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Beyond the victim number: faunistic and ecological data from a road-mortality study in the Iron Gates Natural Park, Romania

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Abstract

Road mortality is one of the most obvious forms of anthropic impact upon fauna. The Iron Gates Natural Park is an area of great biodiversity, crossed by a 154 km long road running parallel to the Danube River. To estimate the impact of this road upon the fauna, between March 2019 and February 2020 we monitored road mortality on a monthly basis. We recorded 13,230 road-killed animals, belonging to 71 taxa. The greatest proportion of taxa was killed in early summer and the number of individuals killed peaked in autumn. A spring mortality peak was not observed. Cold-blooded animals were killed year-round, even though they should not have been active in winter in Romania. This is a consequence of the warmer climate of the region compared to the rest of Romania, but also the mild winter of 2019/2020. Mitigation measures such as stopping the construction of new roads would prevent the problem of animal road deaths being replicated in other areas. In addition to the ecological, zoogeographical and conservation value of its findings, our study also warns of a cause-effect link between global warming and an increase in road mortality.

Profile

Protected area

Iron Gates Natural Park

Mountain range

Banat and the Mehedinți Mountains

Country

Romania

Introduction

Road mortality, even at moderate rates, may cause a decrease in some animal populations (e.g. Hels & Buchwald 2001; Gibbs & Shriver 2002; Row et al. 2007; Barbosa et al. 2020). Among vertebrates, the number of victims seems high (e.g. Gerow et al. 2010; Garrah et al. 2015; Santos et al. 2016), but this number is even higher among invertebrates (e.g. McKenna et al. 2001; Baxter-Gilbert et al. 2015; Keilsohn et al. 2018). Road-mortality studies, besides establishing the number of carcasses, sometimes provide other insights, for example indicating the distribution of native or invasive species (Schwartz et al. 2020). If other studies have demonstrated that this is true in general, how does it apply specifically to a particular area of remarkable biodiversity? One region of significant biodiversity in Romania is the Danube Gorge, currently a mountain protected area (Iron Gates Natural Park – IGNP). IGNP is one of the largest protected areas in Romania, sheltering numerous protected species (Rozylowicz et al. 2019). Species with various ecological demands (related to warm sub-Mediterranean areas or associated with cold mountain climates) come into contact with each other here (e.g. Paşcovschi 1956; Covaciu-Marcov et al. 2009; Tăușan & Teodorescu 2017). In the Danube Gorge, mountain species descend to very low altitudes (e.g. Paşcovschi 1956; Covaciu-Marcov et al. 2009; Teodor et al. 2019), despite the region having a warmer climate than other areas of Romania (e.g. Stoenescu et al. 1966; Mândruț 2006). Anthropic activities in the region have a long history: it was an important part of the Roman Empire, with the Romans building a bridge over the Danube in the area (e.g. Păunescu & Butușină 2010; Bara & Kaiser 2015; Mehrotra & Glisic 2015). The Danube Gorge has had a wide network of roads since that period (Ilić et al. 2010). To compound the situation, the construction of the Iron Gates Dam I between 1964 and 1972 had a massive impact on the region, increasing the water level and flooding roads, human settlements and islands (e.g. Mihai et al. 2016; Șelău 2018). The current road has therefore been built higher up, cutting through the sometimes steep slopes and threatening neighbouring habitats (Niculae et al. 2014). However, previous studies offer only limited clues on the effect of road traffic on the fauna in the IGNP (Covaciu-Marcov et al. 2005, 2009; Teodor et al. 2019).

Considering the negative effect of road traffic upon fauna in general (e.g. Baxter-Gilbert et al. 2015; Garrah et al. 2015; Ciolan et al. 2017; Keilsohn et al. 2018) and the biodiversity of IGNP (e.g. Rozylowicz et al. 2019), we presumed that the rich diversity of habitats and species in the region would be reflected in patterns of road mortality. The climatic peculiarities of the region (e.g. Stoenescu et al. 1966; Mândruț 2006), and the warmer and drier weather conditions in south-western Romania over the last few years (e.g. Croitoru & Piticar 2013; Prăvălie 2014; Pravalie et al. 2014; Trif & Oprea 2015) led us to suppose that this climate pattern would result in seasonal changes in road-mortality rates, which presumably would be different from the pattern observed in other areas of Romania (e.g. Ciolan et al. 2017; Covaciu-Marcov et al.

Table 1 – The number of road-killed individuals and average number of vehicles per hour by period and section. The sections were distributed along almost the entire length of the gorge (S1-Orșova, S2-Eșelnița, S3-Cazanele Mici Gorge, S4-Ponicova, S5-Liubotina, S6-Șvinița, S7-Cozla, S8-Berzasca, S9-Liborajdea, S10-Gaura cu muscă Cave, S11-Moldova Nouă, S12-Măcești, S13-Radimna, S14-Divici).

