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Can wildlife mortality on a local road tell something general? An answer from a protected area in south-western Romania

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Abstract: Between June 2020 and May 2021, we studied the fauna road mortality on a recently asphalted local road in a protected area in southwestern Romania (Iron Gates Natural Park–IGNP). Despite our expectation of reduced road mortality because of the very low traffic (3.97 cars/hour), numerous animals belonging to a high number of taxa were killed. Animals were killed throughout the entire year, even amphibians in the winter months, which normally should hibernate in that period. Road mortality differences between periods and sectors were obvious. Plant assemblages near the road influence the composition and intensity of road mortality. Diverse habitats near the road have diverse plant assemblages and richer fauna, which are more affected by the road. The highest road mortality intensity was registered in areas with diverse plant assemblages. The possibilities of diverse mitigation measures are also discussed, all implying the decrease of the traffic intensity in the touristic season, which overlaps the road mortality hot time.

Key words: Traffic intensity, habitat peculiarities, plant assemblages, seasonal differences, mitigation measures

1. Introduction

Road mortality is a form of human impact responsible for the direct elimination of a huge number of animals from ecosystems (e.g., Ashley and Robinson, 1996; Seshadri and Ganesh, 2011; Baxter–Gilbert et al., 2015; Garrah et al., 2015; Covaciu–Marcov et al., 2022). Habitats near the roads and plant assemblages have a great influence on road mortality, determining the casualties' composition and frequency (e.g., Glista et al., 2007; Yamada et al., 2010; Matos et al., 2012; Popovici et al., 2018; Lala et al., 2021; Rendall et al., 2021). Roads affect the nearby plant assemblages, a fact that modifies the road mortality intensity of animals related with certain plant species, like birds (Orłowski, 2008), butterflies (e.g., Yamada et al., 2010; Skórka et al., 2013, 2015; Gaudel et al., 2020), or weevils (Teodor et al., 2019). Other habitat characteristics also influence road mortality; for example, wet areas increase road mortality intensity in animals like amphibians (e.g., Mitchell, 2000; D'Amico et al., 2015; Arévalo et al., 2017; Jeganathan et al., 2018; Healey et al., 2020). Besides, the characteristics of roads influence the fauna road mortality, as on roads in poor condition road–killed vertebrates are missing (Miranda et al., 2017). The speed and traffic intensity modify road mortality intensity, as their increase leads to an increase of road–killed animals (e.g., Seshadri and Ganesh, 2011; Jones et al., 2014; Skórka et al., 2015; Tejera et al., 2018; Balčiauskas et al., 2020; Lyamuya et al., 2021; Rendall et al., 2021).

Based on the above–mentioned, we hypothesized that roadside plant assemblages could indicate the extent of road mortality. Roadsides have diverse effects on plant assemblages, both negative and positive, offering favorable conditions for some invasive but also some native species (see in: Lázaro–Lobo and Ervin, 2019). However, the positive effects of roadsides have generally been emphasized in disturbed areas, and the negative effects in more natural zones (see in: Lázaro–Lobo and Ervin 2019). To verify the relation between roadside plant assemblages and road mortality intensity, we chose a recently asphalted local road with reduced traffic in a high-biodiversity area, in the Iron–Gates Natural Park (IGNP), in which previous information regarding road mortality is also available (Covaciu–Marcov et al., 2022). Thus, our hypothesis was that habitat types and the diversity of plant assemblages neighboring the road will influence fauna road mortality. To verify this hypothesis, we set the following objectives: 1. to determine road–killed animals on a recently modernized local road in IGNP, 2. to establish the differences caused by

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periods and neighboring habitats, 3. to analyze the relation between plant assemblages near the road and fauna road mortality, 4. to suggest some possible mitigation measures.

2. Materials and methods

2.1. Studied road

The studied road is situated in the central part of IGNP, in Caraş-Severin County, south-western Romania (between 44º37'12"N/22º00'51"E and 44º39'31"N/22º06'21"E) The road is perpendicular to the Danube and the main road. It heads north, to the village Bigăr (Figure 1), which is the most isolated locality in the Banat region of Romania from a road perspective (Rusu et al., 2013). The road mostly follows the Sirinia valley; it is situated a few meters or tens of m from the valley. The road was asphalted in 2017; thus, it is in a good condition. Because the valley is very narrow, the road is also narrow, with a single lane (Figure 2). Because of the narrow curves and steep slopes, the cars' speed is reduced to only 30–50 km/h. The road crosses compact, mostly beech and hornbeam forests, but in the lower parts, there are also oaks. In the lower areas, there are sectors with steep rocky slopes, covered with natural meadows and shrubs. In the upper part, near Bigăr village there is an open area, with pastures. The road has 17 km. Although the surrounding habitats were generally uniform, the road was divided in six representative sectors, as it could not be walked entirely. Each sector had 1 km length.

2.2. Road sectors

Sector 1 was the most downstream one, located at approximately 200 m from the Danube (44º37'18"N/22º01'05"E). It is surrounded by steep slopes with rare oak and hornbeam forests, but also with rocky areas and meadows. The valley is at maximum 10 m. Sector 2 (44º37'40"N/22º01'57"E) is like the previous one; it is surrounded by beech, hornbeam, and oak forests, but also by a few rocky open areas and meadows. The valley is nearby. Sector 3 (44º38'54"N/22º03'46"E) is situated in a dense beech forest, with steep slopes and the valley near the road. The road is shaded, with mosses growing even on the asphalt. The area has a gorge aspect, even with a small waterfall. Sector 4 (44º38'26"N/22º04'54"E) has similar characteristics, with the valley near the road, but it is less steep, as it is surrounded by a semiopen area with an abandoned walnut plantation. Sector 5 (44º38'52"N/22º06'23"E) climbs a steep slope, while the valley is reduced to some small streams. The area is covered with old beech forests. Sector 6 (44º39'19"N/22º06'25"E), situated upstream, is the most different sector. It is an open area resulted from the deforestation of a forest which was formerly situated westward the locality at the time of its establishment (Salzmann, 1990); now it is used as a pasture. The forest is at tens or hundreds of meters from the road.

