



RESEARCH ARTICLE

Functional Ecology



A trait-based framework for understanding predator–prey relationships: Trait matching between a specialist snake and its insect prey

Edvárd Mizsei^{1,2} | Zoltán Boros³ | Ádám Lovas-Kiss¹ | Csaba Szepesváry^{1,4} |
Márton Szabolcs^{1,4} | Gergő Rák⁵ | János Ujszegi⁶ | Zoltán Gál⁷ |
Szabolcs Lengyel^{1,4} | Gellért Puskás⁸

¹Department of Tisza River Research, Danube Research Institute, Centre for Ecological Research, Hungarian Academy of Sciences, Debrecen, Hungary;

²Department of Ecology, University of Debrecen, Debrecen, Hungary; ³Bio Aqua Pro Ltd., Debrecen, Hungary; ⁴GINOP Sustainable Ecosystems Group, Centre for Ecological Research, Hungarian Academy of Sciences, Tihany, Hungary; ⁵Department of Ecology, University of Veterinary Medicine, Budapest, Hungary;

⁶Lendület Evolutionary Ecology Research Group, Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary; ⁷NARIC, Agricultural Biotechnology Institute, Gödöllő, Hungary and ⁸Department of Zoology, Hungarian Natural History Museum, Budapest, Hungary

Correspondence

Ádám Lovas-Kiss

Email: lovas-kiss.adam@okologia.mta.hu

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Abstract

1. High-quality information on predator–prey relationships is fundamental in understanding food webs, community assembly and ecosystem functioning. Recent analytical advances have made it possible to develop new trait-based approaches to study trophic relationships and evaluate trait matching between predators and prey.
2. Here, we develop a novel analytical approach based on generalized linear mixed-effects models (GLMM) to test the importance of prey availability and to identify the set of prey traits that best explain the occurrence and number of prey in the predator's diet.
3. We demonstrate that the approach by using an extensive dataset on prey availability, prey traits and gut content collected in all known populations of *Vipera graeca*, a little-known, endangered snake of alpine grasslands in the Pindos Mountains of the Balkan Peninsula.
4. We show that *V. graeca* is a unique, venomous snake specialized on bush-crickets and grasshoppers (Orthoptera). Prey selection GLMMs showed that the ideal prey of *V. graeca* is abundant, large-bodied, has poor escape abilities (flightless, slow-moving and bad jumper) and prefers loose grasslands (as opposed to bare ground/rock or closed sward). Vipers restrict their feeding to periods of high Orthoptera abundance in the late summer and need to reach a certain body size to become able to catch large-sized prey.
5. Our analytical approach provides a framework for trait matching between predators and prey and unprecedented fine-scale information on the importance of prey traits in prey selection by a specialist predator. The narrow trophic niche of *V. graeca* likely increases the vulnerability of this cold-adapted snake to extinction.

Lengyel and Puskás contributed equally to this work.

KEYWORDSentomophagy, feeding ecology, grassland, insectivory, trait data, trophic network, *Vipera ursinii*, Viperidae**1 | INTRODUCTION**

Understanding predator–prey relationships is fundamental in several areas of ecology such as trophic networks (Christensen et al., 2014), community assembly and organization (Lavorel et al., 2013; Van der Putten, Macel, & Visser, 2010), ecosystem functioning and stability (Gravel, Albouy, & Thuiller, 2016), evolutionary biology (Yoshida et al., 2007) and conservation (Tylianakis, Laliberté, Nielsen, & Bascompte, 2010). Predator–prey relationships traditionally have been interpreted purely based on taxonomy, that is, which species consumes which species. However, the recent surge of interest and advances in trait-based functional approaches in a variety of fields require a shift of interest to a better understanding of the functional links between predator traits and prey traits (Weigel & Bonsdorff, 2018). Such trait matching (Brousseau, Gravel, & Handa, 2017) has at least three benefits. First, it helps to better predict species interactions which are prohibitively difficult to observe in nature. Second, a deeper knowledge of such functional links contributes to niche theory as it helps our understanding of specialization based on the predators' morphological and behavioural traits. Finally, understanding predator trait–prey trait relationships will enable us to better predict community-level changes in trophic relationships due to climate change (Brousseau et al., 2017).

Although prey trait analysis has been around for a while (e.g. Sánchez-Hernández, Servía, Vieira-Lanero, & Cobo, 2013), this approach mainly concentrated on body size or mass, which has long been known as fundamental in predator–prey interactions (Brose, 2010; Brose et al., 2005; Gravel, Poisot, Albouy, Velez, & Mouillot, 2013). A refocusing of interest, however, will need to extend to functional traits beyond body size/mass such as those related to defence against predators (Eitzinger, Rall, Traugott, & Scheu, 2018). This approach has already been pursued in several empirical studies in aquatic ecosystems (copepods: Kalinoski & DeLong, 2016; fishes: Sánchez-Hernández & Cobo, 2015; fish-zoobenthos: Worischka, Schmidt, Hellmann, & Winkelmann, 2015; Weigel & Bonsdorff, 2018). In terrestrial ecosystems, Brousseau et al. (2017) explored trait matching in a feeding experiment with ground beetles and their prey. Morphological trait matching between resource and consumer species has also been recently applied to study functional relationships between plants with fleshy fruits and frugivorous birds (Bender et al., 2018). For such trait-based approaches, fine-scale information on trait relationships between predators and prey is necessary that help us to understand which predator traits are associated with which prey traits and whether and how trait–trait relationships vary in strength. Such fine-scale information is now possible to obtain as there is an increasing number and depth of (a) databases on predator and prey traits (e.g. Brose et al., 2005), (b) methods in diet tracing

(Nielsen, Clare, Hayden, Brett, & Kratina, 2018) and (c) analytical tools such as multivariate generalized linear mixed-effect models that allow the analysis of non-normally distributed, overdispersed data on prey abundances that are typical in studies of diet or gut content (Wang, Naumann, Wright, & Warton, 2012).

Specialist predators offer an important starting point for developing trait-based approaches because trait matching is probably simpler when traits on either side of the relationship vary little. Snakes (suborder Serpentes) are exclusively predatory reptiles that show great variation in prey specialization (Thomas & Pough, 1979). A likely key innovation in the evolution of the Macrostromatan lineage of snakes is their increased gape, which allows them to feed on large prey, in contrast to lizards and basal snakes (Vincent, Dang, Herrel, & Kley, 2006). Consequently, head size is expected to correlate strongly with prey body size (Glaudas et al., 2019). Snakes represent a significant proportion of total biomass and are fundamental both as predators and as prey in many ecosystems, yet are often underrepresented in studies of trophic ecology (Luiselli, 2008). The foraging mode of snakes ranges from ambush predatory to active searcher strategies (Glaudas et al., 2019; Luiselli, 2006; Schwenk, 2000) and several snakes inject toxins to paralyse and kill prey and to assist digestion. Most snakes consume vertebrates, although there are extreme cases of specialization on other prey such as fish eggs (Voris, 1966). Snake diet often varies by season and age, with juveniles and adults preferring prey of different sizes (Brito, 2004; Greene, 1983; Luiselli, 1996; Shine, 1994). For example, juveniles often consume invertebrates, while adults in most species prefer vertebrates. Specialization on terrestrial arthropods has been reported in only 1.5% of the ca. 3,700 snake species (Table 1). Usually, the main arthropod prey is only known to order level and little is known about finer-scale specialization (Table 1). More generally, we know little on whether and how traits other than body size influence prey selection in snakes or other predators.