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	Total	Average no of vehicles/ hour
March	9	23	19	15	10	16	15	16	26	29	3	55	9	20	**265**	**59.96**
April	65	9	12	9	1	33	65	81	97	16	26	68	19	37	**538**	**65.89**
May	44	66	78	59	32	53	85	90	244	57	37	137	75	57	**1,114**	**89.48**
June	212	72	64	58	75	96	132	239	574	133	38	153	59	27	**1,932**	**134.10**
July	179	59	50	49	48	25	52	63	207	34	47	152	75	65	**1,105**	**183.82**
August	177	61	62	42	23	28	69	85	361	22	118	76	383	170	**1,677**	**125.67**
September	90	27	26	18	26	34	21	20	326	18	48	73	129	99	**955**	**150.37**
October	178	102	64	43	51	106	333	439	455	8	39	262	335	293	**2,708**	**112.27**
November	49	64	16	13	15	267	323	368	358	35	39	216	304	201	**2,268**	**76.77**
December	53	43	36	3	3	19	21	33	123	8	7	21	13	18	**401**	**57.33**
January	18	-	-	1	-	1	5	1	7	-	-	2	-	15	**50**	**64.04**
February	4	2	8	1	1	28	42	40	48	1	5	15	16	6	**217**	**69.41**
Total	**1,078**	**528**	**435**	**311**	**285**	**706**	**1,163**	**1,475**	**2,826**	**361**	**407**	**1,230**	**1,417**	**1,008**	**13,230**	
Average no. of vehicles / hour	**236.12**	**161.50**	**126.74**	**52.89**	**39.85**	**37.40**	**36.73**	**69.70**	**77.64**	**85.85**	**143.61**	**227.19**	**67.55**	**25.78**	**99.18**	

2017; Popovici et al. 2018) or elsewhere in the temperate zone (e.g. Orłowski 2007; Gryz & Krauze 2008; Mollov et al. 2013; Garriga et al. 2017). In order to verify these assumptions, we set the following objectives: 1. Counting and identifying road-killed animals, 2. Establishing seasonal patterns of mortality, 3. Identifying features of taxa composition determined by the climate of the region.

Material and methods

IGNP is situated in south-western Romania, in the Carpathian Mountains. The road which is the focus of the study has a length of 154 km and runs parallel to the Danube River. Due to its length and the diversity of the neighbouring habitats, we chose 14 sections (S1 to S14) each of 500 m in length. The sections were distributed along almost the entire length of the gorge (S1-Orșova, S2-Eșelnița, S3-Cazanele Mici Gorge, S4-Ponicova, S5-Liubotina, S6-Șvinița, S7-Cozla, S8-Berzasca, S9-Liborajdea, S10-Gaura cu muscă Cave, S11-Moldova Nouă, S12-Măcești, S13-Radimna, S14-Divici). They are surrounded by different habitats with varying degrees of disturbance, distance to the Danube, etc. The road has two lanes, is asphalted and is in a good condition. Field trips were conducted monthly between March 2019 and February 2020. To investigate the 14 sections, two consecutive days were necessary each month; each section was surveyed once a month. The road sections studied were walked simultaneously by several people. The carcasses were identified *in situ*. Most of the vertebrates, especially herpetofauna, were identified to species level, while invertebrates and other badly damaged vertebrates were assigned to supra-species taxonomic levels, as in other studies (e.g. Ciolan et al. 2017; Popovici & Ile 2018). We counted the passing vehicles, expressed later as an average number of vehicles per hour for each section and period of time. Data was collected by month and section. For each data subset, we calculated the relative abundance of road-killed taxa (ratio of the number of road-killed animals belonging to a certain taxon to the total number of individuals) in the sections and periods studied. We thus calculated the relative abundance separately in each section studied for each taxon (with respect to the total number of individuals per section), and separately in each month studied for each taxon (with respect to the total number of individuals per month). Relative abundance is a measure often used in road-mortality studies (e.g., Cicort-Lucaciu et al. 2016; Ciolan et al. 2017; Covaciu-Marcov et al. 2017; Popovici & Ile 2018; Popovici et al. 2018). Taxa diversity was estimated using the Shannon index.

Results

In total, we found 13,230 road-killed animals on the seven kilometres of road studied in IGNP, of which approximately 16% were vertebrates. The number of victims differed by period and section (Table 1). The largest number of road-killed individuals was identified in October (2,708 road-killed animals), and the smallest in January (50 road-killed animals). The section on which the most victims were recorded was S9 (2,826 individuals) and the least S5 (285 individuals). The only instances in which we did not find any carcasses were on six sections (S2, S3, S5, S10, S11, S13) in January (Table 1). The average number of vehicles per hour across all sections over the year was 99.18. The greatest number of vehicles per hour was recorded in the summer and at the beginning of autumn, and

Table 2 – Number of road-killed taxa and taxa diversity (Shannon index – H) in the periods and sections studied. The sections were distributed along almost the entire length of the gorge (S1-Orşova, S2-Eşelniţa, S3-Cazanele Mici Gorge, S4-Ponicova, S5-Liubotina, S6-Şviniţa, S7-Cozla, S8-Berzasca, S9-Liborajdea, S10-Gaura cu muscă Cave, S11-Moldova Nouă, S12-Măceşti, S13-Radimna, S14-Divici).

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	Total	H
March	5	9	4	7	4	7	5	6	8	4	2	9	3	7	**23**	**2.37**
April	9	6	7	5	1	7	8	9	10	5	5	7	7	10	**25**	**2.15**
May	20	19	20	23	14	16	19	13	22	16	12	20	16	15	**45**	**2.66**
June	27	22	18	22	17	16	17	19	15	17	9	27	10	13	**50**	**2.53**
July	19	18	16	16	15	11	11	13	13	11	14	14	12	14	**40**	**2.46**
August	16	17	10	13	11	7	16	12	14	10	9	15	13	14	**43**	**2.05**
September	12	8	10	7	10	8	7	8	15	7	15	17	12	9	**35**	**2.19**
October	20	12	15	14	13	17	21	20	17	5	16	14	20	23	**43**	**2.38**
November	10	5	5	9	7	18	19	18	22	11	14	18	15	18	**42**	**2.3**
December	17	13	14	3	2	7	8	6	13	4	5	11	8	7	**34**	**2.81**
January	2	-	-	1	-	1	4	1	5	-	-	1	-	5	**16**	**2.1**
February	3	1	1	1	1	5	6	3	5	1	1	5	4	5	**12**	**1.47**
Total	**47**	**41**	**37**	**47**	**32**	**32**	**44**	**36**	**48**	**28**	**37**	**45**	**36**	**36**	**71**	**2.78**
H	**2.49**	**3.07**	**2.87**	**3.27**	**2.85**	**2.42**	**2.47**	**2.31**	**2.18**	**2.47**	**2.68**	**2.46**	**2.20**	**2.48**		

on the sections situated near the two towns in IGNP, Orșova and Moldova Nouă (Table 1).

