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Spatial and temporal distribution of amphibian road mortality with a *Rana dalmatina* and *Bufo bufo* predominance along the middle section of the Târnava Mare basin, Romania

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Abstract. Here we present the results of the first extensive Transylvanian road mortality survey of *Bufo bufo* and *Rana dalmatina*. Fieldwork was carried out between February 15 and May 03, 2007 in the middle section of the Târnava Mare basin. In a total of seventeen surveys in 2007 altogether 83.3 km of roads were repeatedly investigated. 1437 *R. dalmatina*, 833 *B. bufo* and 30 other amphibian individuals were found on the road. Both predominant species are common for the area, and had a larger migration intensity during rainy nights. Most (over 85%) *B. bufo* individuals were recorded to cross roads in main migration corridors while this ratio was under 50% for *R. dalmatina*, which has important conservation consequence in the protection of these species from road traffic. The average width of main migration routes crossing roads was 188.8 m (Min-Max: 50-350m, SD = 101.11). These were localized for both predominant species using GPS, in order to allow further studies and assessment for conservation. As proved by the site visits in 2008, these localities remained stable over the two studied years.

Key words: road mortality, *Bufo bufo*, *Rana dalmatina*, Romania.

Introduction

Road construction is an essential part of infrastructure developement. Besides having a positive, direct effect on the economy, roads may also cause significant decrease in biodiversity both at a local and at a landscape – regional scale. Their effects on biodiversity are multiple and often synergistic, ranging from direct killing, habitat loss and fragmentation to barriers of animal movements, noize and pollution (Trombulak & Frissell 2000, Shepard et al. 2008). Amphibians seem to be more affected by road traffic than other vertebrates (see for example Gryz & Krauze 2008) and reviews on amphibian road mortality giving a global (Puky 2006) and a European (Elzanowski et al. 2008) overview were recently published emphasizing the importance of this topic. Roads reduce the size of amphibian populations exposing them to stochastic environmental (such as climatic) variations (Hartel 2008), negatively affecting habitat occupancy (Gibbs & Shriver 2005), behaviour (Mazerolle 2004), increasing the likelihood of inbreeding (Lesbarreres et al. 2003) and finally leading to a decline or disappearance of populations (Bressi 1999, Fahrig et al. 1995, Landmann et al. 1999, Cooke 1995, Vos & Chardon 1998). Pond breeding amphibians have a series of eco-physiological and behavioral characteristics that make them sensitive to roads: (i) they have complex life cycles and require a high level of complementing landscape elements, (ii) their annual life cycle is characterized by periodical migrations among the most important habitats (breeding site, summer habitat, overwintering area), (iii) they are ectotherms and have low vagility compared to other vertebrates and (iv) they are sensitive to microclimatic conditions because of their permeable skin that also acts as an osmo-regulatory and respiratory organ.

Amphibian road mortality studies are largely lacking from Romania (Demeter & Hartel 1999). Assessing the spatial and temporal extent of amphibian road mortality may help the identification of those road transects where mass mortalities occur due to the fact that roads cross major migration routes of amphibians (Vogel & Puky 1995). As all amphibian species are protected by national and international (European) laws and conventions in Romania, there is a legal background on which environmental protection agencies may intervene to decrease the frequency of road casualties, using various measures from putting out temporary road signs to building permanent mitigation measures. The assessment of road casualties associated with landscape characterizations may also play a crucial role in creating reference databases for assessing the time lags between anthropogenically induced changes in the landscape and their (potentially) negative effect (Lövenhaft et al. 2004, Findlay & Bourdages 2000).

In this paper we present the results of the first assessment on the spatial pattern of road mortality with special emphasis on two anuran species, *Rana dalmatina* and *Bufo bufo*, the commonest species among road-killed amphibians in the middle section of the Târnava Mare basin, Romania. Both species are common in the Târnava Mare basin (Hartel et al. 2009a), they have large migration radius and are frequently killed on transportation infrastructure, their breeding population abundances being negatively affected by high traffic volume roads (Hartel et al. 2008, 2009b). The main aims of this paper are:

(i) to present the intensity of amphibian breeding migration in the Târnava Mare basin with special focus on the two predominant species, *R. dalmatina* and *B. Bufo*, crossing the roads,

(ii) to investigate how this is related to the distribution of breeding habitats nearby,

(iii) to provide distribution maps and geographic coordinates (identified with Global Positioning System) of transects with significant amphibian migration to provide basic data for further conservation and research.

