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Last meal: food composition of road-killed *Lacerta viridis* (Reptilia: Lacertidae) from Romania

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Abstract. Food composition of road-killed *Lacerta viridis* was studied on 41 individuals from the scientific collection of the University of Oradea, Romania. They were collected from different roads in the country between 1998-2019. All lizards had stomach contents. Although in most cases the stomach contents were partially digested, the preys could be identified at a taxonomical level comparable with the one achieved in other lizard feeding studies. The analysed *L. viridis* individuals had consumed 275 preys, which belong to 25 taxa, as well as inorganic elements and vegetal remains. The most important preys were Orthoptera, Araneidae and Coleoptera. The differences in lizards` food composition according to geographic origin were not significant. Our results confirm that this species food composition can be studied on road killed individuals, but also proves the uniformity of its food, at least in the Balkan Peninsula.

Keywords: road ecology, feeding, European green lizard, region, invertebrates, human impact.

Introduction

Nowadays, road mortality affects countless animals, including lizards (e.g. Meek, 2009; Tok *et al.,* 2011; Mollov *et al.,* 2013; D'Amico *et al.,* 2015; Covaciu-Marcov *et al.,* 2017). The European green lizard *Lacerta viridis* (Laurenti, 1768) is a species of a relatively large size (Fuhn and-Vancea, 1961). In Europe, it is distributed in the south-eastern part of the continent (Sillero *et al.,* 2014). Similarly to many other lizard species, *L. viridis* is frequently killed by cars on roads in different regions of its distribution range, but the number of road-killed individuals is generally low (e.g. Tok *et al.,* 2011; Kambourova-Ivanova *et al.,* 2012; Cicort-Lucaciu *et al.,* 2016; Rassati, 2016; Ciolan *et al.,* 2017). Analysing the food composition of road killed lizards in a relatively good condition had started many years ago (Angelici *et al.,* 1997). Road killed amphibians were also used in feeding studies (Kolenda *et al.,* 2019). Recent data confirms that road mortality studies and the victims of this anthropogenic impact can provide numerous useful information (see in: Schwartz *et al.,* 2020). Also, recent studies confirm the importance of museum preserved individuals even for clarifying the status of extinct taxa, with an unclear status for a long time (e.g. Kehlmaier *et al.,* 2020). The food composition of *L. viridis* was studied in the past using preserved individuals from museum (Sagonas *et al.,* 2018) or university collections (Mollov *et al.,* 2012). Other reptiles from different collections were also analysed from this point of view (e.g. Castilla *et al.,* 1991; Mollov, 2010; Mollov and Petrova, 2013; Sagonas *et al.,* 2015). The feeding ecology of *L. viridis* is still not studied comprehensively (e.g. Korsós, 1984; Mollov *et al.,* 2012; Sagonas *et al.,* 2018). In Romania, to our knowledge, there is only old information on this subject in a volume of the Romanian Fauna referring to reptiles (Fuhn and Vancea, 1961). However, data do exist about lizards` diet, but for other species (e.g. Castilla *et al.,* 1991; Hodar *et al.,* 1996; Angelici *et al.,* 1997; Gvoždík and Boukal, 1998; Sagonas *et al.,* 2015). Starting from the information presented above, we presumed that *L. viridis* individuals killed by vehicles in Romania can offer information about the species` feeding traits. Since in the last several years some road-killed *L. viridis* individuals were collected for educational purposes and were stored in the collection of the Department of Biology, Faculty of Informatics and Sciences, University of Oradea, we could use them in this study. Therefore, the objectives of this study were: **1.** to establish the food composition of the species *L. viridis* killed by cars in Romania, **2.** to observe if there are different food consumption patterns of this species in different areas of the country.