The aim of this study was to explore predator–prey relationships at the trait level by evaluating the importance of predator traits and prey traits in prey selection. We developed a novel analytical framework based on generalized linear mixed-effects models to test the importance of prey availability and prey traits in explaining the occurrence and number of prey in the predator's diet. We demonstrate the approach by using detailed natural history-based field data from a predator–prey system involving *Vipera graeca*, a rare snake species as predator and bush-crickets and grasshoppers (Orthoptera) as prey in alpine meadows of the Balkan Peninsula in Southeast Europe. We used an extensive, fine-scale dataset assembled from gut content sampling in all known predator populations, from sampling of prey availability in all study sites and from detailed laboratory measurements of prey traits. We specifically addressed four questions:

TABLE 1 List of snake species known to consume terrestrial arthropods and proportion and taxonomic identity (to the lowest level reported) of arthropods in their diet. Species with diets in which the proportion of arthropods exceeds 95% are highlighted in bold

Family	Species	Number of prey items	Arthropod % of Diet	Main arthropod prey	References
Anomalepididae	<i>Liotyphlops ternetzii</i>	^a	^a	Isoptera	Franca, Mesquita, Nogueira, and Araújo (2008)
Colubridae	<i>Gyalopion canum</i>	34	100.0	Araneae	Parga (2018)
	<i>Opheodrys a. aestivus</i>	14	100.0	Lepidoptera larvae	Baldwin (2007)
	<i>Opheodrys aestivus</i>	^a	100.0	Orthoptera	Thomas (2014)
	<i>Opheodrys vernalis</i>	21	100.0	Lepidoptera larvae	Baldwin (2007)
	<i>Symphimus mayae</i>	84	100.0	Orthoptera	Stafford (2005)
	<i>Tantilla coronata</i>	222	100.0	Chilopoda	Todd, Willson, Winne, Semlitsch and Gibbons (2008)
	<i>Tantilla hobartsmithi</i>	19	100.0	Araneae	Parga (2018)
	<i>Tantilla melanocephala</i>	129	100.0	Chilopoda	Marques and Puerto (1998)
	<i>Sonora semiannulata</i>	49	97.9	Araneae	Parga (2018)
	<i>Eirenis modestus</i>	41	97.6	Coleoptera	Cicek and Mermer (2007)
	<i>Opheodrys aestivus</i>	443	97.5	Lepidoptera larvae	Plummer (1981)
	<i>Tantilla nigriceps</i>	23	95.6	Araneae	Parga (2018)
	<i>Coluber constrictor mormon</i>	267	91.4	Orthoptera	Shewchuk and Austin (2001)
	<i>Tantilla gracilis</i>	152	85.5	'Larvae'	Cobb (2004)
	<i>Coluber constrictor</i>	96	60.0	Orthoptera	Thomas (2014)
	<i>Coronella girondica</i>	65	26.2	Chilopoda	Luiselli, Pleguezuelos, Capula, and Villafranca (2001)
	<i>Natriciteres variegata</i>	35	17.1	'Arthropods'	Akani and Luiselli (1999)
	<i>Thelotornis capensi</i>	56	8.9	'Insect remains'	Shine, Harlow, Branch and Webb (1996)
	<i>Hierophis viridiflavus</i>	52	7.7	Orthoptera	Rugiero and Luiselli (1995)
	<i>Natriciteres fuliginoides</i>	28	7.1	Chilopoda/ Arachnida (1-1)	Akani and Luiselli (1999)
	<i>Philodryas patagoniensis</i>	92	4.3	Orthoptera	Soledad López and Giraudo (2008)
	<i>Heterodon simus</i>	27	3.7	Hemiptera	Beane, Graham, Thorp, and Pusser (2014)
	<i>Psammophis phillipsii</i>	174	1.7	Mantodea	Luiselli et al. (2004)
	<i>Dendrelaphis punctulata</i>	75	1.3	Gryllidae	Shine (1991)
	<i>Coluber constrictor foxii</i>	^a	^a	'Invertebrates'	Lennon (2013)
	<i>Eirenis coronella</i>	5	^a	Araneae	Shwayat, Disi, and Amr (2009)
<i>Eirenis decemlineata</i>	6	^a	Orthoptera	Shwayat et al. (2009)	
<i>Eirenis lineomaculata</i>	1	^a	Araneae	Shwayat et al. (2009)	
<i>Eirenis rothi</i>	2	^a	Chilopoda	Shwayat et al. (2009)	
<i>Oligodon cinereus</i>	^a	^a	Orthoptera	Meggitt (1931)	
Dipsadidae	<i>Pseudablades agassizii</i>	25	96.0	Araneae	Marques, Sawaya, Stender-Oliveira, and Franca (2006)
Elapidae	<i>Drysdalia coronoides</i>	32	3.1	Cicadoidea	Shine (1981)
	<i>Echiopsis curta</i>	54	1.9	Blattodea	Shine (1982)
Lamprophiidae	<i>Psammophis schokari</i>	29	6.9	Coleoptera	Cottone and Bauer (2009)
	<i>Psammophis phillipsi</i>	120	1.7	Mantodea	Akani, Eniang, Ekpo, Angelici, and Luiselli (2003)
Leptotyphlopidae	<i>Leptotyphlops fuliginosus</i>	^a	^a	'Insectivor'	Franca et al. (2008)

(Continues)

TABLE 1 (Continued)