Matherials and Methods

Surveys were carried out along different road transects with a total length of 83.3 km in the Târnava Mare basin. Road categories covered international roads (E60, 20.2 km length, Sighişoara - end of Saschiz), national roads (DN14, 34.9 km length, Sighişoara - entrance to Buzd), county roads (DJ106, 25.2 km, Sighişoara - Brădeni, and a road near Dumbrăveni) and village roads (3 km, Buzd) (Figure 1). Traffic intensity of the investigated sections can be seen in Figure 1.

Field studies were carried out between February 15 and May 03, 2007, till the end of the breeding migration season of *Bufo bufo* and *Rana dalmatina*. Seventeen night surveys were made between February 15 and April 05 (i.e. in the main migration period of amphibians, especially *B. bufo* and *R.*

dalmatina); the number of surveys per road transect for this period was 12. Between April 05 and May 03, three more night surveys were made but no migration of the two predominant species was found. Roads were investigated by car driving with approximately 20 - 50 km/h, which is around the suggested speed (Puky 2001), frequently used in similar studies (see Puky 2003 for a review of Central European studies). The car was stopped each time amphibians were observed on the road in order to allow species identification and counting. When at least five individuals were found, count data were also recorded, and the location was noted using the Global Positioning System, with 4-7 m accuracy. In such cases the length of the road transect between the animals observed furthest from each other was also measured. Before starting the night surveys, air temperature was recorded and the activity of amphibians was checked in a less impacted landscape near Sighişoara (Şercheş area), a town with central location within the study area, to make sure they move around during the night. Surveys were started at around 20^{30} and finished between 01^{00} and 02^{00} at night till amphibian activity decreased due to low temperature. Surveys were carried out in rainy periods as well. Most transects were surveyed twice per night. To avoid sampling bias due to possible changes in the migration intensity of amphibians during the night, surveys were started from different endpoints of the investigated road network. Daylight surveys were also carried out in the peak migration periods of both anurans on roads to record possible migration during that part of the day. In 2008 main migration routes were re-visited to check their constancy during years.

Amphibians found on the road were classified into three categories, according to their status and location on roads: (i) animals in the danger zone, when they were on the road more than 1 m away from the side of the road towards which they went and (ii) escape zone, when the amphibians were at <1m distance from passing the road and finally (iii) the individuals killed by traffic. Traffic intensity was measured for 5 minutes for every road type for every hour between 20^{30} and 02^{00}.

Parallel with the road transect surveys, breeding ponds near the investigated road sections were also checked to determine the presence, breeding activity and estimated population size of amphibian species. The relationship between the population sizes in the ponds and the number of killed individuals on the road near them was tested with Pearson correlation.

Results and discussion

Species composition and abundance of amphibians on the road

Six amphibian taxa were recorded during night road surveys: *Rana dalmatina* (62.47%), *Bufo bufo* (36.21%), *R. temporaria* (0.91%), *B. viridis* (0.17%), *R. esculenta* complex (0.13%) and *Hyla arborea* (0.08%). We did not find migrating *P. fuscus* or newts (*Triturus cristatus*, *T. vulgaris*) although these species were also present in the ponds adjacent to the roads (Hartel et al. 2007, 2009a). This result might be explained by alternative migration routes, which do not cross roads, and/or might be a side-effect of sampling bias because of low detectibility especially with newts, that was also recorded elsewhere, for example in Slovenia and the United Kingdom (Denac 2003, Evans 1989, 1992). The species composition and abundance of amphibians found on the roads are in line with other European studies made for example in Poland (Elzanowsky et al. 2008, Gryz & Krauze 2008), Hungary (Csapó et al. 1999), the Czech Republic (Mikátová & Mojmir 2002), Italy (Scoccianti 2001) and Spain (Santos et al. 2007). We also recorded a large percentage of animals with long migration radius, large size and abundance, i.e. common toads and brown frogs in amphibian road kills. The low occurrence of *R. temporaria*, a species common in road kills in other ares on Târnava Mare roads, may be explained by the fact that it prefers higher altitude and temporary forest ponds for reproduction in the region (Hartel et al. 2009a). Moreover, the permanent pond use

