

Modeling road mortality hotspots of Eastern Hermann's tortoise in Romania

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Abstract. Road-associated mortality can lead to local declines of wildlife populations, and management agencies are actively implementing mitigation measures, especially focused on potential road mortality hotspots. In this study we used a spatially-explicit simulation modeling approach to estimate the hotspots of road mortality for the Eastern Hermann's tortoise (*Testudo hermanni boettgeri*) within its distribution range in Romania. Using a field experiment, we first evaluated velocities while crossing roads. Adult male tortoises moved faster than females (3.98 m/min vs. 2.51 m/min) which led to higher individual probabilities for females being killed on high-traffic roads (0.61 for females vs. 0.44 for males at traffic levels of 7000 vehicles/day). Both males and females had similar road mortality probabilities for traffic levels <1000 and >35 000 vehicles/day. Our spatially explicit model suggests that, within the entire Romanian distributional range, the tortoises have an overall risk of road mortality 1.6%, which may have a negative impact on tortoise populations. Using the Getis-Ord Gi statistic, we identified road mortality hotspots with mortality rates of 5-30%, in areas bisected by high-traffic national and European-level roads. Our research is timely in that many low-traffic roads are predicted to have increased traffic associated with tourism activities, thus increasing the overall risk of mortality. We suggest that mitigation measures such as signage and roadside fences associated with underpasses have the potential to limit road mortality of this threatened species within predicted current mortality hotspots.

Keywords: movements, road mortality, Romania, spatial statistics, *Testudo hermanni boettgeri*.

Introduction

The negative effects of roads on wildlife populations have become pervasive over the past several decades (Trombulak and Frissell, 2000; Forman et al., 2003; Coffin, 2007), and mitigating road mortality is a central focus for many environmental and wildlife management agencies (Gunson et al., 2009b; Gunson et al., 2011). A plethora of research has documented both direct road effects resulting in wildlife mortality (Gibbs and Shriver, 2005), habitat loss (deMaynadier and Hunter, 2000; Cushman, 2006), decreased habitat connectivity (Bowne et al., 2006; Shepard et al., 2008), as well as indirect

effects, such as behavioral changes (Andrews and Gibbons, 2005; Frair et al., 2008), and impacts from deicing salt (Karraker et al., 2008) and tire debris (Camponelli et al., 2009) on embryonic development.

Herpetofauna species are especially sensitive to road effects (Hels and Buchwald, 2001; Andrews et al., 2008), and the expansion of transportation infrastructure has led to rapid declines of local populations (e.g., Gibbs and Shriver, 2005; Roe et al., 2006), affected sex ratios (Steen et al., 2006), and recruitment (e.g., for aquatic species breeding in roadside pools; Karraker et al., 2008; Karraker and Ruthig, 2009). An array of mitigation methods have been proposed and implemented, such as signage, driver education, temporary road closures, underpasses and overpasses and, roadside fencing (Glista et al., 2009; McCollister and Van Manen, 2010). In some instances, teams of volunteers may help animals cross roads during mass migrations (e.g., spring amphibian migrations towards breeding ponds; Bonardi et al., 2011). Various techniques have been used to predict

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the hotspots of road mortality, which can inform mitigation measures and help draft effective conservation strategies (Beaudry et al., 2008; Gunson et al., 2009a; Mountrakis and Gunson, 2009). For example, GIS models have been used for assessing habitat matrix permeability across landscapes fragmented by roads (Ray et al., 2002; Joly et al., 2003) and for calculating indices of occurrence of amphibians and reptiles on roads (Patrick et al., 2012). Hotspot models have also been used to identify linkage areas along high-traffic roads (Clevenger et al., 2002; Gunson et al., 2009b) and determine factors explaining road mortality (Langen et al., 2009; Cureton and Deaton, 2012).