Family	Species	Number of prey items	Arthropod % of Diet	Main arthropod prey	References
Viperidae	<i>Vipera graeca</i>	356	100.0	Orthoptera	this study
	<i>Vipera ursinii ursinii</i>	626	99.7	Orthoptera	Baron (1992)
	<i>Vipera renardi</i>	^a	98.2	Orthoptera	Kovalyenko (1952)
	<i>Vipera renardi</i>	423	91.2	Orthoptera	Fomina (1965)
	<i>Vipera ursinii ursinii</i>	104	88.5	Orthoptera	Agrimi and Luiselli (1992)
	<i>Echis carinatus</i>	17	64.7	Scorpiones	Barlow, Pook, Harrison, and Wüster(2009)
	<i>Echis pyramidum</i>	60	56.7	Scorpiones	Barlow et al. (2009)
	<i>Echis ocellatus</i>	35	42.9	Chilopoda	Barlow et al. (2009)
	<i>Agkistrodon contortrix</i>	101	34.6	Lepidoptera larvae	Garton and Dimmick (1969)
	<i>Macrovipera schweizeri</i>	12	33.3	Coleoptera	Adamopoulou, Valakos and Anastasios (1997)
	<i>Sistrurus miliarius</i>	20	20.0	Centipedes	Hamilton and Pollack (1955)
	<i>Agkistrodon piscivorus</i>	81	14.8	Coleoptera	Vincent, Herrel and Irschick (2004)
	<i>Bothrops neuwiedi pauloensis</i>	69	14.5	Chilopoda	Valdujo, Nogueira and Tartins (2002)
	<i>Echis coloratus</i>	40	7.5	Scorpiones	Barlow et al. (2009)
	<i>Crotalus enyo</i>	63	6.3	Scolopendridae	Taylor Emily (2001)
	<i>Vipera ammodytes</i>	64	6.3	'Insects'	Dilian and Raichev (2009)
	<i>Vipera latastei</i>	179	4.5	Scolopendridae	Santos et al. (2007)
	<i>Bothrops pubescens</i>	80	2.5	Chilopoda	Hartmann, Hartmann, Cechin, and Martins (2005)
	<i>Trimeresurus stejnegeri</i>	105	1.9	Orthoptera	Creer, Chou, Malhotra and Thorpe (2002)
	<i>Calloselasma rhodostoma</i>	177	1.7	'Arthropods'	Daltry, Wolfgang and Thorpe (1998)
	<i>Bothrops moojeni</i>	144	0.7	Chilopoda	Nogueira, Sawaya and Martins (2003)
	<i>Cerrophidion tzotzilorum</i>	^a	^a	'Arthropods'	Jadin (2007)
<i>Gloydium rubromaculatus</i>	^a	^a	Noctuidae	Shi et al. (2017)	
<i>Vipera anatolica anatolica</i>	4	^a	Orthoptera	Zinenko et al. (2016)	
<i>Vipera anatolica senliki</i>	^a	^a	Chilopoda, Diplopoda	Göçmen, Mebert, Karış, Oğuz and Ursenbacher(2017)	

^aNumber of prey item and/or % arthropod prey in diet not reported.

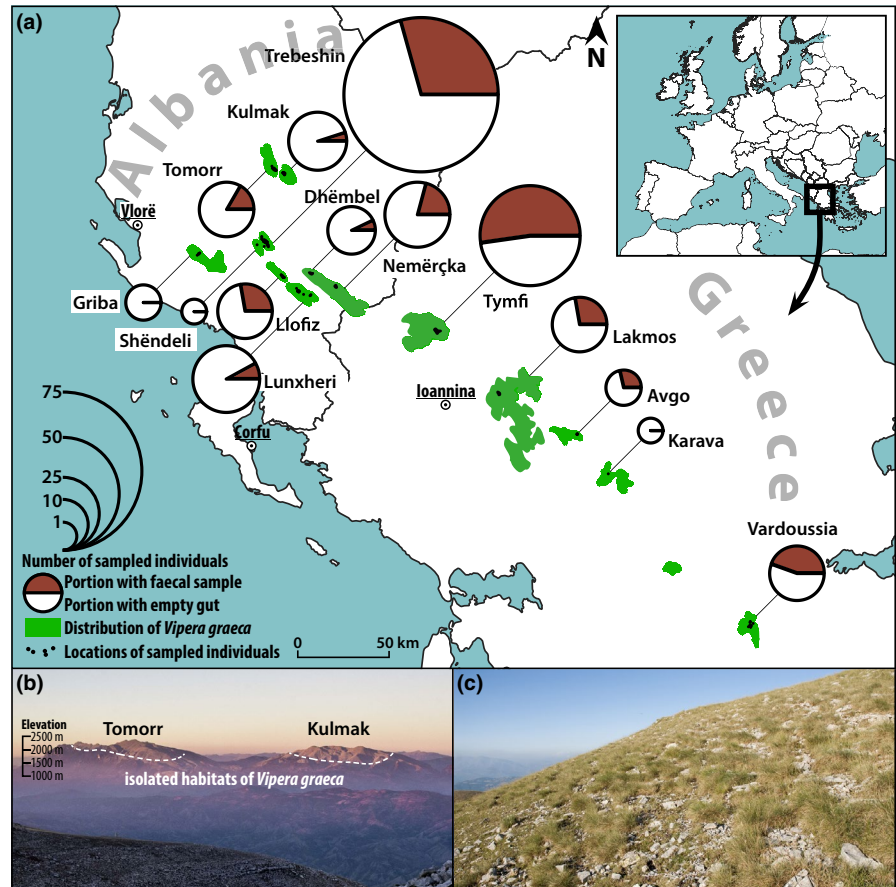
(a) Are there temporal differences in feeding activity throughout the annual cycle? (b) Are there spatial differences in prey selection between populations inhabiting different mountain ranges? (c) Which prey traits influence prey selection? and (d) Do predator traits play a role in prey selection? We hypothesized temporal patterns in feeding activity because snakes are known to time their feeding activity to periods when their prey becomes abundant (Šukalo et al., 2014). Spatial patterns were analysed to explore differences in prey species composition in the diet between predator populations and to identify which prey species explain these differences. We addressed question 3 by testing the hypothesis that prey selection will be related to prey availability and/or prey traits such as the ability of prey to escape from predation. Finally, we hypothesized that prey size selection is constrained by predator morphological traits such as body length or gape size.

2 | MATERIALS AND METHODS

2.1 | Study species

The Greek Meadow Viper, *Vipera graeca* (Nilson & Andrén, 1988), is a poorly known cold-adapted snake living in alpine meadows between 1,600 and 2,200 m above sea level in the Pindos mountain range in Albania and Greece (Mizsei et al., 2016). Originally described as a subspecies, molecular studies confirmed the lineage as basal to the entire complex (Ferchaud et al., 2012; Nilson & Andrén, 2001) and was subsequently elevated to species level (Mizsei, Jablonski, Roussos, et al., 2017a). *Vipera graeca* is listed as endangered in the IUCN Red List due to its small and severely fragmented distribution, ongoing habitat degradation (mostly by overgrazing), mortality from intentional killing by shepherds and vulnerability to climate change

FIGURE 1 Distribution of *Vipera graeca* and location of samples (a), example for habitats (b) and typical vegetation of habitat (c)



(Mizsei, Szabolcs, Dimaki, Roussos, & Ioannidis, 2018; Mizsei, Szabolcs, et al., 2019).

