

# Road-killed Amphibians and Reptiles on a Local Road in a Protected Area in Western Romania

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**Abstract:** On a local road with light traffic from Carei Plain natural protected area, we recorded 731 dead bodies, belonging to 11 amphibian and five reptile species. Individuals killed on the road belong to 80% of the species of the herpetofauna found in the region. The most affected species was *Pelobates fuscus*. The intensity of road mortality differed between periods, with peak in spring. In summer the number of road-killed individuals and species was reduced but in the autumn it raised again. In 2014, the dry summer and autumn reduced the amplitude of road mortality. Most of the affected species are protected.

**Key Words:** herpetofauna, habitats, period, protected area, road traffic

## Introduction

Road mortality has a negative impact on amphibian populations, causing their decline or even disappearance (FAHRING *et al.* 1995; HELS & BUCHWALD 2001; GIBBS & SHRIVER 2005; GLISTA *et al.* 2007; GARRAH *et al.* 2015). Although there are many studies concerning road impact upon herpetofauna in Europe (e.g. CIESIOLKIEWICZ *et al.* 2006; ORŁOWSKI 2007; SANTOS *et al.* 2007; ELZANOWSKI *et al.* 2009; BRZEZIŃSKI *et al.* 2012; MATOS *et al.* 2012; in Romania there are few data on this issue (HARTEL *et al.* 2009; CICORT-LUCACIU *et al.* 2012; COVACIU-MARCOV *et al.* 2012). One of the studies from Romania was conducted in Carei Plain protected area and found that many protected amphibians were killed on a secondary road (CICORT-LUCACIU *et al.* 2012). As that study was made in autumn (CICORT-LUCACIU *et al.* 2012) when some amphibians like newts are more affected (GRYZ & KRAUZE 2008), its results are difficult to be generalised. In autumn 2013, on another road from Carei Plain, we observed a high amphibian and

reptile road mortality. This finding motivated us to study the road mortality in the entire warm season of the following year, including seasonal variation. Our objective was to find out how many amphibians and reptiles were killed on that road, identifying the points and periods with increased road mortality.

## Materials and Methods

The studied road is situated in north-western Romania (Figure 1), near the border with Hungary, in Carei Plain protected area, between Resighea and Scărișoara Nouă localities. We analysed a road length of 3617 m, between Scărișoara Nouă and Oradea – Satu Mare railway. Near Scărișoara Nouă the road has a short crossroad that leads to a monastery. Although covered with asphalt, the road has a lot of declivities and the cars are travelling with approximately 50 km/h. The road is narrow, the asphalted section does not allow for two cars to pass simultaneously, being at the same

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level with its surroundings. As the road is surrounded by diverse habitats, we divided it in nine sectors with different lengths, according to the length of the surrounding habitats (Table 1).

We made 20 field-trips, two in autumn 2013 and 18 in 2014. In 2014 field-trips were made every two weeks from March to October. The road was inspected by walking on it, a method considered more efficient, especially for small-sized animals (e.g. SLATER 2002; LANGREN *et al.* 2007; BRZEZIŃSKI *et al.* 2012). Alongside amphibian and reptile corpses, we counted the cars that pass in an hour. We calculated the percentage abundance of each species and the number of species and individuals / meter of road. Additionally, we established the frequency of occurrence of each species by sector and period. The Shannon-Weaver diversity was calculated. For the similarity we used the Bray-Curtis index. In estimating the significance of differences between periods or sectors we applied Kruskal-Wallis test and Mann-Whitney U test. For estimating the relationships between the sector length, species number, diversity or individuals we used linear regression, using the Past.3x free software (HAMMER *et al.* 2001).

## Results

The average number of cars/hour was 13.75, the minimum number being ten and the maximum 19. We found 731 dead bodies, belonging to 11 amphibian and five reptile species (Table 2), alongside 11 amphibian corpses which could not be determined. *Pelobates fuscus* had the highest percentage abundance, followed by *B. bombina* (Table 2). *Pelobates fuscus*, *Pelophylax esculentus* and *Natrix natrix* were killed in all sectors (Table 2). Not a single species was killed in all periods, with higher frequency of occurrence were *P. fuscus*, *N. natrix* and *Hyla arborea* (Table 2). Both, species and number of individuals killed by cars, differed between periods (Table 3).