Predictive statistical models are often used to evaluate road mortality within defined road segments (Hartel et al., 2009; Meek, 2009; Brzeziński et al., 2012), and fewer mechanistic models that focus on the persistence and viability of populations by evaluating the impacts of roads across broad-scales exist (e.g., Hels and Buchwald, 2001; Gibbs and Shriver, 2005; Beaudry et al., 2008). Fine-scale studies provide specific information for building effective underpasses and other mitigation structures, but tend to be very site-specific (Clevenger et al., 2002; Gunson et al., 2009b). In contrast, broad-scale models are useful for drafting conservation plans, and evaluating spatial hot and cold-spots of road mortality (Hothorn et al., 2012; Patrick et al., 2012).

In this study we aim at developing a broad-scale model of road mortality for the Eastern Hermann's tortoise (*Testudo hermanni boettgeri* Mojsisovics, 1889) within its Romanian range. Eastern Hermann's tortoise is a species of conservation concern in Europe (2006/105/EC, 2006). The Romanian extent of occurrence (EOO) includes the northern and eastern edges of the species geographic range. Although most of the tortoise's range in Romania is protected by national protected areas (i.e., National and Natural Parks) and Natura 2000 Sites of Community Importance (Ioja et al., 2010), habitats are being altered by uncontrolled development

of tourism infrastructure (Rozyłowicz and Dobre, 2010). Moreover, increased road traffic intensity associated with tourism is forecasted on roads throughout the species range (Romanian National Company of Highways and National Roads, 2010). The species movement ecology is characterized by short daily distance steps that interfere with longer seasonal steps. During the mating season males tend to move longer distances leading to increased interactions with roads while during the nesting season the opposite occurs (Bertolero et al., 2011).

The study objectives are: (a) to estimate the probability of being killed on roads for several road categories, (b) to evaluate the mean annual road-associated mortality within the Romanian range of Eastern Hermann's tortoise, and (c) to identify the hotspots of road mortality within the species range in Romania.

Methods

Study area

Our study focused on the entire range of Eastern Hermann's tortoise in Romania, which covers approximately 4420 km² in the southwestern part of the country (Rozyłowicz and Dobre, 2010). The area is bisected by a road network that totals ~3900 km, of which 94.6 km are high traffic 4-lane roads (i.e., European road E70), 243.5 km are medium traffic 2-lane roads (i.e., National roads), 335.1 km are low traffic local roads, and 637.3 km are paved rural roads. The majority of the road network (2532.2 km) consists of unpaved local roads mainly used for seasonal agricultural activities, logging and tourism. The mean road density (\pm SE) within the study area is 1.17 ± 0.04 km/km².

Velocity experimental design

We tested tortoise velocity on a 5-m wide paved road segment within the study area, where we delineated a 5 × 6 m test arena on the road surface with gridlines spaced 1 m apart. We performed velocity trials with tortoises collected from adjacent habitats during April and May 2012 (up to 5 animals per day). Prior to release in the experimental arena, each animal was kept inside the release box for at least 5 minutes to attenuate the effects of handling, and released by remotely opening of the front door of the box. All trials were recorded by a digital video camera, installed on a 2-m high tripod, placed 5 m outside of the arena. We recorded the time it took an animal to cross each 1-m quadrant within the test arena and obtained an average speed for each animal (m/min). After each velocity trial, we recorded the road

temperature, as well as tortoise weight and sex. Tortoises were only used once, and were released back at the location of collection.

Tortoise velocity was successfully tested for 26 adults (10 males and 16 females). Eleven trials failed because the tortoises did not leave the release box in <15 minutes or did not cross the experimental arena. We tested for gender-specific velocity differences using non-parametric Mann-Whitney *U* test and used a generalized linear model to investigate the relation between tortoises' weight and speed. We evaluated the correlation between the road temperature and the tortoise speed using Spearman's rank correlation.

Estimating mortality in relation to traffic volume

We estimated the probability that a tortoise would be killed as it crosses a road based on an equation adapted from Hels and Buchwald (2001) (Eq. 1). To characterize the spatial variation between different types of roads, we calculated the probability of being killed using Eq. 1 on each road segment within the study area.

$$p_{\text{killed}} = e^{-Na/v} \quad (1)$$

where N is the traffic intensity in vehicles/minute, a is the width of the kill zone on roads in meters and v is the tortoises' velocity on a paved road in meters/minute.