The road-killed animals belong to 71 taxa. The actual number of taxa represented could in fact be considered to be higher: where we found just a few individuals from a particular taxon, we grouped these together under other taxonomic umbrellas. For example, under Chilopoda we included several undetermined individuals along with Geophilidae, *Scutigera* and *Scolopendra*, even though these are distinct in terms of their ecology and zoogeography. Orthoptera includes both Ensifera (Gryllidae and Tettigoniidae) and Caeliferae (*Acrida*). In *Coleoptera others*, besides undetermined individuals, we included taxa with fewer than 10 carcasses (Chrysomelidae, Cantharidae, Elateridae, Buprestidae, Tenebrionidae, Silphidae, Lampyridae, Dytiscidae). We did not group taxa in the case of amphibians and reptiles, because we usually identified them to species level and they have conservation value. The number of taxa varied by period and section (Table 2). By period, the largest number of taxa was killed in June (50 taxa) and May (45 taxa), and the smallest in February (12 taxa). By section, the largest number of taxa was recorded on S9 (48 taxa), S1 (47 taxa) and S4 (47 taxa), and the smallest on S10 (28 taxa). Only five taxa were killed in all 12 months (Oligochaeta Lumbricidae, Gastropoda with shell, Gastropoda Limacidae, Diplopoda and Lepidoptera adults). The diversity varied by period and section (Table 2). By period, the highest taxa diversity among road-killed animals was observed in December (H = 2.81), and the lowest in February (H = 1.47). By section, the highest diversity was recorded on S4 (H = 3.27), and the lowest on S9 (H = 2.18). The total diversity was H = 2.78.

Most of the road-killed individuals belonged to Gastropoda with shell (around 21%), followed by Orthoptera (around 16%) and Oligochaeta Lumbricidae (approximately 9%). The relative abundance varied seasonally (Table 3). Earthworms had high relative abundance in the early summer (in June with a relative abundance of almost 30%) and winter (in February with a relative abundance of almost 50%). Different taxa demonstrated high relative abundances at different times of the year: Orthoptera had high abundance in summer and autumn (representing at its maximum around 30% of the total individuals killed per month), *Bufo bufo* in spring (around 12% to 14%), *Pelophylax ridibundus* in summer (around 25%), *Rana dalmatina* in February and early spring (approximately 26% and 8%). The relative abundance of carcasses on the road through IGNP also varied by section (Table 4). Generally, invertebrates were predominant (Orthoptera with a relative abundance of 41% on S1 and Gastropoda (with shell) with a relative abundance of 38% on S12). However, a high relative abundance of a species of vertebrate was identified in two sections: an amphibian species, *P. ridibundus*, represented more than 30% of the total individuals recorded on S11 and a reptile species, *Natrix tessellata*, made up around 10% of the total victims recorded on S10.

Discussion

The road along the Danube causes the death of numerous animals. However, the number of road-killed animals is probably underestimated due to the relatively low carcass persistence of road-killed invertebrates (Skórka 2016) and small vertebrates (e.g. Santos et al. 2011; Teixeira et al. 2013; Silveira et al. 2018; Cabrera-Casas et al. 2020). On the sections of the road studied in IGNP, mostly invertebrates and, among vertebrates, mostly frogs (including many juveniles) were killed. Victims that adhered to vehicles could not be taken into account, as in other studies (McKenna et al. 2001; Baxter-Gilbert et al. 2015). Underestimation is also related to biodiversity of the surrounding area (e.g. Paşcovschi 1956; Rozylowicz et al. 2019): where the number of potential scaven-

Table 3 – Relative abundance (%) of taxa identified in each month of the year (I-XII – January to December); total number of individuals (n) and relative abundance (%) of each taxon.

