Herpetological Journal

SHORT NOTE

https://doi.org/10.33256/33.1.115

Published by the British Herpetological Society

Extremely low amphibian roadkill probability on busy bicycle paths

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Road mortality can have a significant negative impact on amphibian population survival. Amphibian roadkill and how to avoid it are therefore widely studied, mostly on car roads but limitedly on bicycle paths. We investigated whether amphibian mortality on bicycle paths in Bargerveen, a Dutch Natura 2000 site, was affected by the number of passing cyclists and crossing amphibians. We investigated four transects on a daily basis during most of the amphibian spring migration in 2021. We counted and identified (to species level) all killed amphibians; further, we used cyclist counters and toad fences to assess the number of passing bicycles and crossing amphibians, respectively. We found 11 killed smooth newts *Lissotriton vulgaris*, out of 5,037 that crossed the bicycle paths. Although 11,453 anurans crossed, we found no killed anurans. The occurrence of killed smooth newts was not affected by the number of passing bicycles or crossing newts. The probability of being killed was extremely low for crossing smooth newts (0.22 %) and anurans (0 %), possibly because cyclists successfully avoid cycling over amphibians. Future monitoring should occur from early February to late November to include the complete active period of amphibians including juvenile dispersal, and across multiple successive years because amphibian numbers can vary largely between years. During our study period, however, amphibian mortality on bicycle paths in Bargerveen seems no threat to populations, despite the high numbers of cyclists.

Keywords: road ecology, animal-cyclist collisions, smooth newts, anurans, conservation

Human induced changes to the environment can have a big impact on life histories of animals. One example is road mortality, since it can be a large source of mortality which can negatively impact population survival (Sillero, 2008; Fahrig & Rytwinski, 2009). Several studies found that the mortality rate of amphibians is highest compared to other vertebrates (Glista et al., 2008; Gryz & Krauze, 2008; DeWoody et al., 2010; Attademo et al., 2011; Garriga et al., 2012; Arévalo et al., 2017). Factors determining where and when most amphibian roadkill occurs and possible mitigation measures are therefore widely studied.

Most studies on amphibian roadkill focus on car roads and found complex interactions with adjacent habitats, species composition and more (e.g. Matos et al., 2012; Meek, 2012; Heigl et al., 2017). Higher traffic intensity does not necessarily increase absolute roadkill numbers (Mazerolle, 2004; Orłowski et al., 2008), but it does increase the probability that crossing amphibians will get killed (Fahrig et al., 1995; Hels & Buchwald, 2001; Meek, 2012; Eberhardt et al., 2013; Zimmermann Teixeira et al., 2017). This is because on busy roads many amphibians are killed over time; this leads to a reduction in amphibian populations, which in turn results in lower roadkill numbers (D'Amico et al., 2015). The probability that an amphibian crossing a busy road will get killed, however, remains high (Fahrig et al., 1995; Eberhardt et al., 2013; Zimmermann Teixeira et al., 2017).

Two studies looked into amphibian mortality on bicycle paths. Allain & Smith (2016) and Heigl et al. (2017) found 17 killed smooth newts *Lissotriton vulgaris* and one green toad *Bufo viridis*, respectively, on (sub)urban bicycle paths. To our knowledge, amphibian roadkill has never been studied on bicycle paths in nature reserves, although amphibian mortality on car roads is highest on roads through protected areas, probably because of high local amphibian abundance (Garriga et al., 2012). Besides that, both aforementioned studies monitored roadkill less than once a week, which could lead to inaccurate conclusions, because amphibian roadkill disappears from the road quickly (Santos et al., 2011; 2015). Lastly, neither study corrected for local amphibian abundance, although several studies on car roads find that this affects mortality (Fahrig et al., 1995; Eberhardt et al., 2013; Zimmermann Teixeira et al., 2017).

Biking in nature is popular, resulting in the replacement of small semi-paved bicycle paths by wide concrete or asphalt paths. In 2019 there were 3,617 kilometres of concrete or asphalt bicycle path in Dutch nature areas (Weder, P., Fietsersbond, personal communication, 13 May 2022). These new paths probably attract more cyclists and allow for higher speeds because they are wide and smooth, which may increase the probability

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of amphibian roadkill. This emphasises the need for more research on amphibian roadkill on bicycle paths in nature areas.

