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First data from the new amphibian road-kill hotspot at Vinogradniška pot in Razvanje (NE Slovenia)

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ABSTRACT

There are more than 1500 road segments, where large amphibian mortalities are recorded in Slovenia. One of such road-kill hotspots is Vinogradniška pot at Razvanje (NE Slovenia), where censuses of migrating amphibians were carried out in spring of 2019, 2020, 2021 and 2024 alongside actions to prevent road death that have been taking place since 2018, albeit irregularly. Here, the first data from those censuses are presented. Four amphibian species were recorded migrating over the road, specifically common toad (*Bufo bufo*), common frog (*Rana temporaria*), agile frog (*R. dalmatina*) and Italian crested newt (*Triturus carnifex*). The most numerous was B. bufo, of which 500–1000 individuals migrated over the road annually and represented about 99% of individuals of all recorded amphibian species. Male B. bufo outnumbered females by 4–12:1 and species mortality was fairly low (between 5 and 12%), but was deliberately artificially reduced. Amphibian activity was lower later in the evening and had a significant moderate positive correlation with the air temperature. The data presented indicate the importance of amphibian spring migration monitoring also over low-traffic roads, which are rarely the focus of similar studies.

IZVLEČEK

Prvi podatki s črne točke na Vinogradniški poti v Razvanju (SV Slovenija)

V Sloveniji je trenutno registriranih več kot 1500 odsekov, kjer prihaja do množičnih povozov dvoživk. Ena izmed takšnih črnih točk je cesta na Vinogradniški poti v Razvanju (SV Slovenija), kjer smo popise selečih se dvoživk opravljali v letih 2019, 2020, 2021 in 2024 v sklopu akcije prenašanja dvoživk čez cesto, ki v naši lastni režiji poteka od leta 2018, a ne redno. Tukaj predstavljamo prve podatke iz popisnih let. Na lokaciji smo zabeležili štiri vrste selečih se dvoživk, in sicer navadno krastačo (*Bufo bufo*), sekuljo (*Rana temporaria*), rosnico (*R. dalmatina*) in velikega pupka (*Triturus carnifex*). Najštevilčnejša je bila navadna krastača, s 500–1000 selečimi se osebki na leto z okoli 99 % osebkov vseh zabeleženih vrst dvoživk. Samci so v populaciji prevladovali nad samicami v razmerju 4–12:1, smrtnost pa je bila relativno nizka (med 5 in 12 %), a se je v sklopu akcije ta namerno zmanjšala. Aktivnost dvoživk je skozi večer upadla in je korelirala zmerno pozitivno, a statistično značilno s temperaturo zraka. Podatki prikazujejo pomen monitoringov spomladanskih selitev dvoživk tudi čez manj prometne ceste, ki so redko vključene v podobne raziskave.

KEY WORDS:
amphibian spring migration, road-kill hotspot, Razvanje, *Bufo bufo*

KLJUČNE BESEDE:
spomladanska selitev dvoživk, črna točka, Razvanje, *Bufo bufo*

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INTRODUCTION

Construction of transport infrastructure represents one of the most notable environmental alterations by humans. As a result of our modern lifestyle, which requires ever better and more sophisticated traffic connections, the problem is also increasing with time (Podloucky 1989; Vos & Chardon 1994), which has a large impact on the ecosystems in the aspect of habitat degradation and fragmentation (Trombulak & Frissell 2000). Among all terrestrial vertebrates, populations of amphibians and reptiles were found to suffer the highest impact by transport infrastructure, due to roadkill and habitat fragmentation (Rytwinski & Fahring 2012). Roads can affect amphibians on three different levels, specifically at the level of an individual, level of a population and the level of connectivity between populations (Vos & Chardon 1994). In Slovenia, as many as 1500 road-kill hotspots, where large numbers of amphibians are being run-over annually, have been identified so far (Strašek 2012). Roads in Slovenia pose the greatest threat to the populations of the common toad (*Bufo bufo*) and common frog (*Rana temporaria*), which belong into the group of so-called »explosive breeders«, where almost all sexually mature individuals migrate to their spawning site simultaneously (Poboljšaj et al. 2018).

In order to protect the migrating amphibians from being run-over, an action of transporting amphibians over the road has been conducted along Vinogradniška pot at Razvanje near Maribor (NE Slovenia) since 2018, however, systematic data on migrating amphibians were only gathered in 2019, 2020, 2021 and 2024. Here, we would like to present the data gathered during those years, since, to our knowledge, this road-kill hotspot has not been registered yet.

