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Research article

Diet composition of the lizard *Lacerta viridis* (Laurenti, 1768) (Reptilia: Lacertidae) in Bulgaria confirm its generalistic feeding behaviour

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Abstract: The eastern green lizard (*Lacerta viridis*) is a mostly insectivorous species, based on multiple studies from across its range. However, for Bulgaria the published data of such kind are limited to five publications. We investigated faecal samples from a total of 60 individuals of free-ranging *L. viridis* obtained from two localities in western Bulgaria. Our aim was to clarify the diet regarding its taxonomic composition, as well as some physical characteristics of the prey like hardness and evasiveness. For one of the study sites we compared the realised trophic niche (prey items from the faecal pellets) with the fundamental niche (invertebrates collected via pit-fall traps exposure). In our results, two invertebrate taxa had the largest share (both in frequency and abundance) in the food spectrum of *L. viridis:* Araneae and Coleoptera), respectively 21.2% and 17.6%. Regarding specific prey selectivity, analysis showed that Lepidoptera are most preferred (E* = 0.68), and Formicidae – most avoided prey items (E* = -0.79). We registered several cases of saurophagy (in four samples) and keratophagy (in two samples), two types of dietary items, which have not been reported for *L. viridis* so far.

Keywords: Balkan Peninsula, diet, keratophagy, Sauria, saurophagy

Introduction

The family Lacertidae represents the most diverse group among the lizards in Europe. Generally, lacertids feed on a wide variety of arthropods, mostly insects, and could be considered generalist predators (Arnold, 1987; Carretero, 2004).

The eastern green lizard *Lacerta viridis* (Laurenti, 1768) is relatively large in size [it can reach up to 150 mm SVL (see Vacheva et al., 2022)] and shaping the distribution and habitat use of many of the other smaller lizard species (Maura et al., 2011; Kovács & Kiss, 2016). *L. viridis* occurs in central and southern Europe (from eastern Germany and central Poland in the north, to southern Greece in the south, and from central Austria in the west, to eastern Ukraine in the east), as well as in northern Asia Minor (Ananjeva et al., 2006; Sillero et al., 2014). In Bulgaria, *L. viridis* is widespread from the sea level up to ca. 1200, and in some places up to 1600–1800 m a.s.l. (Stojanov et al., 2011). It inhabits open landscapes or meadows with sparse bush vegetation and forest edges, but also areas densely covered with bushes or sparse forests (Stojanov et al., 2011; Vacheva et al., 2020). *Lacerta viridis* is considered as a mostly insectivorous species and there are several studies on its diet from different parts of its range (e.g. Korsós, 1984; Arnold 1987; Sagonas et al., 2018). In Bulgaria, the diet of the species has been studied on the basis of stomach content by Angelov et al. (1966, 1972), Donev (1984), Donev et al. (2005), and Mollov et al. (2012). Taken together, these five publications give a fairly good idea of the *L. viridis* diet in Bulgaria, but it should be noted that they are based on material collected more than 40 years ago; furthermore, they do not provide comparisons of the food spectrum with the potential food resource in the habitats.

The main purpose of the present work was to collect up-to-date data on the feeding of *Lacerta viridis* in two regions of Bulgaria using a non-invasive

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method, as well as a comparison between consumed prey and the available food resources.

Material and methods

Field work was conducted in two sites, situated in western Bulgaria, as follows: (1) the area of Gabrovitsa Village, Sredna Gora Mts (N42.2602°, E23.9208°, 430–570 m a.s.l.; the site was visited periodically in the spring (April–June) and summer (July–September) of 2017–2018) (a total of 25 field days); (2) the eastern shore of the Ogosta Reservoir, Predbalkan Mts (N43.3739°, E23.2086°, 180–240 m a.s.l.; the site was visited sporadically in 2013–2016) (28 field days in total). Detailed descriptions of the studied sites are provided by Vacheva et al. (2020). In both sites, the lizards were captured by hand, measured [snout-vent length (SVL) to the nearest 1 mm via transparent ruler] and then placed individually in plastic boxes and kept in the laboratory until defecation (up to ca. 48 hours); individuals were then released at the place of capture. Age class was determined based on size, external morphology and colouration. We considered adult SVL to be > 85 mm according to Tzankov (2007) and juveniles and subadults grouped in a single category (immatures), which were <84.9 mm. The faecal pellets thus obtained were preserved in Eppendorf tubes with 75% ethanol. In Gabrovitsa, we assessed the potential prey availability for *L. viridis* by placing 24 pit-fall traps for collecting invertebrates. The pit-fall traps (plastic containers, 9.5 cm wide and 12 cm deep, filled with propylene glycol) were situated in different microhabitats (four series of six traps, 10 metres apart) and were exposed for a total of 79 days (2017: 23 days in spring (May and June) and 16 in summer (August and September); 2018: 17 and 23 respectively). Collected material was preserved in 75% ethanol.

