30 cm), both for females and males. Although females are usually larger than males and mature at a larger size, this allowed us to involve snakes in analyses when sex determination was not possible due to the bad condition of specimens. Because *N. natrix* is a widespread species with differences in body size attributable to local

Comentado [BT3]: Consider providing a more concise description of the study area.

Comentado [BT4]: I didn't understand if you were interested only in the two Natrix species and the other snakes were randomly recorded. But, as your first specific question of your aim is about the composition of the species, I believe you were not interested only in the Natrix. Presenting this information about the data collected here gets me confused, and I suggest removing this part from here and only presenting it in the results sections.

environmental factors (Madsen 1983; Borczyk 2007), our age categorisation relied primarily on the work of Ciesiołkiewicz et al. (2006), who studied *N. natrix* in a region in Southern Poland climatically similar to our study area, and who categorised snakes based on total body length as hatchlings (<19 cm), yearlings (25-30 cm) and adults (>50 cm for males). Our age categorisation, which was based on SVL rather than total body length, was also supported by measurements of the smallest sexually mature male snake observed in mating balls (32 cm SVL) by us in the study region. We determined sex of adult specimens by visually examining the cloacal region and tail because males have thicker tail base. *Natrix tessellata* is also a geographically variable species (Ajtić et al. 2013). Because of the overall similarity of the two species and the low numbers of *N. tessellata* found, we used the same age categorisation for this species as well.

Variables and statistical analyses. For analyses, we divided the roads into 1-km-long sections and summed the number of snakes per section per survey as primary datapoints. There were a total of 36 sections in the flooded and 22 sections in the flood-protected area. We used the R 4.0.2. statistical environment (R Core Team 2021) for statistical analyses and QGIS 3.18 for spatial analyses and visualisations.

We compared the number of roadkilled snakes found on road sections in each study year by Mann-Whitney U tests. We next compared the number snakes between years in the flooded and the flood-protected area by ANOVA. To assess monthly patterns in mortality, we compared the number of snakes per month to a random equal distribution across months using χ^2 tests. To assess temporal and spatial patterns, i.e., whether certain months or certain road sections had higher mortality than others, we estimated the repeatability of mortality by calculating the intraclass correlation coefficient (ICC) either across months or across road sections in the flooded vs. the flood-protected area. Because between-year and monthly patterns in monthly road mortality were similar and information on age and sex were available only from a subset of the individuals, we did not analyse temporal differences by age categories and sexes. Finally, to assess snake mortality on road sections, we used a generalized linear mixed-effects model (GLMM) with year as random factor and road section as fixed factor for both *N. natrix* and *N. tessellata*. We then repeated this GLMM based on the subset of data where the age and sex categories of the snakes was known to analyze possible interactions between road sections on one side and age or sex categories of the individuals on the other.

Finally, we analysed the effects of environmental drivers, i.e., landscape structure and traffic intensity, on roadkill ~~mortality~~. To evaluate the effect of landscape structure and habitat types on snake road mortality, we used the Ecosystem Map of Hungary (Ministry of Agriculture of Hungary 2019; Tanács et al. 2019) in raster format (cell size: 20×20 m). We reduced the number of habitat types from 39 to 22 by merging similar habitat types (e.g. forests of different age structure into forests) and calculated the proportion of habitat types in 2-km buffer zones around the 1-km road sections. While the home range of these species might be smaller (Wisler et al. 2008) we chose this distance to include most of the known and assumed hibernating sites in the foothills and the foraging sites such as oxbows and channels in lower-lying areas and to allow the monitoring of possible movements beyond the home range (e.g. seasonal migration to/from winter hibernating and summer feeding sites). To characterize traffic intensity, we used the most recent data on daily traffic volume from the website of Hungarian Public Roads (2021) and assumed no change in traffic volume between years in the study period. Traffic data were available only in 5 to 10-km sections, and one such section covered several of our road sections. When road section borders in the traffic data fell within one road section in this study, we averaged the two values for that road section. Traffic volume was significantly higher in the flood-protected area (mean 5375 ±

Comentado [BT5]: This is a bit confusing, I couldn't understand what size reference was used to classify the age of the animals. I suggest that you review this part and keep only the references to the measurements used to avoid potential confusion about age classification.