Material and methods

We analysed the stomach content of 41 *L. viridis* specimens, collected between 1998-2019. Initially, the lizards were collected without any intention related to feeding study, but for educational purposes, and they were road-killed specimens identified by chance in different areas of Romania. However, in recent years, as we initiated studies on road mortality (Cicort-Lucaciu *et al.,* 2012, 2016; Ciolan *et al.,* 2017; Covaciu-Marcov *et al.,* 2017; Teodor *et al.,* 2019; Ile *et al.,* 2020) and because of the multiple uses of road traffic victims have started to appear (e.g. Kolenda *et al.,* 2019; Schwartz *et al.,* 2020), we paid more attention to these corpses. The lizards come from 12 counties of Romania (Bihor, Arad, Satu-Mare, Alba, Hunedoara, Caraș-Severin, Mehedinți, Gorj, Dolj, Ialomița, Tulcea, Constanța), including the western, central (Transylvania) and southern part (including Dobruja). Seven individuals collected long time ago for educational purposes did not have data about the region of origin. The rest of 34 lizards had labels with the collecting date, locality and county. The specimens were preserved in alcohol and they are deposited in the scientific collection of the Department of Biology, Faculty of Informatics and Sciences, University of Oradea.

The preserved lizards were dissected, and their stomach was extracted. Although we also tried to analyse the intestinal and cloacal content, the preys were too digested to be determined. The stomachs were dissected in a Petri dish, and the preys were identified using a stereomicroscope, as in other cases (e.g. Angelici *et al.,* 1997; Mollov, 2010; Sagonas *et al.,* 2018). Preys were determined to higher taxonomic level (family, order, etc), as many of them were in an advanced degree of digestion. This assignment to taxonomic groups was also used in other studies on lizards` feeding pattern (e.g. Castilla *et al.,* 1991; Angelici *et al.,* 1997; Mollov *et al.,* 2012; Sagonas *et al.,* 2015, 2018). The data has been processed both for the total and according to the region of origin (northwest, southwest and southeast). We calculated the average number of preys/specimen, percentage abundance and frequency of occurrence for each taxa. The prey diversity was calculated with the Shannon index (Shannon, 1948), the homogeneity with the Pielou index (Pielou, 1966). The significance of differences between the feeding of lizards from the three zones was calculated with help of the Kruskal Wallis test. The calculations were made in Microsoft Office Excel and Past software (Hammer *et al.,* 2001).

Results

None of the lizards were with empty stomachs. The 41 individuals of *L. viridis* had consumed 275 preys in total, from 25 invertebrate taxa (Tab. 1). The average number of prey / individual was 6.70, the number of preys consumed by an individual varied between 1 and 17. Besides preys of animal origin, in the stomachs of the lizards we found vegetal fragments and inorganic particles (Tab. 1). The largest percentage abundance had Orthoptera (19.27%) and Araneida (18.90%). Among Orthopterans, alongside plenty grasshoppers and locusts in a very advanced digestion stage, we identified one *Gryllotalpa* sp. The third place by percentage abundance was occupied by undetermined Coleoptera (15.27%). But if we take the Coleoptera families altogether, their percentage exceeds 25%, thus, this taxon becomes the most favoured by lizards. By frequency, the first place is occupied by Araneida, who were consumed by 68.29% of lizards, followed by Coleoptera and Orthoptera, both with a frequency of 60.97%. Vegetal fragments were found in 70.73% and inorganic elements in 17.07% of the stomachs.

Table 1. Percentage abundance, frequency of occurrence, Shannon diversity and Pielou homogeneity of prey taxa consumed by *L. viridis* in Romania (NW – northwestern Romania, SW – southwestern Romania, SE – southeastern Romania, * includes both individuals from the three geographic regions and the seven specimens which could not be assigned to any region).