Taxa	III	IV	V	VI	VII	VIII	IX	X	XI	XII	I	II	Total	
	%												n	%
Oligochaeta Lumbricidae	7.55	19.14	14.54	29.50	9.50	2.09	0.63	0.22	0.26	13.97	4.00	48.39	**1,176**	**8.889**
Hirudinea	-	-	-	-	-	-	-	-	0.04	-	-	-	**1**	**0.008**
Gastropoda (with shell)	2.26	10.22	30.16	16.61	28.60	29.46	7.64	27.03	17.77	4.24	2.00	0.92	**2,756**	**20.831**
Gastropoda Limax	1.89	28.62	7.36	4.92	5.61	2.09	4.29	9.79	14.11	19.20	4.00	13.36	**1,167**	**8.821**
Araneidae	2.26	-	1.62	0.57	0.27	0.12	0.21	1.14	1.23	7.23	-	-	**130**	**0.983**
Opilionidae	-	-	0.63	0.05	-	-	-	-	0.18	-	-	-	**12**	**0.091**
Scorpionidae	-	-	-	-	0.18	0.06	0.10	0.07	-	0.50	-	-	**8**	**0.060**
Chilopoda	1.51	0.74	0.27	0.83	0.36	0.30	0.84	0.74	2.07	4.99	2.00	-	**132**	**0.998**
Diplopoda	31.32	0.37	1.44	0.31	0.45	0.12	-	13.11	21.21	4.99	2.00	1.84	**975**	**7.370**
Isopoda Oniscidea	-	-	0.09	0.10	-	-	-	0.04	0.22	0.50	-	-	**11**	**0.083**
Odonata	-	-	-	0.31	0.18	0.24	0.42	0.07	0.22	-	-	-	**23**	**0.174**
Plecoptera	-	-	-	-	-	-	0.10	-	-	-	-	-	**1**	**0.008**
Blattodea	-	-	0.18	0.31	-	-	-	0.07	-	-	-	-	**10**	**0.076**
Orthoptera	1.13	-	0.72	10.56	21.90	22.12	31.83	15.88	21.69	8.73	2.00	-	**2,090**	**15.797**
Dermaptera	-	-	-	-	-	0.06	0.10	0.66	0.62	2.24	-	-	**43**	**0.325**
Mantodea	-	-	-	-	-	-	0.10	0.07	0.04	-	-	-	**4**	**0.030**
Coleoptera Carabidae	1.13	0.56	2.69	0.31	0.45	0.18	0.10	0.18	0.75	0.50	-	-	**75**	**0.567**
Coleoptera Scarabeidae	-	0.37	1.53	0.52	1.72	-	0.21	0.15	0.09	0.50	-	-	**58**	**0.438**
Coleoptera Lucanidae	-	-	0.99	0.26	1.00	0.18	0.31	-	-	-	-	-	**33**	**0.249**
Coleoptera Coccinellidae	0.38	-	0.63	0.47	0.18	0.06	-	3.62	5.25	2.99	18.00	-	**258**	**1.950**
Coleoptera Staphylinidae	-	-	0.36	0.10	-	-	-	0.04	0.22	1.00	-	-	**16**	**0.121**
Coleoptera Cerambycidae	-	0.19	0.90	-	0.45	-	-	-	0.04	-	-	-	**17**	**0.128**
Coleoptera Curculionidae	-	-	0.18	0.05	-	-	-	0.07	0.04	1.75	-	-	**13**	**0.098**
Coleoptera Meloidae	3.02	0.19	-	-	-	-	-	-	-	0.25	-	0.46	**11**	**0.083**
Coleoptera others	1.89	-	0.81	0.72	0.36	0.24	0.10	0.59	1.01	4.74	-	-	**95**	**0.718**
Panorpata	-	-	0.72	0.10	-	0.06	-	-	-	-	-	-	**11**	**0.083**
Trichoptera	-	-	-	0.05	-	-	-	0.04	0.13	-	-	-	**5**	**0.038**
Lepidoptera adults	0.38	0.93	1.44	8.95	5.79	2.15	3.77	0.37	0.53	4.74	2.00	0.92	**375**	**2.834**
Lepidoptera larvae	6.04	-	7.45	2.48	1.45	1.85	1.88	0.85	2.03	6.73	-	2.30	**313**	**2.366**
Diptera Brachycera	0.38	-	3.59	2.38	3.17	1.91	1.26	0.89	0.84	0.75	2.00	-	**213**	**1.610**
Diptera Brachycera Tabanidae	-	-	-	1.29	2.62	1.31	-	-	-	-	-	-	**76**	**0.574**
Diptera Brachycera larvae	-	-	-	-	-	-	-	-	-	-	-	1.84	**4**	**0.030**
Diptera Nematocera	-	-	-	0.16	-	0.06	-	-	-	-	-	-	**4**	**0.030**
Hymenoptera Apidae	5.28	1.86	7.54	1.71	1.99	1.43	0.84	0.48	0.26	0.50	40.00	-	**236**	**1.784**
Hymenoptera Bombus	0.38	-	0.90	0.16	0.09	0.06	-	-	-	-	-	-	**16**	**0.121**
Hymenoptera Vespidae	-	-	1.89	1.29	2.35	2.21	5.86	4.25	1.46	0.25	-	-	**314**	**2.373**
Hymenoptera Formicidae	-	-	0.36	0.93	0.27	0.06	0.10	0.07	0.75	-	-	-	**46**	**0.348**
Hymenoptera others	-	-	1.26	0.36	0.54	0.60	0.31	0.07	-	0.50	-	-	**44**	**0.333**
Homoptera Cicadidae	-	-	0.27	0.16	-	-	-	0.22	0.13	-	-	-	**15**	**0.113**
Heteroptera Pyrrhocoris	9.81	-	1.08	0.36	1.36	0.78	0.73	0.85	0.04	1.00	-	-	**108**	**0.816**
Heteroptera others	-	-	-	0.21	0.45	0.30	1.05	7.68	1.41	1.00	2.00	-	**269**	**2.033**
Salamandra salamandra	0.38	0.56	0.09	-	-	-	-	-	-	-	2.00	-	**6**	**0.045**
Lissotriton vulgaris	-	0.37	0.09	-	-	-	-	-	-	0.25	-	-	**4**	**0.030**
Triturus cristatus	-	-	-	-	-	-	-	-	0.04	-	-	-	**1**	**0.008**
Bombina variegata	-	-	-	-	-	-	-	-	0.04	-	-	-	**1**	**0.008**
Bufotes viridis	-	-	-	-	-	0.06	-	-	-	-	-	-	**1**	**0.008**
Bufo bufo	12.45	14.50	1.71	0.31	0.09	0.06	-	0.04	0.26	0.25	2.00	1.84	**151**	**1.141**
Pelobates fuscus	-	0.19	-	-	-	-	-	-	-	-	-	-	**1**	**0.008**
Hyla arborea	-	0.19	-	-	-	-	-	-	-	-	-	-	**1**	**0.008**
Pelophylax ridibundus	-	9.11	1.26	4.40	2.62	25.52	26.91	6.91	2.29	2.00	6.00	-	**1112**	**8.405**
Rana dalmatina	8.68	6.32	0.09	0.72	0.18	-	0.31	0.26	2.03	0.50	-	26.27	**189**	**1.429**
Rana temporaria	-	-	-	-	0.09	-	-	-	-	-	-	-	**1**	**0.008**
***Anura* undetermined**	-	-	0.27	0.05	0.09	0.06	-	0.30	-	-	4.00	-	**16**	**0.121**
Emys orbicularis	-	-	0.18	0.05	-	0.06	-	-	-	0.25	-	-	**5**	**0.038**
Ablepharus kitaibelii	-	-	-	0.10	-	0.06	-	0.11	-	-	-	-	**6**	**0.045**
Podarcis muralis	0.75	0.93	0.45	0.88	0.54	0.54	1.57	0.37	0.18	-	-	-	**73**	**0.552**
Lacerta viridis	-	0.74	0.99	3.11	2.90	2.50	4.61	1.37	0.04	-	-	-	**231**	**1.746**
Anguis fragilis	-	-	0.18	0.05	-	-	-	-	-	0.25	-	-	**4**	**0.030**
Natrix natrix	-	1.30	0.45	0.62	0.54	0.18	0.21	0.15	0.04	-	-	-	**40**	**0.302**
Natrix tessellata	0.38	2.04	2.33	2.07	0.54	0.30	2.41	0.89	0.26	1.75	-	0.46	**150**	**1.134**
Zamenis longissimus	-	0.19	0.09	-	0.18	-	-	-	-	-	-	-	**4**	**0.030**
Dolichophis caspius	-	-	0.09	0.21	0.09	-	-	0.04	-	-	-	-	**7**	**0.053**
Coronella austriaca	-	-	-	0.10	-	0.06	0.21	0.07	-	-	-	-	**7**	**0.053**
Vipera ammodytes	-	-	0.09	0.05	-	-	0.10	-	-	-	-	-	**3**	**0.023**
Reptilia undetermined	-	-	-	0.05	0.18	0.12	-	-	-	-	-	-	**5**	**0.038**