2.2 | Study area and faecal sample collection

We characterized the diet of *V. graeca* by visual analysis of faecal samples collected from individuals in all known populations of the species in Albania and Greece between 2013 and 2018 (Figure 1). Although new techniques for diet tracing (stable isotopes, fatty acid analysis and DNS-based methods) have advanced considerably recently, visual analysis has several advantages such as the simplicity of sample collection, storage and processing, and the possibility of obtaining information on the species identity, life stage, age or sex of prey and the number and relative abundance of prey consumed, thus still widely used in trophic studies and monitoring programs (Nielsen et al., 2018). In each population, we intensively searched for snakes (total sampling effort c. 5,700 person-days) during the vipers' active season ranging from April to September (Mizsei et al., 2016). The captured individuals were kept in separate textile bags for 2–4 days (min. 1 day, max. 5 days) to allow them to produce faecal samples. Most of the captured individuals, however, defecated while we were handling them to measure body size variables (e.g. snout-vent length, head width and photography for scale counts). Before releasing the snakes, we carefully investigated individuals that did not defecate by abdominal palpation to confirm the absence of any gut content; these individuals were classified in the 'empty gut' group.

After measurements, we released all individuals at the exact site of their capture. Faecal samples were stored in 96% ethanol.

2.3 | Prey availability and prey traits

We expected a diet dominated by Orthoptera (crickets, grasshoppers and locusts) for *V. graeca*, because its sister lineages in the *V. ursinii-renardi* complex are well known Orthoptera consumers (Baron, 1992; Filippi & Luiselli, 2004; Table 1). To characterize prey availability in the habitat of each study population, we sampled the Orthoptera community in all sites where vipers were captured. Sampling was conducted by standardized sweep-netting in 5 × 5-metre quadrats and was supplemented by manual collection for 15 min in each site. Orthoptera community samples were stored in 96% ethanol until processing. For identification, we used the keys of Harz (1969, 1975), Ramme (1951), Willemse (1985) and Willemse, Helversen and Odé (2009) and the comparative material of the Hungarian Natural History Museum. To quantify prey availability, we calculated the relative abundance of specimens in three age-sex groups: adult males, females and nymphs for each Orthoptera species.

We used the Orthoptera community reference material to measure prey traits relevant in predator–prey relationships. We photographed every Orthoptera specimen collected in the prey availability material from a lateral view, which is a likely view of the prey by the predator and which also allows the measurement of several important prey traits on the photographs, with a Nikon

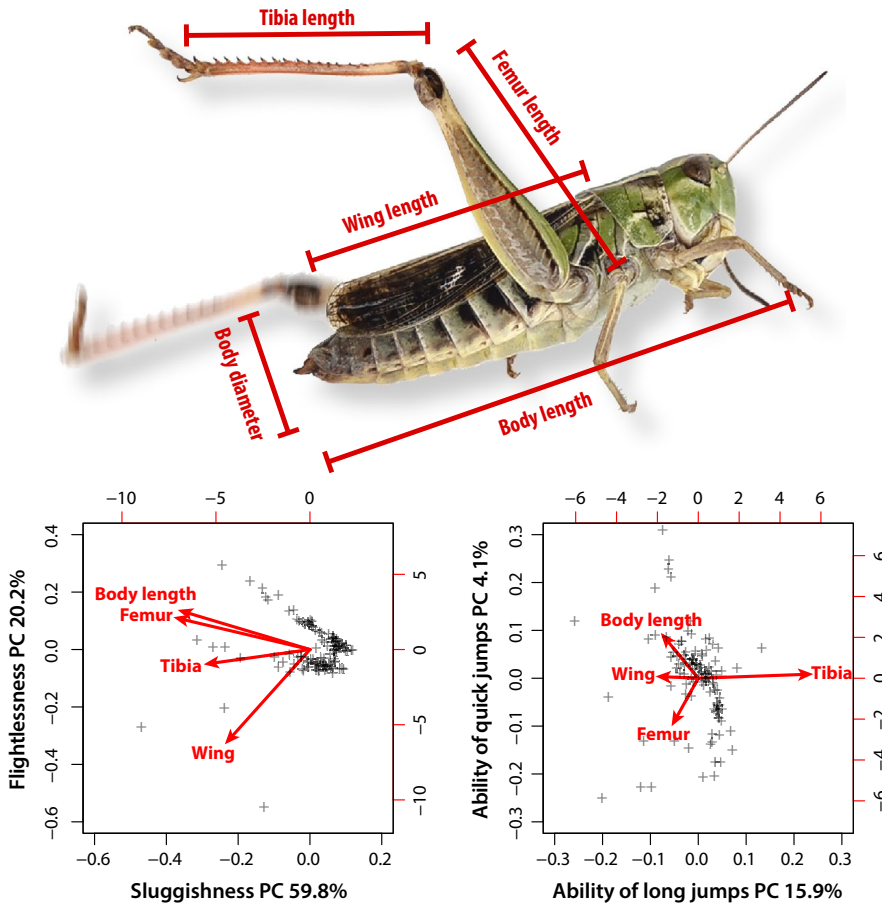


FIGURE 2 Measured traits, biplots of trait principal components and their explained variance

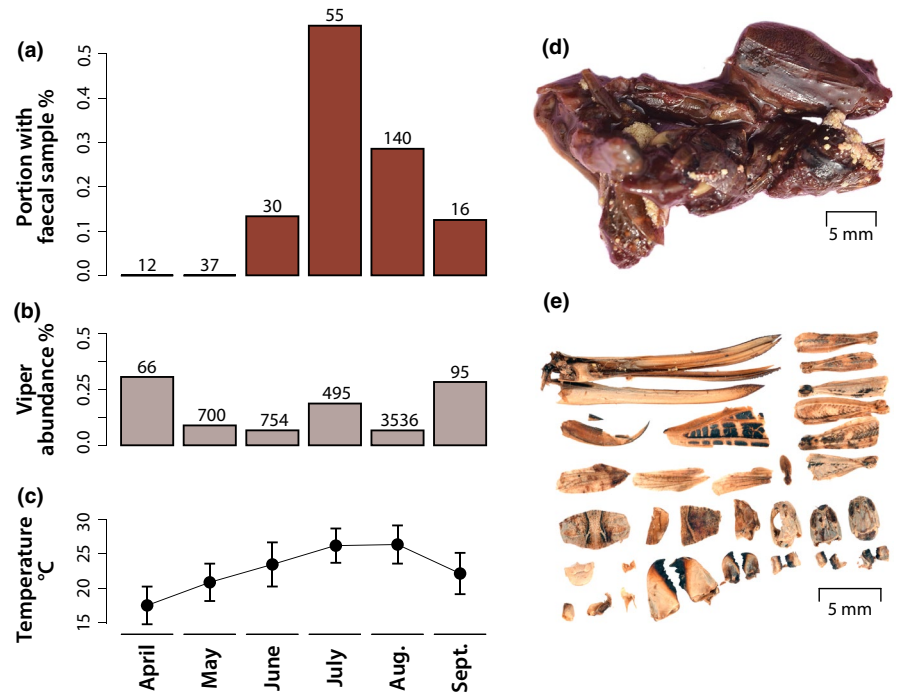
D600 DSLR and a Micro-Nikkor 55 mm f2.8 lens. To characterize prey body size, we measured the area of visible surface (AVS) on the photographs using the LeafArea package in R (Katabuchi, 2017) (accuracy $\pm 0.0001 \text{ mm}^2$) for each individual and averaged them for each age/sex category of each species. To estimate the ability of Orthoptera specimens to escape from predator attack, we measured (a) total body length, (b) wing length, (c) femur length and (d) tibia length of the hind leg on the photographs and averaged the measurements for each age/sex category of each species (Figure 2). We reduced these body size variables into four non-correlating principal components and interpreted them based on their correlations with the original variables (Figure 2). We interpreted PC1 as 'sluggishness' because it was negatively correlated with all four body size variables related to escape behaviour and PC2 as 'flightlessness' as it negatively correlated with wing length (Figure 2). Considering that longer legs allow longer jumps, whereas shorter legs allow quicker jumps in bush-crickets (Burrows & Morris, 2003), we interpreted PC3 as 'ability of long jumps' because it positively correlated with tibia length and PC4 as 'ability of quick jumps' as it negatively correlated with femur length (Figure 2). We characterized the microhabitat preferences of Orthoptera species by classifying them into categories based on Rácz (1998) and Stevaev & Nikitina (1976). This system consists of three main types (chortobiont: species of closed swards that are influenced mainly by vegetation microclimate, geobiont: species of bare ground or rock surfaces influenced mainly by ground microclimate and thamnobiont: species of high dry, shrubby or woody