Fixed material was examined under a stereomicroscope with a magnification of 10–40×. Both prey remains from the faecal pellets and invertebrates from the pit-fall traps were identified to the lowest possible systematic level and grouped into “operational taxonomic units” (hereafter OTU/OTUs). The OTUs from the faecal pellets were also categorised regarding their evasiveness [sedentary (E1), intermediate (E2), and evasive (E3)] and hardness [soft (H1), intermediate (H2), and hard (H3)] according to Verwaijen et al. (2002) and Vanhooydonck et al. (2007).

Correlation between abundance and frequency of the prey items, found in the faecal pellets, was described and tested via Spearman’s rank correlation coefficient (Rho). Chi-square ($\chi 2$) test was used for a comparison between adults and immatures regarding the categories of evasiveness and hardness of the prey items. Statistical procedures were performed using PAST 4.07 (Hammer et al., 2001). Prey selection (for the samples from Gabrovitsa) was analysed on the basis of a comparison between relative abundance of the OTUs in the faecal pellets and in the pit-fall traps using the electivity index (E*) of Vanderploeg & Scavia (1979). The index represents a modification of the Ivlev’s forage ratio, but has better theoretical justification (Lechowicz, 1982). It takes values from -1 to +1 and can be explained as a measure of deviation from random feeding (E* = 0). In this study, the index values were interpreted as follows: E* > 0.5 (preferred OTUs); 0.5 ≥ E* ≥ -0.5 (neutral OTUs); E* < -0.5 (avoided OTUs). The OTUs represented by low relative abundance (< 0.2%) were excluded, because according to Lechowicz (1982) the index is vulnerable to sampling errors for food types that are rare.

Results

A total of 60 faecal samples from *Lacerta viridis* were collected: respectively 49 from Gabrovitsa [15 from adult (SVL > 85 mm) and 34 from immature lizards (SVL<84.9 mm)] and 11 from Ogosta (from immature lizards only). The remains of invertebrates in the faecal pellets were attributed to 278 individuals belonging to 18 OTUs (respectively 238/14 for the sample from Gabrovitsa and 40/10 for those from Ogosta; Supplementary material 01: Appendix 1). The average number of (individual) invertebrate remains per the individual faecal pellets was 4.63 (from Sample from Gabrovitsa between 1 and 14, mean4.86 and from Ogosta between 1 and 11, mean 3.64).

In the samples from Gabrovitsa, four of the OTUs (Coleoptera, Araneae, Auchenorrhyncha, and Orthoptera) were found in above 30% of the faecal pellets and three of them (Araneae, Coleoptera and Auchenorrhyncha) also prevailed in number of individual remains (over 10%). Divided the lizards into age categories (although samples are not equal in number), in the adults the predominant OTU (both in frequency and in abundance; respectively 60.00 and 30.88%) was the order Coleoptera, while in the im-