We investigated amphibian roadkill on bicycle paths in the raised bog Natura 2000 site Bargerveen, the Netherlands (52.678267, 7.023264). In this area, all semi-paved bicycle paths have been replaced by concrete paths between 2017 and 2019. Our research aims were to investigate:

i) how many amphibians are killed on bicycle paths in this nature area,
ii) what percentage of path-crossing amphibians are killed, and
iii) whether traffic intensity and the number of crossing amphibians explain roadkill occurrence.

We defined four study transects, with a total length of 5,093 metres. On 25 February 2021 we removed all roadkill from our transects, except from the transect where we started counting later. In the period 26 February to 30 May 2021, which covers most of the amphibian spring migration, we collected the following data:

i) We counted amphibian roadkill at the end of each day (somewhere between 1600 hours and 30 minutes before sunset) because we expected most roadkill to occur during day time when most cyclists pass. We counted roadkill by walking the transects, because walking results in the most accurate monitoring (Puky, 2005). We noted the species and exact location for all amphibian roadkill and then removed them from the bicycle path. We counted daily on transects 1–3 (except, by force majeure, on transects 1 and 2 on 18 March and on transect 3 on 8 March, 10 March and 10 May). On transect 4 we counted on average twice per week during the period 24 March to 23 April and daily during 24 April to 30 May (except 13 May). On this transect we found no roadkill on the set-up day (24 March), when we would have removed all present roadkill, so this day is included in our analysis.
ii) On each transect we placed two 25-metre-long toad fences, both on opposite sides of the bicycle path, parallel to, but not opposite of each other. We left a gap of 5 metres between the fences to minimise the effect of one toad fence on the other and to prevent a small area of increased amphibian crossings, possibly leading to deviating mortality patterns. On both sides of every fence three buckets were dug in the ground, one on each end and one in the middle, to trap passing amphibians (Supplementary Fig. 2). Every morning we counted all caught amphibians and then released them on the side of the bicycle path they were headed to.
iii) We used VT300 cyclist counters to collect data on traffic intensity. Two transects were adjacently on the same path, so data from one counter was used for both transects. The counter on one transect was malfunctioning, so we have traffic data for three transects. Vehicles were counted between 4 March and 30 May 2021.

Table 1. Total number of amphibian roadkill, total number of amphibians caught in the toad fences and estimation of the total number of amphibians that crossed on all study transects, between 26 February and 30 May 2021.

	Number of roadkill	Number of caught amphibians	Estimation of number of crossing amphibians
Smooth newts	11	250	5,037
Anurans	0	562	11,453

Supplementary Figure 1 shows the location of the toad fences, the study transects on which we counted amphibian roadkill and the roadkill that we found.

In total we counted 11 killed amphibians, all smooth newts (mean per day per transect ± SD = 0.034 ± 0.20). A total of 250 smooth newts (mean per day per transect ± SD = 0.66 ± 1.85) and 562 anurans (mean per day per transect ± SD = 1.49 ± 4.46) were caught in the toad fences. We estimated total crossings on the study transects based on the counted crossings at the toad fences. We estimated that 5,037 smooth newts (mean per day per transect ± SD = 15.5 ± 42.0) and 11,453 anurans (mean per day per transect ± SD = 35.3 ± 102.0) crossed the transects. Table 1 gives an overview of the numbers of killed and crossed amphibians. The cyclist counters counted 30,843 bicycles (mean per day per transect ± SD = 123.9 ± 146.2) and 13,149 other vehicles (mean per day per transect ± SD = 52.8 ± 46.2), such as electric scooters and people on rollerblades.

We analysed, on a daily basis, the relationships between the number of passing bicycles and crossing smooth newts, and the presence of roadkill. We used roadkill presence instead of roadkill number, because roadkill number was mostly zero or one. We performed tests with the total number of passing vehicles and with cyclists only, and since the tests yield similar results, we only present results on cyclists. First, we used a generalised linear model using a Poisson distribution, as we analysed count data, to test if the numbers of passing bicycles and crossing smooth newts were correlated. These were positively correlated (Fig. 1a). Since there were many days without crossing newts, we tested the correlation excluding these days and the correlation holds (estimate = 0.0021, $N = 64$, $p < 0.001$). Second, we used a generalised linear model using a binomial distribution to test the effect of the number of passing bicycles on roadkill presence, but found no effect (Fig. 1b). Third, we tested the effect of the number of crossing smooth newts on roadkill presence with a generalised linear model using a binomial distribution. We removed an outlier with 441 crossings and one roadkill, which was just after a sudden rise in temperature leading to massive amphibian migration. We again found no effect (Fig. 1c).