The highest number of individuals was killed at the beginning of April and the highest number of species at the end of April (Table 3).

There were significant differences of road mortality between periods (Kruskal-Wallis test  $p=0.002$ ). Analysing individually the differences between periods, they were in many cases significant according to Mann Whitney U Test. Thus, there were significant differences between April and summer (April 9 / July 16:  $p=0.012$ , April 23 / July 16:  $p=0.006$ ) and autumn months (April 9 / September 16:  $p=0.010$ , April 23 / September 16:  $p=0.004$ ) and also between the first date from October 2013 and summer (October 10, 2013 / July 16, 2014:  $p=0.018$ ) and autumn 2014 (October 10, 2013 / September 16, 2014:  $p=0.014$ ). We recorded differences in similarity between periods (Bray-Curtis index) from the last date from October 2014, with no dead bodies owing to the very cold period.

The highest number of individuals was killed in sectors 3 and 2, and the lowest in sectors 7 and 8. The highest number of species (12 species) was killed in sectors 5 and 6 (Table 4). The differences between the sectors were not significant (Kruskal-Wallis test  $p=0.197$ ). Analysing individually the differences between sectors with Mann Whitney U Test, we found no significant difference except for sector 1 and sector 5 ( $p=0.016$ ) and 6 ( $p=0.035$ ).

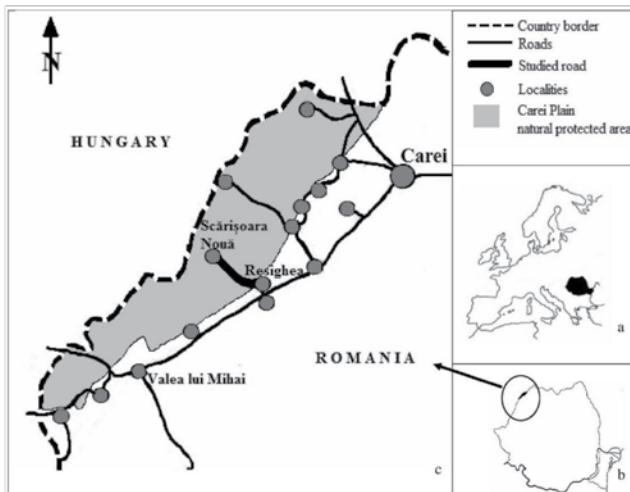
The overall diversity was  $H=1.38$ . The highest diversity was in sector 5 (Table 4) and at the beginning of June (Table 3). The average number of species killed/m of road was 0.0044, differing between sectors (Table 4). There was a moderate positive relation between the sectors length and the number of killed species ( $r=+0.55$ ) even if statistically it did not prove to be significant ( $p=0.12$ ). The number of individuals killed/m of road was 0.202, differing between sectors (Table 4). There wasn't a relation between the sectors' length and the number of killed individuals ( $r=+0.21$ ,  $p=0.57$ ).

**Table 1.** Description of the examined road sectors

Sector	Length (m)	Aspect	Description
1	301	diverse	agricultural areas, sandy areas, wet areas
2	341	diverse	wet areas, agricultural areas, sandy areas
3	475	diverse	wet areas, sandy areas, agricultural areas,
4	218	uniform	black locust plantation on a sand dune
5	220	diverse	partially dried out swamp, sandy areas, black locusts
6	1002	uniform	open areas with grassy vegetation on sandy soil, pastures
7	249	uniform	black locust plantation on a sand dune
8	311	uniform	open areas with grassy vegetation on sandy soil, pastures
9	500	diverse	partially dried out swamp, sandy areas, black locusts

**Table 2.** Percentage abundance (P) and frequency of occurrence by sectors (Fs%) and periods (Fp%) of the identified species