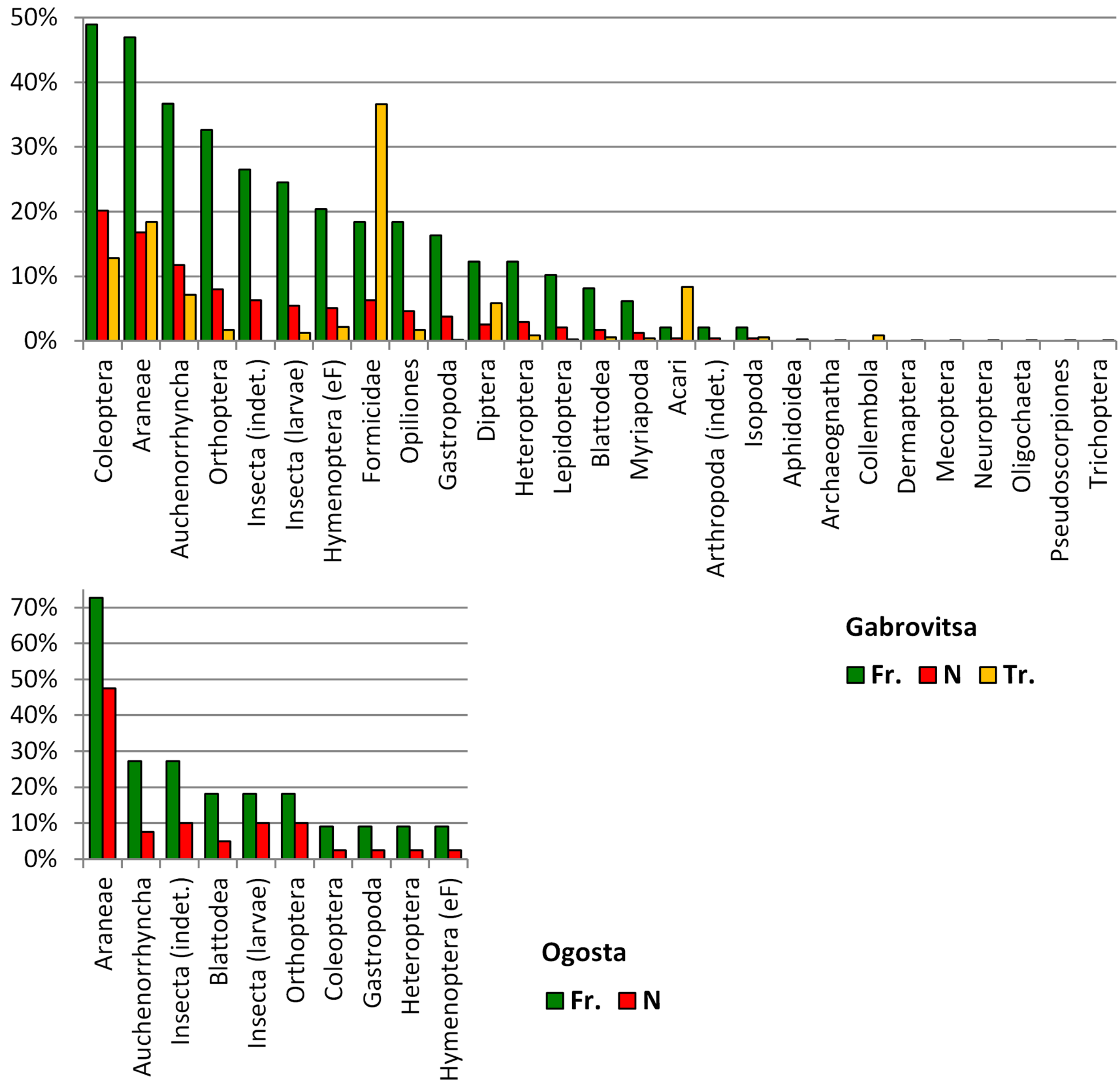

Fig. 1. Percentage share of the OTUs according to: number of faecal samples of *L. viridis* in which the OTU was found (Fr.); number of specimens registered in the faecal samples (N); number of specimens, collected by pit-fall traps (Tr.).

matures it was the order Araneae (67.65 and 23.53% respectively). In the samples from Ogosta, the only predominant OTU (both in frequency and abundance) was Araneae (Fig. 1), but it should be kept in mind that this sample consisted only of immature lizards. Correlation between the abundance and frequency of occurrence of OTUs in the faecal pellets was positive with a high level of significance in both samples (Gabrovitsa: Rho = 0.987, $p < 0.001$; Ogosta: Rho = 0.912, $p = 0.008$). The invertebrates collected by the pit-fall traps from Gabrovitsa were attributed to 25 OTUs, with Formicidae and Araneae being the most abundant (Fig. 1; Supplementary material 01: Appendix 2). According to the electivity index values (Table 1) three of the OTUs were categorised as preferred prey of *L. viridis* (Lepidoptera, Orthoptera, and insect larvae), two as avoided (Formicidae and Diptera), and the rest as neutral.