vegetation) and four transitional types (chorto-thamnobiont: species found both in closed swards and in high shrubby/woody vegetation; geo-chortobiont: primarily ground-dwelling species that also occur in vegetation; geo-psammo-chortobiont: primarily ground-dwelling species specialized on sandy surfaces and thamno-geobiont: primarily vegetation-dwelling species that also occur on bare ground or rock surfaces). Finally, to characterize body size of prey that can be swallowed by snakes (question 4), we also measured the maximum body diameter of Orthopterans and calculated averages for each age/sex category of each species.

2.4 | Faecal sample processing and identification

We processed the faecal samples under a stereomicroscope to find and identify all remains of potential prey specimens (Figure 3). Visual examination of the faecal samples allows the detection of bones and teeth of vertebrates, non-digestible hair of mammals, feathers of birds, scales of reptiles, chitinised cuticles of arthropods and other body parts (e.g. earthworm bristles) and usually also allows species-level identification of prey items (e.g. Angelici, Luiselli, & Rugiero, 1997, Shine, Harlow, Keogh, & Boeadi, 1998 and Pérez-Mellado, Pérez-Cembranos, Garrido, Luiselli, & Corti, 2011). We used the Orthoptera community samples as a reference in the identification of partly digested gut content material to the lowest taxonomic level possible, following the methodology described by Luiselli and Amori (2016). We counted the number of prey by species in each faecal

FIGURE 3 Temporal pattern of feeding activity of *V. graeca* per month with the number of examined individuals above the bars (a), effort-corrected relative abundance of individuals per month with temporal sampling effort in person-days (b), monthly mean (\pm SD) temperatures at meteorological stations nearest to the study sites (<25 km) in the study years (2010–2018) from the Global Surface Summary of the Day database (Sparks, Hengl, & Nelson, 2017) (c), examples for faeces (d) and prey remains found in a faecal sample (e)



sample, and if the condition of the digested prey allowed, we determined the age (nymph/adult) and sex of the prey specimens.

2.5 | Dataset and statistical analyses

We evaluated the temporal pattern in feeding activity throughout the annual cycle (question 1) by building Generalized Linear Mixed Models (glmm) with binomial error distribution using the lme4 package (Bates, Mächler, Bolker, & Walker 2014) in R. The binary dependent variable was presence/absence of any gut content of a viper individual, while the fixed factors were Julian date (number of days after Jan 1 in each year), sex and snout-vent length of vipers, and sampling site was a random factor to control for the spatial nonindependence of the observations.

To assess spatial differences in prey selection (species composition of the diet) between populations (question 2), we prepared a dataset of abundances of Orthoptera species in the diet, with abundance pooled across ages and sexes by species. We analysed differences in species composition of the diet between viper populations by fitting Bayesian Ordination and Regression Analysis (boral) models using the boral package in R with default parameters for controlling the Markov chain Monte Carlo sampling (Hui, 2016, 2018). To find species explaining differences among populations in species composition of the diet, we used Generalized Linear Models for Multivariate Abundance Data (manyglm) models with negative binomial error distribution and a log link, with unknown overdispersion parameter, using the mvabund package in R (Wang et al., 2012). Species for which deviance estimated by the manyglm was significant were inferred to cause the differences in species composition of the diet between the populations.

To analyse how prey traits influence prey selection by the viper (question 3), we first prepared a dataset as follows: (a) we listed

Orthoptera species separately for each viper population, (b) then, we added age/sex information to each Orthoptera species as was found in the reference material; (c) then, we joined each viper individual studied to each of the age/sex categories of Orthopterans, (d) added presence/absence information (0 or 1, respectively) to each line based on whether the Orthopteran age/sex category was found in the faecal samples or not, (e) added the abundance information to each Orthopteran age/sex category based on the number of specimens found in faecal samples of each viper individual and finally, (f) joined the traits of the Orthopteran species and the viper predators to each observation. Prey traits included the relative abundance of species in the Orthoptera community reference material, mean AVS, trait principal components PC1-4 and microhabitat preference. Predator traits included sex, snout-vent length, head width and Julian date of sampling.

We analysed how prey traits influence prey selection by building two similar models, a glmm for the presence/absence of prey in the diet and a manyglm for the abundance of prey in the diet. In the glmm, presence/absence of prey in the diet was the binary dependent variable, and prey traits were fixed explanatory variables, while the identity of vipers was a random factor to control for the nonindependence of observations of different prey items from the same viper individual. We fit the glmm with binomial error distribution using the lme4 package (Bates et al., 2014). After fitting the glmm, we calculated the relative importance of explanatory variables in a model selection approach to identify models with substantial empirical support based on Akaike differences ($\Delta_i = AIC_i - AIC_{min} < 2.0$) in an information-theoretic framework (Burnham & Anderson, 2002) using the MuMIn package in R (Bartoń, 2018). In the manyglm, we specified negative binomial error distribution and a log link function with an unknown overdispersion parameter. The abundance of prey in the diet was the continuous dependent variable, and prey traits were fixed explanatory variables. We used the mvabund package for manyglm

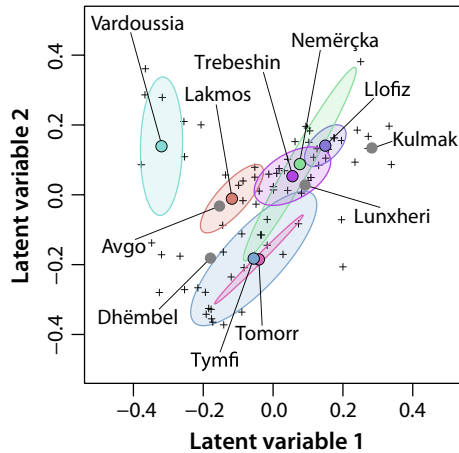


FIGURE 4 Bayesian ordination of viper diet samples, estimated centroids and confidence intervals (95%) of the studied populations

and applied the ANOVA.manyglm function to compute an analysis of deviance table for the manyglm models (Wang et al., 2012).