Comentado [BT6]: The reason behind this sentence is not clear to me.

Comentado [BT7]: Here it mentions age after referring to sex classification. I suggest that this be included together with the description of age classification. And for sex classification, it should be explicitly stated that the same method was used for all species - as I understood it was done.

Comentado [BT8]: I didn't understand which data regarding age/sex was used to perform this analysis and why it was not used in temporal analysis.

Comentado [BT9]: What do you mean by these terms? I suggest you use land classes/land uses because when I read 'habitat types,' I initially think of specific land classes that are habitat to the species. However, I believe you are not solely using habitat classes in the analyses.

Comentado [BT10]: I don't think I got the idea here. How would you monitor the possible movements?

Comentado [BT11]: Do you really believe that the traffic volume was the same between the years you surveyed? I was wondering if COVID-19 didn't affect the traffic volume in 2020. Don't you have the traffic volume by year?

Comentado [BT12]: I wonder if 5 sections of 1km with different roadkill counts but the same traffic volume will provide enough information regarding the influence of traffic on mortality. Why not use larger sections, such as those from traffic data, as the road sections for the monitored roads, at least for this analysis?

S.D. 2982.1 vehicles per day, median 5150.5, n = 22 sections) than in the active floodplain (3838 ± 3016.7, 3328, n = 36 sections) (Mann-Whitney U = 249, p = 0.018).

We assessed the similarity of the habitat types of the flooded and the flood-protected areas by comparing the proportion of habitat types in the buffer zones around road sections between the two floodplain types with ANOVA. To model the effect of landscape structure on snake road mortality, we further reduced the 22 habitat types into four latent variables (LV1-LV4) in a Bayesian ordination approach using the 'boral' package of R (Hui 2016). Latent variables have advantages over principal components from a PCA in that they are not linear combinations of the original variables yet they still reduce variance inflation arising from multicollinearity among the original independent variables (Mizsei et al. 2020). For the interpretation of results, we calculated Pearson correlation coefficients between LV1-4 and the original data on the proportion of habitat types. These correlations showed that LV1 represented a gradient from rural to urban habitats, with significantly negative correlations with habitats for example Sessile oak-hornbeam forests, Fruit plantations and Arable land and significantly positive with habitats like Buildings, Paved roads and Urban forests. With the same logic, LV2 represented a gradient from dry to wet habitats from Dirt roads, Arable lands and Railways (negative correlations) to Rivers, Riverine willow-poplar forests and Mixed woodlands (positive correlations). LV3 represented a gradient from artificial to natural habitats with Complex cultivation patterns, Vineyards and Other artificial areas (negative) to Tall marsh vegetation standing in water, Wet meadows with periodic flooding and Lakes (positive), and LV4 was a gradient from closed (woody) to open (treeless) habitats from Urban forests, Urban green areas and Mixed woodlands to Arable land, Closed mountain grasslands and Lakes (Fig. 2.).

We assessed the effect of environmental drivers (landscape structure and traffic volume) on snake road mortality by constructing a generalized linear mixed-effects model for each species (GLMM) using the 'lme4' package of R (Douglas et al. 2015). We built GLMMs with the number of individuals per section per survey as discrete dependent variable, and flood regime (flooded vs. flood-protected as binary variable), traffic volume, survey week and the four latent habitat variables (LV1-LV4) as fixed explanatory variables and by specifying Poisson error distribution with logit link function. Road section ID and survey year were used as random effects to control for spatial and temporal non-independence of road sections and survey dates, respectively. We then used a model selection approach in an information-theoretic framework (Burnham and Anderson 2002) to identify models with substantial empirical support based on Akaike differences ($\Delta i = AIC_i - AIC_{min} < 2.0$) and to find the best set of explanatory variables for road mortality of each species. We then performed model-averaging of the best models using the 'MuMIn' package of R for each species (Bartoń 2019). When either of the LVs was significant in the averaged model, we ran simple linear regressions to evaluate which habitat type had direct positive or negative effect on the number of road-killed snakes. In these analyses, we only tested variables that showed the three strongest correlations with the number of road-killed snakes either in the positive or the negative direction (Fig. 2.), and we adjusted significance levels for multiple comparisons by the False Discovery Rate method (Benjamini and Hochberg 1995).