Table 1. Electivity index values (E*) for the sample from Gabrovitsa; symbols [>], [=], and [>] denote preferred, neutral, and avoided prey, respectively.

OTU	E*
Araneae	-0.23 [=]
Auchenorrhyncha	0.06 [=]
Blattodea	0.35 [=]
Coleoptera	0.04 [=]
Diptera	-0.54 [<]
Formicidae	-0.79 [<]
Heteroptera	0.43 [=]
Hymenoptera (eF)	0.23 [=]
Insecta (larvae)	0.51 [>]
Lepidoptera	0.68 [>]
Myriapoda	0.35 [=]
Opiliones	0.31 [=]
Orthoptera	0.53 [>]

Regarding the evasiveness of the prey, in both study sites the sedentary prey predominated in the faecal samples (Fig. 2). In terms of hardness, hard prey items predominated in Gabrovitsa, and soft – in Ogosta (Fig. 2). When dividing the sample from Gabrovitsa into adults and immatures, the remains of prey with a medium degree of evasiveness and high degree of hardness predominated in the adults (respectively 50.00 and 67.19% of the identified invertebrates), while in the immatures the remains of low evasiveness and low hardness prevailed (respectively 51.90 and 48.73%). Differences between adults and immatures in this regard were statistically significant (evasiveness: $\chi2 = 12.131$, df = 2, $p = 0.0023$; hardness: $\chi2 = 15.286$, df = 2, $p = 0.0005$).

Besides invertebrates, remains of lizard body parts (an evidence for expression of saurophagy) and lizard shed skin parts (i.e. expression of keratophagy) were also found in the faecal pellets of *L. viridis* from Gabrovitsa. Saurophagy was recorded in 4 immature individuals: remains of *Ablepharus kitaibelii* Bibron & Bory de Saint-Vincent, 1833 in the faecal sample of a juvenile *L. viridis* (SVL = 36 mm) and remains of lacertid lizards (undefinable to species level) in the faecal pellets of 3 subadults. Keratophagy was observed in two immatures (subadult females). Plant remnants were observed in the faecal pellets of 6 immature and 3 adult individuals.

Discussion

Our results confirmed that *Lacerta viridis* is an active predator, whose diet includes a wide variety of invertebrates. Observed feeding activity (4.63 ingested invertebrate individuals on average) was higher than in previous studies of the species, where the given values ranged between 3.2 and 4.2 (Angelov et al., 1966, 1972; Donev, 1984; Donev et al., 2005; Sagonas et al., 2018). According to our results, the order Araneae and order Coleoptera have the largest share in the

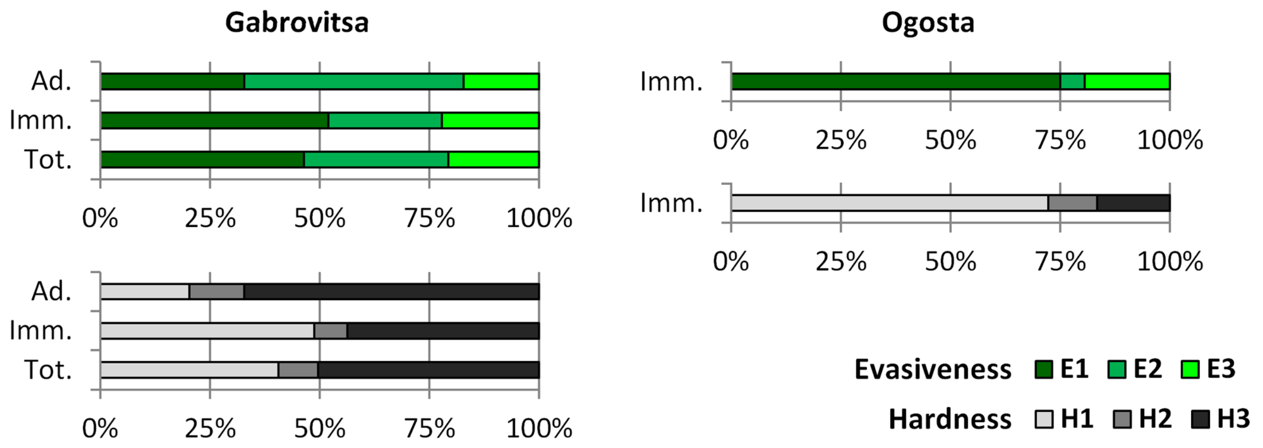

Fig. 2. Percentage share of the categories of evasiveness and hardness according to the number of categorised prey items from the faecal samples of *L. viridis* (Ad. = adults; Imm. = immatures; Tot. = the entire sample).