Taxa	III	IV	V	VI	VII	VIII	IX	X	XI	XII	I	II	Total	
	%												n	%
Aves	-	0.19	0.09	-	0.54	0.24	0.42	0.15	0.04	0.50	6.0	1.38	**29**	**0.219**
Mammalia Rodentia	-	0.19	-	0.05	0.09	0.06	0.10	-	-	0.50	-	-	**7**	**0.053**
Mammalia others	-	-	-	-	-	-	0.31	-	0.04	-	-	-	**4**	**0.030**
Mammalia Vulpes	0.75	-	-	-	-	0.06	-	0.04	0.04	-	-	-	**5**	**0.038**
Mamalia Erinaceus	-	-	-	0.05	-	-	-	-	-	-	-	-	**1**	**0.008**
Mamalia Chiroptera	-	-	-	-	-	0.06	-	-	-	-	-	-	**1**	**0.008**
Sum	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	**13,230**	**100%**

Table 4 – Relative abundance (%) of taxa identified in the 14 sections studied (S1–S14). The sections were distributed along almost the entire length of the gorge (S1-Orşova, S2-Eşelniţa, S3-Cazanele Mici Gorge, S4-Ponicova, S5-Liubotina, S6-Şviniţa, S7-Cozla, S8-Berzasca, S9-Liborajdea, S10-Gaura cu muscă Cave, S11-Moldova Nouă, S12-Măceşti, S13-Radimna, S14-Divici).