Results

Numbers of roadkilled snakes. We found a total of 1715 snakes, of which 1441 were *Natrix natrix*, 214 were *Natrix tessellata*, 45 were *Zamenis longissimus*, 14 were *Coronella austriaca* and one was *Vipera berus*. We found 1180 individuals of *N. natrix* and 210 individuals of *N. tessellata* on 36 km of road in the flooded area (corresponding to 38.6 snakes*km^{-1} in three

Comentado [BT13]: I think it would be less confusing if you present only here the other species you found. You can present all the data you collected in the surveys and then mention that for both Natrix species, more analyses were performed.

years combined) and 261 individuals of *N. natrix* and 4 individuals of *N. tessellata* on 22 km of road in the flood-protected area (12.0 snakes*km^{-1}).

The median OBSERVED number of *N. natrix* and *N. tessellata* was significantly higher in the flooded than in the flood-protected area in 2012 and 2020 (Table 1, Mann-Whitney tests, $W \geq 150$, $p < 0.02$), but not in 2013, when the number of *N. natrix* was similar in the two areas (Table 1, $W \geq 328$, $p \geq 0.732$). Because there were very few *N. tessellata* individuals in the flood-protected area, we did not further analyse data on this species in this area.

Temporal patterns. The total number of roadkilled snakes ranged between 391 and 512 for *N. natrix* and between 44 and 118 for *N. tessellata* for year in the three years and did not differ between years (*N. natrix*, $F_{2,24} = 0.115$, $p = 0.892$; *N. tessellata*, $F_{2,24} = 0.920$, $p = 0.412$). The number of *N. natrix* also did not differ between years either in the flooded area ($F_{2,24} = 2.957$, $p = 0.071$) or in the flood-protected area ($F_{2,24} = 0.483$, $p = 0.623$). There were two peaks in road mortality in each year (Fig. 3.), which differed significantly from a random equal distribution of roadkilled snakes across the months (*N. natrix*: flooded sections, $\chi^2 = 660.02$, df = 8, $p < 0.0001$; flood-protected sections, $\chi^2 = 141$, df = 8, $p < 0.0001$; *N. tessellata*: flooded sections, $\chi^2 = 137.93$, df = 8, $p < 0.0001$). The first peak of road mortality was in May and June, and the second peak was in September and October, and these patterns were similar in each year (Fig. 3.). The repeatability of the number of roadkilled snakes across months was relatively high for *N. natrix* (ICC = 0.503) and slightly lower for *N. tessellata* (ICC = 0.495).

Comentado [BT14]: I suggest providing more specific information about which months (one, two...?) are being considered as peaks in this case.

Comentado [BT15]: Even in Figure 3B? Wouldn't it be just one peak in September? How were the peaks identified?

Comentado [BT16]: Is there any recommended threshold that indicates a high value for N. natrix but a low value for N. tessellata? Can we consider 0.50 to be different from 0.49?

It was possible to measure the length and to assess the age of 461 roadkilled individuals of *N. natrix* (247 adults, 66 subadults, 148 juveniles) and 111 individuals of *N. tessellata* (57 adults, 16 subadults, 38 juveniles). The number of adults showed a smaller spring peak and a larger autumn peak in both *N. natrix* and *N. tessellata* (Fig. 4a,b). There were more young in the spring than in the autumn in *N. natrix*, whereas there were more *N. tessellata* young in the autumn than in the spring (Fig. 4a,b). Within adults, sex ratio was 1:1.44 females to males in *N. natrix* and 1.3:1 females to males in *N. tessellata*. There were more males than females in the autumn in *N. natrix*, whereas there were no monthly differences in sex ratio in *N. tessellata* (Fig. 4c,d).