2.3. Study methods

The road mortality study lasted 12 months, between June 2020 and May 2021. In each of the 12 months, we made one field trip as in a previous study in the IGNP (Covaciu–Marcov et al., 2022). We made pedestrian surveys, as previously (Covaciu–Marcov et al., 2022). The study began in the morning, at 10 a.m., and lasted depending on the season (in spring–summer, it lasted more, as we found more casualties on the road; in the cold season, the survey lasted less, as we found fewer casualties). The road–killed animals were determined on the field, to the lowest taxonomic level possible, as in other cases (Healey et al., 2020). Therefore, we came to different taxonomic levels depending on the group; in vertebrates we generally

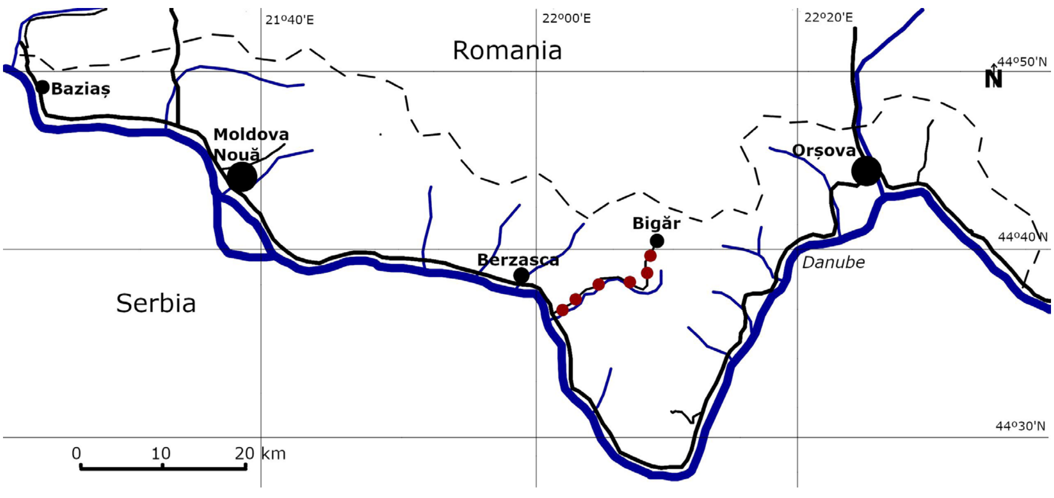

Figure 1. Study area (blue line–rivers, black line–roads, discontinuous line–Iron Gates Natural Park limits, black dots–localities, red dots–the six studied sectors on the road to Bigăr).

could identify the species (Figure 2), but in invertebrates we usually stopped at higher taxonomic levels, as in other cases (e.g., Cicort–Lucaciu et al., 2016; Popovici et al., 2018; Pop et al., 2020; Covaciu–Marcov et al., 2022). For the identification of road–killed animals, we used various resources (e.g., Radu & Radu 1967–for invertebrate taxa, Fuhn 1960 for amphibian species, Fuhn & Vancea 1961 for reptile species). We counted the passing cars, calculating the average number of cars/hour, as in other cases (e.g., Ciolan et al., 2017; Popovici et al., 2018; Covaciu–Marcov et al., 2022). In spring and autumn, we identified on the field the plant species neighboring the road sectors, on a width of 5 m on both sides of the road.

2.4. Statistical analysis

Road mortality data was processed both totally and separately depending on sectors and months. In each case, we calculated the percentage abundance of each taxon and the frequency with which they were killed in the studied sectors and months. The diversity was calculated with the Shannon index, and the similarity with the Jaccard index. The significance of differences between periods and sectors was calculated with the Kruskal–Wallis test, with the Mann–Whitney test as post–hoc test. The Pearson correlation was applied between different parameters (number of victims and number of taxa, number of victims and number of plant species, number of victims and number of invasive plant species, etc.). The statistical analysis was performed with the free Past v. 3.26 software (Hammer et al., 2001).

3. Results

The road to Bigăr has a low traffic. It is passed by an average of only 3.97 cars/hour/year. The number of cars/hour differed both between sectors and months (Tables 1, 2). The highest number of cars/hour was registered in September (6.58 cars/hour), and the lowest in February (1.37 cars/hour). We determined 247 plant species. The number of plant species differed between sectors, varying between 76 species and 120 species (Table 1). The highest number of plant species was identified in sectors 2 and 4 (120 species and 119 species), and the lowest in sectors 6 and 5 (76 species and 91 species).

Totally, on the road to Bigăr, we identified 2981 road-killed animals, belonging to 64 taxa. Even though this is a very high number, we must take into account the fact that we observed this number on a cumulated road

Figure 2. The studied road in Sector 2 (up left) and Sector 3 (up right), and two road–killed vertebrates identified on the road: *Salamandra salamandra* (down left) and *Talpa* sp. (down right).

Table 1. Road–killed taxa percentage abundance on sectors (S1–S6 = Sector 1–Sector 6). Total percentage abundance (P%) and frequency of occurrence (f%). Number of road-killed individuals and taxa, road–killed taxa diversity, no. of plant species, number of cars/hour.