Species	P (%)	Fs%	Fp%
<i>Triturus dobrogicus</i>	0.958	33.33	25
<i>Lissotriton vulgaris</i>	0.137	11.11	5
<i>Bombina bombina</i>	13.95	66.67	55
<i>Hyla arborea</i>	3.83	88.89	60
<i>Pelobates fuscus</i>	63.61	100	90
<i>Bufo bufo</i>	0.547	44.44	10
<i>Bufo viridis</i>	0.137	11.11	5
<i>Rana dalmatina</i>	1.778	55.56	35
<i>Pelophylax ridibundus</i>	0.274	22.22	5
<i>Pelophylax esculentus</i>	5.198	100	45
<i>Pelophylax lessonae</i>	0.547	44.44	20
<i>Emys orbicularis</i>	0.684	44.44	15
<i>Lacerta agilis</i>	1.642	44.44	35
<i>Zootoca vivipara</i>	0.137	11.11	5
<i>Podarcis tauricus</i>	2.462	66.67	35
<i>Natrix natrix</i>	4.104	100	65



**Fig. 1.** Study area: Romania (a), the Carei Plain Protected Area in Romania (b) and the studied road between Resighea and Scărișoara Nouă in the Carei Plain (c)

## Discussion

The local road between Scărișoara Nouă and Resighea has a reduced traffic, the number of cars/hour being lower than in other cases (e.g. MATOS *et al.* 2012). Nevertheless, the road mortality of herpetofauna is higher than for other roads (e.g. TOK *et al.* 2011, BORKOVCOVÁ *et al.* 2012; MATOS *et al.* 2012; DE CARVALHO *et al.* 2014). This is probably a consequence of the pedestrian survey of the road, which allowed us to observe even the smallest dead bodies, while other studies were done from moving cars (TOK *et al.* 2011; BORKOVCOVÁ *et al.* 2012; MATOS *et al.*

*et al.* 2012; DE CARVALHO *et al.* 2014). In other studies conducted by walking on the road, the number of encountered corpses was high, even much higher than in our study (e.g. ASHLEY & ROBINSON 1996; GRYZ & KRAUZE 2008; GOLDINGAY & TAYLOR 2006).

Regardless of the low traffic, from the 20 herpetofauna species recorded in Carei Plain (COVACIU-MARCOV *et al.* 2008 a, b, 2009; SAS & CICORT-LUCACIU 2012) specimens belonging to 80% of the species were found killed by the traffic on this road, more than in other regions (SOUZA *et al.* 2015). The most affected species are common in Carei Plain (COVACIU-MARCOV *et al.* 2009). Although *Bufo bufo* is frequently killed on roads (e.g. SANTOS *et al.* 2007; MATOS *et al.* 2012; BRZEZIŃSKI *et al.* 2012), on the studied road its percentage abundance was lower than 1%. *Pelobates fuscus* was killed more often, as it is more abundant in the region (COVACIU-MARCOV *et al.* 2009). It prefers sandy soils (FUHN 1960), which are common near the road. This nocturnal species (COGALNICEANU *et al.* 2000) is probably active even in drier periods, which explains why it was killed almost during all periods. *Natrix natrix* is one of the common reptiles in the region (COVACIU-MARCOV *et al.* 2008 a, b, 2009) and was also frequently killed. *Podarcis tauricus* occurs in the area owing to the presence of sandy soil, and in western Romania the species is distributed only here, at its northern distribution limit (e.g. COVACIU-MARCOV *et al.* 2009). Being present on the sandy areas close to the road it is exposed to road mortality. *Zootoca vivipara*, one of the less affected species is very rare in Romanian plains, where it is recorded near wet areas (e.g. COVACIU-MARCOV *et al.* 2009). Thus, usually it was further away from the road and only one killed individual was recorded.

There are important differences in road mortality between the studied road and the one previously analysed in Carei Plain (CICORT-LUCACIU *et al.* 2012). We encountered less newt corpses than in the past (CICORT-LUCACIU *et al.* 2012), probably because of the higher distance between the road and the large wet areas. Moreover, there were differences between autumn 2013 and 2014: in 2013 the intensity of road mortality was higher than but these differences were not significant ( $p=0.18$ ). The lower road mortality in autumn 2014 was likely due to the very dry summer and autumn, as compared with 2013. The dry period from 2014 probably diminished some species' activity (*T. dobrogicus*, *L. vulgaris*, *B. bombina*, *R. dalmatina*), leading to an underestimation of the road mortality. Earlier studies had observed differences between different years (ASHLEY & ROBINSON 1996; MOLLOV *et al.* 2013).