	Percentage abundance (%)				**Frequency of occurrence (%)**			
	NW	**SW**	**SE**	**Total***	**NW**	**SW**	**SE**	**Total***
Vegetal fragments					80.00	69.23	50.00	70.73
Inorganic elements					6.66	30.76	16.66	17.07
Gastropoda	-	2.27	-	0.72	-	15.38	-	4.87
Isopoda	-	-	6.45	2.18	-	-	16.66	7.31
Diplopoda	2.10	1.13	-	1.09	13.33	7.69	-	7.31
Araneida	20.00	22.72	9.67	18.90	73.33	76.92	33.33	68.29
Odonata	-	-	3.22	0.36	-	-	16.66	2.43
Dermaptera	-	-	-	0.36	-	-	-	2.43
Orthoptera	22.10	12.50	12.90	19.27	66.66	53.84	50.00	60.97
Homoptera	2.10	-	-	0.72	13.33	-	-	4.87
Heteroptera	6.31	5.68	16.12	7.63	26.66	30.76	50.00	34.14
Coleoptera, Silfidae	-	-	-	0.36	-	-	-	2.43
Coleoptera, Scarabeidae	-	-	-	1.09	-	-	-	4.87
Coleoptera, Cicindelidae	1.05	-	-	0.36	6.66	-	-	2.43
Coleoptera, Curculioniae	-	-	3.22	1.09	-	-	16.66	4.87
Coleoptera, Carabidae	4.21	2.27	3.22	3.27	20.00	15.38	16.66	19.51
Coleoptera, Chrysomelidae	1.05	-	-	0.36	6.66	-	-	2.43
Coleoptera, Cerambicidae	3.15	3.40	-	2.54	6.66	23.07	-	12.19
Coleoptera, Elateridae	1.05	2.27	-	1.09	6.66	15.38	-	7.31
Coleoptera (larvae)	1.05	-	-	0.36	6.66	-	-	2.43
Coleoptera (undeterm.)	10.52	20.45	16.12	15.27	53.33	61.53	66.66	60.97
Lepidoptera	1.05	1.13	-	1.09	6.66	7.69	-	7.31
Lepidoptera (larvae)	11.57	6.81	12.90	8.00	40.00	15.38	50.00	29.26
Diptera, Brahicera	2.10	7.95	9.67	4.36	13.33	30.76	50.00	21.95
Diptera, Nematocera	1.05	3.40	3.22	1.81	6.66	7.69	16.66	7.31
Hymenoptera, Formicidae	8.42	7.95	3.22	7.27	40.00	23.07	16.66	31.70
Hymenoptera (undeterm.)	1.05	-	-	0.36	6.66	-	-	2.43
Shannon diversity	**2.34**	**2.26**	**2.29**					
Pielou homogeneity	**0.81**	**0.85**	**0.92**					

The food diversity was H=2.46, and the homogeneity J'=0.76. From the 34 individuals which could be assigned to a geographical region, the majority came from north-western Romania, then from the south-western- and only a small number from the south-eastern part of the country (Tab. 1). The number of preys and the number of consumed taxa varied from region to region. The largest number of preys was consumed in south-western Romania, and the largest number of taxa was consumed in north-western Romania (Tab. 1), but, the number of taxa / lizards was high in the south-eastern region. Differences between regions were observed also in the vegetal and inorganic elements consumption. Thus, vegetal remains were ingested more frequently by lizards from the north-western region, and the inorganic elements by lizards from the south-western region (Tab. 1). In each region, Orthoptera and Coleoptera registered high percentage abundance, but the taxa percentage abundance varied (Tab. 1). In the case of frequency of occurrence, also Orthoptera and Coleoptera were consumed in each region by at least 50% of the lizards. Nevertheless, in the north-western and south-western regions, spiders registered a frequency of occurrence above 70% (Tab. 1). The highest food diversity was registered in the north-western region and the lowest in the south-western region, where also the number of lizards was small (Tab. 1). The differences of the food consumed by *L. viridis* between the three studied regions were not significant (p=0.11).