Road–killed taxa	S1	S2	S3	S4	S5	S6	P%	f%
Oligochaeta Lumbricidae	21.35	30.74	12.98	9.94	8.08	23.67	17.04	100
Gastropoda (with shell)	20.19	5.50	12.55	5.04	4.33	5.79	9.29	100
Gastropoda (without shell)	13.74	10.81	11.68	6.82	3.74	1.44	8.78	100
Scorpionidae: *Euscorpius carpathicus*	0.99	0.37	0.86	0.14	0.78	–	0.57	83.33
Opilionidae	0.16	0.94	1.51	0.74	0.19	–	0.63	83.33
Araneidae	0.16	0.18	0.43	2.07	1.38	2.41	1.00	100
Isopoda Oniscidea	–	–	0.43	0.74	–	–	0.23	33.33
Chilopoda	0.16	0.18	0.43	–	–	–	0.13	50.00
Diplopoda	0.66	2.08	6.92	8.75	1.77	3.38	4.09	100
Ephemeroptera	–	–	0.21	–	1.38	–	0.26	33.33
Odonata	0.16	–	–	–	0.19	–	0.06	33.33
Blattodea	–	–	–	–	0.19	–	0.03	16.66
Plecoptera	–	–	–	0.14	–	–	0.03	16.66
Orthoptera	7.94	11.38	2.81	3.26	3.55	7.72	5.93	100
Dermaptera	–	–	–	–	–	0.48	0.03	16.66
Homoptera Cicadidae	–	–	0.64	0.29	–	0.48	0.20	50.00
Heteroptera Pyrrhocoris	2.31	0.37	0.86	–	0.39	0.96	0.80	83.33
Heteroptera others	–	–	2.16	0.74	0.98	0.48	0.70	66.66
Coleoptera Carabidae	1.65	2.84	3.03	2.37	2.76	2.89	2.51	100
Coleoptera Cetonia	1.15	2.65	3.46	2.07	1.97	0.48	2.07	100
Coleoptera Geotrupidae	0.82	7.21	6.27	10.68	30.57	2.89	10.23	100
Coleoptera Melolontha	0.16	0.18	0.64	1.92	0.98	0.48	0.80	100
Coleoptera Cantharidae	–	–	–	0.59	0.98	–	0.30	33.33
Coleoptera Staphylinidae	–	–	–	0.14	0.19	0.96	0.13	50.00
Coleoptera Lampyridae	0.16	–	–	0.44	0.19	–	0.16	50.00
Coleoptera Cerambycidae	–	–	0.64	0.14	0.19	–	0.16	50.00
Coleoptera Coccinellidae	0.16	–	0.64	–	0.59	4.83	0.57	66.66
Coleoptera Chrysomelidae	0.66	0.75	0.43	2.96	1.38	0.48	1.27	100
Coleoptera Lucanidae	0.99	2.65	4.11	1.33	1.38	–	1.84	83.33
Coleoptera Curculionidae	–	–	0.21	0.14	–	2.89	0.26	50.00
Coleoptera others	0.49	0.56	0.64	0.44	0.59	1.44	0.60	100
Panorpata	–	–	0.21	0.14	0.39	0.48	0.16	66.66
Trichoptera	–	0.56	0.43	0.44	–	–	0.26	50.00
Lepidoptera adults	0.82	2.08	1.51	0.74	0.59	2.89	1.24	100
Lepidoptera larvae	1.65	1.32	0.86	1.48	0.98	10.14	1.91	100
Diptera Brachycera	0.49	0.94	2.81	15.43	9.46	6.28	6.23	100
Diptera Nematocera	–	–	–	0.44	0.19	0.48	0.16	50.00
Hymenoptera Bombus	0.66	0.56	0.64	0.14	0.59	0.96	0.53	100
Hymenoptera Apidae	2.31	2.08	9.30	3.26	5.91	3.86	4.29	100
Hymenoptera Vespidae	2.15	1.32	4.97	1.92	1.38	3.86	2.38	100
Hymenoptera Formicidae	6.45	2.46	0.86	9.19	2.76	–	4.42	83.33

Table 1. (Continued).

Hymenoptera others	0.16	0.18	0.21	–	–	0.48	0.13	66.66
Salamandra salamandra	0.99	0.94	0.64	2.67	5.32	2.41	2.14	100
Bombina variegata	–	–	0.21	–	0.19	–	0.06	33.33
Bufo bufo	4.30	2.46	0.86	0.29	0.39	0.96	1.64	100
Rana dalmatina	0.16	–	0.43	0.29	0.39	–	0.23	66.66
Rana temporaria	–	–	0.43	0.29	0.98	–	0.30	50.00
Pelophylax ridibundus	0.49	2.08	0.21	–	–	–	0.50	50.00
Anura others	–	0.18	–	0.14	–	–	0.06	33.33
Ablepharus kitaibelii	0.16	–	–	–	–	–	0.03	16.66
Podarcis muralis	0.49	0.18	–	0.14	0.19	–	0.20	66.66
Lacerta viridis	2.48	0.94	–	–	–	0.48	0.70	50.00
Anguis (fragilis) colchica	0.16	–	–	–	0.19	0.48	0.10	50.00
Natrix natrix	0.66	0.75	0.21	0.29	0.39	–	0.43	83.33
Natrix tessellata	0.16	0.37	–	–	–	–	0.10	33.33
Coronella austriaca	–	–	–	–	–	0.96	0.06	16.66
Zamenis longissimus	0.16	–	–	0.14	–	–	0.06	33.33
Vipera ammodytes	0.33	0.37	–	–	–	–	0.13	33.33
Ophidia others	0.16	–	–	–	0.19	–	0.06	33.33
Aves	–	0.18	–	–	–	–	0.03	16.66
Aves: *Motacilla cinerea*	–	0.18	–	–	–	–	0.03	16.66
Mammalia Rodentia	0.33	–	–	0.29	0.19	–	0.16	50.00
Mammalia Insectivora Talpa	–	0.18	0.43	0.14	–	–	0.13	50.00
Mammalia Insectivora Sorex	–	–	–	–	0.39	0.96	0.13	33.33
No. of individuals	**604**	**527**	**462**	**647**	**507**	**207**	**2981**	
No. of taxa	**42**	**38**	**42**	**44**	**45**	**34**	**64**	
Taxa diversity	**2.61**	**2.64**	**2.98**	**2.95**	**2.79**	**2.88**	**3.08**	
No. of plant species	**101**	**120**	**105**	**119**	**91**	**76**	**247**	
Cars/hour	**4.75**	**4.23**	**3.15**	**2.48**	**3.66**	**5.56**	**3.97**	