Finally, we tested the influence of predator traits (question 4) by using a piecewise regression to assess the relationship between body size variables (snout-vent length, body mass and vipers' head width) as independent variables and prey body diameter as dependent variable. We used changepoint estimation and fitted nonlinear least squares to visualize the relationship using the colf (Boutaris, 2017) and splines2 (Wang & Yan, 2018) packages in R. All statistics and figures were produced in R 3.5 (R Core Team, 2018).

3 | RESULTS

We captured 290 individuals of *Vipera graeca* and collected faecal samples from 78 individuals (21 juveniles, 15 males and 42 females) in 14 of the known 16 populations. The remaining 212 individuals (73%) had empty guts (41 juveniles, 66 males and 105 females). We found a temporal pattern in feeding activity because the proportion of individuals that had food in their stomach increased from June, peaked in July at 56% and decreased afterwards (Figure 3; glmm-1: $Z = 3.054, p = .002$).

We detected 356 prey items in the faecal samples, of which 96.3% was Orthoptera, 3.1% Araneae (*Drassodes* sp., Gnaphosidae; *Alopecosa* sp., Lycosidae, *Thanatus coloradensis*, Philodromidae), and we found one specimen of Coleoptera (*Otiorhynchus* sp., Curculionidae; 0.3%) and Scorpiones (*Euscorpis* sp.; 0.3%). No vertebrate prey was found in the samples. In the Orthoptera community reference material, we collected 1,190 specimens of 55 species as potential Orthoptera prey. With the aid of the reference material, we were able to identify 35 Orthoptera species from the faecal samples.

Viper diet was dominated by Orthoptera in all study populations; however, species composition in the diet differed between sampling sites (Figure 4). The manyglm-based deviance analysis showed that differences in the diet between populations were explained by the presence of *Psorodonotus macedonicus* (dev = 39.370, $p = .001$), *Chorthippus*

willemsei (dev = 34.869, $p = .001$), *Modestana ebneri* (dev = 33.917, $p = .002$), *Peripodisma llofizi* (dev = 31.364, $p = .003$), *Decticus verrucivorus* (dev = 26.093, $p = .011$), *Parnassiana coracis* (dev = 25.787, $p = .016$) and *Stenobothrus rubicundulus* (dev = 23.859, $p = .031$). In general, these species were more frequent in the vipers' diet, whereas eight other species (mostly *Chorthippus* spp.) were less frequent than could be expected based on their frequency in the Orthoptera community reference material (Figure 5). The two largest sized bush-cricket species (*Decticus verrucivorus* and *Psorodonotus macedonicus*) were more common in faecal samples than in the reference material, whereas the three most abundant species (all small-bodied: *Euchorthippus declivus*, *Chorthippus mollis* and unidentified *Chorthippus* nymphs) were almost missing from the diet of *V. graeca* (Figure 5).

The GLMM model selection on the presence/absence of prey in the diet found no better model than the full model, in which all explanatory variables had significant effects (Table 2, glmm). A similar model on the abundance of prey in the diet showed similar results (Table 2, manyglm). In both models, body size (AVS), 'sluggishness' and 'flightlessness' positively affected prey selection, while 'ability of quick jumps' and the 'geo-chortobiont' and 'geobiont', indicating a microhabitat preference for bare ground and rock surfaces, negatively affected prey selection. Prey availability had significant positive effects, whereas 'ability of quick jumps' and 'geo-psammo-chortobiont' microhabitat preference had a significant negative effect on the presence of prey in the diet. There was a positive correlation between values predicted by the presence/absence-based and the abundance-based models for each age/sex category of each species (Figure 6a). This analysis confirmed a set of preferred species that was similar to that found by the deviance-based manyglm analysis (Figure 6a; *Parnassiana coracis*, *Psorodonotus macedonicus*, *Stenobothrus rubicundulus*, *Chorthippus willemsei* and *Modestana ebneri*). When we averaged these predicted values across viper populations, the Vardoussia population emerged as richest in preferred viper prey availability, followed by Lakmos and Trebeshin and the rest of all were at the lower end of the preference spectrum (Figure 6b).

The body size distribution of Orthoptera prey items was bimodal (Figure 7) with two main size categories: (a) small- and average-sized grasshoppers (e.g. *Omocestus haemorrhoidalis*, *Chorthippus* spp.) and small bush-cricket (e.g. *Modestana ebneri*, *Platycleis* sp.) with a mean \pm SE body diameter of 5.5 ± 0.15 mm and (b) large bush-cricket (e.g. *Decticus verrucivorus* and *Psorodonotus macedonicus*) with a mean \pm SE body diameter of 13.0 ± 0.75 mm. We found a nonlinear positive relationship between vipers' head width and body diameter of their Orthoptera prey (Figure 7), indicating that vipers become able to prey on larger prey when they reach 10.5 mm in head width ($p < .001$). We found no other sign of ontogenetic shift in the diet as *V. graeca* preys on Orthoptera at all ages (results not shown).

4 | DISCUSSION

The main novelty of this study is that it provides evidence that prey traits can be used to predict prey selection in a specialist predator

FIGURE 5 Relative abundance of prey species found in the diet of *Vipera graeca* and their availability in the habitat

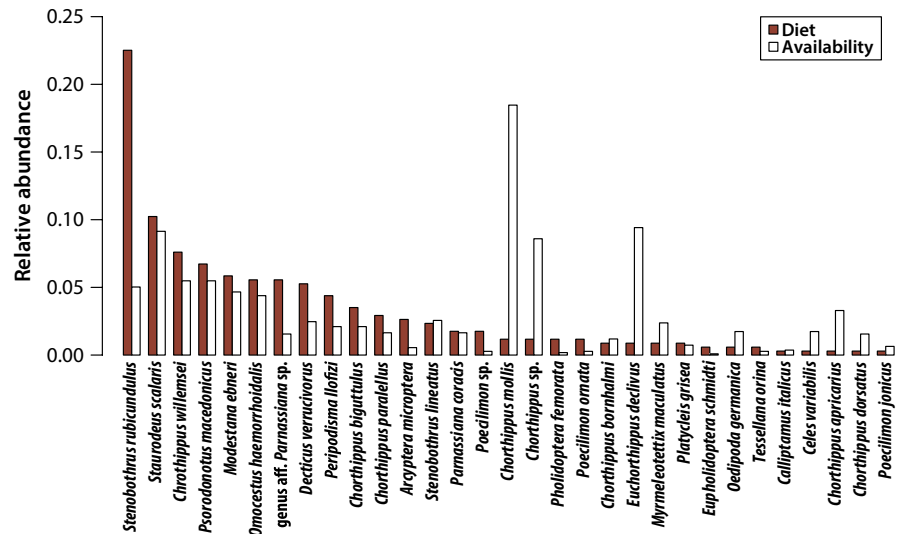


TABLE 2 Parameter estimates of prey selection models. Significant parameter estimates are highlighted in bold letters