Comentado [BT17]: Are these differences significant?

Spatial patterns. The number of snakes in the 1-km sections ranged from 0 to 202 in *N. natrix* and 0 to 53 in *N. tessellata*. Road mortality showed large differences among the sections and was significantly different from a random, i.e., equal, distribution of individuals in road sections in both species (*N. natrix*: flooded, $\chi^2 = 60.5$, $p < 0.0001$, flooded-protected, $\chi^2 = 15.609$, $p = 0.008$; *N. tessellata*: flooded, $\chi^2 = 69.5$, $p < 0.0001$) (Fig. 5.).

The repeatability of the number of roadkilled snakes on certain road sections was higher in the flooded area (ICC = 0.489) than in the flood-protected area (ICC = 0.241) for *N. natrix* and was low for *N. tessellata* in the flooded area (ICC = 0.147).

The number of *N. natrix* individuals differed significantly between the road sections in both areas (GLMM with year as random factor; flooded, $F_{40,80} = 4.000$, $p < 0.0001$; flood-protected, $F_{16,32} = 2.889$, $p = 0.005$) and so did the number of *N. tessellata* in the flooded area ($F_{40,80} = 1.567$, $p = 0.045$). In the flooded area, *N. natrix* numbers were highest on sections between km 2 to 8 and on sections km 18 to 24 in each year and on sections 28 to 31 in 2012 (Fig. 5a), and numbers of *N. tessellata* showed a similar pattern (Fig. 5b). In the flood-protected area, the number of *N. natrix* individuals varied more and was higher on sections km 8 to 15 than on other sections in 2013, whereas it was more equally distributed in 2012 and 2020 (Fig. 5c). There was no interaction between road sections and either the age categories (GLMM, *N. natrix*, $F_{2,226} = 1.508$, $p = 0.224$; *N. tessellata*, $F_{2,102} = 0.884$, p =

0.416) or sex (*N. natrix*, $F_{2,112}$ = 0.757, p = 0.386; *N. tessellata*, $F_{1,68}$ = 0.094, p = 0.761) of the individuals, indicating that road mortality influenced age categories and sexes similarly on all sections.

Environmental drivers of road mortality. The proportion of habitat types did not differ significantly between the flooded and the flood-protected area ($F_{1,42}$ = 0.0001, p = 0.992) (Fig. 6.). The GLMM models showed that the number of roadkilled *N. natrix* was significantly positively influenced by the closed to open LV4 and week of the year, and significantly negatively by the dry to wet LV2 and the artificial to natural LV3, while flood regime had a marginally non-significant positive effect, and traffic volume and the rural to urban LV1 had no effect (Table 2). The number of roadkilled *N. tessellata* was significantly positively influenced by flood regime and week of the year, and significantly negatively by traffic volume (Table 2~~.~~) but we did not find significant relationships with any of the latent variables.

Finally, the simple linear regression analysis showed that the number of road-killed snakes was positively influenced by the area of Mixed woodlands, Riverine willow-poplar forests, Tall marsh vegetation standing in water, and Complex cultivation patterns, and negatively by the area of Railways, Dirt roads, Urban green areas, Arable lands, and Vineyards (Fig. 7~~.~~, Table 3).

Discussion

This study provided four key results. First, we found that the road mortality of snakes was unexpectedly high and rather similar across years and months, with a spring and an autumn peak in both the flooded and the flood-protected areas. Second, and most importantly, mortality was higher in the flooded than in the flood-protected area, even though traffic was more intense in the flood-protected than in the flooded area. Third, there were differences in mortality across road sections: χ^2 test and GLMM results as well as ICC values each indicated that certain road sections involved higher risks of mortality for *N. natrix* (but less so for *N. tessellata*) than other road sections. Finally, these differences arose because road mortality of snakes was higher in sections surrounded by more natural habitat types and lower in sections surrounded by urban or agricultural areas.