Discussions

The European green lizards killed by cars in Romania can be used to establish the food composition of this species, like in the case of some related lizard (e.g. Angelici *et al.,* 1997), or snakes species (e.g. Daltry *et al.,* 1998; Brito, 2004). Because the analyzed lizards were not collected for food composition studies, generally their preservation was not done immediately. However, their stomach contents, although relatively digested, could be determined at least to a taxonomic level comparable with other lizard food composition studies (e.g. Korsós, 1984; Angelici *et al.,* 1997; Mollov *et al.,* 2012; Sagonas *et al.,* 2015, 2018). Nevertheless, the stomach contents level of digestion probably depends less on the time between collecting and preservation of lizards and more on the time elapsed between killing and collecting. Even in the case of lizards from museum or university collections, the taxonomic level of prey identification was not more detailed (e.g. Mollov *et al.,* 2012; Sagonas *et al.,* 2015, 2018). The number of road killed *L. viridis* individuals observed in the last years was far higher than the one analysed here, because most lizards were repeatedly crushed by cars, and thus could not be used for studies. For example, in some road mortality studies (Cicort-Lucaciu *et al.,* 2016; Ciolan *et al.,* 2017) all road killed *L. viridis* found were too crushed by cars to be analysed. Probably, the road killed individuals in good condition were hit only once. In some individuals the head was squashed, as in other species (Jackson and Lemn, 2009). Monitoring roads surrounded by favourable habitats for this species would be useful from this perspective. At least on this way, road-kills could offer information on the ecology of some species, moreover because the intensity of this anthropogenic impact is increasing (e.g. Philcox *et al.,* 1999; Kazemi *et al.,* 2016; Hill *et al.,* 2020).

The 41 road-killed European green lizards gave us information about their last meal. Their food consisted entirely from invertebrates, although some large sized Lacertidae also feed on small vertebrates (e.g. Busack and Visnaw, 1989; Castilla *et al.,* 1991; Angelici *et al.,* 1997; Christopoulos *et al.,* 2020). Generally, the food of this species consisted in invertebrates, while the consumed taxa are almost the same (e.g. Mollov *et al.,* 2012; Sagonas *et al.,* 2018). However, because in the case of Coleoptera we generally managed to determine the family, the number of prey taxa and the food diversity were higher than in other cases (Sagonas *et al.,* 2018). At least two important prey taxa for the European green lizards from Romania (Coleoptera and Orthoptera) were also important in Greece and Bulgaria, although in those cases Araneida had lower percentage abundance (Mollov *et al.,* 2012; Sagonas *et al.,* 2018). The number of preys/individual was higher than in other cases (Mollov *et al.,* 2012). As well as in Romania, vegetal elements were found in the stomachs of the green lizards in other regions too (Sagonas *et al.,* 2018). This indicates that the species diet does not differ much from region to region, at least in the Balkan Peninsula, *L. viridis* mainly eating the available insects. Coleoptera had the largest number of species on the planet (e.g. Radu and Radu, 1967; Nielsen and Mound, 1999), thus the fact that they were consumed in large number was not surprising, as they are important prey for other lizards too (e.g. Castilla *et al.,* 1991; Pérrez-Melado *et al.,* 1991; Burke and Mercurio, 2002; Sagonas *et al.,* 2015). Orthoptera are insects that prefer dryer steppe areas (e.g. Radu and Radu, 1967), and *L. viridis* also likes a dryer substratum with vegetation, mainly composed of shrubbery and bushes (e.g. Fuhn and Vancea, 1961; Korsós, 1984; Strijbosch *et al.,* 1989; Heltai *et al.,* 2015), but also move frequently in grassy areas for feeding (Arnold, 1987). Thus, the contact between predator and prey is facilitated, a fact that explains the high consumption of Orthoptera by *L. viridis*. At the same time, the large size of the European green lizards facilitates the consumption of Orthoptera, as in the case of large sized amphibians (Cicort-Lucaciu *et al.,* 2013). Nevertheless, there are some differences in this species feeding between different areas. Thus, sometimes inorganic elements were not recorded (Sagonas *et al.,* 2018), although in Romania they were relatively frequently consumed.