We used traffic intensity values for different road categories from the average annual daily traffic data for European, national and county roads recorded in 2010 by the Romanian National Company of Highways and National Roads (Romanian National Company of Highways and National Roads, 2010). No such data existed for paved local roads, thus we simulated traffic intensity from a uniform random distribution between 10 vehicles/day and 200 vehicles/day. For unpaved local roads, we assumed a mean traffic of 10 vehicles/day. We removed 20% of the daily traffic since it occurred outside of the tortoises' daily cycle between 06:00 AM and 06:00 PM (Festin, 1996).

We estimated the width of the kill zone (a) for each road type as two-times tire width per lane plus twice the carapace length (Hels and Buchwald, 2001). Tire width was calculated as a weighted average of the killing width of each type of vehicle (i.e., cars = 0.39 m, vans = 0.50 m, tractor trailers = 1.40 m; buses = 0.70 m, and agricultural tractors = 0.80 m), and the percentage of the traffic intensity recorded for that type of vehicle (Romanian National Company of Highways and National Roads, 2010). We used a mean tortoise carapace length of 18.9 cm (Rozyłowicz and Pătroescu, 2004).

Estimating hotspots of road mortality

We estimated annual road-associated mortality (d_{road}) at the population scale using an equation derived by Gibbs and Shriver (2002) (Eq. 2).

$$d_{\text{road}} = 1 - (1 - p_{\text{killed}})^{n_{\text{crossings}}} \quad (2)$$

where p_{killed} represents the probability of being killed during a road crossing event and $n_{\text{crossings}}$ represents the

mean number of the road crossings an adult tortoise can accumulate during its annual movements.

To obtain the number of crossings ($n_{\text{crossings}}$), we used a correlated random walk simulation model (Bartumeus et al., 2005). The model simulated independent random movement steps drawn from the daily distance and turning angle distributions that were determined empirically (Beyer, 2011). Rozyłowicz and Popescu (2013) found that movement ecology of Hermann's tortoises within our study area is characterized by frequent short-distance movements with rare longer-distance steps (daily mean \pm SE = 31.18 \pm 1.59 m). The daily distance movements recorded by Rozyłowicz and Popescu (2013) fits a negative exponential distribution (Kolmogorov-Smirnov $Z = 1.09$, $P = 0.19$). Therefore, we simulated the daily movement steps from a negative exponential distribution with a rate parameter of 0.026 (Forbes et al., 2010). The turning angles were drawn from a uniform random distribution between 0 and 6.2831 radians (Gibbs and Shriver, 2002).

We started the simulations from 791 occurrences within the tortoise range, collected using systematic sampling during 2000-2009 (Rozyłowicz and Dobre, 2010). The simulated paths followed 199 daily steps of the annual activity period observed for Eastern Hermann's tortoise in Romania (i.e., April 15th to October 31st; Rozyłowicz and Dobre, 2010). We forced the movement paths inside simulated home range boundaries (i.e., circular neighborhoods centered on each starting location). Simulated home range areas were drawn from a uniform random distribution between 0.53 ha and 10.82 ha (Rozyłowicz and Popescu, 2013). We overlaid the simulated paths with the road layer digitized from 2005 aerial imagery (0.5 m resolution), and extracted the number of road crossings ($n_{\text{crossings}}$). We then estimated the relationship between the predicted number of road crossings per km² and the road density using least-squares linear regression with the intercept forced through zero (Gibbs and Shriver, 2002).