food spectrum of *L. viridis*. The main role of Coleoptera as prey of *L. viridis* has been highlighted in other studies (Angelov et al., 1966, 1972; Shcherbak & Shcherban, 1980; Donev, 1984; Donev et al., 2005; Sagonas et al., 2018). However, there are also studies according to which Lepidoptera larvae (Korsós, 1984) or Orhtoptera (Mollov et al., 2012) have the largest share in the diet of the species, but in both cases the second place falls to Coleoptera. Order Araneae have not been identified as a major component in the diet of *L. viridis,* while in our case exactly the Araneae were consumed the most both in frequency and in numbers (summarised for both samples). This is most likely due to the fact that our sample consisted mainly of immature lizards, whereas most similar studies seem to have been based mainly on adults.

The diet of young *L. viridis* differs from that of adults, with juveniles preferring softer and smaller prey (Sagonas et al., 2018). Our data also showed a predominance of soft (and sedentary) prey in immature as opposed to adult lizards. To a large extent, this is determined by the amount of spider remains in the faecal pellets: Araneae was the most abundant and frequent OTU in immatures, but completely absent in adults. Differences in diet between adult and young lacertids may result from morphological differences, i.e. the smaller size of the immatures, especially in terms of head size, determines the smaller bite force (Herrel & O'Reilly, 2006; Urošević et al., 2013). Ontogenetic differences in feeding undoubtedly depend also on a number of ecological factors, e.g. microhabitat choice, thermal preferences, etc. (Herczeg et al., 2007).

The comparison between consumed prey and available food resources (done for the sample from Gabrovitsa) showed that at least two of the OTUs are subjects to active selection (positive or negative) by *L. viridis:* Lepidoptera, as preferred prey, and Formicidae, as avoided prey. Such kind of analysis (using electivity indices) has not been done regarding the feeding of *L. viridis* (at least to our knowledge), so we cannot compare our data, but it may serve as a basis for more detailed studies in this direction.

In addition to habitual feeding on invertebrates, our results also indicated feeding behaviours that seem to have not been recorded in *Lacerta viridis* so far: saurophagy and keratophagy. Cases of saurophagy are known for some species of *Lacerta,* but they have only been observed in adults (Angelici et al., 1997; Christopoulos et al., 2020). In our case evidence for this phenomenon were found only in immatures. It is unclear whether the latter is due to the significant difference in sample size between adults and immatures (15:34 for Gabrovitsa) or reflects a real difference between age groups in feeding behaviour. Keratophagy has been found in a number of lizard species (Mitchell et al., 2006), but specifically for *Lacerta,* it is known only for *L. agilis* Linnaeus, 1758 (Gvozdik, 1997). We registered the manifestation of keratophagy in only two individuals (4% of the sample from Gabrovitsa), therefore it can be assumed that in *L. viridis* this phenomenon occurs rarely. Plant remains were recorded by us in 18% of the examined faecal pellets from Gabrovitsa, both in adult and immature lizards (in contrast to Sagonas et al. 2018, who recorded plant remains only in adult *L. viridis*). The origin of these plant parts is not clear; it is possible that they were accidentally ingested simultaneously with other food components or contained in the stomachs of ingested invertebrates. On the other hand, the presence of plant components in lacertids diet is not uncommon, especially in large species, as an addition to their basic food (Van Damme, 1999; Carretero, 2004; Sagonas et al., 2015; 2018). It should be noted that no evidence of saurophagy and keratophagy or ingestion of plant material was found by us from Ogosta, which could be explained by the significantly smaller size of this sample (11 individual faecal pellets from Ogosta vs 49 from Gabrovitsa).

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Supplementary materials

01
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