Comentado [BT18]: It is not clear what and why exactly you don't expect.

It is important to note that the large number of roadkilled snakes found should be considered as a minimum estimate and that snake mortality is likely to be much higher in reality than reported here. The two-week monitoring interval used here enabled us to obtain a relative measure of road mortality across months and road sections, but is insufficient to estimate absolute numbers. Santos et al. (2011) found that most snake carcasses disappear from the road after one day on median and only some remain there for a maximum of 14 days. Weather conditions in the days prior to survey days may also affect the number of roadkills (Gravel et al. 2012). More optimal conditions, as sunny, warm weather may facilitate snake movement and subsequent road mortality. Thus, if we are to measure the true number of roadkilled snakes, we would need to monitor the road every day or at least several times a week, or use an alternative method, e.g. collecting live snakes along fences set up to run parallel to the road.

Comentado [BT19]: The observed roadkill numbers collected in the surveys may not represent even the minimum number of roadkill on each road. This is because, in addition to the issue of carcass persistence mentioned here, there is also the searcher efficiency to be considered in the estimation (as the observer may not detect 100% of the available carcasses on the road).

Comentado [BT20]: The reason behind this sentence is not clear to me. Is it related to carcass persistence or is it related to the possibility of more roadkill in these conditions?

Comentado [BT21]: Even if it were possible to survey every day, it would be just an attempt to control the effect of carcass persistence on the observed numbers. However, even with daily monitoring, it would not be possible to identify all available carcasses due to the imperfect detection of the observer. Additionally, there are possibilities to estimate these errors without the need for daily monitoring, such as conducting experiments to estimate them. However, I didn't understand the intention of mentioning this. I suggest rephrasing the sentence to make it clearer.

Higher numbers of road-killed snakes in the flooded than in the flood-protected area can be explained by at least three factors: traffic volume, landscape structure, and higher snake abundance. If traffic intensity were the main explanatory factor, flood-protected areas, where traffic was more intense, would have had higher road mortality. In fact, even though traffic intensity was 40% higher in the flood-protected area, the number of roadkilled snakes was three times higher in the flooded area (38.6 snakes*km^{-1}) than in the flood-protected area (12.0 snakes*km^{-1}). Our results thus clearly show that traffic intensity alone was not sufficient to explain the differences found. With regard to large-scale landscape structure, we expected that there will be more roadkilled snakes in less modified landscapes, i.e., the flooded area, than in more modified landscapes, i.e., the flood-protected area. However, the proportion of habitat types did not differ between the flooded and the flood-protected area (Fig. 6), thus, large-scale landscape structure was also unlikely to explain the differences found. Despite the similarities in habitat structure, the higher abundance of snakes may also explain their higher mortality in the flooded area. For instance, even though landscape structure or the proportion of habitat types did not differ, it is still possible that resources such as food were more abundant in the flooded than in the flood-protected areas. Differences in flood regime may lead to differences in species assemblages (Haugaasen and Peres 2005) and larger biomass production in favour of flooded areas (Hawes et al. 2012), among other factors. Personal observations collected in the area showed that amphibian species which are the main prey of *N. natrix* were the same in both areas, although we did not quantify their abundance. Nonetheless, it is possible that the presumably larger biomass production in the flooded area from the same amphibian species may indirectly lead to more snakes via the higher availability of resources.

Comentado [BT22]: What is the purpose of this paragraph? The factors described here do not seem to explain the roadkill.