length of 6 km on the 12 study days. If we extrapolate this to the total length of the road (approximately 17 km), it results in a number of 8446 victims. If we extrapolate this for a year, it would mean 256,904 victims, which is a huge number. Both the smallest number of taxa (34 taxa) and the smallest number of carrions (207 carions) were registered on sector 6 (Table 1). The highest number of victims (647 victims) was registered on sector 4, and the smallest one (207 victims) on sector 6 (Table 1). The highest number of road-killed animals was registered in August (661 animals), and the smallest in December (25 animals). The highest number of taxa were killed in June (45 taxa), and the lowest in January and March (only 4 taxa). The total diversity of road–killed animals was H = 3.08, but it varied between months and sectors (Table 1, 2). Totally, Lumbricida registered the highest percentage abundance (17.04%), followed by Coleoptera Geotrupidae (10.23%) and Gastropoda with shell (9.29%). However, if we reunite Gastropoda with and without shell, they register a percentage abundance of 18.07%, surpassing earthworms. Earthworms and snails represented together more than 35% of the road-killed animals. Most of the taxa had percentage abundance smaller than 1% (45 taxa, from which 6 with values lower than 0.03%). Just two vertebrate taxa had percentage abundance higher than 1% (*Salamandra salamandra* had a percentage abundance of 2.14%). Great percentage abundance differences were registered between sectors (Table 1). Even more obvious differences were registered between months. Although earthworms were killed in all 12 months, their percentage abundance was higher in cold and wet periods and lower in drier months: August, June, September, and May (Table

Table 2. Road-killed taxa percentage abundance in the studied months (VI – V, months from June to May). Total frequency of occurrence.

Taxa	VI	VII	VIII	IX	X	XI	XII	I	II	III	IV	V	f%
Oligochaeta Lumbricidae	3.67	22.72	2.26	4.92	12.43	19.69	60.00	84.26	30.32	88.82	46.23	6.62	100
Gastropoda (with shell)	13.23	11.53	6.50	2.60	3.24	1.51	–	–	–	–	13.97	27.62	66.66
Gastropoda (without shell)	0.91	8.74	2.72	13.62	2.70	12.12	4.00	12.35	66.45	7.05	5.37	6.07	100
Scorpionidae: *Euscorpius carpathicus*	1.47	–	0.90	0.57	–	–	–	–	–	–	–	0.27	33.33
Opilionidae	2.20	–	0.75	0.57	–	–	–	–	–	–	–	–	25.00
Araneidae	0.73	–	1.96	–	0.54	–	–	–	–	–	2.15	2.76	41.66
Isopoda Oniscidea	0.18	–	0.30	0.28	–	–	–	–	–	–	–	0.82	33.33
Chilopoda	–	–	0.15	0.28	–	3.03	–	–	–	–	–	–	25.00
Diplopoda	1.28	0.34	8.01	11.59	1.62	1.51	–	–	–	–	8.60	2.48	66.66
Ephemeroptera	0.55	–	0.30	–	–	–	–	–	–	–	–	0.82	25.00
Odonata	–	0.34	0.15	–	–	–	–	–	–	–	–	–	16.66
Blattodea	0.18	–	–	–	–	–	–	–	–	–	–	–	8.33
Plecoptera	0.18	–	–	–	–	–	–	–	–	–	–	–	8.33
Orthoptera	0.91	0.69	9.83	24.92	7.56	4.54	4.00	–	–	–	–	0.27	66.66
Dermaptera	–	–	–	–	0.54	–	–	–	–	–	–	–	8.33
Homoptera Cicadidae	0.36	0.69	0.30	–	–	–	–	–	–	–	–	–	25.00
Heteroptera Pyrrhocoris	2.75	–	0.90	–	0.54	–	–	–	–	–	–	0.55	33.33
Heteroptera others	–	0.34	2.42	1.15	–	–	–	–	–	–	–	–	25.00
Coleoptera Carabidae	4.04	0.69	3.32	3.76	2.70	1.51	–	–	–	–	1.07	2.48	66.66
Coleoptera Cetonia	5.33	3.49	3.17	–	–	–	–	–	–	–	1.07	0.27	41.66
Coleoptera Geotrupidae	10.11	3.84	17.39	3.18	29.72	4.54	4.00	1.12	–	–	2.15	14.08	83.33
Coleoptera Melolontha	1.65	–	–	0.57	–	–	–	–	–	–	–	3.59	25.00
Coleoptera Cantharidae	–	–	1.36	–	–	–	–	–	–	–	–	–	8.33
Coleoptera Staphylinidae	0.18	–	0.15	–	0.54	1.51	–	–	–	–	–	–	33.33
Coleoptera Lampyridae	0.36	–	–	0.28	–	–	–	–	–	–	–	0.55	25.00
Coleoptera Cerambycidae	0.73	–	0.15	–	–	–	–	–	–	–	–	–	16.66
Coleoptera Coccinellidae	1.10	–	0.75	–	2.70	–	–	–	–	–	1.07	–	33.33
Coleoptera Chrysomelidae	0.36	–	2.57	2.89	–	3.03	–	–	–	–	1.07	1.65	50.00
Coleoptera Lucanidae	2.02	4.19	4.38	0.57	–	–	–	–	–	–	–	0.27	41.66
Coleoptera Curculionidae	0.18	–	–	–	–	–	–	–	–	–	–	1.93	16.66
Coleoptera others	1.10	0.69	0.60	–	1.62	–	–	–	–	–	2.15	0.27	50.00
Panorpata	–	–	0.30	0.57	–	–	–	–	–	–	–	0.27	25.00
Trichoptera	1.28	–	–	0.28	–	–	–	–	–	–	–	–	16.66
Lepidoptera adults	3.12	2.09	0.90	0.86	1.08	–	8.00	–	–	–	–	0.27	58.33
Lepidoptera larvae	0.91	1.04	1.36	4.34	9.72	7.57	–	–	–	–	1.07	0.27	66.66
Diptera Brachycera	16.36	1.04	5.90	4.92	2.16	1.51	–	–	–	–	–	9.11	58.33
Diptera Nematocera	–	–	–	–	–	–	–	–	–	–	–	1.38	8.33
Hymenoptera Bombus	1.65	–	0.75	0.57	–	–	–	–	–	–	–	–	25.00
Hymenoptera Apidae	6.61	26.92	0.90	0.86	1.08	–	–	–	–	–	–	1.10	50.00
Hymenoptera Vespidae	0.55	–	7.26	5.50	0.54	–	–	–	–	–	–	–	33.33
Hymenoptera Formicidae	9.92	0.34	7.86	5.21	–	–	–	–	–	–	–	1.93	41.66
Hymenoptera others	–	–	0.15	0.28	0.54	–	–	–	–	–	–	0.27	33.33