MATERIAL AND METHODS

Amphibians were surveyed during spring migration rescue activity along the road Vinogradniška pot at Razvanje, about 2 km southwest of Maribor (NE Slovenia). The surveyed path consisted of an 800 m long road segment in all years and an additional 600 m long segment since 2020 (Fig. 1). The latter ran parallel to the first one and was added mainly to reduce amphibian mortality along that road. Coordinates of the amphibian spawning site are: 46°31'02.8"N, 15°37'45.9"E. The road is asphalted, 3 m wide and located in an urbanised area. The pond in which amphibians spawn is surrounded by vineyards, intensively farmed meadows and mixed forests. Meadows east of the pond are ploughed in certain years for the purpose of maize production.

Amphibian surveys were conducted in 2019, 2020, 2021 and 2024. The annual fieldwork started when the first amphibians were spotted on the road and ended when no more amphibians were found for at least three consecutive evenings. During that time, surveys were conducted every evening, except for three in 2019 and two in 2024. Hence, the annual surveys lasted approximately as long as the spring migration itself. Their starting and ending dates are presented in Tab. 1 and

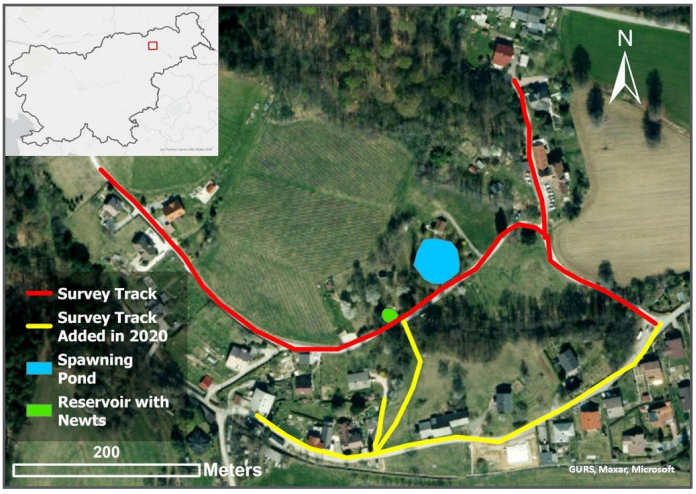

Figure 1. Survey track with the pond that serves as the spawning site for Anurans. Italian crested newts (*Triturus carnifex*) spawn in a smaller water reservoir southwest of the pond.
Slika 1. Popisna pot z ribnikom kot mrestiščem za žabe in krastače. Veliki pupki (*Triturus carnifex*) mrestijo v manjšem vodnem zajetju jugozahodno od ribnika.

individual survey dates are listed in the supplementary material (Tab. S1). Evening fieldwork began at 7:30 pm (8:30 pm when daylight savings changed) and ended after the entire survey path was walked twice. The minimum amount of time spent in the field was set at one hour, but the survey time varied depending on the number of migrating individuals and could last up to 2.5 h. In 2019, the time of the beginning was not consistent and the path was only walked once. In 2020, a third walk was sometimes carried out, however, the data from that one were not used in any analysis except in comparison between the number of individuals found during different walks.

Table 1. Beginning and ending dates of amphibian surveys during the amphibian spring migration over the road at Razvanje (NE Slovenia), according to a specific study year.
Tabela 1. Začetni in končni datum popisov dvoživk med spomladansko selitvijo čez cesto v Razvanju (SV Slovenija), glede na popisno leto.

Study Year	Beginning Date	Ending Date
2019	5. 3.	30. 3.
2020	1. 3.	1. 4.
2021	12. 3.	5. 4.
2024	25. 2.	14. 3.

Every evening, the exact time of the start and end of the survey were noted, together with the weather conditions and air temperature. The latter was measured on site, using a stationary wet-bulb thermometer, positioned at approximately 1 m above ground. Amphibians were searched for using a headlamp, over the whole width of the road, as well as within about 1 m distance from each side of the road. Detected individuals were carried to the spawning pond and counted separately by sex and species and ran-over individuals were also counted separately by sex and species, according to Veenvliet & Veenvliet (2003). Due to their known aggressive mating behaviour, male *B. bufo* were transported in a separate bucket from females and amplexi, and other species in a separate bucket from *B. bufo* to prevent them from being harmed by toxins produced by the toads (Kowalski et al. 2018; Bolčina et al. 2021). No pro-

tective fence for amphibians was used during any of the study years.