At a smaller, road-section scale, however, the number of roadkilled *N. natrix* was significantly affected by habitats surrounding the road, as it was positively related to a gradient from closed to open and negatively to gradients from dry to wet and from artificial to natural habitat types (Table 2.). The analyses of latent variables indicated fewer roadkilled snakes on roads running through wetter and more natural habitat types in general, which can be surprising because we expected more snakes on roads bordered by more natural habitat types. This apparently also contradicts the results from the simple linear regressions where we found that more natural habitat types such as Riverine willow-poplar forests or Tall marsh vegetation standing in water positively, while more artificial types such as Arable land or Urban green areas negatively influenced road mortality. The differences can be explained in three ways. First, *N. natrix* is a rather generalist species (Reading and Jofré 2009), which can occur in many different habitats. Second, most of the studied landscapes are modified, with many areas converted to Arable land or Vineyards (Fig. 1.), thus it is not surprising that the proportion of these two habitat types fundamentally influenced our calculations. Finally, it is also possible that snakes find their habitats easier or faster in road sections surrounded by wetter and more natural habitat types than in sections surrounded by drier and more artificial habitat types, where they thus spend more time on the road and have higher risks of being run over by cars.

Comentado [BT23]: Consider to clarify this sentence for a better comprehension.

The spatial pattern in the number of roadkilled *N. natrix* was more repeatable in the flooded area than in the flood-protected area, which can be explained if the habitat use of snakes is more opportunistic in the flood-protected area, for example, if sites suitable for hibernation and/or feeding are patchy in space and non-stationary in time. Overall, our results on spatial patterns of road mortality support the view that the local presence and/or the proximity of natural habitat types near the road, mostly forests suitable for snake hibernation as well as rivers and wetlands suitable for feeding and reproduction, largely influenced the abundance and road mortality of water snakes at the local, road-section scale. We found no such effects on the number of roadkilled *N. tessellata* individuals, even though flooding had a positive effect and traffic had a negative effect on the number of roadkilled individuals of this species as well. However, mortality in this species was also confined to specific road sections (Fig. 4), which suggests that the lack of significant effects of neighbouring habitat is likely due to low sample size.

The monthly variation in the number roadkilled snakes was similar in both areas and can be explained based on the timing of major activities such as hibernation and foraging. The spring peak in mortality is likely related to the end of the hibernating period, when snakes

Comentado [BT24]: I would say that Figure 3B has a different pattern than A and B, the peak in May is not clear in B.

migrate to their lower altitude foraging grounds in rivers and wetlands, and the autumn peak is related to their migration from the feeding areas to higher altitude foothill areas used as hibernating sites (Fig. 3). Similar monthly patterns, with higher mortality in spring and autumn and limited mortality in summer also occur in *Natrix* species in Central Europe (Heigl et al. 2017; Anđelković and Bogdanović 2022). Such movements, when snakes move larger distances one time compared to their usual daily movements in the active season, ~~is~~ are known in several snake species living in temperate areas (e.g. for *Thamnophis sirtalis*, Gregory and Stewart 1975 or *Agkistrodon piscivorus*, Glaudas et al. 2007). Reptile body temperature changes with environmental temperature, thus they need to hibernate to maintain their life functions in cold climates. The availability of good hibernating sites known as hibernacula can also be more limited with increasing latitudes (Rudolph et al. 2007). The temporal patterns in road mortality were similar in both species, suggesting that road mortality is predominantly mediated by the movement of snakes in and out of hibernacula, and not by activities related to reproduction such as mate-searching in males, egg laying in females, or the dispersal of juveniles because in these cases one would expect mortality patterns skewed towards particular age or sex categories (Bonnet et al. 1999). The monthly differences between age categories and sexes were non-significant in this study, although we found more juveniles killed in spring (Fig. 4a) and more adults killed in autumn in both sexes in *N. natrix*, while there were more juveniles and adults killed in autumn in *N. tessellata* (Fig. 4.a,b). One possible explanation for lower spring mortality is if snakes move fast to the foraging grounds to refuel their energy storages after hibernation as quickly as possible (Costanzo 1989). A similar pattern is known in birds where autumn migration is more prolonged and days are spent much actively with feeding than with travelling (Nilsson et al. 2013). The seasonal mortality of juvenile *N. natrix* individuals was different because there were more roadkilled juveniles in the spring than in the autumn (Fig. 4.a), which may be related to higher predation on juveniles than on adults during the summer.