We used the Getis-Ord G_i^* statistic to identify local clusters with mortality rates significantly higher than expected, by comparing the local mean of mortality with the population mean (Ord and Getis, 1995). In our case, this statistic uses the local context of spatial features (i.e., adjacent cells in a raster or adjacent vector features) to identify aggregations of high and low values of mortality probability by assigning Z_{scores} to each areal unit ($Z_{\text{scores}} > 1.96$ denote significant hot spots of mortality). First, we generated a 1 \times 1 km cell size grid within the study area and estimated the mean mortality probability for each cell, by averaging the probabilities of all road segments inside that particular cell. The $n_{\text{crossings}}$ within a grid cell was estimated as an average of $n_{\text{crossings}}$ of all tortoises in that cell. Second, we calculated the annual road-associated mortality for each cell using Eq. 2. Lastly, we performed a Getis-Ord G_i^* test (Ord and Getis, 1995; ESRI, 2009) in ArcGIS Desktop 10 (ESRI, Redlands, CA) using a threshold distance of 1414.2 m, in order to include all the neighbouring cells in calculation (Chainey, 2010).

Results

Tortoise velocity

The Hermann’s tortoise speed of locomotion on paved surfaces ranged from 0.96 to 7.64 m/min, with a mean speed (\pm SE) of 3.07 ± 0.36 m/min. Males moved faster than females (Mann Whitney: $Z = 39.00$, $P = 0.03$, $\text{mean}_{\text{males}} = 3.98$ m/min; $\text{mean}_{\text{females}} = 2.51$ m/min).

The speed of locomotion decreased linearly with increasing tortoises’ weight according to the following equation: $\text{speed} = -2.813 \times \text{weight} + 7.142$ ($R^2 = 0.24$, $P < 0.01$, $n = 26$). Road crossing speed was positively correlated with road surface temperature (Spearman $\rho = 0.49$, $P < 0.01$).

Road mortality in relation to traffic volume

Road mortality was positively associated with traffic volume. There was <0.001 probability that a tortoise would be killed on unpaved, local roads, and this probability increased up to 0.185 for high traffic, four-lane national roads (table 1). The maximum road mortality probability estimated for Hermann’s tortoises in Romania was 0.529 for the European E70 road which crosses the eastern part of the range (fig. 1). Moreover, the differences in velocity between males and females resulted in females being more likely to be killed on roads. For low traffic intensity (10 vehicles/day), both males and females were not likely to experience road mortality (probability < 0.001) but for traffic values of 6830 vehicles/day, such as ones recorded on the European road E70, the difference between

Table 1. Mean probabilities of an individual Hermann’s tortoise being killed on roads (p_{killed}) in Romania. N = average traffic intensities for different road categories (vehicles/day); a = width of kill zone in m.

Road type	N	a	p_{killed}
European	6830	1.22	0.529
National	2180	1.22	0.185
County	611	1.09	0.042
Paved local roads	93	1.09	0.006
Unpaved local roads	10	0.78	<0.001

sexes was 0.16. The mortality probability was 1 for both sexes, when traffic intensities exceeded 35 000 vehicles/day (fig. 2).

Hotspots of road mortality

The number of predicted road crossings per km^2 increased linearly with road density ($n_{\text{crossings}} = 10.83 \times \text{road}_{\text{density}}$; $R^2 = 0.49$, $P < 0.001$). Almost half (49.6%) of the simulated movement paths intersected the road network at least once during a given year, and we estimated that an adult tortoise can accumulate an average of 7.37 road crossings per year. Based on this estimate, the predicted mean annual road-associated mortality (d_{road}) for the entire range was 1.6%.

Furthermore, we predicted high road mortality rates (5-30%) for several populations bisected by European or National roads (fig. 1). A higher than expected mortality rate (Getis-Ord $G_i^* Z_{\text{score}} > 1.96$) occurred in populations bisected by the E70 European road (i.e., Gura Văii, Valea Cernei, and Şimian), as well as in populations bisected by the national road DN57 (i.e., Sviniţa, Baziaş sites). Other isolated mortality hotspots were predicted at Balota and Malovăţ (fig. 1).