Shapiro-Wilk test showed that the distribution of the number of found living individuals during every successive evening walk deviated significantly from normality for the first walk ($W = 0.692$, $p < 0.001$), the second walk ($W = 0.634$, $p < 0.001$) and for the third walk ($W = 0.667$, $p < 0.001$). Hence a non-parametric Kruskal-Wallis test with a post-hoc Dunn's test was used to find out whether the difference in the number of found living individuals between every successive evening walk was statistically significant. Spearman's correlation coefficient was calculated between the average evening air temperature and the number of all living individuals of amphibians observed in a single evening. Only alive individuals were used in the analysis, since the dead ones could have been run-over the night before. Spearman's correlation was chosen, since the data on the number individuals was again not following the normal distribution according to the Shapiro-Wilk test ($W = 0.710$, $p < 0.001$). Using the same data set, a linear regression analysis was conducted between the number of all living individuals of amphibians observed in a single evening and the evening air temperature. In order to obtain the proportion of variance explained, the value of R2 was calculated.

RESULTS AND DISCUSSION

During the census years, four amphibian species were found migrating over the road, specifically the common toad (*Bufo bufo*), common frog (*Rana temporaria*), agile frog (*R. dalmatina*) and Italian crested newt (*Triturus carnifex*) (Tab. 2). *B. bufo* greatly outnumbered the other three species and represented around 99% of individuals of all species of migrating amphibians. Including past observations, there have been five findings of migrating *T. carnifex*, specifically a living female and a dead male in 2018, a dead female in 2020 and two living males in 2021. *T. carnifex* were deliberately searched for in the pond where Anurans spawn, using a single Ortmann's funnel trap, baited with beef (Drechsler & Bock 2010). The trap was moved around the pond during the last week of July in 2020, so as to remain at a single location for one day and one night. Despite the effort newts were never found, until a smaller water reservoir with lush vegetation was discovered west of the pond. Since most individual newts were also found migrating along that path, it is assumed that they spawn in that particular reservoir, rather than in the larger pond (Fig. 1). The reservoir also went dry in the summer of 2024. One of the reasons why newts were not found by the original search, however, might also have been that July is already a bit late in the season for censusing that species (Mazej Grudnik & Triglav Brežnik 2015). In the summer evenings, the European tree frog (*Hyla arborea*) can be heard singing by the pond and the fire salamander (*Salamandra salamandra*) also occurs in the nearby forests.

The study area is evidently important for *B. bufo*, where between 500–1000 individuals migrate over the road every year (Tab. 2). However, our survey does present several limitations. Unfortunately, the counts are not very comparable over the years, since only in 2020 and 2021 the surveys were carried out every evening during the migration and the two days when the census was not conducted in 2024 could have been crucial, since those were the two days when the migration might have reached its peak. In 2019, only the northern road segment was surveyed and the shorter, parallel southern segment was only added in 2020. In 2019, the time of the beginning of the surveys was also variable and only one walk was conducted every evening. Furthermore, the annual fluctuations in amphibian population sizes have to be taken into account. Mavrič et al.

Table 2. Number of individuals of different amphibian species migrating over a road towards the spawning pond during spring migration at Razvanje (NE Slovenia) (according to sex and survival) between study years.
Tabela 2. Število osebkov različnih vrst dvoživk med spomladansko selitvijo čez cesto v Razvanju (SV Slovenija) na poti v ribnik, kjer mrestijo, ločeno glede na spol in glede na to, ali so bili najdeni živi ali povoženi med različnimi leti popisov.

Species	Male	Female	Male Dead	Female Dead	Total
		Year 2019			
B. bufo	790	67	71	9	937
R. temporaria	3	1	0	0	4
R. dalmatina	3	2	0	0	5
T. carnifex	0	0	0	0	0
		Year 2020			
B. bufo	1059	166	66	10	1301
R. temporaria	1	5	0	0	6
R. dalmatina	5	3	0	1	9
T. carnifex	0	0	0	1	1
		Year 2021			
B. bufo	407	97	17	8	529
R. temporaria	2	5	0	0	7
R. dalmatina	2	0	1	0	3
T. carnifex	2	0	0	0	2
		Year 2024			
B. bufo	424	46	31	2	503
R. temporaria	4	4	1	1	10
R. dalmatina	1	0	0	0	1
T. carnifex	0	0	0	0	0