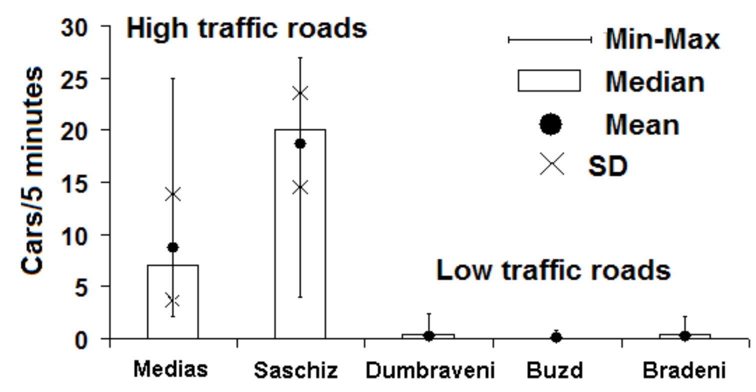

Figure 1. Traffic intensity of the investigated road sections

and persistence of this species is negatively affected by roads in this area (Hartel & Öllerer 2009). As the detectibility of *R. temporaria* and *R. dalmatina* can be considered roughly equal due to their similar shape, size, colour and movement pattern, the ratio of these species along the investigated road sections can be regarded as a valid assumption. On the other hand, the percentage of prolonged and late breeders, such as *B. viridis*, on roads, however, was probably underestimated in our study because the surveys were finished before their main migration period ended as we focussed our investigation on the main migration period of the amphibian community.

Temporal distribution of Rana dalmatina and Bufo bufo migration

1437 *R. dalmatina*, 833 *B. bufo* and 30 other amphibian individuals were found on the roads during the night surveys but no migrating individuals were recorded during day searches. The breeding migration of the two predominant anurans through roads lasted from February 23 to April 03 in 2007. In a previously studied *B. bufo* population undisturbed by roads (Şercheş area, near Sighişoara) the end of the breeding migration was also in the first part of April (April 10), but it started one week later (March 3-11) in 2001 and 2002 (Hartel & Demeter 2005). The peak of the breeding migration seems to be in a constant period, the second part of March, both in the Şercheş population (Hartel and Demeter 2005) and also along the roads (this study). The peak migration in 2007 was in a rainy night but intensive migration was recorded in other nights as well (Figure 2). These observations are similar with those made in the Şercheş population (Hartel & Demeter 2005). No migration on roads was found during daylight in the peak activity period. A previous study (Hartel & Demeter 2005) found that especially females of *B. bufo* may migrate during daylight in warm afternoon hours but it is known to be affected by meteorological conditions, structure of the vegetation and road characteristics (Puky 2006).

The migration of *R. dalmatina* peaked in the period from February 23 to March 03. Another peak was registered on March 22-23. *R. dalmatina* had a larger migration intensity in nights with rain (Figure 2).

The importance of weather conditions for amphibian breeding migration is well-known (Gittins et al. 1980, Wisniewsky et al. 1980, Puky et al. 1990, Hartel & Demeter 2005). Migration intensity and breeding behaviour is strongly influenced by weather conditions (see also Hartel et al. 2007 for a review) because amphibians are ectotherms and their skin is permeable, highly sensitive to local microclimatic conditions (Martin & Feder 1991).

Spatial distribution of Rana dalmatina and Bufo bufo migration

In the peak migration activity most amphibians on the roads were dead and only a small percentage reached the escape zone, from where they can get to the edge of the road (Table 1). Live and dead individuals were plotted together to counter-balance two antagonistic factors biasing the relative frequency of species.