Discussion

Our study showed that Eastern Hermann’s tortoises in Romania are at a relatively low risk from road mortality, with the majority of roads having <0.001 mortality probability. However, the 1.6% overall road mortality predicted across the entire Romanian range may have deleterious consequences on population viability on the long term (e.g., Gibbs and Shriver, 2002). Additionally, we detected several potential hotspots of mortality associated with high traffic roads bisecting tortoise habitat (fig. 1). It is likely that such local populations already underwent declines in abundance due to road mortality, and that predicted increases in traffic volumes on several national and local roads might pose such threats to additional localities predicted by our

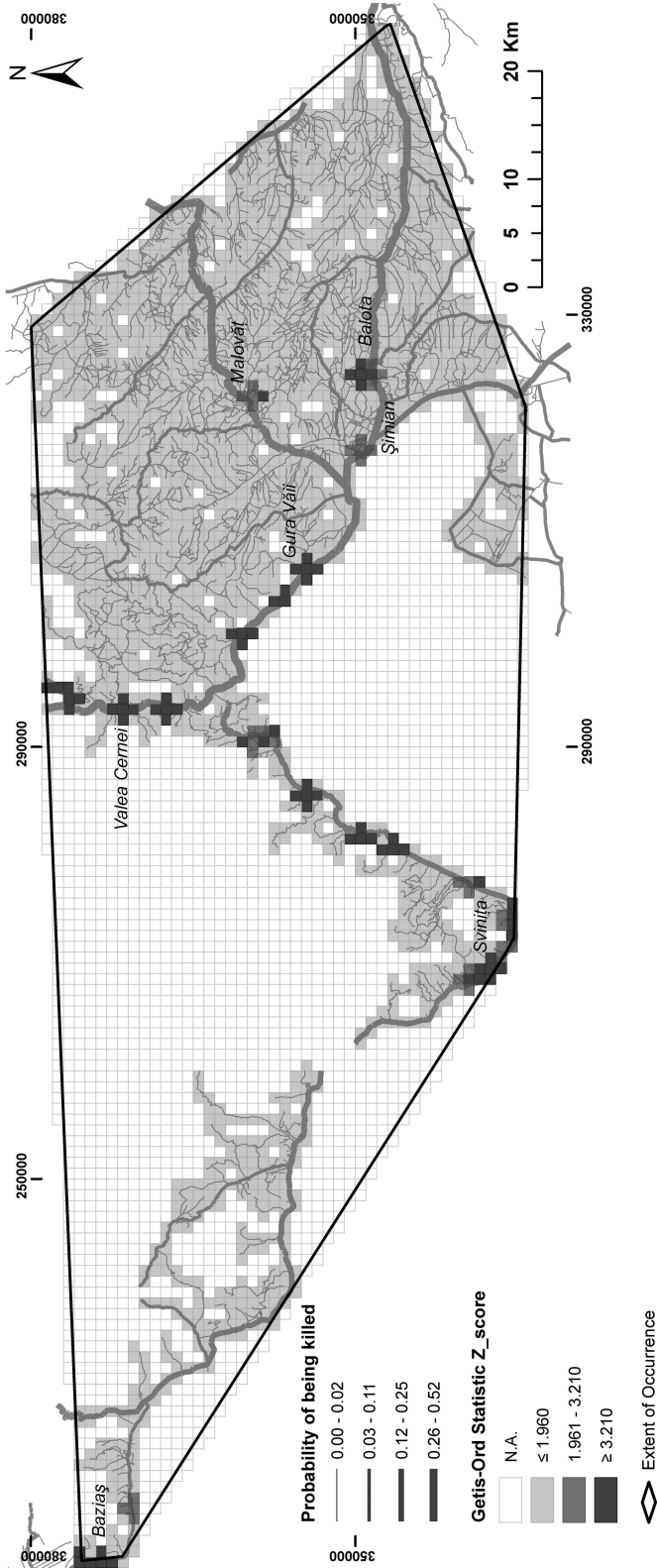


Figure 1. Road mortality hotspots for Hermann's tortoise in Romania. Thicker lines are roads with higher vehicle traffic and higher tortoise mortality. Hotspots of road mortality for Hermann's tortoises in Romania are indicated by darker grid cells. The map highlights the sites with mortality rates significantly higher than expected (Getis Ord Statistic $Z_{scores} \geq 1.96$). The hotspot model ignores the areas with no roads or documented occurrences where no road mortality may occur (N.A. values).