Table 2. (Continued).

Salamandra salamandra	0.55	0.34	–	–	7.56	19.69	12.00	2.24	0.64	0.58	3.22	6.35	83.33
Bombina variegata	0.18	–	–	–	–	–	–	–	–	–	1.07	–	16.66
Bufo bufo	0.91	–	–	0.86	8.64	15.15	8.00	–	0.64	3.52	6.45	–	66.66
Rana dalmatina	–	0.34	0.30	0.57	–	–	–	–	–	–	–	0.55	33.33
Rana temporaria	0.18	1.04	0.15	0.28	–	–	–	–	1.29	–	–	0.27	50.00
Pelophylax ridibundus	0.18	1.39	1.36	–	0.54	–	–	–	–	–	–	–	33.33
Anura others	0.18	–	0.15	–	–	–	–	–	–	–	–	–	16.66
Ablepharus kitaibelii	–	0.34	–	–	–	–	–	–	–	–	–	–	8.33
Podarcis muralis	–	0.69	0.30	0.28	–	–	–	–	–	–	–	0.27	33.33
Lacerta viridis	0.36	4.54	0.60	0.28	–	1.51	–	–	–	–	–	–	41.66
Anguis (fragilis) colchica	0.18	0.34	–	0.28	–	–	–	–	–	–	–	–	25.00
Natrix natrix	0.36	0.34	0.30	0.57	0.54	–	–	–	–	–	–	1.38	50.00
Natrix tessellata	–	–	–	0.57	–	–	–	–	–	–	–	0.27	16.66
Coronella austriaca	–	–	–	–	–	–	–	–	–	–	–	0.55	8.33
Zamenis longissimus	–	–	–	–	0.54	1.51	–	–	–	–	–	–	16.66
Vipera ammodytes	–	–	–	0.57	–	–	–	–	–	–	1.07	0.27	25.00
Ophidia others	0.36	–	–	–	–	–	–	–	–	–	–	–	8.33
Aves	–	–	–	–	–	–	–	–	–	–	–	0.27	8.33
Aves: *Motacilla cinerea*	–	0.34	–	–	–	–	–	–	–	–	–	–	8.33
Mammalia Rodentia	0.18	0.34	–	–	–	–	–	–	0.64	–	–	0.55	33.33
Mammalia Insectivora Talpa	–	–	–	–	–	–	–	–	–	–	–	1.10	8.33
Mammalia Insectivora Sorex	–	–	–	0.28	0.54	–	–	–	–	–	2.15	–	25.00
No. of individuals	**544**	**286**	**661**	**345**	**185**	**66**	**25**	**89**	**155**	**170**	**93**	**362**	
No. of taxa	**45**	**29**	**41**	**36**	**25**	**16**	**7**	**4**	**6**	**4**	**17**	**38**	
Taxa diversity	**2.97**	**2.34**	**2.98**	**2.68**	**2.44**	**2.31**	**1.35**	**0.53**	**0.78**	**0.44**	**1.95**	**2.66**	
Cars/hour	**3.91**	**6.36**	**5.31**	**6.58**	**4.03**	**3.78**	**2.29**	**4.87**	**1.37**	**2.72**	**3.38**	**3.08**	

2). Unlike these, Orthoptera had the highest percentage abundance in September (24.92%). Only 20 taxa were killed in all sectors (Table 1). Only two taxa (Lumbricidae and Gastropoda without shell) were killed in each month, and 11 taxa were killed in just one month (Table 2).