Unfortunately, little is known about the hibernation preferences of *Natrix* species and European Colubrids as a whole. Our limited number of field observations suggest that both species hibernate in sites (soil and rock pits) well above the flood water level. Floods can appear several times a year on river Tisza and Bodrog but they regularly occur at the end of winter and early spring when snow melts in the catchment area, and when temperature is too low for snakes to emerge. Snakes thus need to find hibernacula well above the flood water level, which at least partly explains the autumn and spring peaks in their mortality. Individuals of the closely related North American semiaquatic snake genus *Nerodia* prefer to hibernate above the stream level and are known to abandon flooded hibernacula (Ultsch 1989). Some other snake species, however, can opportunistically choose to winter underwater, for example, the phylogenetically more distant but similarly semiaquatic *Thamnophis sirtalis* and *T. sauritus* prefer to hibernate submerged if possible and such individuals can spare more energy compared to conspecifics hibernating in dry places (Costanzo 1989; Todd et al. 2009). According to our field observations in the area, *Natrix* species behave similarly to *Nerodia* and *Agkistrodon* (Glaudas et al. 2007) and they try to avoid hibernation sites that may get flooded by water. This is also implied by the bimodal temporal distribution of road mortality as snakes appear on the road in spring, after they came out of hibernaculas and in autumn, when they are moving back to them.

Conservation implications

Because wetlands are abundant in the studied landscape, road mortality of water snakes could occur in several places, yet fortunately it was low in most of the road sections. However, there are a few road sections where road mortality is a serious conservation concern

Comentado [BT25]: To whom is this sentence referring?

Comentado [BT26]: This is a bit confusing for me. Although you mentioned that there are non-significant differences, you discussed a lot of explanations for these differences. I suggest you to rethink these phrases.

Comentado [BT27]: In addition to being somewhat subjective, I find that this paragraph is somewhat disconnected from the purpose of the study. I suggest better relating it to the results or removing it all.

(Fig. 5). Our minimum estimate of the maximum number of roadkilled *N. natrix* in a section was 202 but this number can be in the hundreds annually. More than 50 roadkilled individuals in a section in a given year was not uncommon and there were three sections with more than 100 roadkills in 2020. Our study fortunately will be thus useful in identifying the sections where conservation interventions should be implemented.

There are sections outside of towns where culvert passages with barrier walls under the road could be a feasible solution to reduce snake mortality (Dodd et al. 2004). However, it is important that these are designed properly, e.g. they are wide enough to be used by snakes or other animals because a passage itself does not guarantee that animals will use it (Baxter-Gilbert et al. 2015). Other sections of concern are located in towns, for example, those with the highest mortality in the study are located within the city limits of Tokaj. Although they are located in a green urban space, the landscape is very complex here. There are several buildings, pedestrian crossings, parking lots and other elements which, in our view makes it impossible to construct such road features. Our suggestion is to build one or a series of artificial hibernacula in the way of migrating snakes farther from the road. Snakes often occur in or near artificial structures and such hibernacula are used as a conservation tool to provide additional shelters for them (Zappalorti et al. 2014). We are aware of two hibernacula on the floodplain side of the road, but well above the stream level which are predominantly used by a few hundred *N. tessellata* individuals but individuals of *N. natrix* and *Zamenis longissimus* also occur in them. Mortality in nearby road sections is generally low, suggesting that hibernacula are preventing road mortality. In addition, one of the hibernacula is artificial, as it is made from illegally disposed debris of building materials and other household litter, supporting the view that these snakes are capable to occupy such newly created structures in the future. Artificial snake hibernaculas are usually made from logs and stones covered with soil thick enough to prevent snakes from freezing (Zappalorti et al. 2014). We are not aware of such an intervention to prevent road mortality but there is evidence that artificial nesting sites can reduce road mortality for freshwater turtles (Paterson et al. 2013).

Comentado [BT28]: Is there a reference for this?

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