Analyses and visualisation were performed in R (R Core Team, 2019), using the packages readxl (Wickham & Bryan, 2019), reshape2 (Wickham, 2007), ggplot2

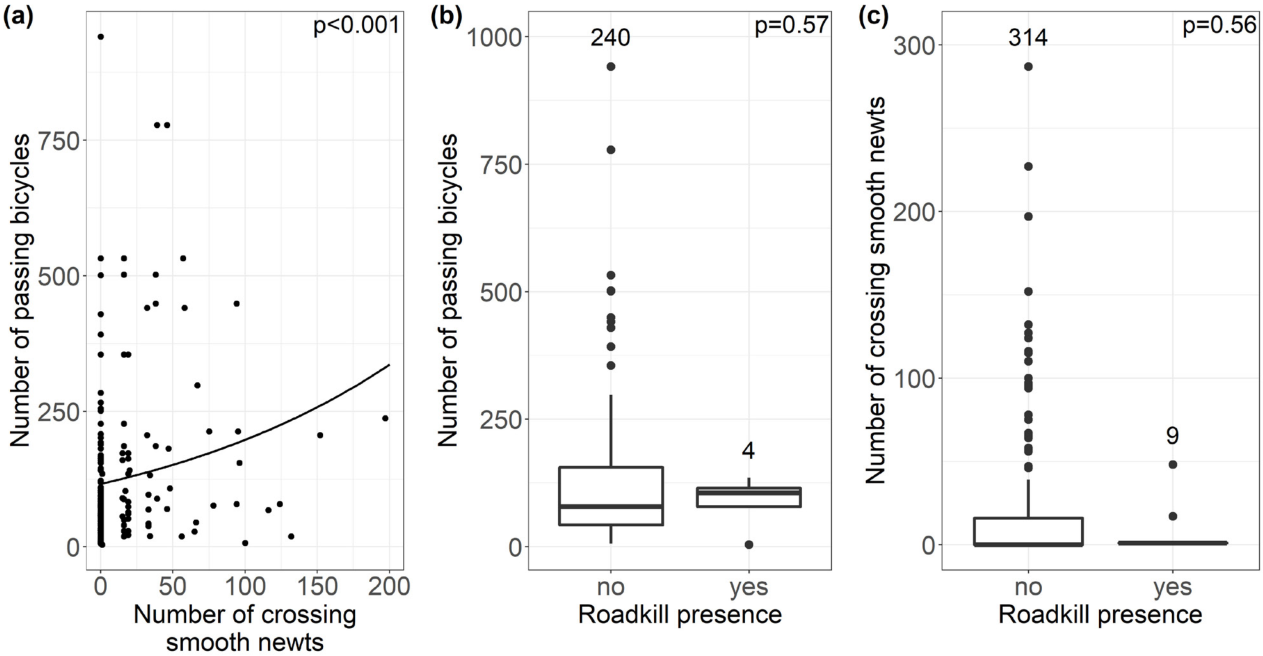

Figure 1. (a) The number of passing bicycles as a function of the number of crossing smooth newts (estimate = 0.0051, N = 244, $p < 0.001$); **(b)** The effect of the number of passing bicycles on roadkill presence (estimate =-0.0042, N = 244, p = 0.57); **(c)** The effect of the number of crossing smooth newts on roadkill presence (estimate =-0.0089, N = 323, p = 0.56). Above the boxplots sample sizes are given.

(Wickham, 2016), plyr (Wickham, 2011) and ggpubr (Kassambara, 2020).