	Presence-absence of prey in diet (glmm)				Abundance of prey in diet (manyglm)			
	Estimate	SE	Z	p	Estimate	SE	Z	p
(Intercept)	-12.931	1.601	-8.078	.000	-11.733	1.535	-7.644	.001
Availability	13.642	6.1	2.237	.025	7.275	5.791	1.256	.213
Area of visible surface	2.159	0.342	6.31	.000	1.924	0.323	5.951	.001
Trait PC								
Sluggishness	3.705	0.751	4.932	.000	3.307	0.718	4.605	.002
Flightlessness	2.442	0.694	3.518	.000	2.083	0.658	3.165	.005
Ability of long jumps	-10.214	2.692	-3.794	.000	-9.068	2.566	-3.534	.002
Ability of quick jumps	-2.404	0.964	-2.493	.013	-1.829	0.928	-1.971	.053
Microhabitat preference								
Chorto-thamnobiont	0.098	0.731	0.134	.893	0.539	0.735	0.734	.455
Chortobiont	-0.704	0.588	-1.197	.231	-0.093	0.601	-0.155	.862
Geo-chortobiont	-1.736	0.637	-2.725	.006	-1.488	0.649	-2.294	.017
Geo-psammo-chortobiont	-1.38	0.653	-2.114	.034	-0.854	0.659	-1.296	.198
Geobiont	-4.023	1.159	-3.471	.001	-4.016	1.185	-3.389	.001
Thamno-geobiont	-20.149	20,724.5	-0.001	.999	-11.676	188.9	-0.062	.950
Thamnobiont	-0.512	0.885	-0.579	.563	-0.021	0.861	-0.024	.978

(question 3). Beyond body size, several prey traits, related to the ability to escape from predators (sluggishness, flightlessness and ability for long/quick jumps) as well as prey microhabitat preference, were found to influence the presence/absence and number of prey in the predator's diet. To our knowledge, this is the first field-based empirical evidence of trait matching between prey and predators in a terrestrial predator-prey system. Our results show that the optimal prey of *V. graeca* is abundant, large-bodied, has poor escape abilities (slow-moving, flightless, bad jumper) and prefers loose grasslands as opposed to bare ground/rock or dense closed sward. Our work provides evidence on trait matching involving traits other than body size. Such trait matching was first reported in an experimental feeding trial of 20 carabid beetles and 115 of their prey species

(Brousseau et al., 2017), which found matching between predator bite force and prey cuticular toughness.

Our study is the first to present data on the diet of *V. graeca*. This species is unique, as it is an obligate insectivorous snake specializing on Orthoptera bush-crickets and grasshoppers in all known populations. The diet of other meadow vipers is also based on Orthoptera, but they also feed on lizards and rodents (Baron, 1992; Filippi & Luiselli, 2004; Starkov, Osipov, & Utkin, 2007). Although lizards (*Podarcis muralis*, *P. tauricus* and *Lacerta agilis*) and rodents (e.g. *Chionomys nivalis*) are present in *V. graeca* habitats (Mizsei, Jablonski, Végvári, Lengyel, & Szabolcs, 2017b; Stolarik, Gula, & Jablonski, 2017), the absence of vertebrate remains in faecal samples suggests that *V. graeca* is a dietary specialist on terrestrial arthropods.

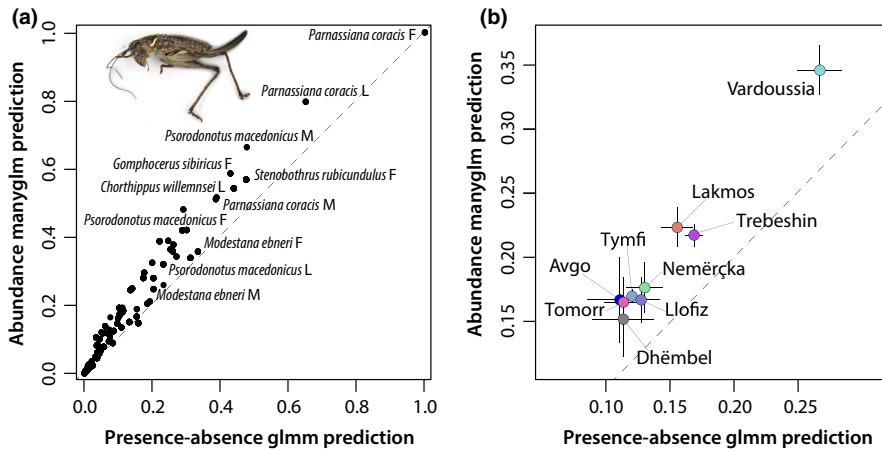


FIGURE 6 Predicted prey suitability values for each age/sex category of Orthoptera species (a) and averaged predictions (mean and SE) by Orthoptera species composition of the studied populations (b)

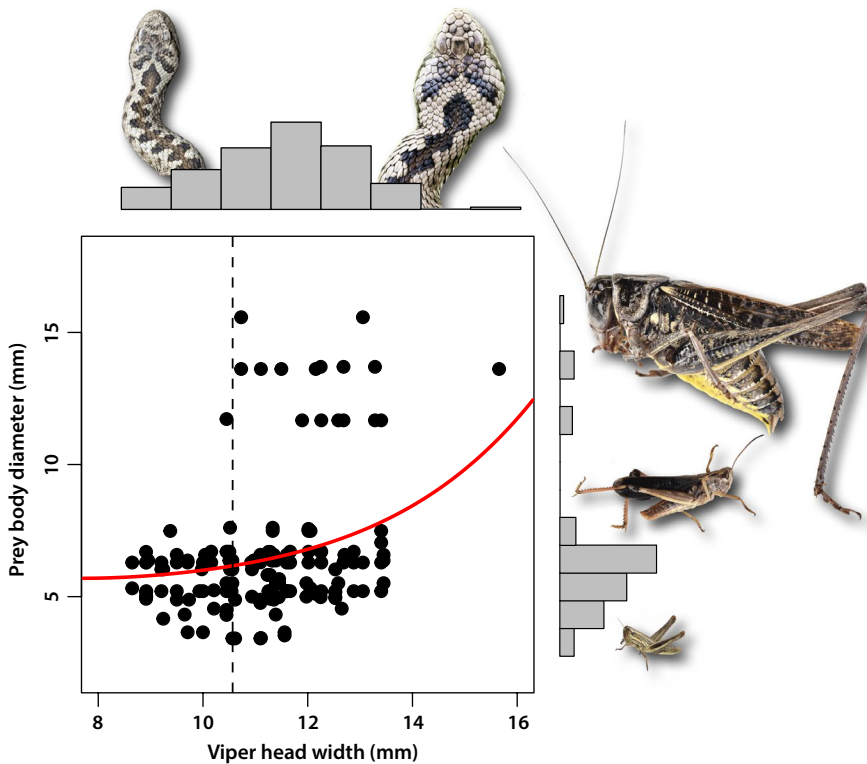


FIGURE 7 Prey body diameter as a function of viper head width; vertical dashed line indicates breakpoint of piecewise regression