Taxa	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14
	%													
Oligochaeta Lumbricidae	3.15	4.36	10.80	8.04	9.47	12.32	12.90	10.98	15.96	10.80	1.72	6.34	1.48	2.48
Hirudinea	-	-	-	0.32	-	-	-	-	-	-	-	-	-	-
Gastropoda (with shell)	7.98	7.58	8.51	5.14	3.16	8.07	24.59	15.66	27.81	6.65	3.93	38.54	33.45	21.83
Gastropoda Limax	4.17	11.93	5.52	1.93	1.40	20.54	13.93	9.08	10.69	2.49	5.16	7.48	7.34	5.56
Araneidae	3.06	2.27	1.38	2.25	0.70	1.42	1.46	1.56	0.32	-	0.74	0.57	-	0.10
Opilionidae	-	-	-	2.57	-	-	0.09	0.20	-	-	-	-	-	-
Scorpionidae	-	-	0.92	0.64	-	-	0.17	-	-	-	-	-	-	-
Chilopoda	0.83	0.76	0.92	1.61	1.75	1.70	3.27	0.34	0.57	1.66	0.00	0.24	0.92	1.19
Diplopoda	1.02	1.14	4.60	6.43	5.96	5.81	5.76	30.51	1.63	9.14	1.23	2.44	14.18	2.78
Isopoda Oniscidea	0.28	0.19	-	0.32	-	-	0.09	0.27	0.04	-	-	-	-	-
Odonata	-	-	0.23	-	-	-	-	0.14	-	0.28	1.72	0.57	0.07	0.40
Plecoptera	-	0.19	-	-	-	-	-	-	-	-	-	-	-	-
Blattodea	0.56	-	0.23	0.32	-	-	-	0.14	-	-	-	-	-	-
Orthoptera	41.00	16.48	15.86	16.08	21.40	27.05	17.28	11.25	8.28	5.82	12.04	8.86	9.32	27.58
Dermaptera	0.28	0.95	-	0.64	-	-	0.34	0.20	0.32	-	1.23	-	0.35	0.69
Mantodea	-	-	-	-	-	-	0.09	-	0.04	0.28	0.25	-	-	-
Coleoptera Carabidae	0.46	0.76	0.92	4.82	1.40	1.13	0.26	0.68	0.07	0.83	0.25	0.65	0.21	0.50
Coleoptera Scarabeidae	0.56	0.95	1.61	1.61	1.75	0.28	0.17	-	0.25	1.39	0.98	0.49	-	0.40
Coleoptera Lucanidae	0.37	0.76	0.23	3.54	2.81	0.14	-	-	-	0.55	-	-	0.07	0.10
Coleoptera Coccinellidae	0.56	3.79	1.15	0.64	-	0.28	0.43	0.41	4.25	-	0.98	0.24	0.35	7.94
Coleoptera Staphylinidae	0.09	0.19	0.23	0.32	-	-	0.09	0.07	0.07	0.28	0.25	0.41	0.07	-
Coleoptera Cerambycidae	0.09	0.38	0.23	1.29	0.70	0.42	0.17	0.07	0.04	-	-	-	-	-
Coleoptera Curculionidae	0.09	1.52	-	-	-	0.14	-	0.07	-	-	0.25	-	0.07	-
Coleoptera Meloidae	-	-	-	0.64	-	0.14	-	0.07	0.11	-	-	0.24	-	0.10
Coleoptera others	1.86	3.41	0.46	3.22	0.70	0.85	0.43	0.07	0.11	1.66	2.46	0.41	0.07	0.60
Panorpata	0.19	-	-	1.29	0.35	-	0.09	-	-	-	-	0.08	0.07	0.10
Trichoptera	-	-	-	-	-	0.28	0.09	-	0.04	-	-	0.08	-	-
Lepidoptera adults	1.48	1.52	2.53	3.22	11.93	3.40	1.38	2.85	1.38	29.09	6.39	2.03	0.64	0.99
Lepidoptera larvae	3.15	3.60	12.87	3.54	3.51	1.42	1.20	3.39	1.27	0.83	1.72	2.03	1.48	1.69
Diptera Brachycera	1.76	4.36	1.61	4.50	6.32	1.56	0.17	1.22	0.18	2.22	6.14	2.76	1.27	1.09
Diptera Brachycera Tabanidae	2.13	0.95	1.15	0.96	1.40	0.42	0.60	0.14	0.04	-	0.98	1.22	0.07	0.30
Diptera Brachycera larvae	-	-	-	0.32	-	-	0.09	-	0.04	-	-	0.08	-	-
Diptera Nematocera	0.09	-	-	-	-	-	-	-	-	0.28	-	0.08	0.07	-
Hymenoptera Apidae	3.62	2.46	0.69	0.96	1.75	0.42	0.43	0.81	0.07	1.39	3.44	3.82	2.68	4.66
Hymenoptera Bombus	0.09	1.70	0.23	0.64	-	-	-	-	0.04	-	-	0.08	-	0.10
Hymenoptera Vespidae	3.06	5.87	2.53	3.54	3.51	2.12	0.95	3.53	0.78	1.94	5.16	2.76	2.19	2.48
Hymenoptera Formicidae	0.19	0.95	-	5.79	0.35	-	0.69	0.47	0.04	-	0.25	0.24	-	-
Hymenoptera others	0.09	0.57	0.23	2.25	1.40	0.14	-	0.14	0.04	0.28	0.98	1.14	0.28	0.10
Homoptera Cicadidae	0.28	-	0.23	0.32	-	0.42	-	-	0.04	-	-	0.08	0.21	0.20
Heteroptera Pyrrhocoris	0.83	3.79	6.21	0.96	5.96	0.14	0.86	0.07	0.14	-	-	0.08	0.28	1.09
Heteroptera others	5.84	3.98	2.53	1.61	3.86	1.98	1.29	0.47	0.35	0.28	0.49	1.14	2.82	5.46
Salamandra salamandra	-	0.19	-	0.64	-	-	-	-	-	0.83	-	-	-	-
Lissotriton vulgaris	0.19	-	-	-	-	-	-	-	0.04	-	-	0.08	-	-
Triturus cristatus	0.09	-	-	-	-	-	-	-	-	-	-	-	-	-
Bombina variegata	0.09	-	-	-	-	-	-	-	-	-	-	-	-	-
Bufotes viridis	-	-	-	-	-	-	-	-	-	-	0.25	-	-	-
Bufo bufo	1.02	-	0.46	0.64	1.05	-	0.69	0.68	0.04	-	0.25	7.07	0.28	2.18
Pelobates fuscus	-	-	-	-	-	-	-	-	-	-	0.25	-	-	-
Hyla arborea	-	0.19	-	-	-	-	-	-	-	-	-	-	-	-
Pelophylax ridibundus	-	1.14	0.23	0.32	1.75	1.84	4.82	2.24	20.03	8.03	30.96	0.24	16.58	3.77
Rana dalmatina	5.66	1.33	-	2.25	-	-	0.17	-	1.52	-	2.95	3.33	0.99	0.20
Rana temporaria	-	-	-	0.32	-	-	-	-	-	-	-	-	-	-
Anura undetermined	0.83	-	-	-	0.35	-	0.26	-	-	-	0.74	-	-	-