As the presented data suggest, in spite of the faster possible crossing speed of *R. dalmatina*, under the studied conditions this species may spend more time sitting on the road also because of its more favourable characteristics e.g. warm surface resulting in a significantly larger ratio of dead individuals as well as a smaller ratio of live animals reaching the escape zone. On the other hand, *B. bufo* may cross with a slow but steady speed leading to a higher ratio and even to a higher absolute number of live individuals as well as more animals reaching the escape zone. The great majority of *B. bufo* individuals migrated alone and all the individuals that got into the escape zone of the roads were single males and females. A higher vagility of single males compared to those individuals that migrated in amplexus was also assumed by Csapó et al. (1989). Also, according to their study, larger individuals, i.e. females bearing eggs, may be more exposed to road traffic than smaller ones, most probably due to differences in their vagility. Moreover, such differences in the survival rates of the two species may also partly be caused by different behavioural responses to roads due to light, road surface temperature, humidity, etc. conditions (Csapó et al. 1989). We have repeatedly observed males of *B. bufo* displaying mate searching behaviour on roads (see also Orlowski 2007); this may further expose them to road mortality. However, in rainy nights, *R. dalmatina* may also be „blocked" more by car noize and flashlight (Mazerolle et al. 2005) leading to a higher death rate with that species as compared to *B. bufo*.

In both species the number of individuals safely crossing the roads was higher after 22^{30}, which can be explained by significantly lower traffic intensity after that hour. The population size of the breeding ponds is negatively affected by high traffic volume roads in both species (Hartel et al. 2008, 2009b).

The 2007 survey proved the presence of altogether nine migration routes with at least 30 migrating individuals for *R. dalmatina* and three such routes for *B. bufo* (see Table 2 and Figure 3 for the distribution of these areas along the roads). Their stability was also proved during the fieldwork carried out in 2008 (Hartel and Moga, *personal observations*). The average width of main migration routes was 188.8 m (Min-Max: 50-350m, SD = 101.11). *B. bufo* migrated mainly

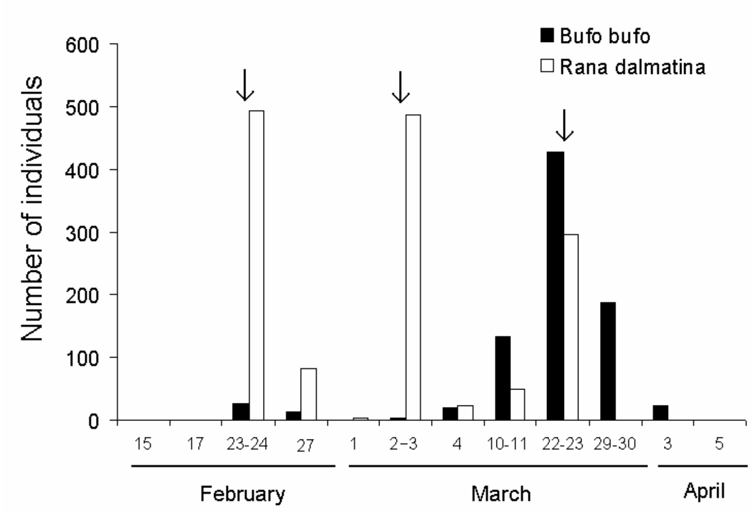

Figure 2. The intensity of breeding migration across roads (cumulated data). Arrows represent rainy nights

Table 1. Status and location of amphibians on roads in the peak migration period

	Rana dalmatina %	*Bufo bufo* %
Danger zone	15.57	35.53
Escape zone	2.72	9.57
Killed	81.70	54.89

in clearly distinguishable routes (800 individuals on such routes *versus* 33 individuals that appeared in other sections individually) but no such pattern was found with more than 50% of *R. dalmatina* individuals (684 individuals in clearly distinguishable routes *vs* 750 individual appearances on roads). Road transects with the most intensive migrations are close to a potential breeding pond. The use of these ponds for reproduction was 100% for *R. dalmatina* and 75% for *B. bufo*. These differences may be explained by the breeding habitat selection of the two species: *R. dalmatina* uses a high variety of ponds for reproduction, ranging from permanent to temporary ones such as puddles on earth roads and drainage ditches (Hartel et al. 2006), whereas *B. bufo* is more conservative with respect to its breeding habitat selection preferring more permanent ponds and showing high pond fidelity (Sinsch 1991). Some of the animals breeding in those ponds, however, might not migrate across the roads. Large numbers of amphibians (particularly *R. dalmatina*) were also detected within the villages Şaeş and Saschiz (Table 2). In both cases, wetlands are present in a radius of 1 km.