(2019) report that in Ljubljana, *B. bufo* numbers during spring migration follow a three-year cycle pattern. The number of migrating individuals is also most likely an underestimation since, due to the absence of the protective fence, several individuals most likely crossed the road unnoticed. Nevertheless, the results show that numbers of migrating individuals are comparable or even higher than in some well-known road-kill hotspots in Slovenia, such as Zaton-Petanjci (Gorički & Strah 2021), yet species diversity is much higher there than at Razvanje. The reason for such large dominance of *B. bufo*, besides them being explosive breeders, could also have been the presence of fish in the spawning pond, which pose a greater threat to other amphibian species. *B. bufo* tadpoles contain toxins that avert fish predation (Manteifel & Reshetnikov 2002; Bókony et al. 2016).

The sex structure in the population of *B. bufo* shows a large surplus of males over females (between 4–12:1), concordant with observations at other road-kill hotspots (Stanković et al. 2010; Bolčina et al. 2021). Mortality rate of *B. bufo* was not very high (between 5% and 12%), considering no protective fence, which is often implemented at road-kill hotspot surveys (e.g. Bolčina et al. 2021). Relatively low mortality can also be attributed to low amounts of overall traffic and curfew during covid pandemic in 2021. Mortality, however, could still have been reduced by placing the protective fence for amphibians during the time of migration or at least by a traffic sign that would alert the drivers about the presence of amphibians on the road during spring migration.

Migration pattern of *B. bufo* varied between years (Fig. 2). In 2019, migration lasted for 26 days and had two distinctive peaks both during migration towards and from the pond. Next year, it lasted for 21 days with one distinctive peak and another smaller one when individuals were returning. In the following year, the migration was repeatedly interrupted by low temperatures, as the first amphibians were spotted already on the 24th of February and the migration was again interrupted by low temperatures and snow between the 15th and 24th of March. In 2024, the migration lasted for 16 days, however, the peak was most likely missed.

It has been suggested that during migration amphibians show highest activity at dusk, during the first hours of night (Sinsch 1989). With every successive walk in 2020 and 2024, a generally lower number of amphibian individuals were found (Fig. 3), and according to the Kruskal-Wallis Test, the difference was statistically significant ($\chi 2(2) = 13.03$, $p = 0.001$). A post-hoc Dunn's test, performed with a Bonferroni-corrected alpha level of 0.025 revealed a statistically significant difference between the first and the second walks ($Z = 3.40$, $p = 0.0003$), as well as the first and third walks ($Z = 2.13$, $p = 0.017$). Differences between the second and third walks were not statistically significant ($Z = 0.22$, $p = 0.414$). However, as the third walk was carried out only nine times, it is hard to distinguish whether such a result represents a genuine effect or it is just an artefact of a low number of replicates. The reason for the decline in amphibian activity through the night could have been the decline in temperature. According to the Spearman's correlation coefficient, there has been a moderate positive correlation between the air temperature and the number of amphibian individuals, which was statistically significant ($\rho(87) = 0.66$, $p = 2.03*10^{-12}$). Linear regression, however, explained only 23% of the variance in the data ($R^2 = 0.23$) (Fig. 4).