Regarding the similarity between sectors, sector 6 was the most different, and sectors 1 and 2 were the closest, while sectors 3, 4, and 5 formed a distinct group (Figure 3). According to the Kruskal-Wallis test, the differences were not significant ($p = 0.234$). Comparing the sectors two by two, the Mann-Whitney test indicated that generally, the differences were not significant ($p > 0.05$), with the exceptions of the differences between sectors 6 and 4 ($p = 0.022$) and 6 and 5 ($p = 0.031$). In the case of periods, the winter months (December–March) formed a cluster clearly separated from the rest of the months; October, November, and April were different from the other months (May–September) (Figure 4). According to the Kruskal-Wallis test, the road mortality differences between periods were significant ($p < 0.0001$). Also, by analyzing the differences two by two with the Mann–Whitney test, the differences were in most cases significant ($p < 0.05$). Between the number of road-killed animals and the number of taxa, there was a strong positive and significant correlation ($r = 0.954$, $p = 0.0008$). Between the number of road–killed animals and the number of plant species, there was a strong positive and significant correlation ($r = 0.983$, $p < 0.0001$). Between the number of road-killed taxa and the number of plant species, there was a strong positive and significant correlation ($r = 0.926$, $p = 0.002$).

4. Discussion

Although the road to Bigăr has a very reduced traffic, the road mortality intensity was high. Thus, the 2981 road-killed animals are a large number of victims. Even if there were fewer road-killed animals compared with the main

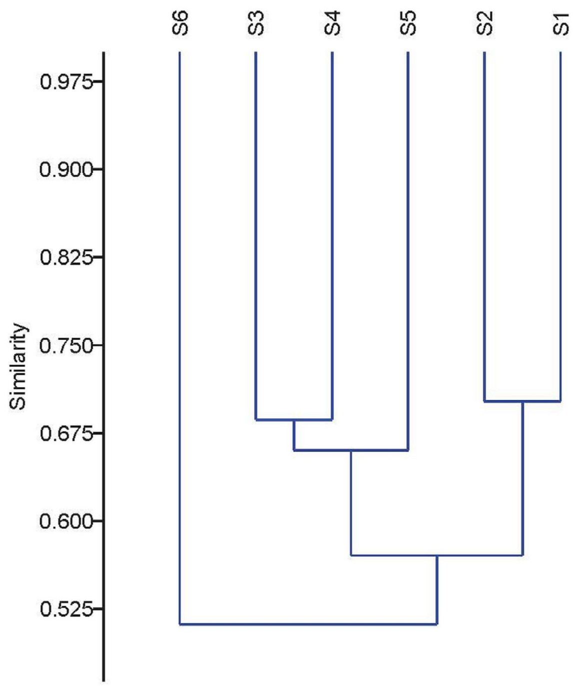

Figure 3. Similarity between sectors (S1–S6–studied sectors).

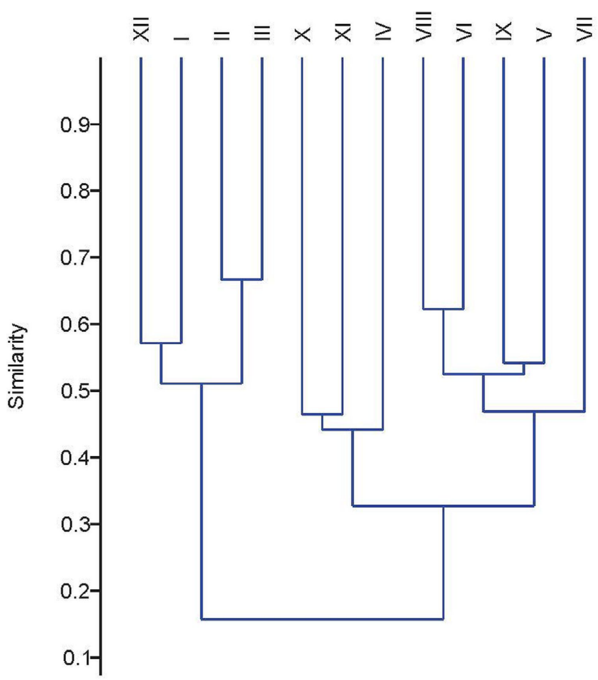

Figure 4. Similarity between periods (I–XII–studied months).

(national) road parallel to the Danube, where 13,230 animals were killed (Covaciu–Marcov et al., 2022), the differences between the traffic intensity of the two roads were obvious. Thus, on the road to Bigăr there were only 3.97 cars/hour, in contrast to the almost 100 cars/hour on the main road (Covaciu–Marcov et al., 2022), and the road to Bigăr is bordered with more uniform habitats compared with the main road, which is surrounded by diverse habitats (Covaciu–Marcov et al., 2022). The differences are explainable, as road mortality is influenced by roadside habitats (e.g., Glista et al., 2007; Yamada et al., 2010; Matos et al., 2012; Popovici et al., 2018; Lala et al., 2021; Rendall et al., 2021). Moreover, the increase in traffic intensity also increases road mortality intensity (e.g., Jones et al., 2014; Ruiz–Capillas et al., 2015; Miranda et al., 2017; Lyamuya et al., 2021), and on the road to Bigăr the traffic was very reduced. The speed also counts, as at higher speeds there are more road-killed animals (e.g., Jancke and Giere, 2011; Jones et al., 2014; Miranda et al., 2017; Rendall et al., 2021), and on this road, the speed is reduced because of the very narrow curves and steep slopes. Moreover, the main road has two lanes, and wider roads are more difficult for animals to cross (e.g., Baur and Baur, 1990; Rico et al., 2007; Miranda et al., 2017). Thus, on narrow roads, there are fewer road–killed animals compared with wider roads (Lyamuya et al., 2021).