A significant positive relationship (r = 0.51, $P < 0.05$) was found between the number of egg masses deposited by *R. dalmatina* in the ponds adjacent to roads and the number of individuals killed on nearby road sections, though the correlation was significant only after we excluded two ponds with large populations: the marshy area of Şaeş (with up to 5,000 *R. dalmatina* egg masses) and the fishponds from Brădeni (up to 3,000 egg masses). Orlowski (2007) reported positive relationship between *B. bufo* roadkills and wetland size, whereas Santos et al. (2007) reported that the largest counts of road mortality in *B. bufo* were associated with the presence of streams crossing the road, low slope of the roadside and semi-natural vegetation. All these results suggest that the occurrence of amphibians on roads is not random. There is a series of population, landscape and habitat features that may positively predict the occurrence and number of road casualties. These may be used to predict significant amphibian loss in newly planned roads and help managers and decision makers to mitigate the effects of roads on amphibians more efficiently.

The localization of road transects where intensive migration was recorded (i.e. main migration routes) and also the total number of individuals counted on these road sections is presented in Table 2. The migration of amphibians was sometimes facilitated by tunnels constructed for drainage under roads. In these tunnels often small, temporary ponds are formed, where *R. dalmatina* reproduction takes place. Such tunnels were especially efficient along DN14, probably because they created humidity gra-

Table 2. Geographic coordinates (identified with GPS) of migration routes with at least five individuals recorded and the total number of *B. bufo* and *R. dalmatina* individuals counted in them

Location name	No.	N	E	*B. bufo* (number of individuals)	*R. dalmatina* (number of individuals)	Presence of at least one large pond (within 1000m radius)
Attila Berg (1)	1	46.21978	24.75503	4	25	+
Attila Berg (2)	2	46.22244	24.75378	5	64	+
Attila Berg (3)	3	46.22472	24.74688	22	73	+
After Daneş (1)	4	46.2166	24.64141	0	38	+
After Şaroş (1)	5	46.19966	24.48552	0	171	+
After Vânători	6	46.24036	24.94046	7	25	+
Attila Berg (4)	7	46.2251	24.74427	1	30	+
After Şaroş (2)	8	46.19855	24.48391	9	21	+
Near Brădeni fishponds	9	46.07007	24.80647	0	5	+
Saschiz	10	46.20393	24.96489	0	30	+
After Daneş (2)	11	46.22138	24.68055	0	32	+
Dumbrăveni ponds (1)	12	46.25296	24.57019	119	21	+
Dumbrăveni ponds (2)	13	46.25633	24.56848	535	82	+
Şaeş village	14	46.14945	24.76981	98	31	+
Total				**800**	**648**	