During our study period, the probability that a crossing amphibian got killed was extremely low (0.22 % for smooth newts and 0 % for anurans). This was despite substantial cyclist numbers passing on the same days that amphibians were migrating in large numbers, which appeared from the positive correlation between passing bicycles and crossing newts (Fig. 1a). The roadkill probabilities may have been underestimated, because roadkill is often eaten by scavengers. These probabilities may also have been overestimated, since some amphibians may have escaped from, or may have been predated from our buckets, and higher numbers of caught amphibians result in lower roadkill probabilities. Gibbs & Shriver (2005) stated that annual roadkill probabilities of 10 % or lower result in stable populations for adult spotted salamanders *Ambystoma maculatum*. Although we studied other amphibian species and despite uncertainty around our roadkill probabilities, mortality on bicycle paths in the Bargerveen area seems no threat to amphibian populations during our study period.

However, we did not monitor during the complete active period of amphibians, so it is unknown if roadkill on bicycle paths is a threat to populations outside our study period. For example, on 25 February, the day before monitoring started, we removed 29 smooth newt mortalities. More than 50 % of amphibian roadkill disappears from the road within 24 hours (Santos et al., 2011), so the number of newts that were killed before monitoring started was probably much higher than 29.

The roadkill probability being extremely low, and roadkill presence being independent of the number of passing cyclists and crossing amphibians, could be explained by several factors. First, cyclists pass mostly during the day and amphibians migrate mostly during the night (Hels & Buchwald, 2001; Zhang et al., 2018; RAVON, n.d.c). However, a substantial part of smooth newts migrates diurnally (Jarvis et al., 2019; RAVON, n.d.c) so this is only a partial explanation. Second, amphibians migrate mostly during rainy periods (Meek, 2012; RAVON, n.d.a) which is not favourable weather for cyclists. The positive correlation between crossing smooth newts and passing cyclists (Fig. 1a), however, indicates that newts and cyclists are active on the same days, so this explanation can only be true if they are active at different hours of the day. Third, amphibians could possibly not cross bicycle paths when it is busy with cyclists. Fourth, cyclists could successfully avoid cycling over amphibians. Maybe, the chance for an individual cyclist to avoid an amphibian successfully increases with the number of cyclists present, because many cyclists could see an amphibian earlier. These group effects could possibly explain our lack of correlation between cyclists' traffic intensity and roadkill presence. Cyclists avoiding amphibians can also explain the absence of a positive correlation between crossing newts and roadkill presence (Fig. 1c) because cyclists possibly pay more attention to avoiding amphibians when many amphibians are crossing. For drivers of motorised vehicles it is harder to avoid amphibians, as shown in multiple studies (Fahrig et al., 1995; Meek, 2012; Eberhardt et al., 2013; Zimmermann Teixeira et al., 2017). Cyclists are probably able to avoid amphibians due to their lower speed. The light colour of the concrete bicycle paths in our study area might also help, since it makes amphibians on the path more visible.

As all roadkill were smooth newts, while 69 % of crossing amphibians were anurans, it could be that newts are more likely to be killed while crossing a bicycle path than anurans. Several reasons could explain this. First, newts are flatter and thinner than anurans, which makes them less conspicuous on bicycle paths; however, newts have similar body length (the length that could be struck) as anuran species common in our study area (up to 11 cm for smooth newt, common frog *Rana temporaria* and common toad *Bufo bufo* and 8 cm for moor frog *Rana arvalis* (RAVON, n.d.b)) (Allain & Smith, 2016). The least visible species having the highest mortality probability advocates that cyclists successfully avoid killing amphibians. Second, newts move slower than anurans, so they take longer to cross bicycle paths (Hels & Buchwald, 2001). Third, smooth newts migrate earliest in the year, as shown by our toad fence catches and the literature (RAVON, n.d.a), implying that newts migrate when it is dark early in the evening. Since migration generally starts from dusk (Puky, 2005), the overlap between migration and passing cyclists is probably higher for smooth newts than for anurans.

Even though our study has clear results, long term research is necessary to make hard conclusions. Future monitoring should occur from early February to late November, including the complete active period of all amphibian species (RAVON, n.d.a). It is specifically important to include the period when juvenile amphibians migrate, June–October (RAVON, n.d.a), because juvenile survival is in many cases the driving factor for population stability (Petrovan & Schmidt, 2019) and because juveniles may be more likely to become roadkill, as they are less visible and migrate more diurnally, when most cyclists pass (Vos & Chardon, 1994; Petrovan & Schmidt, 2019). Furthermore, amphibian numbers show large natural variation between years. Thus, monitoring should occur for at least 3 years in a row to draw solid conclusions on roadkill (Puky, 2005). Moreover, it may be interesting to analyse the effects of speed and group size of passing cyclists on amphibian mortality, as well as the time of the day in which cyclists pass.