Taxa	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14
	%													
Emys orbicularis	-	-	-	-	-	-	0.09	-	-	-	-	0.24	-	0.10
Ablepharus kitaibelii	-	-	-	0.32	0.35	0.28	-	-	0.07	-	-	-	-	-
Podarcis muralis	0.19	0.95	6.90	0.64	0.35	2.27	1.03	-	0.04	-	-	0.24	0.07	-
Lacerta viridis	1.86	6.63	3.45	1.29	2.11	1.56	1.12	1.22	1.70	0.55	2.46	1.54	0.99	1.59
Anguis fragilis	-	-	-	0.32	-	-	0.17	-	0.04	-	-	-	-	-
Natrix natrix	0.09	0.38	-	-	-	0.14	0.26	0.07	0.25	1.11	1.47	0.24	0.49	0.50
Natrix tessellata	0.19	1.14	3.22	0.32	1.05	1.27	1.20	0.54	0.96	10.80	0.25	0.98	0.28	0.99
Zamenis longissimus	0.09	0.19	-	-	-	-	-	-	0.07	-	-	-	-	-
Dolichophis caspius	-	-	-	-	0.35	-	-	-	-	-	0.98	0.08	-	0.10
Coronella austriaca	-	-	0.23	0.32	-	-	-	-	0.11	0.28	-	-	0.07	-
Vipera ammodytes	-	-	0.23	-	-	-	-	-	-	0.28	0.25	-	-	-
Reptilia undetermined	0.09	-	-	-	-	-	0.26	0.07	-	-	-	-	-	-
Aves	0.19	0.19	0.46	0.32	1.05	-	0.43	0.34	0.07	-	0.25	0.33	0.14	0.10
Mammalia Rodentia	0.09	0.19	-	-	-	-	0.09	-	0.04	-	0.25	0.08	0.07	-
Mammalia others	0.09	0.19	-	-	-	-	-	-	0.04	-	-	0.08	-	-
Mammalia Vulpes	-	-	0.23	-	-	-	0.09	-	0.04	-	-	0.16	-	-
Mamalia Erinaceus	-	-	-	-	-	-	-	-	-	-	-	0.08	-	-
Mamalia Chiroptera	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-
Sum	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%

gers is high, as observed in studies of other areas (Silveira et al. 2018), carcasses are removed quickly (e.g. Antworth et al. 2005; Schwartz et al. 2018). Furthermore, a warm and humid climate accelerates the decomposition of animal carcasses (Santos et al. 2011). A sample taken at any given moment on a section of this road is therefore likely to be reasonably representative of the actual number of animals killed on this section for a much shorter period than the 24 hours suggested by other studies (Santos et al. 2011; Popovici & Ile 2018).

The huge number of road-killed animals raises a difficult dilemma for IGNP. On one hand, it indicates that mitigation measures should be taken, but on the other hand tourism is one of the most important activities for local human communities (e.g. Ökrös et al. 2014; Brad et al. 2018). Tourism (and the number of vehicles) increases in summer, coinciding with the animals' main period of activity. Increased road mortality caused by an influx of tourists has been observed in other studies (e.g. Seshadri & Ganesh 2011; Jeganathan et al. 2018). Curbing modernization of other roads in IGNP might be the most effective solution and would not affect the equilibrium between the local community and the park. This would at least mean that undisturbed areas could be maintained, as road modernization in forest areas can lead to negative impacts on biodiversity (Ciolan et al. 2017). The high biodiversity of habitats in the vicinity of the road, the road's significant length and its variety of uses across space and time make it difficult to implement any blanket mitigation measures.

We found some road-killed animals in the winter that should not have been active in the cold season. Even if the number of road-killed individuals and taxa was relatively low in the winter, it was larger in December than in March. This is a consequence of a mid-December period which was warmer than March. We even recorded carcasses in January, when night temperatures and sometimes even daytime temperatures were below zero. Moreover, the carcasses belonged predominantly to cold-blooded groups, such as invertebrates, amphibians and reptiles, rather than warm-blooded animals (birds and mammals). Although amphibians and reptiles hibernate in the winter in Romania (e.g. Fuhn 1960; Fuhn & Vancea 1961), amphibians were killed by vehicles in all three winter months and reptiles in December and February. In February, on a rainy day with temperatures of between 7 °C and 8 °C, we identified 57 carcasses of *Rana dalmatina*. In January, a *Salamandra salamandra* was killed; in February, although not on the sections studied, we found another road-killed individual and in December a live one. Thus, in the Danube Gorge in the winter of 2019 / 2020, these animals did not hibernate continuously, instead alternating between dormant and active every few days, exhibiting an activity pattern characteristic of the species in areas further south (Rebelo & Leclair 2003). Dice snakes were killed in December and February in areas with limestone rocks. While *S. salamandra* is a species common in hilly and mountain areas (Fuhn 1960) and which withstands cold conditions (Catenazzi 2016), *Natrix tessellata* is rare even in north-eastern Romania (Cogălniceanu et al. 2013), and yet it was active during two winter months. In addition to herpetofauna, invertebrates such as *Euscorpius carpathicus* and *Scolopendra* were also active in the winter. Winter activity has been observed over recent years in Europe, both in the case of reptiles (Zuffi et al. 1999) and amphibians (Jablonski 2013; Kaczmarek et al. 2018), but this has been in the Mediterranean region or in urban habitats.