We also found that (a) the feeding activity of *V. graeca* is highly seasonal, with a peak in late summer, when Orthopterans are most abundant, (b) the species composition of the diet differed between the predator populations, mostly because several preferred prey species occurred only in a subset of the populations and (c) predator traits related to body size, such as head width, are important because they are directly related to the maximum gape width of the snake. Differences in diet composition among populations are likely explained by biogeographic differences in prey species composition among the populations. For example, *Parnassiana coracis* was found only on Vardoussia, whereas *Psorodonotus macedonicus* was found only on Avgo, Lakmos and Trebeshin mountains.

We found that feeding is almost or entirely paused for *V. graeca* during the spring months as individuals did not produce faeces under the same conditions as individuals did in the summer. Our results suggest that vipers start feeding in June, coinciding with the period

when Orthopteran nymphs start to develop into adults. The energetically profitable period for feeding is probably the second half of the summer (July and August), when large-sized Orthopterans are abundant. A low proportion of individuals with food in their gut, for example 10%–50% of several hundred individuals, is not unusual among snakes (Šukalo et al., 2014). Studies which used the palpation-regurgitation method to produce samples showed that the proportion of snakes containing food can vary considerably among seasons, with a low prevalence of food usually in spring (Brito, 2004) and that it also varies between populations in relation to food availability (Šukalo et al., 2014). Thus, we assume that when a snake did not produce faeces, it usually did not have food in its gut and that this reflects true dietary patterns.

Despite our results on diet and prey selection of *V. graeca*, still little is known on the foraging strategy of the species. Glaudas et al. (2019) found that ambush foragers feed on a wide range of prey

size, while active foragers tend to be more specialized in terms of prey size and determined the sister species *V. ursinii* as ambush forager as well as other *Vipera* species. However, our results show that prey selection of *V. graeca* significantly differs from that expected based only on the abundance of potential prey species, suggesting that it follows a more active foraging strategy on the ambush-active foraging mode gradient. We have only one direct observation of a *V. graeca* individual swallowing a locust relatively far from a typical hiding place; thus, we cannot confirm the exact foraging mode. Several personal observations (EM) of *V. ursinii rakosiensis* that showed hunting behaviour in captivity suggest an active foraging strategy for that subspecies. The high frequency of large prey with poor jumping ability in the diet of *V. graeca*, however, suggests that its predatory strategy includes a strike attempt to reach the focal prey before the prey can jump away, similarly to the case of rattlesnakes and kangaroo rats (Higham, Clark, Collins, Whitford, & Freymiller, 2017).

Our results regarding the importance of predator traits in prey selection match those of several previous studies. Our finding that the area of visible surface of prey positively influenced prey selection suggests that *V. graeca* prefers prey of larger size than what could be expected by chance. However, younger and smaller snakes are usually able to consume smaller prey only, and larger snakes consume large prey items, a pattern that appears general in snakes (Vincent et al., 2006). The positive nonlinear relationship between prey size and predator head width also shows that prey selection is constrained by the ability to swallow large prey (Shine et al., 1998). Baron (1992) also found that the smallest Orthoptera eaten by *V. ursinii* were 16 mm in total length, regardless of the size of the snake. Successful capture of prey larger than this probably requires larger gape size in snakes (King, 2002), which is supported by our finding that *V. graeca* has to reach a head size >10.5 mm to be able to swallow large bush-crickets. However, our data also show that large individuals still consume smaller prey (e.g. Dugan & Hayes, 2012). It has to be noted that we cannot exclude the possibility that small/juvenile insects or larvae, which have less chitinised cuticles than adult insects, are digested more thoroughly and are more difficult to detect in faecal samples (Pincheira-Donoso, 2008).

Our study used several methodological advances that may be useful in future studies. We developed a prey trait database from actual measurements of traits using the reference material collected in the field (see Supplementary Material); thereby, we minimized the possibility of bias that might have resulted from using literature data on traits. We used a noninvasive method to collect faecal samples and no animal was harmed or killed during this project, whereas regurgitation induced by palpation sometimes causes mortality to snakes. Finally, our application of generalized linear mixed-effects models to study the effects of prey availability and prey traits on prey selection led to biologically plausible relationships between prey traits and prey selection.

Beyond the significance of our results in feeding ecology, our findings also have conservation relevance. Snakes with dietary specialization are more prone to extinction than are generalists

(Filippi & Luiselli, 2000; Reed & Shine, 2002). Understanding dietary specialization should thus be added to relevant life history traits to develop an evidence-based, successful conservation management plan for this species. For example, populations whose habitat contains more of the preferred prey species (Figure 6) should enjoy higher conservation attention, while habitats with low quality in prey availability could be managed to enhance prey availability.

In conclusion, our study provides a novel analytical framework for studying trait matching between predators and their prey, which can be applied relatively easily in other predator-prey systems. Our results also reveal unprecedented details in understanding feeding activity and prey selection in a previously little-known snake. Our analysis of factors influencing prey selection supported the importance of prey availability, body size and other traits related to defence/escape from predators and prey habitat preference. Our results imply that *V. graeca* tends to select prey that are large, cannot effectively escape and live in loose alpine grasslands. In addition, our study also showed that predator body size and, particularly, head width is fundamental in determining prey selection and those vipers need to reach a certain body size to become able to catch feed on large prey. The analytical framework used here will hopefully serve as a model for future studies, and this study will thus contribute to the advancement of trait-based methods in functional and feeding ecology by improving our understanding of the matching of traits between predators and their prey.

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AUTHORS' CONTRIBUTIONS

E.M., Á.L.K. and G.P. conceived the ideas and designed methodology; all authors collected the data; E.M. and Á.L.K. analysed the data; E.M., Á.L.K. and S.L. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT

All data used in the analyses will be available from Dryad Digital Repository <https://doi.org/10.5061/dryad.5nj70ks> (Mizsei, Boros, et al., 2019).

R scripts are provided in the Supporting Information.

ORCID

Edvárd Mizsei  <https://orcid.org/0000-0002-8162-5293>

Ádám Lovas-Kiss  <https://orcid.org/0000-0002-8811-1623>

Márton Szabolcs  <https://orcid.org/0000-0001-9375-9937>

Szabolcs Lengyel  <https://orcid.org/0000-0002-7049-0100>

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