We found that mortality probabilities for amphibians on bicycle paths were very low, despite substantial cyclists' traffic intensity, indicating that road mortality is, in our study period, no threat to amphibian populations.

ACKNOWLEDGEMENTS

We thank everybody who contributed to this publication. More than 60 volunteers and students collected data. Richard Struijk (RAVON) gave useful suggestions for improvement of the research design. The municipality Emmen provided the cyclist data. Two anonymous reviewers and Sara Raj Pant provided useful improvements of the manuscript. Staatsbosbeheer Veenland realised the research financially.

Data Accessibility

Data and R code are available via https://doi.org/10.34894/KYY0OX.

REFERENCES

Allain, S.J.R. & Smith, L.T. (2016). Newt mortalities on an urban cycle path. *The Herpetological Bulletin*, 138, 27–28.

Arévalo, J.E., Honda, W., Arce-Arias, A. & Häger, A. (2017). Spatiotemporal variation of roadkills show mass mortality events for amphibians in a highly trafficked road adjacent to a national park, Costa Rica. *Revista de Biología Tropical*, 65(4), 1261–1276. https://doi.org/10.15517/rbt.v65i4.27903.

Attademo, A.M., Peltzer, P.M., Lajmanovich, R.C., Elberg, G., Junges, C., Sanchez, L.C. & Bassó, A. (2011). Wildlife vertebrate mortality in roads from Santa Fe Province, Argentina. *Revista Mexicana de Biodiversidad*, 82(3), 915–925. http://dx.doi.org/10.22201/ib.20078706e.2011.3.783.

D'Amico, M., Román, J., De los Reyes, L. & Revilla, E. (2015). Vertebrate road-kill patterns in Mediterranean habitats: Who, when and where. *Biological Conservation*, 191, 234–242. https://doi.org/10.1016/j.biocon.2015.06.010.

DeWoody, J.A., Nogle, J.M., Hoover, M. & Dunning, B. (2010). Monitoring and predicting traffic induced vertebrate mortality near wetlands. Purdue University, West Lafayette, USA. https://doi.org/10.5703/1288284314256 [not peer reviewed].

Eberhardt, E., Mitchell, S. & Fahrig, L. (2013). Road kill hotspots do not effectively indicate mitigation locations when past road kill has depressed populations. *The Journal of Wildlife Management*, 77(7), 1353–1359. https://doi.org/10.1002/jwmg.592.

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(3), 177–182. https://doi.org/10.1016/0006-3207(94)00102-V.

Fahrig, L. & Rytwinski, T. (2009). Effects of roads on animal abundance: an empirical review and synthesis. *Ecology and Society*, 14(1), 21. https://doi.org/10.5751/ES-02815-140121.

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(11), 2761–2774. http://dx.doi.org/10.1007/s10531-012-0332-0.

Gibbs, J.P. & Shriver, W.G. (2005). Can road mortality limit populations of pool-breeding amphibians? *Wetlands Ecology and Management*, 13(3), 281–289. http://dx.doi.org/10.1007/s11273-004-7522-9.

Glista, D.J., DeVault, T.L. & DeWoody, J.A. (2008). Vertebrate road mortality predominantly impacts amphibians. *Herpetological Conservation and Biology*, 3(1), 77–87.

Gryz, J. & Krauze, D. (2008). Mortality of vertebrates on a road crossing the Biebrza Valley (NE Poland). *European Journal of Wildlife Research*, 54(4), 709–714. http://dx.doi.org/10.1007/s10344-008-0200-0.

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(1), 1–11. https://dx.doi.org/10.1186%2Fs12898-017-0134-z.

Hels, T. & Buchwald, E. (2001). The effect of road kills on amphibian populations. *Biological Conservation*, 99(3), 331–340. http://dx.doi.org/10.1016/S0006-3207(00)00215-9.

Jarvis, L.E., Hartup, M. & Petrovan, S.O. (2019). Road mitigation using tunnels and fences promotes site connectivity and population expansion for a protected amphibian. *European Journal of Wildlife Research*, 65, 27. https://doi.org/10.1007/s10344-019-1263-9.