Table 3. Percentage abundance of individuals and species' diversity in different periods

Year	2013												2014											
	X		III		IV		V		VI		VII		VIII		IX		X							
Month	10	23	12	26	09	23	07	21	04	17	01	16	28	19	21	03	16	02	16	31				
<i>T. dobrogicus</i>	2.60	6.66	-	0.85	-	0.94	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
<i>L. vulgaris</i>	0.86	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
<i>B. bombina</i>	29.56	53.33	75.00	17.94	2.84	6.60	30.55	-	-	-	58.82	-	8.33	-	11.11	-	-	-	-	3.57	-			
<i>H. arborea</i>	0.86	-	25.00	1.70	3.97	2.83	5.55	3.22	-	-	11.76	-	16.66	-	11.11	25.00	-	-	-	17.85	-			
<i>P. fuscus</i>	48.69	33.33	-	76.92	83.52	69.81	50.00	38.70	22.22	37.50	17.64	25.00	33.33	66.66	55.55	25.00	33.33	81.81	67.85	-	-			
<i>B. bufo</i>	0.86	-	-	2.56	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
<i>B. viridis</i>	-	-	-	-	-	-	-	-	-	-	-	-	8.33	-	-	-	-	-	-	-	-			
<i>R. dalmatina</i>	1.73	-	-	-	2.27	1.88	2.77	0.64	5.55	-	-	-	-	-	-	-	33.33	-	-	-	-			
<i>R. ridibunda</i>	-	-	-	-	1.13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
<i>R. esculenta</i>	11.30	-	-	-	2.27	-	-	25.80	33.33	-	5.88	-	25.00	16.66	-	-	-	4.54	3.57	-	-			
<i>R. lessonae</i>	-	-	-	-	0.56	-	-	-	5.55	-	5.88	-	-	-	-	-	-	-	-	3.57	-			
<i>E. orbicularis</i>	-	-	-	-	1.13	1.88	-	3.22	-	-	-	-	-	-	-	-	-	-	-	-	-			
<i>L. agilis</i>	-	-	-	-	-	3.77	2.77	6.45	5.55	-	-	50.00	8.33	-	-	-	-	4.54	-	-	-			
<i>Z. vivipara</i>	-	-	-	-	-	0.94	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
<i>P. tauricus</i>	-	-	-	-	-	9.43	-	3.22	11.11	12.50	-	-	-	-	11.11	25.00	-	9.09	-	-	-			
<i>N. natrix</i>	3.47	6.66	-	-	2.27	1.88	8.33	12.90	16.66	50.00	-	25.00	-	16.66	11.11	25.00	33.33	-	-	-	-			
Percentage abundance by date	15.73	2.05	0.54	16.01	24.08	14.5	4.92	4.24	2.46	1.09	2.32	0.54	1.64	0.82	1.23	0.54	0.41	3.01	3.83	-	-			
Diversity	1.36	1.06	0.56	0.71	0.77	1.19	1.28	1.67	1.72	0.97	1.2	1.04	1.63	0.87	1.3	1.39	1.1	0.66	1.05	1.05	0			