Although *L. viridis* seems to be more trophic specialized than *L. agilis*, in Hungary it also consumes a large number of Coleoptera, even if caterpillars were its main food (Korsós, 1984). Caterpillars were also consumed in Romania, but in a lower percentage abundance and frequency of occurrence, although they are slow prey, which can be easily captured by lizards. Even if it seems that *L. viridis* adults avoid eating small prey like ants (Sagonas *et al.,* 2018), we found ants only in the stomachs of large lizards. *L. viridis* individuals that feed on ants consumed inorganic elements too (pebbles). Probably inorganic elements were ingested by accident, because *L.viridis* is a lizard that only uses its jaws to catch the prey-(e.g. Bels and Goosse, 1990; Urbani and Bels, 1995). Thus, it is difficult for lizards to separate the ants from the substratum on which they were captured. Unlike this, locusts were probably captured from the vegetation, not with the substratum. The consumption of inorganic elements was also registered in other lizard (e.g. Roth, 1971; Castilla *et al.,* 1991) and amphibian species diet (e.g. Cicort-Lucaciu *et al.,* 2011, 2013). The consumption of vegetal remains by *L. viridis* seems to be recorded only recently (Sagonas *et al.,* 2018), albeit in other species it was well-known (e.g. Busack and Visnaw, 1989; Castilla *et al.,* 1991; Hodar *et al.,* 1996; Sagonas *et al.,* 2015). Although it seems that *L. viridis* juveniles consume vegetal elements intentionally (Sagonas *et al.,* 2018), it is possible that they are ingested accidentally, just like the inorganic elements, together with the prey, as it was previously indicated in amphibians (e.g. Mollov, 2008; Cicort-Lucaciu *et al.,* 2013).

The food composition of *L. viridis* is alike the one consumed by other large lizards, like *L. trilineata* (Mollov and Petrova, 2013; Sagonas *et al.,* 2015), which consume even vegetal elements (Sagonas *et al.,* 2015). Also, in Italy *L. bilineata* consumed numerous Coleoptera, but in that case there were some differences, because the percentage abundance of Isopoda was higher (Angelici *et al.,* 1997) than in Romania. This is probably a consequence of a different region and period, because in some cases some amphibians, for example, consume a large number of isopods (e.g. Covaciu-Marcov *et al.,* 2012; Çiçek *et al.,* 2017; Pafilis *et al.,* 2019). Probably the different period affects the percentage of Orthoptera too, as the previous study was done between March and July (Angelici *et al.,* 1997), and most of our lizards were collected in summer and the beginning of autumn. In a smaller sized lizard (*L. agilis*), even if the study was made in summer, the percentage abundance of Orthoptera was low, but Coleoptera registered a higher percentage (Gvoždík and Boukal, 1998). However, in autumn, *Lacerta lepida* from Spain feed almost only on Orthoptera and Coleoptera (Castilla *et al.,* 1991), although this species generally consumes numerous Coleoptera (Busack and Visnaw, 1989; Castilla *et al.,* 1991). Even smaller lizards consumed predominantly Coleoptera (Pérrez-Melado *et al.,* 1991). Most preys consumed by *L. viridis* were common taxa, well represented in their habitats. Beside the prey taxa, in the stomachs of two lizards we found eight nematodes. Probably they were parasites, because this species is known to be infested by different types of Nematodes (Yildirimhan *et al.,* 2020).

Although in other cases there were seasonal variations in lizards feeding (e.g. Castilla *et al.,* 1991; Sagonas *et al.,* 2015, 2018), we analysed only few specimens, almost all collected in summer and early autumn, thus we did not register seasonal variations of feeding. As well as other related species, like *L. trilineata* (Sagonas *et al.,* 2015), *L. viridis* seems to change its food composition depending on habitats, fact indicated by the slight differences between the three regions of origin. In the case of another lizard species, *L. agilis*, it seems that specimens from a southern, warmer area are more active than those situated in more northern areas with less warm days (Pačuta *et al.,* 2018). Such differences probably exist between the three regions of Romania, but they are too small to modify noticeably the feeding pattern of *L. viridis*.

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