Kassambara, A. (2020). ggpubr: 'ggplot2' based publication ready plots. R package version 0.4.0. https://CRAN.R-project.org/package=ggpubr.

Matos, C., Sillero, N. & Argaña, E. (2012). Spatial analysis of amphibian road mortality levels in northern Portugal country roads. *Amphibia-Reptilia*, 33(3–4), 469–483. http://dx.doi.org/10.1163/15685381-00002850.

Mazerolle, M.J. (2004). Amphibian road mortality in response to nightly variations in traffic intensity. *Herpetologica*, 60(1), 45–53. http://dx.doi.org/10.1655/02-109.

Meek, R. (2012). Patterns of amphibian road-kills in the Vendée region of Western France. *The Herpetological Journal*, 22(1), 51–58.

Orłowski, G., Ciesiołkiewicz, J., Kaczor, M., Radwańska, J. & Żywicka, A. (2008). Species composition and habitat correlates of amphibian roadkills in different landscapes of south-western Poland. *Polish Journal of Ecology*, 56(4), 659–671.

Petrovan, S.O. & Schmidt, B.R. (2019). Neglected juveniles; a call for integrating all amphibian life stages in assessments of mitigation success (and how to do it). *Biological Conservation*, 236, 252–260. https://doi.org/10.1016/j.biocon.2019.05.023.

Puky, M. (2005). Amphibian road kills: A global perspective. In *Proceedings Of The 2005 International Conference On Ecology And Transportation*. Irwin C.L., Garrett P. & McDermott K.P. (Eds.). North Carolina State University, Raleigh, USA.

RAVON. (n.d.a). Amfibieëntrek [Amphibian migration]. Downloaded on 1 July 2021. https://www.padden.nu/Amfibieentrek.aspx.

RAVON. (n.d.b). Amfibieën [Amphibians]. Downloaded on 7 July 2021. https://www.ravon.nl/Soorten/Amfibie%C3%ABn.

RAVON. (n.d.c). Beïnvloeden verkeer [Affect traffic]. Downloaded on 26 October 2021. https://www.padden.nu/Oplossingen/BeinvloedenVerkeer.aspx.

R Core Team. (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Santos, S.M., Carvalho, F. & Mira, A. (2011). How long do the dead survive on the road? Carcass persistence probability and implications for road-kill monitoring surveys. *PLoS ONE*, 6(9), e25383. https://doi.org/10.1371/journal.pone.0025383.

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. http://dx.doi.org/10.1016/j.jenvman.2015.07.037.

Sillero, N. (2008). Amphibian mortality levels on Spanish country roads: descriptive and spatial analysis. *Amphibia-Reptilia*, 29(3), 337–347. https://doi.org/10.1163/156853808785112066.

Vos, C.C. & Chardon, J.P. (1994). Herpetofauna en verkeerswegen; een literatuurstudie [Herpetofauna and roads; a literature study]. Instituut voor Bos- en Natuuronderzoek, Wageningen, the Netherlands.

Wickham, H. (2007). Reshaping data with the reshape package. *Journal of Statistical Software*, 21(12), 1–20. http://dx.doi.org/10.18637/jss.v021.i12.

Wickham, H. (2011). The split-apply-combine strategy for data analysis. *Journal of Statistical Software*, 40(1), 1–29. http://dx.doi.org/10.18637/jss.v040.i01.

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

Wickham, H. & Bryan, J. (2019). readxl: read excel files. R package version 1.3.1. https://CRAN.R-project.org/package=readxl.

Zhang, W., Shu, G., Li, Y., Xiong, S., Liang, C. & Li, C. (2018). Daytime driving decreases amphibian roadkill. *PeerJ*, 6, e5385. http://dx.doi.org/10.7717/peerj.5385.

Zimmermann Teixeira, F., Kindel, A., Hartz, S.M., Mitchell, S. & Fahrig, L. (2017). When road-kill hotspots do not indicate the best sites for road-kill mitigation. *Journal of Applied Ecology*, 54(5), 1544–1551. http://dx.doi.org/10.1111/1365-2664.12870.

Accepted: 20 September 2022

Please note that the Supplementary Material for this article is available online via the Herpetological Journal website: https://thebhs.org/publications/the-herpetological-journal/volume-33-number-1-january-2023