The road mortality is lower in sectors with black locust plantations, which are habitats with reduced diversity in other groups too. The road mortality differences were significant among periods, as frequently demonstrated by earlier studies (e.g. ASHLEY & ROBINSON 1996; GRYZ & KRAUZE 2008; KAMBOUROVA-IVANOVA *et al.* 2012; MOLLOV *et al.* 2013; WANG *et al.* 2013; GARRAH *et al.* 2015). The highest number of species and individuals was killed in spring. The differences between the road mortality intensity are known to be a result of the victims' activity and reproduction cycle (GRYZ & KRAUZE 2008; CHITTAREGI & HOSETTI 2014; GARRAH *et al.* 2015), depending on meteorological conditions. Amphibians are more active in spring, when they are reproducing (COGALNICEANU *et al.* 2000). The road mortality intensity rises again in autumn, a similar pattern being observed in other south-eastern European areas (MOLLOV *et al.* 2013). Nevertheless, the intensity in autumn was much lower than in spring, at least in 2014, because of the drought. In other regions this second peak is much stronger (GRYZ & KRAUZE 2008; MOLLOV *et al.* 2013). The number and diversity of corpses are higher in areas where the road is surrounded by more diverse habitats, especially near wet areas. The road mortality is lower both in sectors with black locust plantations and habitats with lower diversity of other groups (FERENȚI *et al.* 2012). The same situation was previously observed in Carei Plain (CICORT-LUCACIU *et al.* 2012), the aspect of the road surroundings influencing the road mortality (e.g. ASHLEY & ROBINSON 1996; GRYZ & KRAUZE 2008). Not the sectors length but the habitats near them shape the diversity of the road-killed herpetofauna.

Almost all road-killed species are protected by the Romanian legislation (O.U.G. 57/2007). The high number of killed species and individuals is of concern, because of the protection status of the region, but especially because the road is local, with low traffic and speed, while the road mortality being positively correlated with the traffic volume (GIBBS, SHRIVER 2005; DE CARVALHO *et al.* 2014). Probably any enlargement of the road will increase the road mortality, as it was previously indicated in Carei Plain (CICORT-LUCACIU *et al.* 2012) and in other regions (JONES *et al.* 2014). The low speed should be maintained by installing calming devices. Other conservative actions, like under crossing and fences were already proposed for Carei Plain (CICORT-LUCACIU *et al.* 2012). Preventive measures should be considered in any future enlargement of the road. The local roads should not be linked with other major roads, because this will increase the traffic volume and the pressure upon herpetofauna.

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**Table 4.** Percentage abundance of individuals by sectors, individuals/meter, species/meter and diversity

Sectors	S1	S2	S3	S4	S5	S6	S7	S8	S9
<i>T. dobrogicus</i>	-	-	0.44	-	5.45	-	-	-	4.83
<i>L. vulgaris</i>	-	0.63	-	-	-	-	-	-	-
<i>B. bombina</i>	9.75	32.48	11.11	-	5.45	-	3.33	-	29.03
<i>H. arborea</i>	2.43	3.18	3.11	2.53	5.45	2.56	6.66	-	9.67
<i>P. fuscus</i>	82.92	58.59	76.00	73.58	43.63	66.66	56.66	66.66	25.80
<i>B. bufo</i>	-	-	0.44	-	1.81	1.28	-	-	1.61
<i>B. viridis</i>	-	-	-	-	-	1.28	-	-	-
<i>R. dalmatina</i>	-	-	-	1.88	9.09	1.28	6.66	-	6.45
<i>R. ridibunda</i>	-	0.63	-	-	-	1.28	-	-	-
<i>R. esculenta</i>	2.43	3.18	4.44	1.88	9.09	5.12	3.33	6.66	14.51
<i>R. lessonae</i>	-	0.63	-	-	1.81	1.28	-	3.33	-
<i>E. orbicularis</i>	-	-	0.44	-	2.35	1.28	-	3.33	-
<i>L. agilis</i>	-	-	1.33	7.54	7.27	1.28	-	-	-
<i>Z. vivipara</i>	-	-	0.44	-	-	-	-	-	-
<i>P. tauricus</i>	-	-	-	5.66	1.81	6.41	13.33	10.00	3.22
<i>N. natrix</i>	2.43	0.63	2.22	5.66	5.45	10.25	10.00	10.00	4.83
Individual / m	0.136	0.460	0.473	0.243	0.250	0.077	0.120	0.096	0.124
Species / m	0.016	0.023	0.021	0.032	0.054	0.011	0.028	0.019	0.018
Percentage abundance by sector	5.60	21.48	30.78	7.25	7.52	10.67	4.10	4.10	8.48
Diversity (H)	0.65	1.03	0.94	1.02	1.96	1.32	1.41	1.14	1.86

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