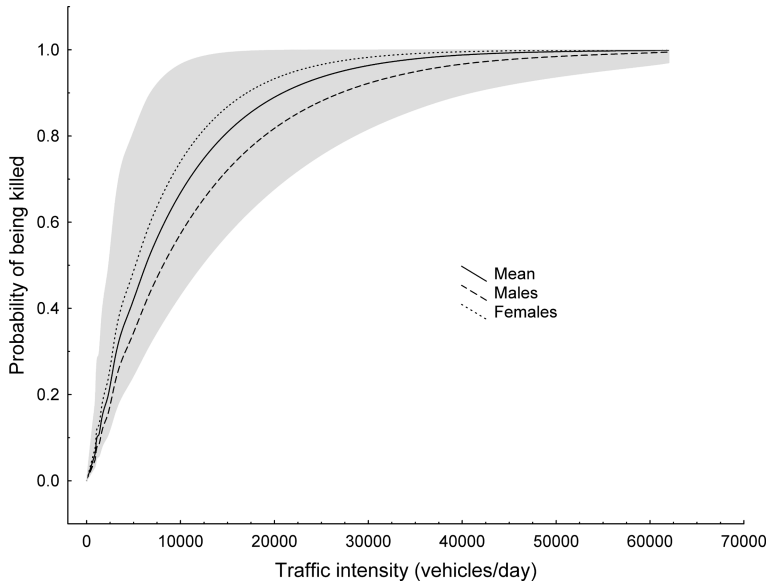


Figure 2. Gender-specific differences in probability of being killed on roads for Eastern Hermann's tortoise, estimated from an equation derived by Hels and Buchwald (2001). The shaded area represents the predictions within the range of empirically-determined tortoise velocities: 0.96-7.64 m/minute.

model. Road mortality might act synergistically with other current threats, such as poaching, nest predation, afforestation of abandoned hay-fields, and increase in fire frequency (Rozylowicz and Dobre, 2010; Bertolero et al., 2011). A further Population Viability Analysis is required to determine the impact of additional adult mortality from roads on population persistence and viability.

Females had lower road-crossing velocities, which were associated with the overall larger female body size. This association led to higher road mortality probability estimates, which could bias sex ratios towards males in local populations affected by road mortality (i.e., in the vicinity of high traffic roads; Steen and Gibbs, 2004; Steen et al., 2006). The tortoise carapace acts as a defense structure, hence tortoises may adopt a defensive behavior on roads by moving inside the carapace in response to passing vehicles. This behavior might thus lead to lower effective road crossing speeds, which in turn may result in higher than expected mortalities (Gooley, 2010). Thus, the variation in tortoise velocity increases the uncertainty around

the estimates of probability of being killed on roads (see fig. 2). Despite several limitations of our experiment stemming from latent factors that influence the speed of locomotion in the real world, including stress from handling and variation in individual behavior in relation to road traffic (Wren et al., 1998; Claussen et al., 2002), our velocity estimates corroborate other studies on terrestrial turtles and tortoises. For example, the average speed of locomotion of 3.07 m/min recorded in this study was similar to those recorded for *Terrapene carolina carolina* (3.6 m/min; Muegel and Claussen, 1994) and *Terrapene ornata* (3.0 m/min; Claussen et al., 2004).

When compared with amphibians, Hermann's tortoises have lower road mortality probabilities. For example, slower velocities of caudates and anurans (i.e., *Triturus vulgaris* = 0.50 m/min, *Rana temporaria* = 2.00 m/min) were associated with road mortality probabilities > 0.90 (Hels and Buchwald, 2001). *Ambystoma maculatum* had a probability of being killed on roads of nearly 0.40 for a road with traffic intensity of 900 vehicles/day (Gibbs

and Shriver, 2005). Such high mortalities for amphibians are mostly related to mass migrations or dispersal movements (deMaynadier and Hunter, 2000).