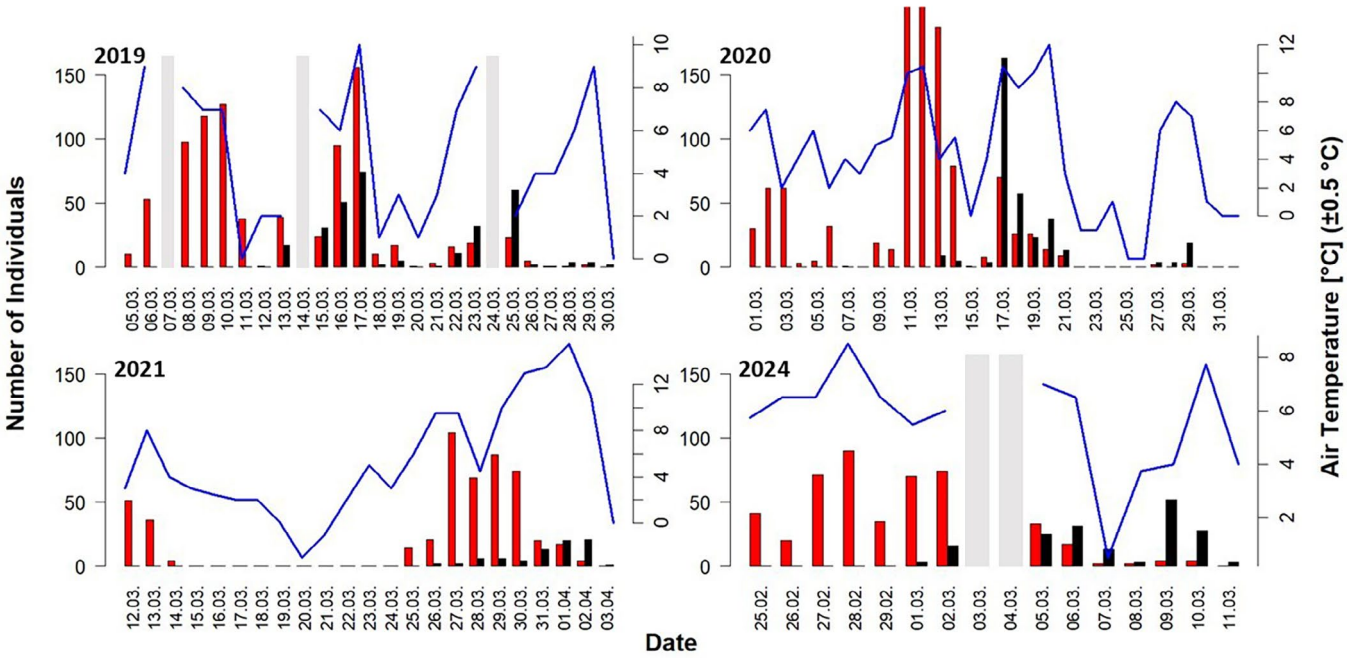

Figure 2. Common toad (*B. bufo*) migration pattern during spring migration over a road at Razvanje (NE Slovenia) in 2019 (top left), 2020 (top right), 2021 (bottom left) and 2024 (bottom right). Blue line represents the air temperature (measured on site, 1 m above ground), red bars are individuals on the way into the spawning pond and black bars represent individuals returning back from the spawning pond. Shaded are days when the survey was not carried out.

Slika 2. Selitveni vzorec navadne krastače (*B. bufo*) med spomladansko selitvijo čez cesto v Razvanju (SV Slovenija) v letih 2019 (zgoraj levo), 2020 (zgoraj desno), 2021 (spodaj levo) in 2024 (spodaj desno). Modra črta ponazarja temperature zraka (merjene na lokaciji, 1 m nad tlemi), rdeči stolpci so osebki na poti v ribnik, ki služi kot mrestišče, črni stolpci pa ponazarjajo osebke na vračanju iz ribnika. Osenčeni so dnevi, ko popis ni bil opravljen.