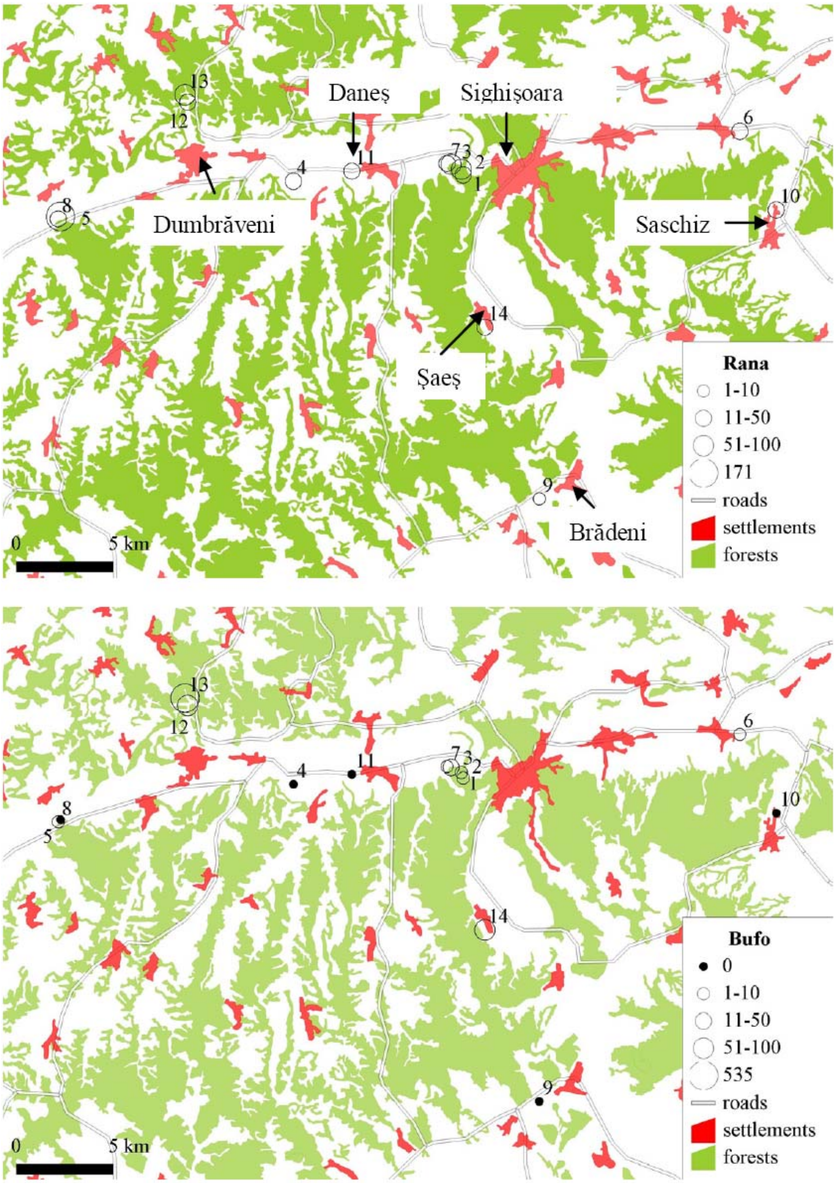

Figure 3. The distribution of the main migration routes of *R. dalmatina* (upper map, *Rana*) and *B. bufo* (lower map, *Bufo*). Circle size is proportional to the number of individuals. The numbers represent the point number from Table 2.

Photo 1. Tunnel under the road DN14 (lower figure) and its landscape surroundings (upper figure). In the surroundings of this tunnel (around 50 m) many dead specimens of *B. bufo* and *R. dalmatina* were counted. In the pond formed in the wetland area of the tunnel 38 *R. dalmatina* egg masses were counted on April 01, 2007.

dients that attracted amphibians (Photo 1); but even in these cases, a large number of amphibians were killed on the road around these tunnels. Similar results were reported by Santos et al. (2007) for *B. bufo* in Spain.

Conclusions

A large number of amphibians can potentially be killed during the breeding migration by road traffic in the Saxon landscapes of Southern Transylvania. New available tools (such as the Global Positioning System and the possibility for visualization on electronic maps) help researchers to locate, visualize and monitor the spatial pattern of roadkills and analyse them from a landscape context. We suggest that if short and relatively inexpensive surveys such as ours are applied in wider areas, this will help environmental protection agencies and conservationists in Romania in developing mitigation measures along roads to lower amphibian casualties. Tunnels already existing under roads should be more carefully evaluated and maintained, also with a conservation approach, as they may represent useful ways for mitigating the effects of roadkills on amphibians. As International experience (Mata et al. 2008, Yanes et al. 1995) demonstrates that amphibians, as well as other animals, may be directed into these tunnels using fences; and at least three animal groups - amphibians, reptiles and mammals - can safely cross roads through them (Puky et al. 2007).

Our study suggests that amphibian species may have different distribution patterns along roads. These differences need a closer examination because of their direct relation to the conservation measures for amphibians. In our case, if the revealed distribution pattern in *R. dalmatina* and *B. bufo* persist long-term, tunnel systems will be more efficient for *B. bufo* (that migrates primarily in main migration routes) than for *R. dalmatina*.

Also, further studies should test the efficiency of different methods (i.e. driving and walking surveys [Langen et al. 2007]) in detecting different amphibian species and life stages on roads. Surveys should be continued to detect potential shifts in the main migration routes and / or the appearance of new routes. Such projects can play an important role in environmental education as well as serving necessary information for the creation of mitigation measures (Puky & Vogel 2003). Toad-saving events were already organized in this area (Demeter & Hartel 1999), involving local schools (from Sighişoara) and scouting groups, the present study proved that there is a need for further efforts in this respect.

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