On the road to Bigăr earthworms had the highest percentage abundance, because of the high humidity on Sirinia valley, which is heavily forested and shaded. Road–killed earthworms were encountered in other regions too (e.g., Ciolan et al., 2017; Jeganathan et al., 2018; Popovici et al., 2018; Covaciu–Marcov et al., 2022), but on this road their percentage abundance was higher. There were cases when earthworms encountered dead on the road were not considered victims of road traffic, because it was considered that they reached accidentally the road and died by dehydration (Seibert and Conover, 1991). Although, the same was indicated in the case of snails and slugs on railways (Pop et al., 2020, 2023), on the road to Bigăr, earthworms were killed by cars, a fact proved by the condition of carrions and also by direct observation. Even if they were just dehydrated on the road, they are still victims of the road, because if the road was not there, the earthworms would not have died on it. Because the narrow road to Bigăr passes through forests and it is wet even in the summer, the earthworms can cross it and are direct victims of the road traffic. Although earthworms are not protected by law in Romania (O.U.G. 57/2007), in the Carpathian Mountains there are numerous endemic earthworm species (e.g., Pop et al., 2010; Csuzdi et al., 2011), thus they have high biogeographic importance.

Plant assemblages from the road's vicinity influence the road mortality of certain animals (e.g., Orłowski, 2008; Yamada et al., 2010; Skórka et al., 2013, 2015). In human-affected areas, road verges preserve a diverse vegetation, thus they may represent refuges for biodiversity (Arenas et al., 2017). Nevertheless, this is not the situation in Sirinia valley, where the road is inside a wide forest (three sectors are completely surrounded by forests), thus road verges could not offer anything more to animals. The area next to the road (except sector 6) is a huge natural habitat that was recently crossed by the road. Thus, natural plant communities are everywhere, and the number of plant species and the number of road-killed animals are related. As the conditions are more favorable, there are more plant species near the road, and more animals get road killed. In the lowest sectors, there are numerous plant species because in that area there are more diverse habitats. In the lowest sectors, southern, sub-Mediterranean animals were killed by cars (*Ablepharus kitaibelii* and *Vipera ammodytes*). The area has both rocky and open grassy areas with southern oaks, which are typical habitats for these species (e.g., Fuhn and Vancea, 1961). Basically, there is a relation between plant assemblages neighboring the road and fauna road mortality, but both reflect the existence of favorable conditions of the habitats near the road. If a road is surrounded by optimal habitats, it will have more plant species and more animals will be exposed to road traffic.

The highest number of road–killed animals was registered in summer, as in the previous study, confirming the road mortality peculiarities in the region (Covaciu–Marcov et al., 2022). However, the road to Bigăr is perpendicular to the Danube and moves away from the river and from the lower and warmer areas (Stoenescu et al., 1966; Mândruț, 2006). With all these, animals were road-killed in each month, even amphibians in the winter (*Salamandra salamandra* and *Bufo bufo*). The highest road mortality intensity was in summer, although mortality peaks are usually registered in the spring (e.g., Orłowski, 2007; Orłowski et al., 2008; Ciolan et al., 2017; Covaciu–Marcov et al., 2017; Popovici et al., 2018). The peculiar conditions on the Danube Gorge, as an area with sub–Mediterranean climatic influences (e.g., Drugescu and Geacu, 2004; Mândruț, 2006), are also noticeable on this road as in the case of the main road (Covaciu–Marcov et al., 2022). Further south, in Bulgaria, vertebrate road mortality was highest in September (Kambourova–Ivanova et al., 2012). The absence of the road mortality spring peak is probably a consequence of the absence of the amphibians' spring peak, which was important in other cases (e.g., Orłowski, 2007; Orłowski et al., 2008; Covaciu–Marcov et al., 2017). Stagnant aquatic habitats near the road are very rare, thus explosive breeding amphibians which usually use those habitats do not cross the road and do not fall victims to traffic as in other cases (e.g., Mitchell, 2000; D'Amico et al., 2015; Arévalo et al., 2017).

Other amphibians as *S. salamandra* were killed relatively constantly but with the peak at the end of autumn. At least as the number of victims, the most reduced mortality was in December. All these are random, as what matters is not the month, but the fact that in the winter the road mortality is the lowest, while in spring is fluctuant and not very different from winter. The high number of victims registered in summer and autumn is specific to areas with sub–Mediterranean climatic influence, where the autumn is long, warm, and rainy (Drugescu and Geacu, 2004).

Road–killed scorpions were absent only in the last sector. They ascend far from the Danube, but only in forested areas. Terrestrial isopods were killed only in the sectors with dense and humid forests, as in Romania there are many forest species (Radu, 1983, 1985; Tomescu et al., 2011, 2015). Also in other cases, forest terrestrial isopods were killed on roads (Ciolan et al., 2017). Orthopterans were killed everywhere but had a higher percentage abundance in sectors with open areas, as in the case of the main road (Covaciu–Marcov et al., 2022). All these confirm the importance of roadside habitats for road mortality (e.g., Glista et al., 2007; Yamada et al., 2010; Matos et al., 2012; Popovici et al., 2018; Lala et al., 2021; Rendall et al., 2021). Road-killed snails with shells were absent between December and March, but snails without shells were killed in all months (with a percentage abundance of over 66% in February). Scorpions were killed in the warm period (May–September), Diplopoda between April and November, and Orthoptera between May and December. Geotrupida were relatively numerous; some species lay their eggs in the soil and others are coprophagous (e.g., Byk and Piętka, 2018). On other roads, their abundance was related with cattle (Cicort–Lucaciu et al., 2016), but in this case their abundance was smaller in sector 6 where cattle are present, compared with sector 4 and 5 situated in forests. Probably on this road, Geotrupida are attracted by wildlife feces, a fact mentioned in the case of other insect species (Barahona–Segovia, 2021).