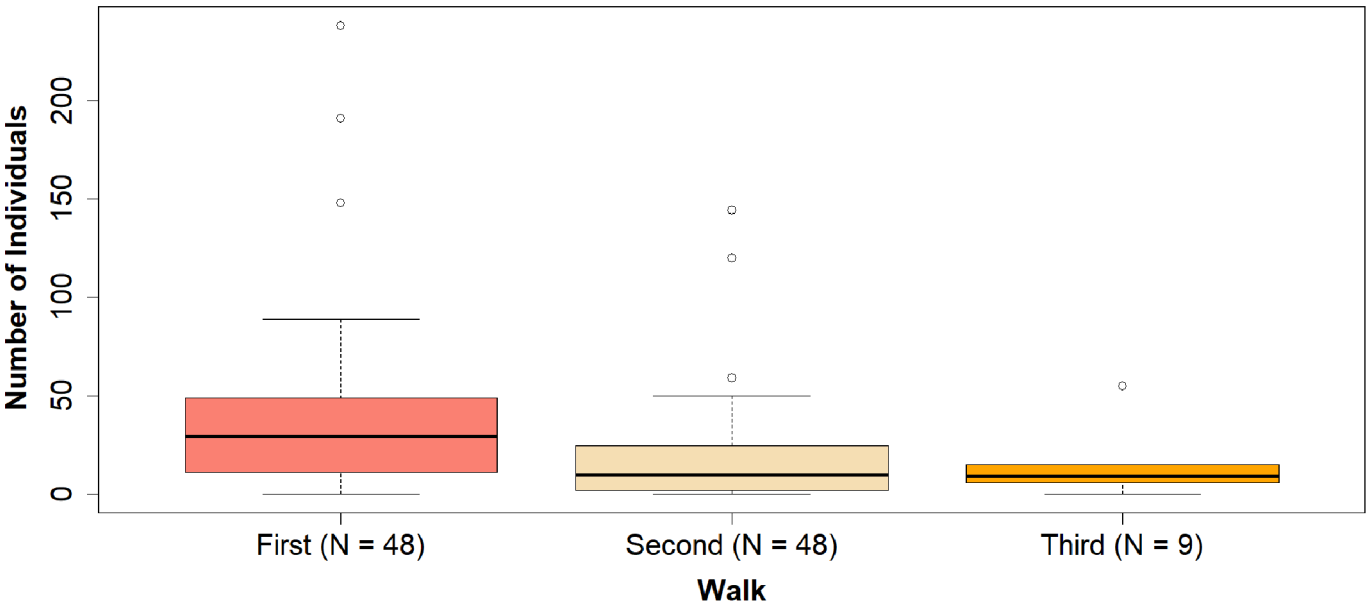

Figure 3. Descriptive statistic of the number of found living individuals of amphibians per consecutive transect walk within a single evening. Line represents the mean, the box is interquartile range, whiskers are maxima and minima and dots are outliers. N = the number of walks included in the data set, out of 69 survey evenings. Only data from evenings in which at least one individual was found were included. Censuses were carried out during spring migration over a road at Razvanje (NE Slovenia) in 2020, 2021 and 2024. Data from censuses from the year 2019 were not included as only one walk was carried out at that time and survey times were not consistent.

Slika 3. Vrednosti opisne statistike števila najdenih živih osebkov dvoživk med zaporednimi obhodi transekta znotraj istih večerov. Vodoravna črta ponazarja povprečje, škatla je medkvartilni razpon in pike so izrazite vrednosti. N = število obhodov, vključenih v set podatkov od skupno 69 popisnih večerov. Vključeni so samo podatki iz večerov, ko je bil najden vsaj en osebek. Popisi so bili opravljeni med spomladansko selitvijo dvoživk čez cesto v Razvanju (SV Slovenija) v letih 2020, 2021 in 2024. Podatki iz popisov iz leta 2019 niso vključeni, ker je bil takrat opravljen le en obhod in čas začetka ni bil konsistenten.