The road-mortality data gathered in IGNP goes beyond demonstrating changes in the ecology of some species. Our results are of faunistic and conservation importance for IGNP, at least in terms of the identification of certain road-killed amphibians which have not been observed in the area for approximately 50 years. These species are *Lissotriton vulgaris* and *Triturus cristatus*. In the Danube Gorge, newts had been assumed to be absent due to the steep slopes (Covaciu-Marcov et al. 2009). The two newt species had not been reported in the area since at least as far back as 1971 (Stănescu et al. 2015). In IGNP, *T. cristatus* had been observed only in Orșova, recorded as a specimen in the local high school's collection (Fuhn 1975). After that date, it was recorded in the Locvei Mountains (Covaciu-Marcov et al. 2005), but this was outside IGNP. Identifying a road-killed *T. cristatus* in November was therefore unexpected, even if it was discovered near the previously mentioned locality of Orșova (Fuhn 1975). This shows that habitats favourable to newts still exist near Orșova. Furthermore, the presence of *L. vulgaris* in three sections, including those close to the Danube, show that the region should be searched intensively for newts, which are protected species (O.U.G. 57/2007), thus important for IGNP. Without it being our goal, the road-mortality study therefore led to new records of newts in IGNP. *Ablepharus kitaibelii* was recorded on S9 at Liborajdea, approximately 50 km west of its westernmost known location in Romania as indicated by previous distribution records (Cogălniceanu et al. 2013). Our record therefore became its current westernmost distribution locality in the country, proving its continuous distribution in the region. *Ablepharus kitaibelii* was killed in summer and early autumn on sections surrounded by rocky and forested habitats.

The peculiarities of the Danube Gorge in terms of the presence of certain mountain animals at low altitudes (e.g. Pașcovschi 1956; Covaciu-Marcov et al. 2009; Teodor et al. 2019) were also noticeable. *Salamandra salamandra*, *Bombina variegata* and *Rana temporaria* were killed even below an altitude of 100 m, which is lower than the altitude at which they usually occur in Romania (Fuhn 1960). Some road-killed species are protected (O.U.G. 57/2007), including almost all herpetofauna species, certain invertebrates (*Lucanus cervus*), etc. Others, such as *E. carpathicus*, are endemic to Romania (Fet & Soleglad 2002). Anthropically favoured species, such as bees, were also killed on sections surrounded by beehives, as was the case in other studies (Cicort-Lucaciu et al. 2016). Bees were killed even in January: despite temperatures falling below zero at night, the sun warmed the hives enough during the day to activate some bees.

The usual two-peak pattern (spring and autumn) of road-mortality rates (e.g. Gryz & Krauze 2008; Ciolan et al. 2017; Garriga et al. 2017) was not observed in IGNP. Fluctuations in the number of individuals and taxa killed had different periodicity. The number of individuals identified reached a maximum in the autumn (October and November), while the maximum number of taxa was recorded in June. A second, less pronounced peak was observed in the number of individuals in June and in the taxa richness in October and November. Both the number of individuals and the number of taxa killed increased between the cold and the warm season. However, unusually the number of road-killed individuals did not exhibit a spring peak, which is normally the most significant (e.g. Orłowski 2007; Orłowski et al. 2008; Ciolan et al. 2017). In IGNP, the autumn peak was driven by the deaths of snails and orthopterans, which are affected in different ways by the same local meteorological conditions. As snails prefer humidity, they were activated by the warm autumn rains, and because they move slowly and cover only short distances, they were killed in large numbers. Increased road mortality of snails following rainy days has also been observed in other areas (Jeganathan et al. 2018). Orthopterans, for their part, like heat; they are commonly associated with steppe areas (Radu & Radu 1967) and are particularly prone to traffic deaths in summer (Ciolan et al. 2017). The grasslands and high temperatures in IGNP helped keep them active in the autumn and there were even road-killed orthopterans recorded in December. Atypical mortality peaks were observed in some other taxa too. Among amphibians, the usual spring peak (e.g. Orłowski 2007; Orłowski et al. 2008; Ciolan et al. 2017) shifted to summer, when many *P. ridibundus* juveniles were killed. This is a result of the road's vicinity to the Danube.

In addition to highlighting the huge number of road-kill victims in IGNP, the results demonstrate the necessity for road-mortality monitoring, at least in this area, even in the cold season. The large number of victims recorded, along with the negative impact on wildlife, shoud provide motivation for new studies. The road through IGNP may be a useful focus for other studies which examine the carcasses of road-killed animals. Such uses of road-killed vertebrates' carcasses have been mentioned recently in studies concerning feeding ecology, parasites, morphology and age structure (e.g. McAllister et al. 2016; Kolenda et al. 2019a,b; Vafae Eslahi et al. 2017; Ile et al. 2020; Maier et al. 2020). The road could also be used as a source of samples in the case of invertebrates, including rare species, such as *E. carpathicus*. Another conclusion that can be drawn from the study is that the mild winter exposed animals to road mortality in a period in which they should not have been exposed. In the case of salamanders, the findings of this study seem to support the suggestion that global warming will affect their activity period (Catenazzi 2016). This highlights another, hitherto ignored, negative facet of climate change, namely the increased exposure of animals to road mortality. The extension of their activity period due to this warming will expose more animals to traffic over a longer period. We cannot predict the exact effect of these milder winters on this fauna, which has already been impacted by road mortality. The points discussed in this study demonstrate the important part played by road-mortality studies in various contexts and the many conclusions that can be drawn in relation to human-nature interaction.

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