Although many vertebrates were killed on the road to Bigăr, they had a percentage abundance of only 7.38%, lower than in other cases (e.g., Ciolan et al., 2017; Jeganathan et al., 2018; Covaciu–Marcov et al., 2022). This lower percentage abundance is probably a consequence of the cars' reduced speed. Thus, larger and faster vertebrates could avoid cars easier than invertebrates. *Salamandra salamandra*, the most frequent road–killed vertebrate, is a slow amphibian (Fuhn, 1960). It was killed on all sectors, and it was missing only in two months. Even if it would be expectable that those were cold months, *S. salamandra* was not killed on the road in August and September (warm and dry months). This fact confirms its tolerance to low temperatures, its sensibility to high temperatures, and its vulnerability to climate changes (Catenazzi, 2016). At the same time, it confirms the fact that in the Danube Gorge, this species is active in the winter (Covaciu–Marcov et al., 2022). During the study year *S. salamandra* did not hibernate, but only estivated two months at the end of summer and at the beginning of autumn, as in 2020 in June and July the weather was rainy, although warm. The high number of road–killed fire salamanders is a consequence of the forests, and the winter activity is a consequence of the peculiarities of the Danube Gorge, where this species feeds intensely even at low temperatures (Maier et al., 2020, 2022). *Salamandra salamandra* is a protected species (O.U.G. 57/2007) and the region is a natural park, thus the high number of victims is alarming and confirms that these slow animals (Fuhn, 1960) are exposed to road mortality (e.g., Manenti et al., 2010; Matos et al., 2012; Rassati, 2016; Ciolan et al., 2017). The entire region is populated by salamanders, which are active even in the winter, therefore, they become exposed to road traffic. Furthermore, our study indicates that the more southern the region is, the more animals are killed on roads. At least in the case of railway mortality, in a northern area of western Romania, no victims were registered in the winter (Pop et al., 2020). Therefore, there are spatial changes in seasonal variation patterns, noticeable even in small territories, like western Romania. For this reason, in road–mortality studies, spatial particularities must be considered (even more in protected areas), and the data obtained should not be generalized or extrapolated (the pattern of one region is not mandatorily repeated in the same way in another region). At the same time, these differences also modify the usual pattern of the road mortality intensity peak. The abovementioned should be considered in the planning and design of other road-mortality studies in areas that are important by their zoogeography and conservational status and should be taken into account when discussing mitigation measures.

Most road-killed taxa include slow and nonflying animals, both in the case of invertebrates and vertebrates. This fact is a reflection of the neighboring habitats and conditions, but also of the road aspect and traffic speed. Road mortality similarities also reflect the roadside habitats. Sector 6, an open area replacing a beech forest, was the most different. Thus, the lowest number of road-killed animals, taxa and plant species was registered in this sector. Sectors 1 and 2 had similar road mortality, as both are situated downstream in more open rocky areas. Also, the similarity of road mortality between the winter months is very clear, compared to the similarity between the spring and autumn months, and also between the warm months.

Road mortality in Sirinia valley clearly indicates that even an extremely low traffic causes a high fauna road mortality, affecting numerous species, even with zoogeographic and conservation importance. Thus, we registered road-killed animals with conservation value

(*Lucanus cervus, S. salamandra, Bombina variegata*, etc). In this context, it is probably impossible to further reduce the traffic intensity on the road without isolating Bigăr village. At least in winter, the traffic reduces only to locals and forestry equipment, and animals still die on the road. The decrease in road traffic under a certain limit is not sustainable, and a road with less than one car for every 15 min is probably a minimum in this direction. This confirms that a reduced traffic intensity does not always decrease road mortality (Abraham and Mumma, 2021). Probably the most efficient method remains to stop constructing new roads, a fact already mentioned (Ciolan et al., 2017; Covaciu–Marcov et al., 2022). The increase in traffic intensity in the warm period is caused by tourists who are attracted by Bigăr waterfall, a known and important tourist attraction (e.g., Artugyan, 2017; Arsene et al., 2021), but Bigăr waterfall is not in Bigăr village, as this is just a coincidence of names. If tourists knew that the road does not lead to the waterfall, they would no longer enter the road. Thus, in summer (exactly in the road mortality hot time) a large part of the traffic could be reduced if at the beginning of the road a warning signal with exact information regarding to Bigăr village/waterfall would be installed; this simple mitigation measure could make a difference. Wildlife warning signs have been continuously changing in the last period, in the context in which considering biodiversity in road infrastructure designs gains more and more importance (Tryjanowski et al., 2021). Moreover, some consider these signs a relatively cheap measure for reducing road mortality (Bond and Jones, 2013). Some other warning signs could also be installed, for example, for salamanders, which were the most affected vertebrates on this road. Because the speed of cars is reduced, drivers would be able to notice most of the salamanders and drive more carefully. On the edge of the road, informative signs for different touristic objectives (ruins, gorges, waterfalls, etc.) were already placed. In the same way, warning signs could be installed by the protected area administration or even volunteers, with messages like 'stop by the ruins, visit the cascade, but also pay attention to nature as an asset of the region'. Even though in other cases warning signs did not reduce the road mortality of birds and mammals (Bullock et al., 2011), in our case road particularities could make the difference, all the more as it is known that warning signs for small animals seem to change the drivers' behavior (Collinson et al., 2019). Also, because drivers seem to take into account signs with the number of victims on a road (Bond and Jones, 2013), information from road mortality studies, just like this one, could be useful in this direction. In this manner, raising awareness of the phenomenon of road mortality would exceed the relative limits of scientific study, reaching out to people by which the actual changes start. From another perspective, the results and experience accumulated by studying this road are valuable because the lessons learned here are warning signs themselves and could be useful in the effective management of other protected and nonprotected areas.

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