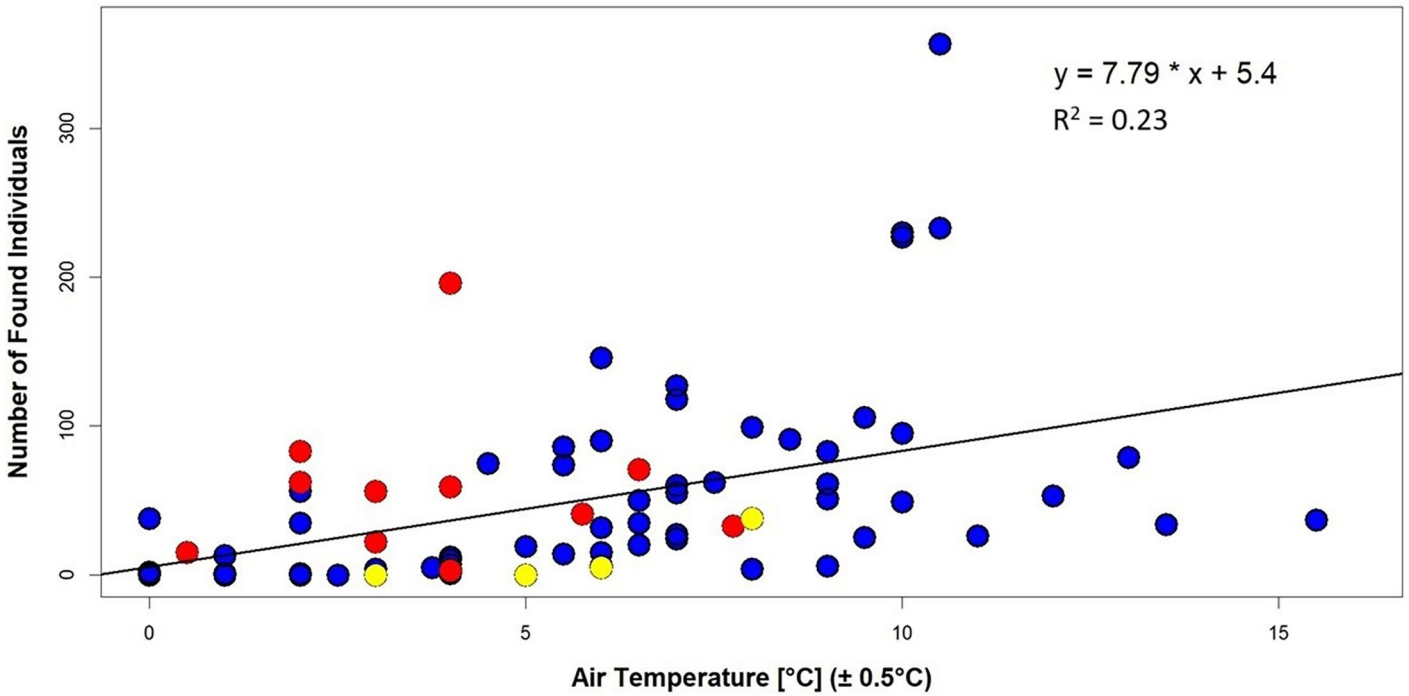

Figure 4. Linear regression of the number of all amphibian individuals found alive during spring migration over a road at Razvanje (NE Slovenia) in 2019, 2020, 2021 and 2024, and the average air temperature (measured on site, 1 m above ground) in a certain survey evening. Red dots represent rainy evenings and yellow dots represent windy evenings.

Slika 4. Linearna regresija med številom vseh živih osebkov dvoživk, najdenih med spomladansko selitvijo čez cesto v Razvanju (SV Slovenija) v letih 2019, 2020, 2021 in 2024, ter povprečno temperaturo zraka (merjeno na lokaciji, 1 m nad tlemi) v določenem popisnem večeru. Rdeče pike ponazarjajo deževne večere, rumene pike pa vetrovne večere.

As a part of the Interreg project »Varstvo dvoživk in netopirjev v regiji Alpe-Jadran«, criteria for classifying road segments as potential road-kill hotspots, have been established. They have been partially adopted from the Austrian Ministry of Transport Infrastructure (RVS 3.04 Amphibienschutz an Straßen) and adjusted to the conditions in Slovenia (Poboljšaj et al. 2018). According to the criteria, the most critical road segments are prioritised according to the number of migrating individuals of amphibians, the number of species and how endangered those are (Poboljšaj et al. 2018). According to the number of individuals, the road segment at Vinogradniška pot belongs into the fourth category (between 1,000 and 3,000) and according to the number of species into the second category (with four species present). In regard to how endangered those species are, the road segment would fall into the first category, since all the species found are listed as vulnerable on the red list (Ur. l. RS 2002). Furthermore, *R. dalmatina* is listed in the Annex IV and *T. carnifex*, is listed both in the Annex II and IV of the Habitats Directive (OJ EC 1992). It has also been suggested that the presence of *T. carnifex* would place the segment into an additional first category, since it is considered one of the most endangered amphibian species in Slovenia (Cipot et al. 2011; Poboljšaj et al. 2018). A road segment is classified as a road-kill hotspot if it belongs into the first category according to any of the three criteria, or if it falls into any of the first four categories according to the number of migrating individuals (Poboljšaj et al. 2018). Thus, the road segment at Vinogradniška pot classifies as a road-kill hotspot. Lastly, the road-kill hotspots that are shorter than 10 km are recognised as the 10% most critical ones (Poboljšaj et al. 2018). The segment at Vinogradniška pot, with its length of about 1.4 km, would also satisfy this criterion.

Our results indicate the importance of monitoring spring migration of amphibians over low-traffic roads, which are often overlooked. Nonetheless, populations of amphibians with significant sizes can be under threat also along low-traffic roads, such as at Razvanje, where between 500–1000 individuals of *B. bufo* migrate to their spawning site every year. Even though the percentage of individuals killed on the road was relatively low, the absolute number of killed individuals is still fairly high, due to the sheer number of migrating individuals; mortality is probably much higher in years when the action is not carried out (due to anti-mortality methodology). The location is also important because of the occurrence of other three amphibian species, which are listed as vulnerable in Slovenia (Ur. l. RS 2002), and especially *T. carnifex*, which is one of the most endangered amphibian species in Slovenia (Cipot et al. 2011; Poboljšaj et al. 2018). The road-kill hotspot satisfies the criteria for a critical road-kill hotspot (Poboljšaj et al. 2018). Unfortunately, no traffic sign has been placed so far at the segment that would alert the drivers of the presence of amphibians during their spring migration.