The overall low value of annual road mortality rate for Hermann's tortoises can be explained by the annual movement pattern that exhibit only occasional long movements for mating and nesting (Mazzoti et al., 2002; Rugiero and Luiselli, 2006), thus reducing the risk of road-kill (Ashley and Robinson, 1996). For example, annual movement patterns that incorporated longer seasonal steps (e.g. 500 m) led to excessive mortality rates for terrestrial and semiaquatic turtles (Gibbs and Shriver, 2002). Seasonal movements rarely exceed 150 m in the case of Eastern Hermann's tortoise (Rozyłowicz and Popescu, 2013), thus limiting the potential number of road crossings performed in a season. Exceptions may occur in home ranges that are bisected by major roads that tortoises may cross more frequently during their daily movements. For freshwater turtles, Gibbs and Shriver (2002) estimated an annual road-associated mortality > 5%. For the salamander *Ambystoma maculatum*, the annual road-associated mortality rate was 25-30%, potentially causing local extinctions in <25 years (Gibbs and Shriver, 2005). A modeling exercise similar to ours focusing on water snakes in Indiana, USA estimated mortality rates of 21% and 5% for *Nerodia erythrogaster neglecta* and *Nerodia sipedon*, respectively (Roe et al., 2006). One caveat for estimating road mortality rates is that the correlated random walk model used in these modeling exercises assumes a homogeneous habitat within individual simulated home ranges, as well as constant behavior through time (Byers, 2001). In order to restrict the habitat heterogeneity, we forced the movement paths inside simulated home range boundaries. Thus, we implicitly assumed that tortoises can select any habitat types in any time during the active season with the same probability. Another caveat is related with seasonal variations expected in traffic intensities, which have the po-

tential to determine hot moments of road mortality (Beaudry et al., 2010). Because we are lacking seasonal traffic data, we use annual averages of traffic intensity. Lastly, our model does not account for driver behavior, which has the potential to either increase or decrease the risk of mortality for tortoises. The drivers are more or less aware of small animals on the road, and there are indications that some drivers intentionally hit the tortoises crossing roads (see Ashley et al., 2007).

The main result of our broad-scale approach for assessing road mortality is the spatial representation of mortality hotspots, which can be used in road mortality mitigation efforts (Hothorn et al., 2012). We identified population groups under no risk of road-associated mortality, mainly due to isolation from the nearest roads or adjacency to low traffic, unpaved roads. At the same time, we predicted mortality rates up to 30% in several sites bisected by National and European roads (i.e., Baziaș-Ribiș and Svinița sites bisected by DN57 National road; Gura Văii and Valea Cernei bisected by E70 European road; fig. 1). For populations at greatest risk of road mortality, a range of mitigation techniques exist and have been implemented for both amphibians and reptiles (Forman et al., 2003; Puky et al., 2007). A variety of crossing structures were designed for herpetofauna (e.g., culverts, underpasses), but their efficiency depend on numerous factors such as temperature, cover type and presence or absence of debris (Yanes et al., 1995; Patrick et al., 2010). Roadside fences reduced annual road mortality to zero for an isolated tortoise population in France (Guyot and Clobert, 1997). Thus, fences in association with underpasses would be the most efficient measure to limit Hermann's tortoise, as well as other herpetofauna mortality on high-traffic roads in Romania. Other roadside obstacles such as guardrails or ditches can prevent reptiles from crossing roads, but they also may act as traps, increasing overall mortality (Dodd et al., 2004; Lovich et al., 2011).

In conclusion, we predicted that excessive road mortality rates may occur locally, especially where high traffic roads bisect highly suitable habitats. The synergy with other stressors such as habitat loss and poaching may lead to or has already led to population declines. Therefore, our predictions of road mortality hotspots have the potential to inform effective conservation strategies for locally threatened populations bisected by high traffic roads.

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