POVZETEK

Zaradi degradacije in fragmentacije njihovega habitata ima lahko gradnja cestne infrastrukture hude posledice za populacije dvoživk (Rytwinski & Fahring 2012). V Sloveniji je bilo do sedaj registriranih več kot 1500 odsekov cest, kjer prihaja do množičnih povozov dvoživk (t.i. črnih točk) (Strašek 2012), ti pa najhuje prizadenejo populacije navadnih krastač (*Bufo bufo*) in sekulj (*Rana temporaria*) (Poboljšaj et al. 2018). V tem prispevku predstavljamo prve podatke o črni točki na Vinogradniški poti v Razvanju (SV Slovenija), ki po našem vedenju še ni bila registrirana kot črna točka.

Seleče se dvoživke smo prenašali čez cesto in šteli ločeno glede na vrsto in spol na 800 m dolgem odseku na Vinogradniški poti v letih 2019, 2020, 2021 in 2024. Od leta 2020 smo pregledovali še dodaten 600 m dolg vzporedni odsek ceste (Sl. 1). Datumsko smo s popisi začeli, ko so bili na cesti opaženi prvi osebki dvoživk, ter zaključili, ko vsaj tri dni zapored na cesti nismo našli več nobene dvoživke. S popisi smo začenjali vsak večer ob 19.30 in opravili dva obhoda, saj zaščitne ograje nismo uporabljali. V letu 2019 ura začetka ni bila konsistentna in vsak večer je bil opravljen le en obhod. Ob začetku in koncu vsakega popisa smo zabeležili še stanje vremena in temperaturo zraka (merjeno na lokaciji, 1 m nad tlemi). Samce navadnih krastač smo prenašali v ločenem vedru od samic in ampleksusov, krastače pa smo prav tako prenašali ločeno od rjavih žab. Izračunali smo Spearmanov korelacijski koeficient in napovedno moč linearne regresije med povprečno temperaturo zraka in številom najdenih osebkov dvoživk v določenem večeru.

Na lokaciji so se čez cesto selile štiri vrste dvoživk, in sicer navadna krastača (*Bufo bufo*), sekulja (*Rana temporaria*), rosnica (*R. dalmatina*) in veliki pupek (*Triturus carnifex*) (Tab. 2). Najštevilčnejša je bila navadna krastača z okoli 500–1000 osebki, selečimi se čez cesto na leto, kar je pomenilo 99 % osebkov vseh najdenih vrst. Samci so prevladovali v populaciji navadne krastače v razmerju 4–12:1, smrtnost osebkov pa je bila dokaj nizka (5–12 %), kar je delno posledica nizke prometnosti ceste in delno uspeha naših reševalnih akcij. Selitev navadne krastače je trajala med 12 in 24 dni, v letu 2021 pa je bila nekajkrat prekinjena zaradi nizkih temperatur (Sl. 2). Leta 2020 smo občasno opravili tudi tretji obhod, a je bilo število najdenih osebkov dvoživk glede na Kruskal-Wallisov test z vsakim obhodom statistično značilno nižje ($\chi 2(2) = 9{,}22$, $p = 0{,}010$) (Sl. 3). Ugotovljena je bila statistično značilna zmerna pozitivna korelacija med številom najdenih osebkov in povprečno temperaturo zraka ($\rho(87) = 0{,}63$, $p = 3{,}62*10^{-11}$). Napovedna moč linearne regresije je bila 23 % ($R^2 = 0{,}23$) (Sl. 4). Odsek ceste na Vinogradniški poti v Razvanju ustreza kriterijem za t.i. »kritično črno točko«, rezultati pa tako poudarijo pomen takšnih monitoringov tudi na manj prometnih cestah, ki so v podobnih raziskavah pogosto spregledane.

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SUPPLEMENTARY MATERIAL

Table S1. Individual amphibian survey dates per study year during amphibian spring migration over a road in Razvanje (NE Slovenia). The table is available as an online supplement at https://doi.org/10.14720/ns.19547.

Tabela S1. Posamezni datumi popisov dvoživk med spomladansko selitvijo čez cesto v Razvanju (SV Slovenija), glede na popisno leto. Tabela je dostopna kot spletni suplement na naslovu https://doi.org/10.14720/ns.19547.